SPRINGER NATURE
Reference

Andreas Sing
Editor

Zoonoses: Infections Affecting Humans and Animals

Second Edition

Springer

Zoonoses: Infections Affecting Humans and Animals

Andreas Sing
Editor

Zoonoses: Infections Affecting Humans and Animals

Second Edition

With 97 Figures and 70 Tables

Springer

Editor
Andreas Sing
Bavarian State Institute of Health II
Dept. of Public Health Microbiology
Bavarian Health and Food Safety
Authority
Oberschleißheim, Bayern, Germany

Ludwig-Maximilians-Universität
Munich, Germany

ISBN 978-3-031-27163-2 ISBN 978-3-031-27164-9 (eBook)
https://doi.org/10.1007/978-3-031-27164-9

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Paper in this product is recyclable.

"To our families, teachers and colleagues – these are the ones on whose shoulders we are standing"

Preface to the Second Edition

Zoonoses: Infections Affecting Humans and Animals – A Focus on Public Health Aspects

"Habent sua fata verba" – at least some words have their own history and success stories, even when starting their career with one or more little errors at their coming to life. A striking example is the term "zoonosis." It was coined in 1855 by the German physician Rudolf Virchow, mainly known as father of scientific pathology, but also as an important political figure in nineteenth-century Germany. Prior to the widespread acknowledgment of microorganisms as causative agents of infectious diseases, Virchow introduced the term – without clearly defining it – basically through the back door, when writing a subchapter in the second volume of the "Handbuch der speciellen Pathologie und Therapie" entitled "Intoxicationen, Zoonosen und Syphilis" on "Infectionen durch contagiöse Thiergifte" and putting the term "Zoonosen" as explanation in brackets. Actually, he not even mentioned the term in his chapter except in its heading. Today we know that the underlying pathogenic concept of "Thiergifte," that is, animal poisons or toxins, as disease agents is mostly wrong as is Virchow's very first sentence stating that luckily the number of diseases transmissible from animals to humans is not very large – a gross underestimation since most infectious disease entities and their agents are in fact zoonotic.

Interestingly, the term "zoonosis" is derived from the Ancient Greek word ζῷον (living being, living creature, animal) with its root in ζῷος (living, alive, vivid). Etymologically, Virchow limited the concept of zoonoses to "animals." More than 100 years later in 1958, the Joint WHO/FAO Expert Committee decided to narrow down the scope of "zoonoses" even more by defining these as "diseases and infections which are naturally transmitted between vertebrate animals and man."

On the other hand – and even from the beginning of zoonoses as an object of scientific research – Virchow and his Canadian disciple William Osler, also a physician by training, very early recognized the need for interdisciplinary collaboration between human and veterinary medicine and also – probably even more importantly – the public health, social and political aspects of zoonotic diseases. Researching zoonoses over the decades consequently not only helped to gain deeper insights into infectious diseases – additionally fostered by enormous progress

especially in molecular typing-based techniques – but also changed the research object and broadened the perspective under which zoonoses were seen. In 1984, Calvin Schwabe, a public health-trained veterinarian, introduced the concept of "One Medicine" (as discipline) and the corresponding "One health" approach not only comprising humans and animals but also the environment in all its facets. Thus, the concept of zoonoses had to be expanded by ecological, environmental, even societal, and economical aspects. In this respect, Virchow's etymological rooting of the term "zoonosis" in ζῷον shifts from the narrow "animal" to the much wider "living being," such as in the constructs of "biosphere" or the more mythical "gaia" model (to stay with Ancient Greek-derived expressions or ideas and deities).

"Habent sua fata libelli" (Not only words, but also books have their history). After the publication of its first edition in 2014, the current One Health-based book became rapidly popular among readers worldwide. Since nature never stops creating new infectious challenges for both mankind and the animal kingdom, it became obvious – at latest during the COVID-19 pandemic with one of its very first epicenters being a live wildlife food sale market in China – that a new and completely revised edition of the current book had to be started. New chapters addressing different animal groups (e.g., camels, birds, and reptiles), additional merging and re-emerging pathogens such as Borna Virus, *Borrelia* spp., Coronaviruses, trichinae, *Chlamydia* spp. or staphylococci, or new perspectives, for example, ancient and reverse zoonoses, were incorporated into the second edition.

The authors were chosen from a variety of academic and professional backgrounds, from the fields of human and veterinary medicine, from universities and public health institutions, and from more than 20 countries of all continents except Antarctica. The underlying idea was not to get an encyclopedic review on all known zoonotic disease entities but to have a forum for identifying or discussing urgent issues of zoonoses under a public health perspective.

Accordingly, the main target groups are the respective scientific communities, medical and veterinary practitioners, their students, public health and veterinary public health practitioners as well as decision-makers in the field of public health and veterinary public health.

Finally, I like to thank all authors for their very valuable chapters written with both heart and brain in the midst of a pandemic in which most, if not all of us, were heavily involved in COVID19-related professional work. We all are very grateful to Dr. Silvia Herold from Springer for initiating the new edition and especially to Neha Thapa and her team from Springer Nature India for her tireless, prudent, and extremely dedicated support throughout the whole publication process.

Preface to the First Edition

Zoonoses are infectious diseases caused by microorganisms passing from animals to humans and vice versa. In the last few decades most emerging and re-emerging diseases were in fact either of zoonotic origin or zoonotic potential.

The term "zoonosis" was coined by the German physician Rudolf Virchow, mainly known as father of scientific pathology, but also as an important political figure in nineteenth-century Germany. Although rooted in a classical faculty-based university system, he and his Canadian disciple William Osler, also a physician by training, very early recognized the need for interdisciplinary collaboration between human and veterinary medicine and also – probably even more importantly – the public health, social and political aspects of zoonotic diseases. While the scientific basis for both of them was pathology, the rise of microbiology as a medical discipline allowed to put the focus on microorganisms as the obvious and easiest walkable bridge between human and animal infectious diseases. This is even more true since the advent of especially DNA-based typing techniques for analyzing microorganisms isolated from different species thus allowing to study their real zoonotic potential.

By incorporating life and social science subdisciplines (e.g., immunology or epidemiology) a systemic paradigm was introduced in medical science thus preparing the ground for inter- and transdisciplinary approaches both in human and veterinary medicine. A striking example for the consequences of this paradigm shift on a population level are the concepts of New Public Health.

Not at last driven by the need for global public health efforts to combat both real and anticipated releases from Pandora's box in an interconnected and globalized world the One Health concept rapidly gained momentum in the last decade after the establishment of the 2004 "Manhattan Principles."

This book is based on the One Health concept with a focus on the public health impacts of zoonoses, both medically and societally. Important aspects in understanding zoonoses are not restricted to more classical issues, for example, their epidemiology in both humans and animals or disease symptoms in the respective two-legged, four-, or more-legged, feathered or unfeathered species, but have to take into account molecularly based epidemiological data and systemic, for example, ecological approaches.

To give an impression of the wide range of zoonotic research issues, the authors of this book were chosen from a variety of academic and professional backgrounds, from the fields of human and veterinary medicine, from universities and public health institutions, and from all continents. The underlying ideas were not to get an encyclopedic review on all known zoonotic disease entities but to have a forum for identifying or discussing urgent issues of zoonoses under a public health perspective. Accordingly, the main target groups are the respective scientific communities, medical and veterinary practitioners, their students, public health and veterinary public health practitioners as well as decision-makers in the field of public health and veterinary public health.

Contents

Volume 1

About the Editor

Andreas Sing Prof. Andreas Sing, MD, PhD, MA, DTM&H, is currently Head of the Bavarian State Institute of Health and the Department of Public Health Microbiology at the Bavarian Health and Food Safety Authority (LGL). He studied Human Medicine at the Albert-Ludwigs-University of Freiburg/Germany and the University of Massachusetts Medical School in Worcester, MA, USA, as well as Sociology & Political Sciences at the University of Freiburg. He obtained a PhD degree in Sociology at the University of Augsburg/Germany. After a post-doc training at the Department of Innate Immunity/LPS at the Max Planck-Institute of Immunobiology, he obtained his DTM&H at the London School of Hygiene and Tropical Medicine in London, UK, habilitated in Medical Microbiology & Hygiene with studies on Yersinia Innate Immunity at the Max von Pettenkofer-Institute of the Ludwigs-Maximilian-University (LMU) of Munich and became board-certified in Medical Microbiology. He teaches Medical Microbiology at the LMU, is Head of the German National Consiliary Laboratory on Diphtheria as well as Deputy Head of the National Reference Lab on Borreliosis in the Robert Koch-Institute-run German reference lab network. He is a member of ESGBOR (European Study Group of Lyme borreliosis) and founder member of ESGPHM (European Study Group of Public Health Microbiology) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and serves as supervisor for the EPIET and EUPHEM fellowship organized by the ECDC (European Centre for Disease Prevention and Control).

Contributors

Fredrick M. Abrahamian David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Faculty, Department of Emergency Medicine, Olive View-UCLA Medical Center, Sylmar, CA, USA

Nikolaus Ackermann Dept. of Public Health Microbiology, Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

Franz Allerberger National Reference Laboratory/Centre for Listeria, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria

Jaffar A. Al-Tawfiq Infectious Disease Unit, Specialty Internal Medicine, and Quality and Patient Safety Department, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia

Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Aline S. de Aluja Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México City, Mexico

Benjamin D. Anderson Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, Gainesville, FL, USA

Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Natural and Applied Sciences, Duke Kunshan University, Kunshan, Jiangsu, China

Adwoa Asante-Poku Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Prince Asare Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Dominique Aubert Department of Parasitology-Mycology, EA 7510, SFR Cap-Santé, UFR Medicine, University of Reims Champagne Ardenne and National Reference Centre on Toxoplasmosis, University Reims Hospital, Reims Cedex, France

Sara Babo Martins Faculty of Medicine, Institute of Global Health, University of Geneva, Geneva, Switzerland

Zoltán Bagó National Reference Laboratory/Centre for Listeria, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria

Amber N. Barnes Department of Public Health, Brooks College of Health University of North Florida, Jacksonville, FL, USA

László Bartosiewicz Department of Archaeology and Classical Studies, Osteoarchaeological Research Laboratory, Stockholm University, Stockholm, Sweden

Markus Bauswein Institute of Clinical Microbiology and Hygiene, Regensburg University Hospital, Regensburg, Germany

Andreas Bauwens National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), Institute of Hygiene, University Hospital Münster, Münster, Germany

Anja Berger Bavarian Health and Food Safety Authority (LGL), National Consiliary Laboratory on Diphtheria, Oberschleißheim, Germany

Merle M. Böhmer Department of Infectious Disease Epidemiology and Surveillance, Data and Modelling Unit, Bavarian Health and Food Safety Authority, Munich, Germany

Jesse Bonwitt Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA

Department of Anthropology, Durham University, Durham, UK

Nicole Borel Institute of Veterinary Pathology, Department of Pathobiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Maria Borowiak Federal Institute for Risk Assessment, Department Biological Safety, National Study Centre for Sequencing in Risk Assessment, Berlin, Germany

Valeria Bortolaia Department of Bacteria, Parasites and Fungi, Statens Serum Institute, Copenhagen, Denmark

Leo Both St George's Medical School, University of London, London, UK

Henri-Jean Boulouis Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, Cedex, France

Enrico Brunetti WHO-Collaborating Centre for Clinical Management of Cystic Echinococcosis, Division of Infectious and Tropical Diseases, University of Pavia, IRCCS San Matteo Hospital Foundation, Pavia, Italy

Felicity Jane Burt Division of Virology, National Health Laboratory Service, Bloemfontein, South Africa

Division of Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

Mathias Büttner Faculty Veterinary Medicine, Institute of Immunology, University of Leipzig, Leipzig, Germany

Simone M. Cacciò Unit of Foodborne and Neglected Parasites, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

Arturo Carpio Escuela de Medicina, Universidad de Cuenca, Cuenca, Ecuador

G.H. Sergievsky Center, Columbia University, New York, NY, USA

Chao-chin Chang Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung, Taiwan

Bruno B. Chomel Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, Davis, CA, USA

Trudi A. Collet Tasmanian School of Medicine, University of Tasmania, Bernie, TAS, Australia

Elizabeth Cook International Livestock Research Institute, Nairobi, Kenya

Carlos L. Correa-Martinez National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), Institute of Hygiene, University Hospital Münster, Münster, Germany

Marialaura Corrente Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy

Scott B. Craig Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia

David A. B. Dance Lao Oxford Mahosot Hospital Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic

Centre for Tropical Medicine, University of Oxford, Oxford, UK

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Filipe Dantas-Torres Department of Immunology, Aggeu Magalhães Institute, Fiocruz, Recife, PE, Brazil

Jeffrey B. Doty Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA

Sandra Essbauer Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, German Centre for Infection Research, Munich, Germany

Christa Ewers Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Giessen, Germany

Anna Fahrion Institute of International Animal/One Health, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany

Caoimhe Nic Fhogartaigh Lao Oxford Mahosot Hospital Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic

Division of Infection, Barts Health NHS Trust, London, UK

Volker Fingerle National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany

Jennie Fischer Federal Institute for Risk Assessment, Department Biological Safety, Unit Food Microbiology, Pathogen-Host Interactions, National Reference Laboratory for Salmonella, Berlin, Germany

J. Ross Fitzgerald The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK

Agnès Fleury Instituto de Investigaciones Biomédicas, Departamento de Medicina Genómica y Toxicología Ambiental, Universidad Nacional Autónoma de México, Instituto Nacional de Neurología y Neurocirugía, Secretaría de Salud, México City, Mexico

Anthony R. Fooks Animal and Plant Health Agency (APHA), Surrey, UK

Dimitrios Frangoulidis Bundeswehr Medical Service Headquarters VI-2, Medical Intelligence & Information (MI2), Munich, Germany

Maria Fredriksson-Ahomaa Faculty of Veterinary Medicine, Department of Food Hygiene and Environmental Health, University of Helsinki, Helsinki, Finland

Conrad M. Freuling Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany

Angelika Fruth Robert Koch Institute, Department of Infectious Diseases, Unit for Enteropathogenic Bacteria and Legionella, National Reference Centre for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany

Andrew P. Gibson Mission Rabies, Cranborne, UK

Dominique Goedhals Division of Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

PathCare Vermaak, Pretoria, South Africa

Ellie J. C. Goldstein David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

R. M. Alden Research Laboratory, Santa Monica, CA, USA

María Ángeles Gomez Morales Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

Jean-Paul Gonzalez Emerging Diseases and Biosecurity, Metabiota Inc., San Francisco, CA, USA

Delia Grace Natural Resources Institute, Chatham Maritime, UK

International Livestock Research Institute, Nairobi, Kenya

Glenn C. Graham Faculty of Health, University of the Sunshine Coast, Sippy Downs, QLD, Australia

Gregory C. Gray Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Infectious Diseases, School of Medicine, University of Texas Medical Branch, Galveston, TX, USA

Luca Guardabassi Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark

Xinrong Guo Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Colby College, Waterville, ME, USA

Hafez M. Hafez Institut für Geflügelkrankheiten, Freie Universität Berlin, Berlin, Germany

Sonja Hall-Mendelin Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Kim Halpin Australian Centre for Disease Preparedness, CSIRO, Geelong, VIC, Australia

Blake M. Hanson Department of Epidemiology, Human Genetics and Environmental Sciences, University of Texas Health Sciences Center, Houston, TX, USA

Barbara Häsler Veterinary Epidemiology, Economics and Public Health Group, Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, UK

Rüdiger Hauck Department of Pathobiology and Department of Poultry Science, Auburn University, Auburn, AL, USA

Anna J. Henningsson Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden

National Reference Laboratory for Borrelia and Other Tick-Borne Bacteria, Department of Laboratory Medicine, Division of Clinical Microbiology, Region Jönköping County, Jönköping, Sweden

Sabrina Hepner National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany

Glen R. Hewitson Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Bernhard Hobmaier Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

Stefan Hörmansdorfer Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

Steliana Huhulescu National Reference Laboratory/Centre for Listeria, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria

Ruwani S. Kalupahana Department of Veterinary Public Health and Pharmacology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka

Iddya Karunasagar Nitte University, Mangalore, India

Ashley Kates Department of Epidemiology, University of Iowa, Lowa, IA, USA

Scott P. Kenney Center for Food Animal Health, Department of Veterinary Preventive Medicine, Department of Animal Sciences, The Ohio State University, Wooster, OH, USA

Cassandra A. Klostermann Department of Epidemiology, University of Iowa, Lowa, IA, USA

Frans van Knapen Faculty of Veterinary Medicine, Institute of Risk Assessment Sciences, Veterinary Public Health, Utrecht University, Utrecht, The Netherlands

Robin Köck National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), Institute of Hygiene, University Hospital Münster, Münster, Germany

Eric Koka University of Cape Coast, Cape Coast, Ghana

Annelene Kossow National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), Institute of Hygiene, University Hospital Münster, Münster, Germany

Ellen Krautkrämer Nephrology Center, University of Heidelberg, Heidelberg, Germany

Marco Lalle Unit of Foodborne and Neglected Parasites, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

Marina C. Lamparter Federal Institute for Risk Assessment, Department Biological Safety, Unit Food Microbiology, Pathogen-Host Interactions, National Reference Laboratory for Salmonella, Berlin, Germany

Anders Rhod Larsen Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark

Jesper Larsen Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark

Shana R. Leopold Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA

Frederic Lohr Mission Rabies, Cranborne, UK

Antina Lübke-Becker Institute of Microbiology and Epizootics, Center for Infection Medicine, Freie Universität Berlin, Berlin, Germany

Veterinary Centre for Resistance Research (TZR), Freie Universität Berlin, Berlin, Germany

Gavin Macgregor-Skinner Department of Public Health Sciences, College of Medicine, The Pennsylvania State University, Hershey, PA, USA

Tommaso Manciulli WHO-Collaborating Centre for Clinical Management of Cystic Echinococcosis, Division of Infectious and Tropical Diseases, University of Pavia, IRCCS San Matteo Hospital Foundation, Pavia, Italy

Gabriele Margos National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany

Mateusz Markowicz AGES - Austrian Agency for Health and Food Safety, Vienna, Austria

Andrea M. McCollum Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA

David B. McKay Faculty of Health, University of the Sunshine Coast, Sippy Downs, QLD, Australia

Jamie L. McMahon Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Bastiaan G. Meerburg Livestock Research, Wageningen University & Research Centre, Wageningen, The Netherlands

Dutch Pest & Wildlife Expertise Centre (KAD), Wageningen, The Netherlands

Alexander Mellmann National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), Institute of Hygiene, University Hospital Münster, Münster, Germany

Ziad A. Memish Director Research Center, King Saud Medical City, Ministry of Health, Riyadh, Saudi Arabia

Al-Faisal University, Riyadh, Saudi Arabia

Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Ute Messelhäusser Bavarian Health and Food Safety Authority (LGL), Oberschleißheim, Germany

Laurence Millon French National Centre for Echinococcoses, Franche-Comté University and University Hospital, Besançon, France

UMR CNRS 6249 Laboratoire Chrono-environnement, Bourgogne-Franche-Comté University, Besançon, France

Frederick A. J. Moore Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Peter R. Moore Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Lapo Mughini-Gras Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

Thomas Müller Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany

Rajeshwari Nair Department of Epidemiology, University of Iowa, Lowa, IA, USA

Yoshinori Nakazawa Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA

Randall J. Nett CDC, Fort Collins, CO, USA

Erdmute Neuendorf Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

Diane G. Newell School of Veterinary Medicine, University of Surrey, Guildford, UK

Alireza Nourian Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran

Stephen Osei-Wusu Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Albert D. M. E. Osterhaus University of Veterinary Medicine Hannover, Hannover, Germany

Isaac Darko Otchere Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Domenico Otranto Department of Veterinary Medicine, University of Bari, Valenzano, BA, Italy

Faculty of Veterinary Sciences, Bu-Ali Sina University, Hamedan, Iran

Paul Overgaauw Faculty of Veterinary Medicine, Institute of Risk Assessment Sciences, Veterinary Public Health, Utrecht University, Utrecht, The Netherlands

Grace Patterson Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Lukas Peintner Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, German Centre for Infection Research, Munich, Germany

Lyle R. Petersen CDC, Fort Collins, CO, USA

Michael Pietsch Robert Koch Institute, Department of Infectious Diseases, Unit for Enteropathogenic Bacteria and Legionella, National Reference Centre for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany

Ariane Pietzka National Reference Laboratory/Centre for Listeria, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria

Sonja Pleininger National Reference Laboratory/Centre for Listeria, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria

Edoardo Pozio Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

Sarah J. Prior Tasmanian School of Medicine, University of Tasmania, Bernie, TAS, Australia

Wolfgang Rabsch Robert Koch Institute, Department of Infectious Diseases, Unit for Enteropathogenic Bacteria and Legionella, National Reference Centre for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany

Leslie A. Reperant Pikado BV, Utrechtse Heuvelrug, The Netherlands

Florence Robert-Gangneux Department of Parasitology-Mycology, University of Rennes 1 – UFR Medicine and CHU de Rennes, INSERM U1085, IRSET (Institut de Recherche en Santé Environnement Travail), Rennes Cedex, France

Lucy J. Robertson Parasitology Laboratory, Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

Hendrik I. J. Roest Animal Supply Chain and Animal Welfare, Ministry of Agriculture, Nature and Food Quality, The Hague, The Netherlands

Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Jairo Alfonso Mendoza Roldan Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy

Paul A. Rota Division of Viral Diseases, Centres for Disease Control & Prevention, Atlanta, GA, USA

Peregrine Rothman-Ostrow Department of Livestock and One Health, Institute of Infection Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK

Chantal P. Rovers Department of Internal Medicine, Radboud Q fever Center of Expertise, Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands

Charles C. Rupprecht LYSSA LLC, Cumming, GA, USA

Jonathan Rushton Department of Livestock and One Health, Institute of Infection Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK

Hanns-Joachim Rziha Interfaculty Institute for Cell Biology, Department of Immunology, University of Tübingen, Tübingen, Germany

Konrad Sachse Department of RNA Bioinformatics and High-Throughput Analysis, Faculty of Mathematics and Computer Science, Friedrich-Schiller-Universität, Jena, Germany

Mo Salman Colorado State University, Fort Collins, CO, USA

Alireza Sazmand Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran

Katharina Schaufler Institute of Pharmacy, University of Greifswald, Greifswald, Germany

Institute of Infection Medicine, Christian-Albrecht University and University Medical Center Schleswig-Holstein, Kiel, Germany

Holger C. Scholz Robert Koch Institut, Berlin, Germany

Edda Sciutto Instituto de Investigaciones Biomédicas, Departamento de Inmunología, Universidad Nacional Autónoma de México, Mexico City, Mexico

Alvaro Aguilar Setién Unidad de Investigación Médica en Inmunología, Coordinación de Investigación, Instituto Mexicano del Seguro Social (IMSS, Mexico), Mexico City, DF, Mexico

Sandra Simon Robert Koch Institute, Department of Infectious Diseases, Unit for Enteropathogenic Bacteria and Legionella, National Reference Centre for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany

Andreas Sing Dept. of Public Health Microbiology, Bavarian Health and Food Safety Authority (LGL), National Consiliary Laboratory on Diphtheria and National Reference Center for Borrelia, Oberschleißheim, Germany

Samuel P. Smith St George's Medical School, University of London, London, UK

Tara C. Smith Department of Epidemiology, University of Iowa, Lowa, IA, USA

Kent State University College of Public Health, Kent, OH, USA

Katie Steneroden Colorado State University, Fort Collins, CO, USA

August Stich Department of Tropical Medicine, Klinikum Würzburg Mitte, Würzburg, Germany

Matthew J. Stuckey Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, Davis, CA, USA

Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, Cedex, France

Snorre Stuen Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Sandnes, Norway

Inga-Marie Sultana Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Dipendra Thapaliya Department of Epidemiology, University of Iowa, Lowa, IA, USA

Thanaporn Thongthum Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Natural and Applied Sciences, Duke Kunshan University, Kunshan, Jiangsu, China

Andrea Toledo Facultad de Medicina, División de Investigación, Universidad Nacional Autónoma de México, Mexico City, Mexico

Sajid Umar Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Natural and Applied Sciences, Duke Kunshan University, Kunshan, Jiangsu, China

Isabelle Villena Department of Parasitology-Mycology, EA 7510, SFR Cap-Santé, UFR Medicine, University of Reims Champagne Ardenne and National Reference Centre on Toxoplasmosis, University Reims Hospital, Reims Cedex, France

Harjeet Singh Virk Center for Experimental and Molecular Medicine, Amsterdam Infection & Immunity Institute, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands

Division of Microbiology, Portsmouth Hospitals University NHS Trust, Queen Alexandra Hospital, Portsmouth, UK

Nadine A. Vogt Department of Population Medicine, Ontario Veterinary College, Guelph, ON, Canada

Dominique A. Vuitton French National Centre for Echinococcoses, Franche-Comté University and University Hospital, Besançon, France

Jaap A. Wagenaar Division of Infectious Diseases and Immunology, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Wageningen Bioveterinary Research, Lelystad, The Netherlands

WHO Collaborating Center for Reference and Research on Campylobacter and Antimicrobial Resistance from a One Health Perspective/WOAH Reference Laboratory for Campylobacteriosis, Utrecht, The Netherlands

Birgit Walther Advanced Light and Electron Microscopy (ZBS 4), Robert Koch Institute, Berlin, Germany

Shylo E. Wardyn Department of Epidemiology, University of Iowa, Lowa, IA, USA

Steven L. Weier Tasmanian School of Medicine, University of Tasmania, Bernie, TAS, Australia

Lothar H. Wieler Robert Koch Institute, Berlin, Germany

Ian Woolsey Parasitology Laboratory, Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

Kush Kumar Yadav Center for Food Animal Health, Department of Veterinary Preventive Medicine, Department of Animal Sciences, The Ohio State University, Wooster, OH, USA

Dorothy Yeboah-Manu Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Sabine Zange Bundeswehr Institute of Microbiology, Munich, Germany

Part I

Zoonoses as Continuous Companions of Mankind and the Animal Kingdom

Ancient Zoonoses

1

"Les Liaisons Dangereuses"

László Bartosiewicz

Contents

Abstract

Archaeozoology is the study of animal–human relationships using the evidence of archaeological finds. Throughout the history of civilization human and animal welfare have become inseparable from each other. Microbiota found in and on all multicellular organisms include pathogens linking animals and humans not only to their environments but also to individuals of their own and other species. Animal paleopathology is traditionally based on the attempted identification of macromorphological symptoms of various infections on the excavated skeletal remains of various species. Osteological lesions caused by past animal disease reveal situated relations with humans, as many of them may be resulting from zoonoses shared between multiple species, including people. Interactive socio-ecological systems giving rise to zoonoses thus involve humans, animals, and pathogens in specific environments. While many such diseases first emerged with the onset of domestication and increasing social complexity, they are also caused by recent human infringements on the natural habitats of wild animals. Understanding animal disease in the distant past is indispensable in developing a

L. Bartosiewicz (✉)
Department of Archaeology and Classical Studies, Osteoarchaeological Research Laboratory, Stockholm University, Stockholm, Sweden
e-mail: laszlo.bartosiewicz@ofl.su.se

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_54

long-term, holistic perspective on zoonotic infections. Contextualizing scarce archaeozoological evidence for zoonoses in epidemiological terms should help identifying the factors that promote disease and understanding their dynamics.

Keywords

Archaeology · Zooarchaeology · Animal paleopathology · Human medicine · Veterinary medicine · Taphonomy · Skeletal lesion · Pathomorphology · Ancient DNA · Ancient RNA · Paleoparasitology · Mycobacterium sp. · Brucella sp. · Yersinia sp. · Bone inflammation · Arthritis · Dog · Horse · Environment

1.1 Introduction

Human and animal health represent integral aspects of history. An increasing number of modern diseases are zoonotic in nature. Today various forms of the common cold and tuberculosis (TB henceforth) are well-known adaptations of strains originating in species other than humans (Benatar 2007). The historical perspective of co-evolving human and animal diseases cannot be studied without better understanding animal disease in archaeology. This short study is a review of evidence related to animal paleopathology.

Human agency in fostering as well as handling and curing animal disease has recently gained center stage in archaeological research illustrating how ancient people and animals coexisted in their shared environments. A driving force behind pathogen evolution seems to be the general human modification of the environment since many emerging zoonoses have been associated with anthropogenic changes (Pearce-Duvet 2006).

Today, major factors contributing to the emergence of new zoonotic pathogens in human populations are linked to the intensification of agriculture (Jones et al. 2013) as well as increased contacts between humans and wildlife in certain regions (Daszak et al. 2001). Aside from the widely publicized consumption of bushmeat in many parts of the world, zoonoses have recently been exacerbated by the encroachment of human activity into shrinking wilderness areas as well as game straying into ever-expanding human habitats. Contemporary observations in North America show that TB in white-tailed deer is increasingly associated with the proximity of infected cattle herds and/or high areal densities of white-tailed deer (O'Brien et al. 2001). In the *British Isles,* badgers are well adapted as the primary host for bovine TB (Gallagher and Clifton-Hadley 2000).

Throughout history several epidemics have been documented or are hypothesized to have taken place. Most of their causative agents still remain speculative. Archaeological and historical data complemented by robust biomolecular methods can be used to demonstrate the significance of zoonoses in these episodes (Spyrou et al. 2019).

1.2 Research Materials and Methods

"Unlike medical and veterinary work on the living, we usually have calcified tissue and little else. In the future, we can perhaps expect increasing support from immunological and DNA evidence related to specific disease, but for the present we are left with little more than bones and teeth" (Brothwell 2008).

Archaeozoology (or zooarchaeology) is a discipline devoted to the study of relationships between humans and animals, typically using osteological evidence recovered from archaeological excavations. Among archaeological finds, lesions on animal remains have regularly been observed for well over a century, as many diseases cause bone involvement thus allowing research using macromorphological methods (e.g., Steenstrup 1870; Shufeldt 1893).

While human paleopathology can directly benefit from groundbreaking achievements in modern medicine, present-day veterinary research is divided between diverse animal species and is focused on diseases of economic impact rather than odd-looking skeletal disorders. Skeletal manifestations of TB have decreased from 5–9% to 0.5–1% in cattle during the first half of the twentieth century (Lignereux and Peters 1999). In addition to improving prophylaxis, this may be explained by changes in exploitation that reduced longevity (intensive fattening for beef, decreasing draught work). In addition, the spread of modern epidemics is routinely prevented by the mass culling of herds before individuals would develop skeletal lesions. The lack of modern clinical data regarding the appearance and frequency of osteological symptoms of zoonoses in animals thus frequently impedes their detection in archaeozoological assemblages (Bartosiewicz 2021a). Some pathomorphological observations on the human skeleton are thus taken as a proxy in the diagnosis of disease in other mammals.

The sheer nature of archaezoological find assemblages poses additional challenges. Taphonomy is the critical study of information loss caused by fragmentation, poor preservation, and partial recovery in excavated materials. Each of these reduces information of vital significance in archaeozoological inquiry (Bartosiewicz 2008a). The bulk of animal bone deposits were generated through ancient butchery, were prone to scavenging, multiple re-deposition, and other destructive taphonomic forces. Lesions are thus typically noted on isolated bone specimens recovered from fragmented and commingled food refuse. This precludes the reconstruction of diseased individuals in the absence of coherent background information on age, sex, and associated skeletal symptoms. These would be readily available in traditional, undisturbed human burials (Bartosiewicz 2002).

In want of complete skeletons, Upex and Dobney (2012) emphasize the need for large syntheses and detailed analyses in traditional, morphology-based animal paleopathology. Analyses of disarticulated animal bones need to concentrate on the identification, quantification, and mapping of bone lesions associated with infection across the skeleton (Bartosiewicz 2008b) and their correlation with locations of clinically documented skeletal involvement. This work should be supported by advanced biomolecular analyses (Bendrey et al. 2019).

Birds (and likewise mobile bats) present a high risk of zoonotic transmission due to the ease with which they can fly between a variety of habitats, including human settlements. In archaeology, however, their small bones are found in numbers only when targeted, high precision recovery techniques are used in the field (fine screening, water-sieving). Thus, the general underrepresentation of bird remains limits the discovery of pathological cases. Due to these difficulties, traditional, morphology-based animal paleopathology in general and avian paleopathology in particular have seen protracted methodological development compared to archaeozoology as a whole or cutting-edge research in human paleopathology (Thomas 2012; Gál 2013).

By the end of the past millennium, the identification of TB, the probably best researched zoonosis in archaeology, entered a new phase thanks to the identification of mycobacterial DNA and lipid biomarkers in human remains at the eighth century AD site of Bélmegyer–Csömöki domb, Hungary (Haas et al. 2000). The development of high-throughput sequencing has stimulated unforeseen progress in the analysis of ancient DNA and RNA (aDNA and aRNA henceforth), offering a subtle way of identifying causative agents in paleopathology and insights in their microbial evolution (Spyrou et al. 2019).

In aDNA research, there has been a continuing emphasis on the analysis of human samples in the study of zoonotic pathogens. Over two decades ago Lignereux and Peters (1999) lamented the financial limitations of using molecular genetics in routinely identifying TB in archaeozoological assemblages. Studies of aDNA and aRNA necessitate sophisticated analytical procedures using costly infrastructure whose funding is more easily justified in human paleopathology: the long-term evolutionary history of microorganisms is relevant to contemporary challenges in public health (Spyrou et al. 2019). History, however, has been shaped not only by pathogens infecting humans, but also those damaging domestic animals and crops (Balloux and van Dorp 2017). Some groundbreaking work in aRNA research has actually been carried out on plants (e.g., Rollo 1985).

Forces of taphonomy also impact biomolecular methods. The terms aDNA and aRNA refer to the surviving nucleic acids isolated from archaeological samples. *Post mortem* decay takes place both through host enzymes, microbiomes, and abiotic factors. Differential preservation can also distort research results. *Mycobacteria*, for example, have a thicker, hydrophobic, and thus more resistant cell wall than that of *Brucella*. The difference in preservation thus causes a bias in recovery, the *M. tuberculosis* complex (MTBC) being better represented in the archaeological record (Recht et al. 2020). The MTBC includes, among others, *M. tuberculosis*, *M. bovis*, and *M. africanum*. Additional DNA damage may be caused by microbial contaminants (Duchêne et al. 2020). The even faster decomposition of RNA limits the study of aRNA genomes, although recent studies suggest that given favorable conditions even RNA remains can survive for millennia (Smith et al. 2019).

As taphonomic factors determining nucleotide preservation vary dramatically by geological and climatic circumstances, there is no predictable correlation between the level of DNA/RNA damage and the chronological age of archaeological samples. For example, human bone preserved in a shell midden in Latvia yielded identifiable molecular evidence of *Yersinia pestis* after 5000 years of deposition

and 150 years of museum storage in Rudolf Virchow's collection in the *Berliner Gesellschaft für Anthropologie, Ethnologie und Urgeschichte* (Susat et al. 2021).

1.3 History of Zoonoses

During early prehistory, Paleolithic communities of hunter-gatherers tended to be small and probably came into contact rarely with one another. They were indubitably exposed to infections of animal origin, albeit episodically (Sabbatini and Fiorini 2015). Such zoonoses may have occurred through the handling and consumption of infected game, but the spread of disease was likely limited. Isolation would have contained epidemics in localized communities, as the propagation and expansion of pathogens depend on the frequency of contacts between individuals.

In the Old World, this modest conduit for human infection by wildlife was significantly amplified by the onset of domestication (Pearce-Duvet 2006) through increased contact with the vectors of animal illnesses: the ecological entanglements of humans and livestock have resulted in regular somatic exchange (Rosenberg 2020), which became a mutual source of infectious diseases (as well as traumatic injury) for both people and their domesticates. On a broader scale, the combined effects of a higher human population density supported by emerging agriculture, accompanied by close and regular animal–human contacts meant exposure to a much richer pool of dangerous pathogens. Archaeozoological analyses and cases of human TB in late Pre-Pottery Neolithic in the Levant and Northern Syria support the hypothesis that close coexistence between early domesticates and humans has contributed to the emergence of this zoonosis since prehistoric times (Horwitz and Smith 2000; Baker et al. 2017).

Urbanization also increased the risk of mutual infections between animals and humans. Tanga et al. (2022) identified predisposing factors for zoonotic diseases in the Roman cities of Pompeii and Herculaneum. In these crowded urban environments, animals played diverse roles. The intensity of animal-related commercial activities contributed to the risk of infections. The climate also favored the proliferation of parasites and pathogens. Basic hygiene was often ignored in food processing and distribution while the proper disposal of human waste remained to be solved. The contamination of drinking water also became inevitable.

Aside from the immediate physical environment, zoonoses may have been indirectly exacerbated by other natural and social cataclysms. The Black Death (1347–1350 AD), for example, can be seen as part of the Late Medieval Crisis (ca. 1300–1350 AD). Changes occurring until 1350 should be seen within the context of previous catastrophes such as the Great Famine (1315–1322), or the Magdalenian Flood around the end of July in 1342 (Paxinos 2017). Recently, Seetah et al. (2020) have developed an exemplary integrated predictive model of Rift Valley fever virus outbreaks by combining climatic data with those on landscape archaeology, historical sources, and human behavior.

The fact that domestication and urbanization have demonstrably increased the risk of zoonoses should not be treated a *topos*. Contemporary outbreaks have

redirected attention to the role wildlife may play in the evolution of pathogens infecting humans. Paleopathologists should consider wild animals – integral agents in past ecosystems – as sources of health hazard for both humans and their domesticates (Bendrey and Martin 2021).

Paleoparasitological research ought to be increasingly integrated in the study of zoonoses. It would be important to work toward a consistent global coverage in this field (Bendrey and Martin 2021). A recent overview of archaeological finds of zoonotic parasites (identified in association with humans) has demonstrated the key role parasites have played in the evolution and spread of new pathogens, particularly from the Neolithic Period onward (Ledger and Mitchell 2019). An emerging diachronic trend is already evident during prehistory: sediment analyses of the Neolithic Els Trocs cave in Spain show a significant increase in the abundance and diversity of parasites as pastoral resources were increasingly exploited with the advancement of time (Tejedor-Rodríguez et al. 2021).

In order to persist in small, early prehistoric human populations, a pathogen either had to cause chronic infection (surviving in the infected host for a longer time), or it had to spread to other species that served as reservoirs. Some obligate pathogens require more than a single host to fulfill their ontogenetic cycle. The actual host, supporting the adult form of the pathogen, is often a vertebrate, while the intermediate host (referred to as a vector) can also be an arthropod or mollusc (Balloux and van Dorp 2017). In intermediate hosts, pathogens could incubate until susceptible hosts were contacted/infected. Parasite remains are informative in investigating past zoonoses as they have diverse life cycles that often involve both animal and human hosts, while some are not host-specific (Ledger and Mitchell 2019).

In addition to pathogens shared by humans with invertebrates (acting as vectors or intermediate hosts for disease), over 3/4 of reservoir species of zoonoses are mammalian and two thirds of zoonoses have their origins in domesticates (Schwabe 1984; Morse et al. 2012). Risks of infection were multiplied by both human and animal contacts between ancient farming communities (Fournié et al. 2017). In Fig. 1a, the number of diseases shared between humans and common domestic animals (McNeill 1976) are plotted against the approximate time of domestication (calBCE, Zeder 2008). A high correlation between the two variables shows that the longer the shared history between an animal species and humans, the longer the list of bilateral infections.

While we share the greatest numbers of diseases with dog, the first ever domesticate, the four artiodactyl species of the so-called “Neolithic package” (livestock that were first domesticated in addition to dog: sheep, goat, cattle, and pig) form a cluster associated with more zoonoses than expected on the basis of their chronological positions (above the trend line in Fig. 1a). It is important to remember that this diagram shows only bilateral infections between humans and each animal species. In reality the picture is far more complex, following multiple pathways of disease. When various combinations between mammals are considered, humans share the most pathogens with even-toed ungulates (c.f. the “Neolithic package”), followed by rodents, carnivores, and primates (Morse et al. 2012). This is shown by the example of the three most commonly occurring *Mycobacterium* species and their

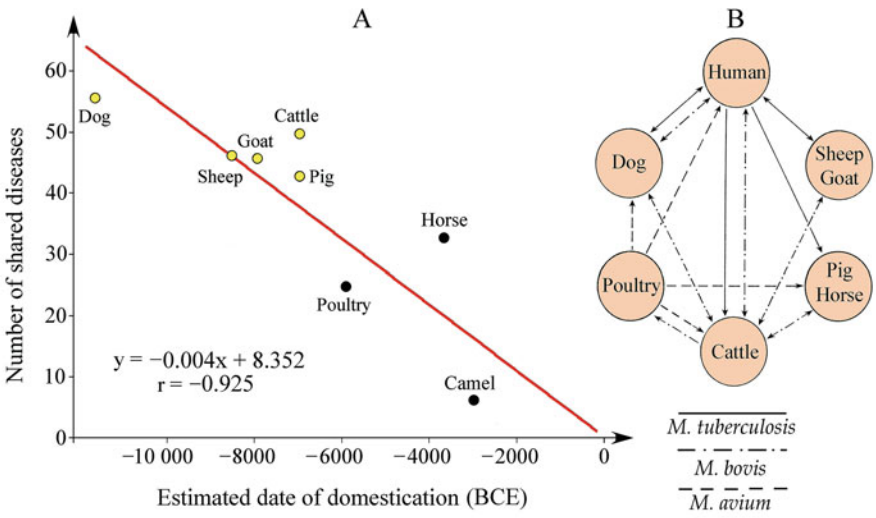

Fig. 1 (**a**) The number of diseases shared between humans and various domestic animals in relation to the time of domestication. Species in the Neolithic Package are marked by light dots; (**b**) Epidemiology of three common forms of *Mycobacterium* infections

hosts (among the best studied in archaeology) summarized in Fig. 1b (Manninger and Mészáros 1975; Lignereux and Peters 1999; Krauss et al. 2003). Based on the worldwide comparison of 14 selected bacterial and viral zoonoses each and four additional (mostly parasitic) zoonoses, Recht and co-workers (2020) warned of inaccuracies and inconsistencies in trying to exactly estimate ever-changing host diversity.

Numerous epidemics, recently recognized as zoonoses, also appear in the documentary record. While a detailed list would be beyond the scope of this chapter discussing archaeolzoological evidence, a few selected examples deserve attention.

In Europe, Thucydides was the first to report, in the *History of the Peloponnesian War*, how the plague of Athens (caused by the bacterium *Yersinia pestis*) devastated the city state in 430 BCE (Balloux and van Dorp 2017). Three major subsequent outbreaks have likewise been documented and even investigated using biomolecular methods on human bone (Wagner et al. 2014).

Cattle plague or *Rinderpest* (*Morbillivirus*) was documented in Asia as early as 3000 BCE, and mentioned in Greek and Roman sources around the beginning of the fourth century BCE (Spinage 2003; Pastoret et al. 2006). Although it is not a zoonotic disease in its modern form, measles likely evolved from cattle plague (Furuse et al. 2010), possibly as early as the fourth millennium BCE (Düx et al. 2020). Viruses seem to mutate at a higher rate than other pathogens thereby adapting to the human host, relying on interpersonal transmission without another reservoir species (Morse et al. 2012). Some experts have suggested that all human viral infections could be originally zoonotic (Benatar 2007).

Another virus infection, rabies (*Rabies lyssavirus*), long known to have spread through dog bites to humans and other animals, was described as early as the thirteenth century by, among others, Albertus Magnus (Walker-Meikle 2012). Archaeological evidence for rapid viral infections in animals is rare as it cannot be visually detected in skeletal remains and their molecular identification is hampered by poor nucleotide preservation.

1.4 Archaeozoological Evidence

Archaeologically, infections can only be identified on animal bones exhibiting a range of secondary macromorphological changes. Several microbes causing zoonoses represent a transition between specific and nonspecific sources of bone inflammation, since after localized beginnings they have a potential to invade the entire skeleton. Within the body, many zoonotic infections are spread by the vascular system through soft tissue foci to the skeleton. This process takes time. Some important zoonoses (e.g., *Erysipelothrix rhusiopathiae* seen mainly in growing pigs) produce acute symptoms rarely visible on the animal skeleton before they cause sudden death. Such diseases cannot be recognized through the visual inspection of archaeological bone finds.

Infections generally affect well-vascularized spongy trabecular bone in vertebral bodies, meta- and epiphyseal ends of long bones, ribs, sternum, and other flat bones, but may also occur under the periosteum (e.g., tuberculous periostitis). Infections by a variety of bacteria can cause tenosynovitis as well as bursitis, leading to osteological changes in the joints affected. However, predilection sites and the character of lesions differ both between pathogens and various host species (Nieberle and Cohrs 1970).

The taphonomic conundrum that symptoms can be observed only on isolated archaeological animal bones limits recognizing ancient MTBC, as only about 5% of known cases are believed to result in osteoarticular changes (Lignereux and Peters 1999; Donoghue et al. 2015). This small proportion shows how intangible even important infectious diseases can remain relying on the evidence of isolated bone finds alone. The macromorphological identification of general zoonotic infections tends to be more reliable in species seldom processed for food, their bodies being buried as a whole. Non-disarticulated skeletons of dogs and horses offer possibilities for better, comprehensive diagnoses.

Studies of human TB show how useful it is to have morphological observations supported by using aDNA (Masson et al. 2013). A case of Pott's disease from Alsónyék-Bátaszék (Köhler et al. 2014) was not only confirmed by the evidence of MTBC aDNA but tests helped detecting three additional humans in the burial group who displayed no visible skeletal lesions (Pósa et al. 2015). As of today, aDNA assays have only been sporadically carried out on animal bone to support macroscopic diagnoses (Rothschild et al. 2001; Bathurst and Barta 2004; Wooding 2010) and the molecular identification of MTBC in archaeozoological assemblages remains elusive. A rare example of testing animal rather than human remains for

microbial aDNA is the Iron Age assemblage from Wetwang Slack, United Kingdom, where aDNA samples from two horses, two cattle, and one pig showing symptoms of infection were tested for both MTBC and Brucella, the primary differential diagnosis for bovine TB (Wooding et al. 2019). Preservation allowing, fetal and neonatal animal bone finds should also be considered for aDNA analyses as possible indicators of abortifacient pathogens (Bendrey and Martin 2021).

1.5 Zoonotic Infections in Archaeology

Tuberculosis, caused by various species of the *Mycobacterium* genus, has been a focal topic in paleopathology. The oldest aDNA evidence for the MTBC in a wild bovine was detected in an American bison dated to ca. 17,000 BP (Rothschild et al. 2001). In the Old World, zoonotic TB infections are at least as old as dairying, a source of food already known in Early Neolithic Europe (Craig et al. 2005; Copley et al. 2003); historically, milk-borne transmission has been responsible for most human *M. bovis* infections (O'Reilly and Daborn 1995). Among livestock it is best known in cattle, its contemporary form being less frequent in sheep and goats. Available aDNA evidence suggests that *M. tuberculosis* in humans and cattle would have gone through a co-evolutionary process. The MTBC genome had a wide time-span to reach a suitable adjustment, co-evolving in geographical environments both at high and low host density (Sabbatini and Fiorini 2015). *M. tuberculosis* is an obligate pathogen that has no environmental reservoir, but numerous mammals and birds can also serve as reservoir hosts. The degree of mutuality and directions of TB infections vary between species as outlined in Fig. 1B. In addition to broadly varying degrees of infection in European livestock (Lignereux and Peters 1999), Mason (1917) reported a 2.8% prevalence of TB in dromedaries at a Cairo abattoir. He concluded that camel TB was caused by *M. bovis* and indicated that the confinement of camels and cattle together may be the source of cross-infection.

In the Americas, mounting pre-Columbian evidence of human TB (Braun et al. 1998; Buikstra 1999) has been indicative of alternative sources for this infection (Lignereux and Peters 1999). The only domestic mammal available to Americans before European contact, dog – as other carnivores – is not highy susceptible to TB (Fröhner and Zwick 1925). Thus it is important that hypertrophic osteopathy caused by TB in a sixteenth century Iroquoian dog skeleton from Southern Ontario, Canada, was directly supported by aDNA evidence (Bathurst and Barta 2004). Pre-Columbian mycobacterial infections in humans also indicate the implication of marine mammals in the zoonotic transmission of the MTBC between continents (Bos et al. 2014).

Avian TB is most often caused by *M. avium* and *M. genovense* (Tell et al. 2001) affecting domestic hen and captive wild birds (Cousins 2008). In the wild it has been observed in wading birds as well as diurnal raptors (Lignereux and Peters 1999). In birds, the lesion first occurs characteristically in the bone marrow, and usually affects the major bones in the leg (Baker and Brothwell 1980).

MTBC is spread through droplet infection and the consumption of infected animal products. When the bacterium is inhaled, pulmonary lesions develop. The aforementioned molecular evidence of TB in several Late Neolithic humans (first half of the fifth millennium BCE; Masson et al. 2013; Pósa et al. 2015) offer the earliest genetically confirmed evidence of TB in Europe. They indirectly support the morphological identification of a "tuberculous" cavern observed macroscopically on a contemporaneous cow metatarsus recovered from the Late Neolithic site of Berettyóújfalu–Herpály in Hungary (Hertelendi et al. 1998).

Human bones from the medieval churchyard of Wharram Percy, England, revealed *M. tuberculosis* aDNA in individuals who also displayed skeletal manifestations of the disease (Mays et al. 2001). Assays aimed at identifying bovine TB (*M. bovis*), however, turned out to be negative, somewhat surprising at this rural settlement where people probably relied on milk and beef. TB infections likely culminated around the industrial revolution when cows were kept even in crowded city centers to provide milk for likewise densely concentrated human populations (Mays 2005). Causing disease in a wide range of mammals, *M. bovis* has the broadest host range of the members of the *MTBC* (O'Reilly and Daborn 1995).

Lignereux and Peters (1999) reviewed the skeletal symptoms attributable to cattle TB, which generally affect well-vascularized trabecular bone in vertebral bodies as primary sites of predilection. Notably, while symptoms of TB commonly occur on both the vertebrae and ribs of animals, they seem unrelated to grave pleural lesions unless infection affects the lymphatic system (Nieberle and Cohrs 1970). In bovids, as in humans, various forms of bone resorption and lysis dominate (e.g., osteomyelitis, osteoporosis, and cavitation). Symptoms of advanced TB also include cavernous, purulent panosteitis with fistulation. Infiltrating TB in cattle may lead to excess bone growth that tends to connect articular surfaces (*arthritis tuberculosa pannosa*; Kardeván 1976) causing ankylosis, as between the three first thoracic vertebrae in a medieval (tenth to eleventh century) horse from Szombathely–Zanat, Hungary (Nyerges 2009). Although TB tends to be manifested in the cervical region in horse (Fröhner and Zwick 1925), this isolated specimen was found among food refuse, thus the involvement of neck vertebrae could not be appraised. The ankylosed vertebrae of this horse also show advanced fistulation (Fig. 2).

A special form, chronic miliary TB results from massive lymphohematogenous dissemination during which bacteria are spread from a single center of infection, producing small tubercles in other parts of the body (Nieberle and Cohrs 1970). A result of this process is the development of tuberculotic fibrous capsules, especially on ribs, reminiscent of callus formation following minor fractures, although these deformations caused by TB have a far softer, spongy structure. In Hungary, such lesions were observed on the ribs of two dogs from the Celtic-Roman village of Balatonlelle–Kenderföld (Daróczi-Szabó 2008) and in a tenth to twelfth century medieval dog in Buda Castle (Csippán and Daróczi-Szabó 2008).

TB usually does not lead to acute arthritis. Its osteological manifestation tends to be infiltrating exudative arthritis rather than acute inflammation in the joints. TB may also be accompanied by osteoporotic inflammation within the bone tissue that is almost always proliferative and leads to the buildup of hypertrophic osseous material

Fig. 2 Fistulized abscess on the ventral surface of three ankylosed thoracic vertebrae of a tenth to eleventh century horse. Cranial and right latero-ventral aspect (Photo: Tibor Tóth)

Fig. 3 First century AD Roman Period pig fifth metacarpus from Budapest–Aquincum, Hungary showing hypertrophic osteopathy. Medial, dorsal, and lateral aspects. (Photo: Alice M. Choyke)

(Wooding 2010). Hypertrophic osteopathy seems present on a Roman Period fifth metacarpal bone of pig found in a late first century pit dwelling in Budapest–Aquincum, Hungary (Fig. 3). Although TB is rarely manifested in the pig skeleton, over one third of the infections may cause skeletal symptoms in present-day pigs. Distal extremity segments (metapodia, including those in vestigial dewclaws) appear commonly affected (Kitt 1900). MTBC infections are capable of inducing this symptom in dogs as well (Bathurst and Barta 2004).

In chronic miliary TB the granulation tissue of the synovial membrane and epiphyseal trabecular bone progressively destroys and replaces the articular cartilage (Nieberle and Cohrs 1970). The concomitant necrosis of articular cartilage was observed in the right hip joint of a young pig from the eighth century, Avar Period settlement of Dunaújváros–Alsófoki-patak, Hungary. The originally clear contours of the bone were rearranged in an amorphous mass within the acetabulum. The new tissue buildup was dense enough to develop a secondary articular surface for the dislocated femur as shown by eburnation on the newly formed mass of bone that took over the acetabulum (Bartosiewicz 2013). Less extreme but similar deformations were noted on two pig pelvis fragments from the Eneolithic levels of Poljanitsa,

Bulgaria (Bartosiewicz et al. 2018). Since neither of the latter specimens was found as part of articulated skeletons, differential diagnoses include traumatic luxation compounded by chronic inflammation.

Advanced MTBC infections may be manifested on the diaphyses of long bones. The aforementioned metatarsus of a small cow from Late Neolithic Berettyóújfalu–Herpály, Hungary, shows proliferative/lytic osteomyelitis attributable to microbial infection. Cavitary osteolysis of this type is frequently accompanied by the formation of a wreath-like osteosclerotic ring around the tuberculous lesion (Fig. 4).

Unfortunately, such proliferative deformations are not specific to offending microorganisms, several could cause lytic anomalies similar in appearance. A definitive diagnosis of TB is not possible without confirming the presence of the pathogen microscopically or by biomolecular tests of a typical lesion.

While present-day brucellosis is the most commonly occurring bacterial zoonosis worldwide (Bendrey and Fournié 2020), it is poorly recognizable in the archaeozoological record. This zoonosis is a threat to all important species of livestock, although the risk of infection varies between *Brucella* species. *B. abortus* is a disease of large bovines (cattle, buffalo, bison), horse, and even

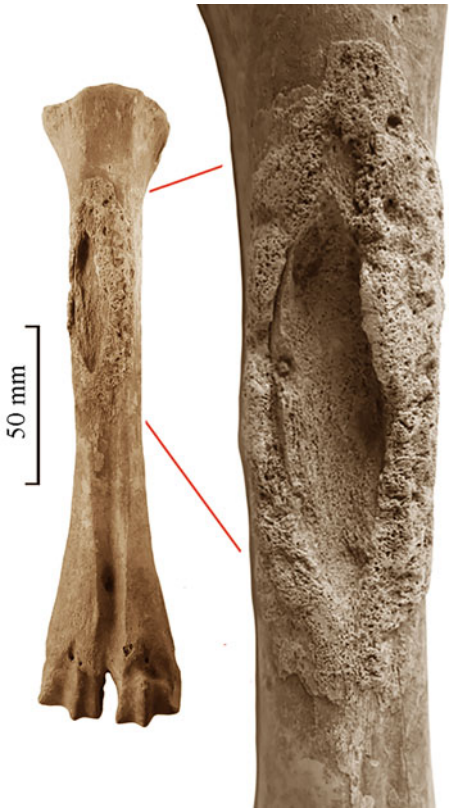

Fig. 4 Osteomyelitic cavern with sclerotic ring on the metatarsus of a gracile Late Neolithic cow from Berettyóújfalu–Herpály, Hungary. Anterior aspect. (Photo: Lajos Sugár)

dog, affecting small stock (pig, sheep and goats) rarely. *B. melitensis* can infect all bovids and camel, but is rarer in horse and pig. *B. suis*, *B. canis*, and *B. ovis* are largely specific to pig, dog, and sheep, respectively. Brucella species generally pathogenic to humans are *B. melitensis, B. abortus*, as well as biovars 1 and 3 of *B. suis* (Corbel 2006).

The impact on bone occurs in the form of focally extensive periosteal formations. Additional bone and joint involvement includes sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis. Osteomyelitis frequently affects vertebral bodies, especially in the lumbar section (Corbel 2006). Such lesions, however, may result from numerous other infections, making the study of aDNA evidence especially important. In a recent summary, Bendrey et al. (2019) cited only five human remains with confirmed biomolecular evidence for Brucella infection, and there are no published definitive cases of animal brucellosis from archaeological sites. Known archaeozoological remains for which brucellosis forms part of the differential diagnosis or as a possible cause are exclusively from horses. In the United Kingdom they originate from the site of Dragonby (Baker and Brothwell 1980), the Late Iron Age/Early Roman sites of Viables Farm and Downlands Farm (Bendrey 2008; Bendrey et al. 2008). A horse skull fragment from Arzhan 1, a site of Early Iron Age Scythian burials in the Tuva Republic, Russia, showed inflammation and necrosis following local infection described as "poll-evil" (Bendrey et al. 2011). Reflecting present-day public health concerns focused on humans, however, aDNA research has been quite anthropocentric in archaeology.

Among the most researched ancient zoonoses bubonic plague is caused by the bacterium *Yersinia pestis*. Although this acute disease kills the victim before leaving visible lesions on the bone, it is a zoonosis (Meerburg et al. 2009) archaeologically identifiable through aDNA sequencing. This bacterium has so far caused three genetically identified human pandemics of catastrophic consequences: the Plague of Justinian (sixth to eighth centuries), the Black Death (fourteenth to seventeenth centuries), and a third plague in the Modern Age (nineteenth to twentieth centuries; Wagner et al. 2014). *Y. pestis* is an obligate parasitic bacterium whose life cycle consists of alternating infections of rodents and fleas, but can infect essentially any mammalian host (Balloux and van Dorp 2017). A dramatic 1532 woodcut from Germany shows plague victims equally including people, a stallion, a dog, a cat, poultry, and even a small songbird (Fig. 5). It seems that the lineage of *Y. pestis* inducing the Plague of Justinian differed from the lineage that caused the next pandemic eight centuries later (Wagner et al. 2014). Recently, *Y. pestis* has also been isolated from a prehistoric (5300–5050 calBP) human skull fragment recovered from the shell midden of Riņņukalns, Latvia, in the late nineteenth century (Susat et al. 2021). This case is the first in a series of ancient strains that evolved following the split between *Y. pestis* and its putative predecessor *Y. pseudotuberculosis* 7000 years ago (Susat et al. 2021). It is suggested that this early form of *Y. pestis* was perhaps less transmissible and virulent than later strains. The Riņņukalns case may be attributable to a single zoonotic event rather than an epidemic. *Y. pestis* infected people in Bronze Age Eurasia three millennia before written records of plague, the highly virulent flea-borne bubonic strain emerged only around 1000

Fig. 5 Plague scene in the 1532 German edition of Francesco Petrarca's "*De remediis utriusque fortunae*" (1492) Woodcut by the anonymous Master of Petrarch

calBCE (Rasmussen et al. 2015). Wagner et al. (2014) confirmed that rodents serve as reservoirs in the recurrence of diverse lineages of *Y. pestis* jeopardizing human populations. This falls in line with Rütimeyer's (1877) observation that beaver, a common host of *Y. pseudotuberculosis*, was the best represented game in the Riņņukalns archaeozoological assemblage (Susat et al. 2021). This is yet another example when aDNA tests carried out directly on archaeological animal bone could confirm and expand the interpretive framework of ancient zoonoses.

1.6 Differential Diagnoses

With a few notable exceptions, animal paleopathology has increasingly been studied by archaeologists specialized in osteology rather than veterinarians with clinical experience (Bartosiewicz 2019). Interpretations thus often lack a broader view on animal morbidity.

Differential diagnoses, however, would be of key importance in evaluating ancient zoonoses (Lignereux and Peters 1999; Lawler 2017; Bendrey and Martin 2021). Conditions potentially caused by multiple pathogens always need to be considered. Analogous osteomorphological manifestations of different diseases are the natural consequence of bone tissue having but a limited repertoire of responses to diverse pathological conditions. Typical zoonoses damaging the skeleton, i.e., visible in archaeozoological assemblages, may be understood in terms of inflammatory diseases caused by general infection (Baker and Brothwell 1980; Bartosiewicz 2013).

Inflammation leading to osteological changes such as bacterial arthritis can be caused by several pathogens not falling under the traditional concept of zoonoses as

they can be part of the normal microbiome inhabiting the oral, nasopharyngeal, and digestive tracts of the host. Lesions caused by bacterial vertebral osteomyelitis and septic arthritis are difficult to distinguish in animals as the primary causative pathogens vary both diachronically and by geographical region, affecting differential diagnosis in archaeozoological studies. Many such bacteria (e.g., *Staphylococcus aureus*, *Pasteurella multocida*) are opportunistic pathogens that can cause endemic disease and have recently been increasingly associated with epizootic outbreaks posing a high risk of becoming zoonotic pathogens (Jaffe 1972; Ho 1993; Rao et al. 2017; Wilson and Ho 2020). General inflammations leading to pyogenous osteomyelitis may also be caused by chronic infection by other bacteria (*Corynebacterium* sp., *Streptococcus* sp.; Lignereux and Peters 1999).

In the case of brucellosis, differential diagnoses of various morphological symptoms observed on Iron Age horses included infections caused by *Trueperella pyogenes*, *Mycobacterium bovis*, *Actinomyces bovis*, *Streptococcus zooepidemicus*, and *Aspergillus* sp. (Bendrey et al. 2008, 2011). In animal paleopathology such infections must be considered whenever localized osteological symptoms can be identified. However, it is in such cases when the scarcity of complete skeletal finds makes it impossible to decide whether a lesion encountered on an isolated bone fragment results from localized or general systemic infection.

Of the best known zoonoses in archaeology, MTBC and brucellosis may both cause cavernous, purulent panosteitis with fistulation. From the viewpoint of differential diagnosis it is of interest that paravertebral abscesses are more common in TB than in brucellosis (Aufderheide and Rodríguez-Martín 1998; Corbel 2006). The involvement of the skeleton is late during the pathogenesis of MTBC. Only 0.5–1% of present-day cattle and 8–9.5% of pigs display bone lesions, in part due to early slaughter. (Osteological manifestations are more common in birds, both wild and domestic, than in mammals: Nieberle and Cohrs 1970). In brucellosis, on the other hand, bone and joint involvement (sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis) may reach 40% of cases in humans (Corbel 2006). Baker and Brothwell (1980) suggest that there is also a greater incidence of periosteal proliferation in brucellosis than TB. Since pulmonary neoplasia may also induce hypertrophic osteopathy, it should also be considered in differential diagnoses of MTBC on dry bone (Snider 1971; Wooding 2010; Bathurst and Barta 2004).

While these difficulties frequently overshadow the visual evaluation of osseous lesions, they are not limited to the macromorphological study of zoonoses. Lawler et al. (2020) warn that potential contribution by soil microbiota should also be considered in differential diagnoses during the aDNA identification of pathogens.

1.7 Concluding Remarks

As with all historical studies, a better knowledge of ancient zoonoses should support informed decisions made both in the present and future (Bendrey and Martin 2021). During the history of civilization human and animal welfare have become inseparable from each other. The study of animal paleopathology shows a rich diversity of attitudes toward animals (Bartosiewicz and Gál 2018). As Benatar (2007) noted,

even if zoonoses seem inevitable, much suffering and damage caused by zoonotic diseases could probably have been prevented if humans had treated animals better.

While the complexities of public health phenomena are far too subtle to be characterized using the patchwork of archaeological evidence offered by animal paleopathology, contemporary epidemics clearly illustrate the large extent to which the health of people and animals are entangled. Studying archaeological occurrences is indispensable in understanding the emergence of zoonoses on a long-term chronological scale. It can also help nurturing a more objective attitude to such contemporary threats, minimizing defensive overreaction by the broader public (Bendrey and Fournié 2020).

Interdisciplinarity has long been a popular slogan in research. Bendrey and Martin (2021) also stress the importance of a holistic approach in the study of past zoonoses. Interpretations must be aimed at capturing the diverse factors influencing infections. Contextualizing scarce archaeozoological evidence for zoonoses in epidemiological terms should help identifying the factors that promote disease. Understanding the time-depth of ever-changing relationships between animals, humans, and their environment should help in generating hypotheses concerning the dynamics of zoonoses. Such hypotheses can then be tested using carefully compiled paleopathological evidence, both morphological and biomolecular, gained from parallel studies of animal and human remains. Archaeological applications of the One Health perspective (Bendrey et al. 2019) acknowledge the essential link between the well-being of animals, humans, and environment in an interdisciplinary setting. On the other side, in archaeology, the emerging posthumanist approach mirrors this integrative endeavor in the form of a widening spectrum of animal studies in humanities (Bartosiewicz 2021b).

References

Aufderheide AC, Rodríguez-Martín C (1998) The Cambridge encyclopedia of human paleopathology. Cambridge University Press, Cambridge

Baker JR, Brothwell D (1980) Animal diseases in archaeology. Academic, London

Baker O, Coqueugniot H, Dutour O, Chamel B, Coqueugniot É, Stordeur D, Le Mort F, Khawam R, Perrin P, Pálfi G, Gourichon L (2017) Prehistory of human tuberculosis: earliest evidence from the onset of animal husbandry in the near east. Paléorient 43(2):35–51

Balloux F, van Dorp L (2017) What are pathogens, and what have they done to and for us? BMC Biol 15:91

Bartosiewicz L (2002) Palaeopathology: similarities and differences between animals and humans. Anthropol Közl 43:29–37

Bartosiewicz L (2008a) Taphonomy and palaeopathology in archaeozoology. Geobios 41(1):69–77

Bartosiewicz L (2008b) Environmental stress in early domestic sheep. In: Miklíková Z, Thomas R (eds) Current research in animal palaeopathology, proceedings of the second animal palaeopathology working group conference. British Archaeological Reports, IS S1844. Archaeopress, Oxford, pp 3–13

Bartosiewicz L (2013) Shuffling nags, Lame ducks. The archaeology of animal disease. Oxbow, Oxford

Bartosiewicz L (2019) Animal palaeopathology: between archaeology and veterinary science. In: Peters J (ed) Animals: cultural identifiers in ancient societies? Verlag Marie Leidorf Gmbh, Rahden/Westfalen, pp 27–36

Bartosiewicz L (2021a) What is a rare disease in animal palaeopathology? Int J Paleopathol 33:13–24. https://doi.org/10.1016/j.ijpp.2021.02.001

Bartosiewicz L (2021b) Herding cats. Curr Swed Archaeol 29:56–71. https://doi.org/10.37718/CSA.2021.07

Bartosiewicz L, Gál E (2018) Introduction: care, neglect and the "osteological paradox". In: Bartosiewicz L, Gál E (eds) Care or neglect? Evidence of animal disease in archaeology. Oxbow Books, Oxford/Philadelphia, pp 1–4

Bartosiewicz L, Nyerges ÉÁ, Biller AZS (2018) Palaeopathology at the eneolithic tell settlement of polyanitsa (NE Bulgaria). In: Bartosiewicz L, Gál E (eds) Care or neglect? Evidence of animal disease in archaeology. Oxbow, Oxford/Philadelphia, pp 23–44

Bathurst RR, Barta JL (2004) Molecular evidence of tuberculosis induced hypertrophic osteopathy in a 16th-century Iroquoian dog. J Archeol Sci 31:917–925. https://doi.org/10.1016/j.jas.2003.12.006

Benatar D (2007) The chickens come home to roost. Am J Public Health 97(9):1545–1546. https://doi.org/10.2105/AJPH.2006.090431

Bendrey R (2008) A possible case of tuberculosis or brucellosis in an Iron Age horse skeleton from Viables Farm, Basingstoke, England. In: Miklíková Z, Thomas R (eds) Current research in animal palaeopathology. British Archaeological Reports, IS S1844. Archaeopress, Oxford, pp 19–26

Bendrey R, Fournié G (2020) Zoonotic brucellosis in long view: can the past contribute to the present? Infect Control Hosp Epidemiol 1–2. https://doi.org/10.1017/ice.2020.270

Bendrey R, Martin D (2021) Zoonotic diseases: new directions in human–animal pathology. Int J Osteoarchaeol. https://doi.org/10.1002/oa.2975

Bendrey R, Taylor GM, Bouwman AS, Cassidy JP (2008) Suspected bacterial disease in two archaeological horse skeletons from southern England: palaeopathological and biomolecular studies. J Archeol Sci 35:1581–1590. https://doi.org/10.1016/j.jas.2007.11.002

Bendrey R, Cassidy JP, Bokovenko N, Lepetz S, Zaitseva GI (2011) A possible case of 'poll-evil' in an early Scythian horse skull from Arzhan 1, Tuva Republic, Central Asia. Int J Osteoarchaeol 21:111–118

Bendrey R, Cassidy JP, Fournié G, Merrett DC, Oakes RHA, Taylor GM (2019) Approaching ancient disease from a one health perspective: interdisciplinary review for the investigation of zoonotic brucellosis. Int J Osteoarchaeol 30:99–108. https://doi.org/10.1002/oa.2837

Bos KI, Harkins KM, Herbig A, Coscolla M, Weber N, Comas I, Forrest SA, Bryant JM, Harris SR, Schuenemann VJ, Campbell TJ, Majander KA, Wilbur K, Guichon RA, Wolfe Steadman DL, Collins Cook D, Niemann S, Behr MA, Zumarraga M, Bastida R, Huson D, Nieselt K, Young D, Parkhill J, Buikstra JE, Gagneux SA, Stone C, Krause J (2014) Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. Nature 514(7523):494–497. https://doi.org/10.1038/nature13591

Braun M, Cook DC, Pfeiffer S (1998) DNA from Mycobacterium tuberculosis complex identified in North American, Pre-Columbian human remains. J Archeol Sci 25:271–277

Brothwell D (2008) Problems of differential diagnosis in Pleistocene mammal pathology. Vet ir Zootech 44(66):88–90

Buikstra JE (1999) Paleoepidemiology of tuberculosis in the Americas. In: Pálfi GY, Dutour O, Deák J, Hutás I (eds) Tuberculosis: past and present. Golden Book Publishers/Tuberculosis Foundation, Budapest, pp 479–494

Copley MS, Berstan R, Dudd SN, Docherty G, Mukherjee AJ, Straker V, Payne S, Evershed RP (2003) Direct chemical evidence for widespread dairying in prehistoric Britain. PNAS 100(4):1524–1529. https://doi.org/10.1073/pnas.0335955100

Corbel MJ (2006) Brucellosis in humans and animals. WHO, Genève

Cousins DV (2008) Avian tuberculosis. In: OIE Biological Standards Commission (ed) Manual of diagnostic tests and vaccines for terrestrial animals. Office International des Epizooties, Paris
Craig OE, Chapman J, Heron C, Willis LH, Bartosiewicz L, Taylor G, Whittle A, Collins M (2005) Did the first farmers of central and eastern Europe produce dairy foods? Antiquity 79:882–894
Csippán P, Daróczi-Szabó L (2008) Animal diseases from medieval Buda. In: Miklíková Z, Thomas R (eds) Current research in animal palaeopathology: proceedings of the second animal palaeopathology working group conference. British Archaeological Reports, IS S1844. Archaeopress, Oxford, pp 74–79
Daróczi-Szabó M (2008) Animal diseases at a Celtic-Roman village in Hungary. In: Miklíková Z, Thomas R (eds) Current research in animal palaeopathology: proceedings of the second animal palaeopathology working group conference. British Archaeological Reports, IS S1844. Archaeopress, Oxford, pp 57–62
Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78(2):103–116. https://doi.org/10.1016/S0001-706X(00)00179-0
Donoghue HD, Spigelman M, O'Gradya J, Szikossy I, Pap I, Lee OY-C, Wu HHT, Besra GS, Minnikin DE (2015) Ancient DNA analysis – an established technique in charting the evolution of tuberculosis and leprosy. Tuberculosis 95(Suppl 1):140–144. https://doi.org/10.1016/j.tube.2015.02.020
Duchêne S, Ho SYW, Carmichael AG, Holmes EC, Poinar H (2020) The recovery, interpretation and use of ancient pathogen genomes. Curr Biol 30(19):1215–1231. https://doi.org/10.1016/j.cub.2020.08.081
Düx A, Lequime S, Patrono LV, Vrancken B, Boral S, Gogarten JF, Hilbig A, Horst D, Merkel K, Prepoint B, Santibanez S, Schlotterbeck J, Suchard MA, Ulrich M, Widulin N, Mankertz A, Leendertz FH, Harper K, Schnalke T, Lemey P, Calvignac-Spencer S (2020) Measles virus and rinderpest virus divergence dated to the sixth century BCE. Science 368(6497):1367–1370. https://doi.org/10.1126/science.aba9411
Fournié G, Pfeiffer DU, Bendrey R (2017) Early animal farming and zoonotic disease dynamics: modelling brucellosis transmission in Neolithic goat populations. R Soc Open Sci 4:160943. https://doi.org/10.1098/rsos.160943
Fröhner E, Zwick W (1925) Lehrbuch der speziellen Pathologie und Therapie der Haustiere II. Seuchenlehre. Enke, Stuttgart
Furuse Y, Suzuki A, Oshitani H (2010) Origin of measles virus: divergence from rinderpest virus between the 11th and 12th centuries. Virol J 7(52):7–52. https://doi.org/10.1186/1743-422X-7-52
Gál E (2013) Pathological changes in bird bones. In: Bartosiewicz L (ed) Shuffling nags, Lame ducks. The archaeology of animal disease. Oxbow, Oxford, pp 217–238
Gallagher J, Clifton-Hadley RS (2000) Tuberculosis in badgers; a review of the disease and its significance for other animals. Res Vet Sci 69(3):203–217
Haas CJ, Zink A, Molnár E, Szeimies U, Reischl U, Marcsik A, Ardagna Y, Dutour O, Pálfi G, Nerlich AG (2000) Molecular evidence for different stages of tuberculosis in ancient bone samples from Hungary. Am J Phys Anthropol 113:293–304
Hertelendi E, Svingor É, Raczky P, Horváth F, Futó I, Bartosiewicz L (1998) Duration of tell settlements at four prehistoric sites in Hungary. Radiocarbon 40:659–667
Ho G (1993) Bacterial arthritis. In: McCarty DJ, Koopman WJ (eds) Arthritis and allied conditions, 12th edn. Lea and Febiger, Philadelphia, pp 2003–2024
Horwitz LK, Smith P (2000) The contribution of animal domestication to the spread of zoonoses: a case study from the southern Levant. Ibex J Mountain Ecol 5:77–84. Anthropozoologica 31. https://sciencepress.mnhn.fr/sites/default/files/articles/pdf/az2000n2a9.pdf
Jaffe HL (1972) Metabolic degenerative and inflammatory diseases of bones and joints. Lea and Febiger, Philadelphia

Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, McKeever D, Mutua F, Young J, McDermott J, Pfeiffer DU (2013) Zoonosis emergence linked to agricultural intensification and environmental change. PNAS 110(21):8399–8404. https://doi.org/10.1073/pnas.1208059110
Kardeván A (1976) A háziállatok kórbonctana 2 [The Pathology of Domestic Animals 2]. Mezőgazdasági Kiadó, Budapest
Kitt T (1900) Lehrbuch der pathologischen Anatomie der Haustiere I. für Tierärzte und Stidierende der Tiermedizin. Enke, Stuttgart
Köhler K, Pálfi G, Molnár E, Zalai-Gaál I, Osztás A, Bánffy E, Kirinó K, Kiss K, Mende B (2014) A late Neolithic case of Pott's disease from Hungary. Int J Osteoarchaeol 24(6):697–703
Krauss H, Weber A, Appel M, Enders B, Isenberg HD, Schiefer HG, Slenczka W, Graevenitz V, Zahner H (2003) Zoonoses. Infectious diseases transmissible from animals to humans, 3rd edn. American Society for Microbiology, Washington, DC
Lawler DF (2017) Differential diagnosis in archaeology. Int J Paleopathol 19:119–123
Lawler DF, Tangredi BP, Widga CC (2020) Organism migration in soils: should we be so comfortable with diagnosing ancient infectious diseases? Int J Osteoarchaeol 30:297–306. https://doi.org/10.1002/oa.2855
Ledger ML, Mitchell PD (2019) Tracing zoonotic parasite infections throughout human evolution. Int J Osteoarchaeol 1–12. https://doi.org/10.1002/oa.2786
Lignereux Y, Peters J (1999) Elements for the retrospective diagnosis of tuberculosis on animal bones from archaeological sites. In: Pálfi GY, Dutour O, Deák J, Hutás I (eds) Tuberculosis: past and present. Golden Book Publishers/Tuberculosis Foundation, Budapest, pp 339–348
Manninger R, Mészáros J (1975) A háziállatok fertőző betegségei [Infectious diseases of domestic animals]. Mezőgazdasági Kiadó, Budapest
Mason FE (1917) Tuberculosis in camels. Agric J Egypt 7:1–11
Masson M, Molnár E, Donoghue HD, Besra GS, Minnikin DE, Wu HHT, Lee OY-C, Bull ID, Gy P (2013) Osteological and biomolecular evidence of a 7000-year-old case of hypertrophic pulmonary osteopathy secondary to tuberculosis from neolithic Hungary. PLoS One 8(10):e78252. https://doi.org/10.1371/journal.pone.0078252
Mays S (2005) Tuberculosis as a zoonotic disease in antiquity. In: Davies J, Fabiš M, Mainland I, Richards M, Thomas R (eds) Diet and health in past animal populations. Current research and future directions. Oxbow Books, Oxford, pp 125–134
Mays S, Taylor GM, Legge AJ, Young DB, Turner-Walker G (2001) Paleopathological and biomolecular study of tuberculosis in a medieval skeletal collection from England. Am J Phys Anthropol 114:298–311
McNeill WH (1976) Plagues and people. Doubleday/Anchor, Garden City
Meerburg BG, Singleton GR, Kijlstra A (2009) Rodent-borne diseases and their risks for public health. Crit Rev Microbiol 35(3):221–270. https://doi.org/10.1080/10408410902989837
Morse SS, Mazet JA, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, Daszak P (2012) Prediction and prevention of the next pandemic zoonosis. Lancet 380:1956–1965
Nieberle K, Cohrs P (1970) Lehrbuch der speziellen pathologischen Anatomie der Haustiere 2. Paul Parey, Stuttgart
Nyerges ÉÁ (2009) Beszámoló egy Szombathely melletti Árpád-kori település állattartásáról [Report on the animal exploitation at an Árpád Period settlement near Szombathely], Paper presented at the 1st conference titled Fiatal Középkoros Régészek I. Konferenciája, Mátyás Király Múzeum, Visegrád, Hungary, 26–28 November 2009
O'Brien DJ, Fitzgerald SD, Lyon TJ, Butler KL, Fierke JS, Clarke KR, Schmitt SM, Cooley TM, Berry D (2001) Tuberculous lesions in free-ranging white-tailed deer in Michigan. J Wildl Dis 37:608–613
O'Reilly LM, Daborn CJ (1995) The epidemiology of Mycobacterium bovis infections in animals and man: a review. Tuber Lung Dis 76(Suppl 1):1–46
Pastoret P-P, Yamanouchi K, Mueller-Doblies UM, Rweyemamu M, Horzinek M, Barrett T (2006) Chapter 5: Rinderpest – an old and worldwide story: history to c. 1902. In: Barrett T, Pastoret PP,

Taylor WP (eds) Rinderpest and Peste des Petits ruminants virus plagues of large and small ruminants. Biology of animal infections. Academic/Elsevier, London, pp 86–104
Paxinos PD (2017) Die Archäozoologie der Pest. Die Auswirkungen des Schwarzen Todes (1347–1350) auf Tierhaltung und Viehnutzung im Gebiet des heutigen Deutschland. Documenta Archaeobiologiae 13. Verlag Marie Leidorf, Rahden/Westfalen
Pearce-Duvet JMC (2006) The origin of human pathogens: evaluating the role of agriculture and domestic animals in the evolution of human disease. Biol Rev 81:369–382. https://doi.org/10.1017/S1464793106007020
Petrarcha F (1532) Von der Artzney bayder Glück / des guoten vnd widerwertigen. Vnnd weß sich ain yeder inn Gelück vnnd vnglück halten sol. Heynrich Steyner, Augsburg
Pósa A, Maixner F, Mende BG, Köhler K, Osztás A, Sola C, Dutour O, Masson M, Molnár E, Pálfi G, Zink A (2015) Tuberculosis in late Neolithic-early copper age human skeletal remains from Hungary. Tuberculosis 95:518–522. https://doi.org/10.1016/j.tube.2015.02.011
Rao RT, Jayakumar K, Kumar P (2017) Bovine origin Staphylococcus aureus: a new zoonotic agent? Vet World 10(10):1275–1280. https://doi.org/10.14202/vetworld.2017
Rasmussen S, Allentoft ME, Nielsen K, Orlando L, Sikora M, Sjögren K-G, Pedersen AG, Schubert M, Van Dam A, Kapel CMO, Nielsen HB, Brunak S, Avetisyan P, Epimakhov A, Khalyapin MV, Gnuni A, Kriiska A, Lasak I, Metspalu M, Moiseyev V, Gromov A, Pokutta D, Saag L, Varul L, Yepiskoposyan L, Sicheritz-Pontén T, Foley RA, Lahr MM, Nielsen R, Kristiansen K, Willerslev E (2015) Early divergent strains of *Yersinia pestis* in Eurasia 5,000 years ago. Cell 163(3):571–582
Recht J, Schuenemann VJ, Sánchez-Villagra MR (2020) Host diversity and origin of zoonoses: the ancient and the new. Animals 10(9):1672. https://doi.org/10.3390/ani10091672
Rollo F (1985) Characterisation by molecular hybridisation of RNA fragments isolated from ancient (1400 BC) seeds. Theor Appl Genet 71:330–333
Rosenberg GN (2020) On the scene of zoonotic intimacies. Jungle, market, pork plant. Transgender Stud Q 7(4):646–656. https://doi.org/10.1215/23289252-8665341
Rothschild B, Martin L, Lev G, Bercovier H, Bar-Gal GK, Greenblatt C, Donoghue H, Spigelman M, Brittain D (2001) *Mycobacterium tuberculosis* complex DNA from an extinct bison dated 17,000 years before the present. Clin Infect Dis 33(3):305–311. https://doi.org/10.1086/321886
Rütimeyer L (1877) Ueber die Thierreste des Rinnekalns. Sitzungsberichte Dorpater Naturforscher-Gesellschaft 4:534–539
Sabbatini S, Fiorini S (2015) L'infezione da micobatteri nell'uomo preistorico. Un cammino parallelo (co-evoluzione) dimostrato già in epoche remote (*Mycobacterium* infection in prehistoric humans: co-evolution in remote ages). Infez Med 23(1):83–93
Schwabe CW (1984) Veterinary medicine and human health, 3rd edn. Williams & Wilkins, Baltimore
Seetah K, LaBeaud D, Kumm J, Grossi-Soyster E, Anangwe A, Barry M (2020) Archaeology and contemporary emerging zoonosis: a framework for predicting future Rift Valley fever virus outbreaks. Int J Osteoarchaeol 30:345–354. https://doi.org/10.1002/oa.2862
Shufeldt RW (1893) Notes on paleopathology. Popular Sci Monthly 42:679–684
Smith O, Dunshea G, Sindling M-HS, Fedorov S, Germonpre M, Bocherens H, Gilbert MTP (2019) Ancient RNA from Late Pleistocene permafrost and historical canids shows tissue-specific transcriptome survival. PLoS Biol 17. https://doi.org/10.1371/journal.pbio.3000166
Snider WR (1971) Tuberculosis in canine and feline populations. Am Rev Respir Dis 104:877–887
Spinage C (2003) Cattle plague. A history. Kluwer Academic/Plenum Publishers, New York
Spyrou MA, Bos KI, Herbig A, Krause J (2019) Ancient pathogen genomics as an emerging tool for infectious disease research. Nat Rev Genet 20(6):323–340. https://doi.org/10.1038/s41576-019-0119-1
Steenstrup JCHR (1870) Oversigt over det Kongelige Danske videnskabernes selskabs forhandlinger [Overview of the affairs of the Royal Danish Academy of sciences]. Bianco Lunos Bogtrykkeri, Kjøbenhavn, pp 17–19

Susat J, Lubke H, Immel A, Brinker U, Macane A, Meadows J, Steer B, Tholey A, Zagorska I, Gerhards G, Schmölcke U, Kalniņš M, Franke A, Pētersone-Gordina E, Teßman B, Tõrv M, Schreiber S, Andree C, Berzinņš V, Nebel A, Krause-Kyora B (2021) A 5,000-year-old hunter-gatherer already plagued by *Yersinia pestis*. Cell Rep 35(13):109278. https://doi.org/10.1016/j.celrep.2021.109278

Tanga C, Remigio M, Viciano J (2022) Transmission of zoonotic diseases in the daily life of ancient Pompeii and Herculaneum (79 CE, Italy): a review of animal–human–environment interactions through biological, historical and archaeological sources. Animals 12(213). https://doi.org/10.3390/ani12020213

Tejedor-Rodríguez C, Moreno-García M, Tornero C, Hoffmann A, García-Martínez de Lagrán Í, Arcusa-Magallón H, Garrido-Pena R, Royo-Guillén JI, Díaz-Navarro S, Peña-Chocarro L, Alt KW, Rojo-Guerra M (2021) Investigating Neolithic caprine husbandry in the Central Pyrenees: insights from a multiproxy study at Els Trocs cave (Bisaurri, Spain). PLoS One 16(1):e0244139. https://doi.org/10.1371/journal.pone.0244139

Tell LA, Woods L, Cromie RL (2001) Mycobacteriosis in birds. Rev Sci Tech 20:180–203

Thomas R (2012) Nonhuman paleopathology. In: Buikstra JE, Roberts CA (eds) The global history of paleopathology. Pioneers and prospects. Oxford University Press, New York, pp 652–664

Upex B, Dobney K (2012) More than just mad cows: exploring human-animal relationships through animal paleopathology. In: Grauer AL (ed) A companion to paleopathology. Wiley-Blackwell, Malden, pp 191–213. https://doi.org/10.1002/9781444345940.ch11

Wagner D, Klunk J, Harbeck M, Devault A, Waglechner N, Sahl JW, Enk J, Birdsell DN, Kuch M, Lumibao C, Poinar D, Pearson T, Fourment M, Golding B, Riehm JM, Earn DJD, DeWitte S, Rouillard JM, Grupe G, Wiechmann I, Bliska JB, Keim PS, Scholz HC, Holmes EC, Poinar H (2014) Yersinia pestis and the Plague of Justinian 541–543 AD: a genomic analysis. Lancet Infect Dis 14(4):319–326. https://doi.org/10.1016/S1473-3099(13)70323-2

Walker-Meikle K (2012) Medieval pets. Boydell Press, Woodbridge

Wilson BA, Ho M (2020) Pasteurella multocida: from Zoonosis to cellular microbiology. Clin Microbiol Rev 26(3). https://doi.org/10.1128/CMR.00024-13

Wooding JE (2010) The identification of Bovine tuberculosis in zooarchaeological assemblages, working towards differential diagnostic criteria. Unpublished PhD thesis, University of Bradford, Bradford

Wooding JE, King SS, Taylor GM, Knüsel CJ, Bond JM, Dent JS (2019) Reviewing the palaeopathological evidence for bovine tuberculosis in the associated bone groups at Wetwang Slack, East Yorkshire. Int J Osteoarchaeol 1–12. https://doi.org/10.1002/oa.2846

Zeder MA (2008) Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and impact. PNAS 105(33):11597–11604. https://doi.org/10.1073/pnas.0801317105

Reverse Zoonotic Transmission (Zooanthroponosis): An Increasing Threat to Animal Health

2

Benjamin D. Anderson, Amber N. Barnes, Sajid Umar, Xinrong Guo, Thanaporn Thongthum, and Gregory C. Gray

Contents

B. D. Anderson (✉)
Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, Gainesville, FL, USA

Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Natural and Applied Sciences, Duke Kunshan University, Kunshan, Jiangsu, China
e-mail: ander88@ufl.edu

A. N. Barnes
Department of Public Health, Brooks College of Health University of North Florida, Jacksonville, FL, USA
e-mail: amber.barnes@unf.edu

S. Umar · T. Thongthum
Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Natural and Applied Sciences, Duke Kunshan University, Kunshan, Jiangsu, China
e-mail: sajid.umar@dukekunshan.edu.cn; thanaporn.thongthum@dukekunshan.edu.cn

X. Guo
Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Colby College, Waterville, ME, USA
e-mail: carolg21@stanford.edu

G. C. Gray
Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China

Division of Infectious Diseases, School of Medicine, University of Texas Medical Branch, Galveston, TX, USA
e-mail: gcgray@utmb.edu

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_59

Abstract

The fundamental premise of the One Health concept is that the collective health of humans, animals, and their shared environments depends upon the interactions between each domain. When it comes to mitigating infectious disease risk at this interface, an integrated approach is necessary. While recognition of the threat of animal-to-human disease transmission (zoonosis) is widely understood, there has been less consideration of the potential harm that humans can have on animal populations through reverse zoonosis. This chapter presents evidence and descriptions of human-to-animal disease transmission spanning viruses, bacteria, parasites, and fungi among companion animals, livestock and poultry, and wildlife as well as recommendations for multidisciplinary strategies for prevention and control. Collaborative efforts between veterinary health, public health, and environmental health professionals are critical to address the anticipated increased risk of reverse zoonotic events.

Keywords

One Health · Zoonoses · Reverse zoonoses · Zooanthroponosis · Epidemiology · Public health · Veterinary health · Environmental health · Animal health · Virus · Bacteria · Fungus · Parasite · Spillover · Spillback · Pandemic · SARS-CoV-2 · H1N1pdm09 virus · MRSA

2.1 Introduction

2.1.1 Healthy Humans, Healthy Animals

Human health and animal health are closely connected. With rapidly growing global human and domestic animal populations, increasing human enchroachment of wildlife habitats, and rapid environmental change, the linkages between human,

animal, and environmental health are becoming more evident. Negative disturbances that impact the health of the environment or biological organisms can affect the equilibrium of their interactions (Alves and Policarpo 2018; Mi et al. 2016). Over the years, many individuals from varied disciplines have worked towards promoting awareness of these risks to mitigate their consequences, which has led to the development of the One Health approach (Europe, A.h. 2017). This multi-disciplinary framework recognizes that the complex relationships that exist at the human-animal-environmental nexus require innovative, holistic, and collaborative approaches to prevent disease threats for all species.

Cross-species transmission of microbes have been observed since humans domesticated animals (Kruse et al. 2004; Reperant et al. 2013). To date, most attention has been placed on the zoonotic movement of pathogens from animals to humans. However, microbes can also move from humans to animals. This reverse zoonosis transmission has two concerns. First, the infected animals can become ill and potentially die; second, the affected population of animals can become a pathogen reservoir from which novel agents may cause spillback into humans resulting in further human disease (Di Marco et al. 2020; Sooksawasdi et al. 2021).

While we have often focused upon zoonotic disease threats to humans, rapidly increasing human population densities and frequent contact with domesticated and wild animals make humans just as likely to be an incubator for pathogens that could be transmitted back to animals. In recent decades, population growth, environmental disruption, and the rise of industrial agriculture have altered the human-animal interface. This change has led to the emergence of several outbreaks of public health and veterinary importance, including influenza A viruses and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since the 1960s, researchers have documented cases of humans infecting wildlife, companion animals, and livestock with a wide range of pathogens, including viruses, fungi, protozoa, and bacteria. Evolutionary analyses of the 2002–2003 SARS outbreak indicated a bidirectional transmission between humans and animals. Studies of the H1N1 influenza pandemic (H1N1pdm09 2009) in 21 countries confirm that sufficient contact between human and non-human species can facilitate human-to-animal transmission (Scotch et al. 2011). Further, several reverse zoonotic events of SARS-CoV-2 in wildlife and companion animals highlight the risk of transmission via close contact with infected humans such as farmworkers (Forgie et al. 2011; Holyoake et al. 2011; Sweetline Anne et al. 2017), pet owners (Zhang et al. 2020; Zhao et al. 2014; Dundon et al. 2010), caretakers, and veterinarians (Martelli et al. 2019; Crossley et al. 2012). As of August 2022, human-adapted SARS-CoV-2 has infected at least 31 unique animals species in 39 countries (https://vis.csh.ac.at/sars-ani/) (Nerpel et al. 2022).

SARS-CoV-2 has demonstrated the potential of reverse zoonoses while also causing great loss to human life, the global economy, and our social and community networks. Several cases of dogs, cats, domesticated animals, and zoo animals testing positive for SARS-CoV-2 have occurred, primarily due to close

contact with infected humans (Kuchipudi et al. 2022; Munir et al. 2020; Santini and Edwards 2020). Transmission of SARS-CoV-2 from infected animals to humans is also suspected (Munir et al. 2020). In addition to human-to-animal direct transmission (e.g., by physical contact with domestic and wild animals), there remains the potential for animal transmission via indirect contact with contaminated human waste (e.g., nasal discharge, phlegm, saliva, blood, urine, and feces) (Franklin and Bevins 2020; He et al. 2021). Also, improper disposal of contaminated personal protective equipment (masks, face coverings, gloves, tissues, and wipes) constitutes a source of SARS-CoV-2 infection for animals. When not managed properly, these items are subject to open dumping, which pose risks of infection for synanthropic and wild animals in surrounding environments (Han and He 2021). Because animals often suffer a similar spectrum of disease as humans, they can serve as "sentinels" for threats within our living or working environments, just as humans may sometimes serve as sentinels for proximal animal health (Alves and Policarpo 2018). Diseases have an important impact on public health and the economy, as well as conservation of wildlife. Animals are an important resource for food, textiles, companionship, and assistance. Besides serving as a source of dietary protein, animal-based products are fundamental ingredients for both traditional and modern medicines. Healthy animals enable farmers to produce more meat, milk, eggs, and fish, with less environmental impact. Many owners depend on their animals for income; therefore, if an animal falls ill, it can create financial difficulties for the household. The veterinary and daily care expenses associated with animals can be cost-prohibitive for poorer households, making it harder to maintain optimal health and custody of the animals. The loss of food animals on account of poor health or disease can create additional public health issues, such as malnutrition, despite no inherent disease transmission risk.

The more than one billion people worldwide who work daily with food animals, or their products, are at particularly high risk for disease transmission. In addition to their personal risks, animal workers can be a bridge population for zoonotic pathogen transmission between animals and the general human population. For example, the spouses of swine workers have demonstrated elevated rates of seropositivity to swine influenza viruses (Gray et al. 2007). The rise in the global human population is also contributing to an increase in the numbers of companion animals. The significance of companion animals speaks to the strength of the human-animal bond. Studies have suggested positive health effects from such attachments, including improvements in physical and mental well-being (Friedmann and Son 2009). However, just like with food animals, good hygiene is vital to prevent the spread of disease to companion animals as well as to people within shared spaces. Maintaining a healthy diet, providing fresh drinking water, creating clean living conditions, and following recommended vaccination schedules can minimize the risk of animal illness (Leeflang et al. 2008).

2.1.2 What Are Zoonoses and Reverse Zoonoses?

Due to the close contact and proximity that humans and animals have had throughout our common history, many pathogenic microorganisms have co-evolved to successfully infect both types of host (Slingenbergh et al. 2004). Today, more than 200 different diseases are shared between people and animals with 60% of the organisms known to be pathogenic to humans classified as zoonotic (Taylor et al. 2001; Cleaveland et al. 2001). In fact, over 77% of pathogens that infect livestock are multiple species pathogens (Cleaveland et al. 2001). Diseases that can be transmitted between animals and people, or zoonoses, or anthropozoonoses, are found in livestock, poultry, wildlife, and companion animals (FAO-OIE-OMS 2019; Messenger et al. 2014). Although zoonotic diseases are primarily described as transmission occurring from an animal reservoir or host to a human, many of our shared pathogens can be spread bidirectionally, meaning contagion can also occur in animals after exposure to an infected human. This is referred to as reverse zoonoses, or zooanthroponosis (Messenger et al. 2014). Zoonotic microorganisms may cause infection in either or both groups with symptoms and severity of illness dependent upon intensity of pathogen exposure, pathogen virulence, and the immune status and overall health of the host. Types of zoonotic and reverse zoonotic pathogens include bacteria, viruses, protozoa, helminths, fungi, and prions (Slingenbergh et al. 2004; Taylor et al. 2001; Cleaveland et al. 2001). Zoonotic pathogens can be transmitted through indirect contact with the contaminated environment (i.e., soil), items (i.e. fomites), vectors (i.e. ticks), airborne particles or by direct contact with an infected host or host bodily fluids (i.e. petting an animal or droplet spread) (FAO-OIE-OMS 2019).

2.1.3 Why Is Knowledge of Reverse Zoonoses Important?

As human population and livestock production continue to expand, the risk of reverse zoonoses will increase as humans and animals have more frequent and closer contact. Knowledge and understanding of the epidemiological triad, which includes transmission factors associated with the host, pathogen, and environment, is essential for devising public health measures to aid in the prevention and control of infectious disease outbreaks. Most epidemiological studies aim to identify clinical and pathological features in infected species to determine host susceptibility (i.e., which species are capable of being hosts and which are susceptible to natural infections) (Sreenivasan et al. 2021). Early and accurate diagnosis could lead to the timely detection of outbreaks and ultimately decrease the impact of epidemics on human and animal populations (Steele et al. 2016). For public health interventions to be effective, knowledge of risk factors, transmission routes, and virulence factors between different species are crucial for estimating the magnitude of outbreak impacts and transmission rates (Kraemer

et al. 2020; Charu et al. 2017). For example, researchers and health providers should understand the probability of transmission of the pathogen, types of interaction between infected humans and recipient animals, the capability of the animal host factors for producing infection, and the suitability of the animal host population for pathogenic persistence (Sooksawasdi Na Ayudhya and Kuiken 2021).

The recognition of the role of humans as a source of pathogens is pivotal to public health measures as it has redefined vulnerable population groups to include animal species. Interspecies transmission may also contribute to an increase of genetic diversity in pathogens by providing more opportunities for genetic reassortment that could drive the evolution of pathogens (Chastagner et al. 2018; Nelson and Vincent 2015). These evolutionary changes in pathogens pose further challenges to the surveillance and outbreak controls such as reducing the efficacy of vaccines (dos Santos 2021; Lemaire et al. 2012) and increasing the possibility of the emergence of new enzootic strains in animal populations (Watson et al. 2015; Simon et al. 2014). Therefore, genomic sequencing studies help revise our understandings of the dynamics of human-animal transmission and contribute to improved forecasting models of outbreak impacts (Jia et al. 2021). Advancing our knowledge of reverse zoonoses is critical to the development of enhanced outbreak surveillance and biosecurity systems.

2.2 Routes of Transmission

The transmission of an infectious disease depends on the type of interactions that occur between humans, animals, and/or their environments. Compared to the better understood transmission routes of zoonotic diseases, human-to-animal transmission still merits more comprehensive investigation (Al-Tawfiq and Memish 2014). Given a global increase in industrial animal production, increasing proximity between humans and animals across multiple settings provides opportunities for humans to spread shared pathogens to animals through reverse zoonoses. Pathogen transmission generally occurs through the inhalation, ingestion, or contamination of mucous membranes or broken skin by respiratory droplets, bodily fluids, secretions, or other excretions (Pantin-Jackwood et al. 2010). Transmission of pathogens to animals may also occur through the ingestion of contaminated feed. The role of vectors in biologic or mechanical disease exposure has been documented but still requires further research (Patel et al. 2022). Ecological and viral characteristics can directly contribute to the transmission of causative pathogens in a host. Pathogens must be able to evade immune response and colonize the host, be nutritionally compatible with the host, be able to reproduce using host resources, and exit and spread to a new host (Alberts 2015). In addition, anthropogenic activities may facilitate favorable

environments for reverse zoonoses. Increasing intensity of interaction and proximity between humans and animals is found to be associated with disease emergence (Despommier et al. 2006).

2.3 Use of New Technology

While basic PCR measures and viral isolation approaches remain key to the surveillance and detection of pathogens, there have been several innovations that have further enhanced novel disease detection capabilities. A large variety of data mapping techniques are now available with disease tracing, which enable individuals to better visualize the spread of disease, susceptible animals, or infected humans with concurrent time and location tags. Such visualization not only assists epidemiologists in researching the movement of the disease but can also be used to educate policymakers and the public.

Compared to a general analysis of the overlap between host animals and human patients, genomic analysis has become more common to distinguish different pathogen strains. For example, genomic sequencing and phylogenic analysis have been widely used in current anthroponotic research. By building up the phylogeny of a core genome (Linz et al. 2018), the evolutionary relationship between different clones can be laid out for researchers and epidemiologists. These methods help to provide more substantial evidence for the transmission pathways of the disease. Such analysis enables health scientists to distinguish between diseases that co-infect animals and humans, zoonotic transmission, and reverse zoonotic transmission. Further analysis of the phylogenetic relationships between disease strains also aids researchers and health practitioners to better predict possible transmission routes, which can then be used for preventative measures.

Besides increased attention to the general phylogenetic relationships, detailed genetic studies have also been implemented. By comparing the results from DNA fingerprinting and repeated PCR results, researchers can recognize and begin to develop responses to mutations that occur during repeated or prolonged transmission (Hasan et al. 2018). This could provide information on highly conservative sequences or key mutations that could lead to improvements in transmissibility, lethality, and antimicrobial resistance (Hosie et al. 2021; Li et al. 2019). By analyzing the conservative sequences, it is possible for researchers to develop innovative testing methods that could remain effective despite the occurrence of further mutations. Sequence data also helps researchers to develop more effective vaccines. The inclusion of research findings examining possible differences among human and animal strains could drive the expansion of more targeted measures. Such information could also help us to predict the harm of specific pathogens or the impact we could expect from their spread to provide more information and urgency for higher-level policymaking.

2.4 Epidemiology of Zoonotic and Reverse Zoonotic Events (Supported with Heat Map and Frequency Map) (Fig. 1)

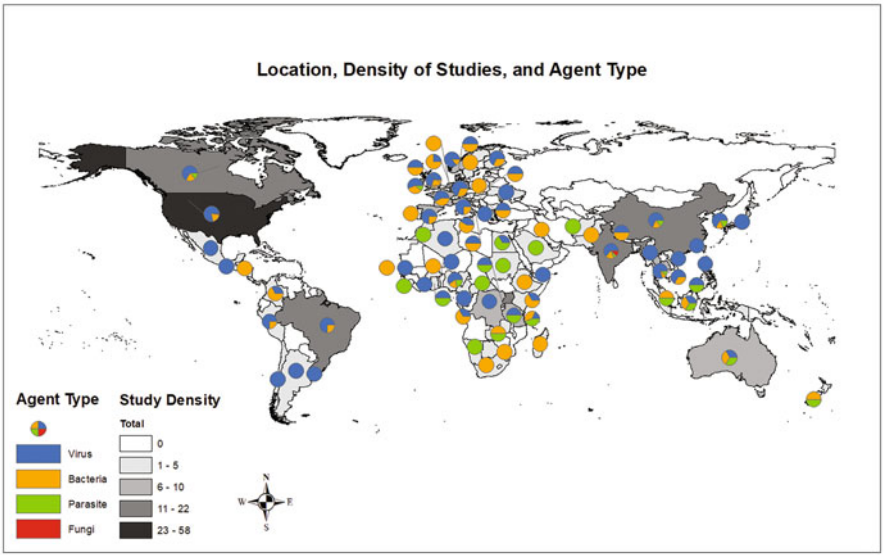

Fig. 1 Map of the density of studies by location and reverse zoonotic agent examined. Study locations without specific country names (ex. Antarctica) were excluded. Country delineation was compiled according to the 2021 World Bank standards. Map created in ArcMap 10.6 (ESRI, Redlands, CA); no copyrighted material was used. ESRI Environmental Systems Research Institute

2.5 Evidence of Reverse Zoonosis of Viruses and Disease Symptoms in Animals and Humans

The rapid increase in the global human population coupled with intensive and frequent traveling for trade, business, and recreational purposes has posed an increased threat regarding the transmission of viruses from humans to animals which may have a negative impact on biodiversity, wildlife conservation, and public health. Human-to-animal virus transmission jeopardizes the health and well-being of wild and domestic animals and the viruses which cause spillback (reverse zoonoses) to animals from humans may cycle back to infect humans again. Several barriers need to be crossed for a virus spillover or species jump between humans and animal species to occur. There must be frequent contact between humans and animals and sufficient compatibility between the virus and the new host to allow for attachment and replication. During the last decade, several reverse zoonoses events among humans, domestic, and wild animals have been reported (Sooksawasdi Na Ayudhya and Kuiken 2021; Messenger et al. 2014; Fagre et al. 2021) (Table 1).

Table 1 Descriptors of reports included in review with documented or probable human-to-animal transmission

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Virus					
Bhatt et al. (1966)	India	Zoo	MeV	Captive wildlife	Monkey
Shishido (1966)	Japan	Lab	MeV	Captive wildlife	Monkey
Potkay et al. (1966)	United States	Zoo	MeV	Captive wildlife	Monkey
Smith et al. (1969)	Thailand	Lab	HSV	Captive wildlife	Gibbon
Emmons and Lennette (1970)	United States	Lab	HSV	Captive wildlife	Gibbon
Levy and Mirkovic (1971)	United States	Zoo	MeV	Captive wildlife	Marmoset
Meléndez et al. (1972)	Guatemala	School of medicine	HSV	Captive wildlife	Monkey
Remfry (1976)	United Kingdom	Quarantine unit	MeV	Captive wildlife	Monkey
MacArthur et al. (1979)	United Kingdom	Safari park	MeV	Captive wildlife	Monkey
McClure et al. (1980)	United States	Zoo	HSV	Captive wildlife	Chimpanzee
Heldstab et al. (1981)	Germany	Zoo	HSV	Captive wildlife	Gorilla
Eberle and Hilliard (1989)	United States, Canada	Zoo	HSV	Captive wildlife	Chimpanzee Gorilla

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Bruno et al. (1997)	Brazil	Wildlife park	HSV	Captive wildlife	Marmoset
Juan-Sallés et al. (1997)	Spain	Clinic	HSV	Captive wildlife	Marmoset
Weissenböck et al. (1997)	Austria	Domestic	HSV	Wildlife	Rabbit
Choi et al. (1999)	South Korea	Zoological park	MeV	Captive wildlife	Monkey
Willy et al. (1999)	United States	Zoo	MeV	Captive wildlife	Monkey
Jones-Engel et al. (2001)	Indonesia	Wildlife park	MeV, IAV, PI	Captive wildlife	Monkey
Allison et al. (2002)	United States	Zoo	HSV	Wildlife	Hedgehog
Huemer et al. (2002)	Austria	Veterinary clinic	HSV	Captive wildlife	Monkey
Sakulwira et al. (2002)	Thailand	Wildlife research center	HSV	Captive wildlife	Gibbon
Wohlsein et al. (2002)	Germany	Lab	HSV	Wildlife	Chinchilla
Kreutzer et al. (2011)	Germany	Captive	HSV	Captive wildlife	Marmoset
Schrenzel et al. (2003)	United States	Captive	HSV	Captive wildlife	Monkey
Kilbourn et al. (2003)	Malaysia	Zoo	hRSV, PI	Captive wildlife	Orangutan
Kik et al. (2005)	The Netherlands	Zoo	HSV	Captive wildlife	Orangutan

Landolfi et al. (2005)	United States	Zoological park	HSV	Captive wildlife	Gibbon
Bermejo et al. (2006)	Gabon, Congo	Zoo	EBOV	Captive wildlife	Gorilla
Jones-Engel et al. (2006)	Nepal	Natural area	MeV	Captive wildlife	Monkey
Mattison et al. (2007)	Canada	Pastoral area	HuNoV	Livestock	Swine Cattle
Williams et al. (2008)	Tanzania	Zoo	EV	Captive wildlife	Chimpanzee
Gozalo et al. (2008)	Peru	Zoo	HSV	Captive wildlife	Monkey
Steyer et al. (2008)	Slovenia	Livestock farm	RV	Livestock	Swine Cattle
Kaur et al. (2008)	Tanzania	National park	hMPV	Captive wildlife	Chimpanzee
Kondgen et al. (2008)	Côte d'Ivoire	Natural area	hMPV, hRSV	Captive wildlife	Great ape
Hanamura et al. (2008)	Tanzania	Zoo	hMPV	Captive wildlife	Chimpanzee
Szentiks et al. (2009)	Germany	Zoo	hRSV	Captive wildlife	Chimpanzee
de Thoisy et al. (2009)	French Guiana	Natural park	DV	Captive wildlife	Various
Barrette et al. (2009)	United States	Livestock farm	EBOV	Livestock	Swine
Hofshagen et al. (2009)	Norway	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Russell et al. (2009)	Canada	Livestock farm	IAV	Livestock	Swine
Nofs et al. (2009)	United States	Zoo	H1N1pdm09 (IAV)	Wildlife	Giant anteater

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Howden et al. (2009)	Canada	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Abe et al. (2010)	Japan	Natural area	RV	Wildlife	Raccoon dog
Dundon et al. (2010)	Italy	Research institute	H1N1pdm09 (IAV)	Companion	Dog Cat
Löhr et al. (2010)	United States	Lab	H1N1pdm09 (IAV)	Companion	Dog
Seiler et al. (2010)	United States	Veterinary clinic	H3N2, H1N1pdm09 (IAV)	Companion	Dog Cat
Sponseller et al. (2010)	United States	Veterinary clinic	H1N1pdm09 (IAV)	Companion	Cat
Weingarti et al. (2010)	Canada	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Pereda et al. (2010)	Argentina	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Nagarajan et al. (2010)	India	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Song et al. (2010)	South Korea	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Swenson et al. (2010)	United States	Household	H1N1pdm09 (IAV)	Wildlife	Ferret
Berhane et al. (2010)	Canada	Livestock farm	H1N1pdm09 (IAV)	Poultry	Turkey
Terebuh et al. (2010)	United States	Livestock farm	H3N2 (IAV)	Livestock	Swine
Köndgen et al. (2010)	Côte d'Ivoire	Zoo	hRSV, hMPV	Captive wildlife	Chimpanzee
Mathieu et al. (2010)	Chile	Livestock farm	H1N1pdm09 (IAV)	Poultry	Turkey
Moreno et al. (2010)	Italy	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Britton et al. (2010)	Canada	Natural area	H1N1pdm09 (IAV)	Wildlife	Skunk
Sreta et al. (2010)	Thailand	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Costa et al. (2011)	Brazil	Natural park	HSV	Captive wildlife	Marmoset
Holyoake et al. (2011)	Australia	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine

Kreutzer et al. (2011)	Germany	Zoo	HSV	Captive wildlife	Monkey
Kim et al. (2011)	South Korea	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Longa et al. (2011)	Brazil	Natural park	HSV	Captive wildlife	Monkey Marmoset
Palacios et al. (2011)	Rwanda	Natural area	hMPV	Captive wildlife	Gorilla
Osbornes et al. (2011)	United States	Multiple sites	CoV	Wildlife	Bat
McCullers et al. (2011)	United States	Veterinary clinic	H3N2, H1N1pdm09 (IAV)	Companion	Cat
Fiorentini et al. (2011)	Italy	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Cat
Said et al. (2011)	Japan	Lab	H3N2 (IAV)	Companion	Dog Cat
Ali et al. 2011 (Reversing itself, appeals court denies trial for blood recipient 1999)	United States	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Cat
Forgie et al. (2011)	Canada	Lab	H1N1pdm09 (IAV)	Livestock	Swine
Schrenzel et al. (2011)	United States	Zoo	H1N1pdm09 (IAV)	Wildlife	American badger
Campagnolo et al. (2011)	United States	Domestic	H1N1pdm09 (IAV)	Companion	Cat
Summa et al. (2012)	Finland	Household	HuNoV	Companion	Dog
Huynh et al. (2012)	North America	Animal Sanctuary/ rehabilitation center	CoV	Wildlife	Bat
Damiani et al. (2012)	Germany	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Dog Cat

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Lin et al. (2012)	China	Veterinary clinic	H1N1pdm09 (IAV)	Companion	Dog
Deng et al. (2012)	Australia	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Reid et al. (2012)	United Kingdom	Veterinary clinic	H1N1pdm09 (IAV)	Poultry	Turkey
Trevennec et al. (2012)	Vietnam	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
McCune et al. (2012)	Peru	Livestock farm	IAV	Livestock	Swine
Bowman et al. (2012)	United States	Agricultural fair	H3N2 (IAV)	Livestock	Swine
Crossley et al. (2012)	United States	Zoo	H1N1pdm09 (IAV)	Captive wildlife	Cheetah
Åkerstedt et al. (2012)	Norway	Natural area	H1N1pdm09 (IAV)	Wildlife	Mink
Njabo et al. (2012)	Cameroon	Farm	H1N1pdm09 (IAV)	Livestock	Swine
Campagnolo et al. (2013)	United States	Pet shop	H1N1pdm09 (IAV)	Wildlife	Ferret
Sharma et al. (2013)	India	Livestock farm	RV	Livestock	Cattle Swine
Unwin et al. (2013)	United Kingdom	Zoo	hRSV	Captive wildlife	Chimpanzee
Xiao et al. (2013)	United States	Livestock farm	Astrovirus	Livestock	Swine
Wolf et al. (2013)	Denmark	Natural area	HuNoV	Wildlife	Rat
Su et al. 2013	China	Lab	H1N1pdm09 (IAV)	Companion	Dog
Ramírez-Martínez et al. (2013)	Mexico	Household	H1N1pdm09 (IAV)	Companion	Dog
Grøntvedt et al. (2013)	Norway	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Rith et al. (2013)	Cambodia	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Rajão et al. (2013)	Brazil	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Sabale et al. (2013)	India	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Killan et al. (2013)	United States	Agricultural fair	H1N1pdm09 (IAV)	Livestock	Swine

Bálint et al. (2013)	Hungary	Veterinary clinic	H1N1pdm09 (IAV)	Livestock	Swine
Kooriyama et al. (2013)	Japan	Research institute	hRSV; hMPV; H3N2, H1N1pdm09 (IAV)	Captive wildlife	Chimpanzee
Goldstein et al. (2013)	Unites States	Animal sanctuary/ rehabilitation center	H1N1pdm09 (IAV)	Wildlife	Seal
Boyce et al. (2013)	Unites States	Animal sanctuary/ rehabilitation center	H1N1pdm09 (IAV)	Wildlife	Sea lion Seals
Buitendijk et al. (2014)	The Netherlands	Zoo	hRSV; hMPV; H3N2, H1N1pdm09 (IAV)	Captive wildlife	Great ape
Gilardi et al. (2014)	Congo	Zoo	HSV	Captive wildlife	Gorilla
Imura et al. (2014)	Japan	Veterinary clinic	HSV	Captive wildlife	Marmoset
Schmitt et al. (2014)	Germany	Natural area	HSV	Captive wildlife	Gorilla
Casagrande et al. (2014)	Brazil	Natural park	HSV	Captive wildlife	Marmoset
Slater et al. (2014)	United States	Zoological department	hMPV	Captive wildlife	Chimpanzee
Sun et al. (2014)	China	University zoological department	H3N2, H1N1pdm09 (IAV)	Companion	Dog
Zhao et al. (2014)	China	Lab	H1N1pdm09 (IAV)	Companion	Cat
Su et al. (2014)	China	Lab	H1N1pdm09 (IAV)	Companion	Dog

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Yin et al. (2014)	China	Veterinary clinic	H1N1pdm09 (IAV)	Companion	Dog
Li et al. (2014)	China	Research institute	H1N1pdm09 (IAV)	Wildlife	Panda
Lin et al. (2014)	Taiwan	Domestic	H1N1pdm09 (IAV)	Companion	Ferret
Meseko et al. (2014)	Nigeria	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Matushima (2014)	Brazil	Captive	HSV	Wildlife	Marmoset
Matushima (2014)	United States	Veterinary clinic	H1N1pdm09 (IAV)	Companion	Cat
Adeola et al. (2015)	Ghana, Nigeria	Slaughterhouse; Research institute	H1N1pdm09 (IAV)	Livestock	Swine
Baudon et al. (2015)	Vietnam	Slaughterhouse	H1N1pdm09 (IAV)	Livestock	Swine
Caddy et al. (2015)	United Kingdom	Veterinary clinic	HuNoV	Companion	Dog
Ducatez et al. (2015)	Togo	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Nelson et al. (2015)	Brazil	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Pauly et al. (2015)	Côte d'Ivoire	Domestic animals	HAdV	Livestock Companion Wildlife	Various
Song et al. (2015)	South Korea	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Dog
Zhang et al. (2015)	China	University zoological department	H3N2, H1N1pdm09 (IAV)	Companion	Cat
Arunorat et al. (2016)	Thailand	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Barnes et al. (2016)	United States	Zoo	HSV	Captive wildlife	Monkey

Er et al. (2016b)	Norway	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Grützmacher et al. (2016)	Congo	Zoo	hRSV	Captive wildlife	Gorilla
Ibrahim et al. (2016a)	United States	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Cat
Kyriakis et al. (2016)	Greece	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Knight et al. (2016)	Canada	University diagnostic service unit	H1N1pdm09 (IAV)	Companion	Cat
Pippig et al. (2016)	Germany	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Araujo et al. (2016)	Brazil	Zoo	HSV	Captive wildlife	Marmoset
Adeola et al. (2017)	Nigeria	Slaughterhouse	H1N1pdm09 (IAV)	Livestock	Swine
Choudhary et al. (2017)	India	Veterinary university	RV	Livestock	Goat Sheep
Boedeker et al. (2017)	United States	Zoo	H1N1pdm09 (IAV)	Wildlife	Sloth bear
Jang et al. (2017)	United States	Veterinary clinic	H3N2, H1N1pdm09 (IAV)	Companion	Dog
Thongyuan and Kittayapong (2017)	Thailand	Natural areas	DV	Companion	Dog
Paungpin et al. (2017)	Thailand	Natural areas	H1N1pdm09 (IAV)	Wildlife	Elephant
Sjurseth et al. (2017)	Norway	Livestock farm	H1N1pdm09 (IAV)	Poultry	Turkey
Wu et al. (2017)	Taiwan	Veterinary clinic; Livestock farm	RV	Livestock	Swine
Demetria et al. (2018)	Philippines	Animal sanctuary/ rehabilitation center	EBOV	Captive wildlife	Monkey

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Kumar et al. (2018)	India	Household	RV	Livestock	Cattle
Grützmacher et al. (2018)	Congo	Natural area	hRSV	Captive wildlife	Bonobo
Ma et al. (2018)	China	Livestock farm	IAV	Livestock	Swine
Patrono et al. (2018b)	Côte d'Ivoire	Zoo	CoV	Captive wildlife	Chimpanzee
Scully et al. (2018)	Uganda	Zoo	Rhinovirus	Captive wildlife	Chimpanzee
Summa et al. (2018)	Finland	Natural park	HuNoV	Wildlife	Rat Bird
Britton et al. (2019)	Canada	Animal sanctuary/ rehabilitation center	H1N1pdm09 (IAV)	Wildlife	Skunk
Chastagner et al. (2019)	France	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Calderon et al. (2019)	Colombia	Natural area	DV	Wildlife	Bat
Dehghan et al. (2019)	United States	Veterinary clinic	HAdVs	Captive wildlife	Bonobo Chimpanzee
Favoretto et al. (2019)	Brazil	Natural area	Zika virus	Wildlife	Monkey
Ghoneim and K.A.A.-M.a.H.S. (2019)	Egypt	Natural area	RV	Wildlife	Cattle Rat
Martelli et al. (2019)	Hong Kong	Animal sanctuary/ rehabilitation center	H1N1pdm09 (IAV)	Wildlife	Panda
Negrey et al. (2019)	Uganda	Natural park	hMPV, HRV3	Wildlife	Chimpanzee

Nelson et al. (2019)	Mexico	Livestock farm	IAVs	Livestock	Swine
Osoro et al. (2019b)	Kenya	Livestock farm	IAVs	Livestock	Swine
Su et al. (2019)	China	Veterinary clinic	H3N2, H1N1pdm09 (IAV)	Companion	Dog
Vieira et al. (2019)	Brazil, Uruguay	Natural area	HBV	Wildlife	Various
Tangwangvivat et al. (2019)	Thailand	Animal shelter	H1N1pdm09 (IAV)	Companion	Cat Dog
Ayim-Akonor et al. (2020)	Germany	Livestock farm	IAVs	Livestock	Swine
Barrs et al. (2020)	China, Hong Kong	Household	SARS-CoV-2	Companion	Cat
Charoenkul et al. (2020)	Thailand	Animal shelter	HuNoV	Companion	Dog
Marcia Helena Braga Catroxo et al. (2020)	Brazil	Wildlife park	HSV	Captive wildlife	Monkey Marmoset
Er et al. (2020)	Norway	Veterinary clinic	H1N1pdm09 (IAV)	Livestock	Swine
Garigliany et al. (2020)	Belgium	Household	SARS-CoV-2	Companion	Cat
Hamer et al. (2020)	United States	Household	SARS-CoV-2	Companion	Dog
Kwasnik et al. (2020)	Japan	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Dog
Mazet et al. (2020)	Rwanda	Zoo	hRSV, hMPV	Captive wildlife	Gorilla
McAloose et al. (2020)	United States	Zoo	SARS-CoV-2	Wildlife	Lion Tiger
Medkour et al. (2020)	Algeria, Congo, Djibouti, Senegal	Zoo	HAdV	Captive wildlife	Monkey Gorilla
Mon et al. (2020)	Myanmar	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Musso et al. (2020)	Italy	Household	SARS-CoV-2	Companion	Cat
Neira et al. (2020)	Chile	Household	SARS-CoV-2	Companion	Cat
Molenaar et al. (2020)	The Netherlands	Livestock farm	SARS-CoV-2	Wildlife	Mink
Oreshkova et al. (2020)	The Netherlands	Livestock farm	SARS-CoV-2	Wildlife	Mink

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Patterson et al. (2020)	Italy	Veterinary clinic	SARS-CoV-2	Companion	Dog Cat
Sailleau et al. (2020)	France	Household	SARS-CoV-2	Companion	Cat
Sit et al. (2020)	China	Household	SARS-CoV-2	Companion	Dog
Segalés et al. (2020)	Spain	Household	SARS-CoV-2	Companion	Cat
Shrivastava et al. (2020)	India	Household	HAdV, cryptosporidium	Livestock	Cattle Goat Sheep
Newman et al. (2020)	United States	Household	SARS-CoV-2	Companion	Cat
Wang et al. (2020a)	United States	Zoo	SARS-CoV-2	Captive wildlife	Tiger
Sojka et al. (2020)	United States	Zoo	hRSV	Captive wildlife	Gibbon
Aguiló-Gisbert et al. (2021)	Spain	Wild	SARS-CoV-2	Wildlife	Mink
Bartlett et al. (2021)	United States	Zoo	SARS-CoV-2	Captive wildlife	Tiger
Fernández-Bellon et al. (2021)	Spain	Zoo	SARS-CoV-2	Captive wildlife	Lion
Ruiz-Arrondo et al. (2021)	Spain	Household	SARS-CoV-2	Companion	Cat
Chaintoutis et al. (2022)	Greece	Household	SARS-CoV-2	Companion	Cat
Dileepan et al. (2021)	United States	Household	SARS-CoV-2	Companion	Cat Dog
Fuentealba et al. (2021)	Argentina	Household	SARS-CoV-2	Companion	Cat
Fritz et al. (2021)	France	Household	SARS-CoV-2	Companion	Cat Dog

Glasser et al. (2021)	Uganda	National park	SARS-CoV-2, hMPV, hRSV	Captive wildlife	Chimpanzee
Gortázar et al. (2021)	Spain	Household	SARS-CoV-2	Wildlife	Ferret
Hosie et al. (2021)	United Kingdom	Household	SARS-CoV-2	Companion	Cat
Karikalan et al. (2021)	India	Zoo	SARS-CoV-2	Captive wildlife	Lion
Klaus et al. (2021)	Switzerland	Household	SARS-CoV-2	Companion	Cat
Kovalenko et al. (2021)	Ukraine	Lab	H3N2, H1N1pdm09 (IAV)	Companion	Dog Cat
Mishra et al. (2021)	India	Zoo	SARS-CoV-2	Captive wildlife	Lion
Mitchell (2021)	United States	Zoo	SARS-CoV-2	Captive wildlife	Tiger
Meisner et al. (2021)	United States	Household	SARS-CoV-2	Companion	Dog
Pagani et al. (2021)	Italy	Veterinary clinic	SARS-CoV-2	Companion	Cat
Senthilkumar et al. (2021)	India	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Smith et al. (2021)	United Kingdom	Household	SARS-CoV-2	Companion	Cat Dog
Stevanovic et al. (2021)	Croatia	Lab	SARS-CoV-2	Companion	Cat Dog
Yoshida et al. (2021)	Congo	Zoo	RSV, PI, IAV	Captive wildlife	Bonobo
Larsen et al. (2021)	Denmark	Livestock farm	SARS-CoV-2	Wildlife farm	Mink
Hammer et al. (2021)	Denmark	Livestock farm	SARS-CoV-2	Wildlife farm	Mink
Oude Munnink et al. (2021)	The Netherlands	Livestock farm	SARS-CoV-2	Wildlife	Mink

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Sun et al. 2021	China	Livestock farm	H1N1pdm09 (IAV)	Livestock	Swine
Shriner et al. (2021)	United States	Wild	SARS-CoV-2	Wildlife	Mink
Calvet et al. (2021)	Brazil	Lab	SARS-CoV-2	Companion	Cat Dog
Abbreviation: *SARS-CoV-2* severe acute respiratory syndrome coronavirus-1, *IAV* influenza A virus, *hRSV* human respiratory syncytial virus, *H1N1pdm09* pandemic H1N1, *PI* parainfluenza, *hMPV* human metapneumovirus, *HuNoV* human norovirus, *RV* rotavirus, *EBOV* Ebola virus, *EV* enterovirus, *DV* dengue virus, *HBV* hepatitis B virus, *HSV* human simplex herpesvirus, *CoV* coronavirus, *HadV* human adenovirus, *MeV Measles morbillivirus*, *HRV3* human respirovirus 3					
Bacteria					
Ackerman et al. (1974)	Brazil	National park	*Mycobacterium tuberculosis*	Captive wildlife	Parrot
Rolland et al. (1985)	Kenya	National park	*Escherichia coli*	Captive wildlife	Baboon
Michalak et al. (1998)	United States	Exotic farm	*Mycobacterium tuberculosis*	Captive wildlife	Elephant
Michel and Huchzermeyer (1998)	South Africa	Veterinary clinic	*Mycobacterium tuberculosis*	Captive wildlife	Marmoset
Washko et al. (1998)	United States	Household	*Mycobacterium tuberculosis*	Captive wildlife	Macaw
Seguin et al. (1999)	United States	Veterinary clinic	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Companion	Horse
Nizeyi et al. (2001)	Uganda	National park	*Cryptosporidium* sp.	Wildlife	Gorilla
Alexander et al. (2002)	South Africa	National park	*Mycobacterium tuberculosis*	Wildlife	Mongoose

Oh et al. (2002)	United States	Private zoo	*Mycobacterium tuberculosis*	Captive wildlife	Elephant
Hackendahl et al. (2004); Erwin et al. (2004)	United States	Veterinary clinic	*Mycobacterium tuberculosis*	Companion	Dog
Prasad et al. (2005)	India	Veterinary clinic	*Mycobacterium tuberculosis, Mycobacterium bovis*	Livestock	Cattle
Kwon et al. (2006)	Korea	Slaughterhouse	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Companion; livestock	Dog
Morris et al. (2006)	United States	Household; Veterinary clinic	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Companion	Cat
Steinnetz et al. (2006)	Switzerland	University zoo	*Mycobacterium tuberculosis*	Captive wildlife	Macaw
Weese et al. (2006)	Canada, United States	Veterinary clinic	*Staphylococcus aureus*	Companion	Cat Dog
Goldberg et al. (2007)	Uganda	National park	*Escherichia coli*	Captive wildlife	Chimpanzee
Goldberg et al. (2008)	Uganda	National park	*Escherichia coli*	Captive wildlife	Assorted primates
Hsieh et al. (2008)	Taiwan	Livestock farm	Oxacillin-resistant *Staphylococcus aureus* (ORSA)	Poultry; livestock	Poultry Cattle Swine
Rwego et al. (2008b)	Uganda	National park	*Escherichia coli*	Wildlife	Gorilla
Berg et al. (2009)	Ethiopia	Slaughterhouse	*Mycobacterium tuberculosis*	Livestock	Cattle
Shrikrisna et al. (2009)	United Kingdom	Household	*Mycobacterium bovis*	Companion	Dog

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Szentiks et al. (2009)	Germany	Private zoo	*Streptococcus pneumoniae;* human respiratory syncytial virus	Captive wildlife	Chimpanzee
Ewers et al. (2010)	Austria, Denmark, France, Germany, Italy, Luxembourg, the Netherlands, Spain	Veterinary clinic	*Escherichia coli*	Companion	Dog
Kottler et al. (2010)	United States	Household; Veterinary clinic	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Companion	Cat Dog
Every et al. (2011)	Australia	University zoology department	*Helicobacter pylori*	Captive wildlife	Dunnart
Rubin et al. (2011)	Canada	Veterinary clinic	*Staphylococcus aureus*	Livestock	Swine
Lin et al. (2011)	United States	Veterinary clinic	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Companion; livestock	Cat Dog Horse Swine
Sunde et al. (2011)	Norway	Livestock farm	*Staphylococcus aureus*	Livestock	Swine
Sutherland et al. (2011)	United States	Coastal area	*Serratia marcescens*	Wildlife	Coral
Gumi et al. (2012)	Ethiopia	Pastoral area	*Mycobacterium tuberculosis*	Livestock	Cattle Camel Goat
Murakami et al. (2012)	Brazil	Zoo	*Mycobacterium tuberculosis*	Captive wildlife	Tapir

Price et al. (2012)	Austria, Belgium, Canada, China, Denmark, Finland, France, French Germany, Guiana, Hungary, Italy, the Netherlands, Peru, Poland, Portugal, Spain, Slovenia, Switzerland, United States	Animal meat for sale	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Livestock	Not specified
Schaumburg et al. (2012)	Uganda, Zambia	National park	*Staphylococcus aureus*	Captive wildlife	Chimpanzee
Špičić et al. (2012)	Croatia	Livestock farm	*Mycobacterium tuberculosis*	Livestock	Cattle
Thakur et al. (2012)	India	Livestock farm	*Mycobacterium tuberculosis, Mycobacterium bovis*	Livestock	Cattle
Wilbur et al. (2012)	Indonesia, Nepal, Singapore, Thailand	Zoo	*Mycobacterium tuberculosis*	Captive wildlife	Macaque
Coscolla et al. (2013)	Switzerland	National park	*Mycobacterium tuberculosis*	Captive wildlife	Chimpanzee
Delannoy et al. (2013)	Colombia, Honduras, Kuwait	Veterinary laboratory	*Streptococcus agalactiae*	Aquatic	Dolphin Fish Frog Seal
Osadebe et al. (2013)	United States	Livestock farm	*Staphylococcus aureus*	Livestock	Swine
Michel et al. (2013)	South Africa	Zoos	*Mycobacterium tuberculosis*	Captive wildlife	Monkey
Nagel et al. (2013)	Gabon	Veterinary laboratory	*Staphylococcus aureus*	Captive wildlife	Gorilla
Obanda et al. (2013)	Kenya	National park	*Mycobacterium tuberculosis*	Captive wildlife	Elephant
Pesapane et al. (2013)	Botswana	National park	*Escherichia coli*	Captive wildlife	Mongoose

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Schaumburg et al. (2013)	Madagascar, Uganda	Natural Park	*Staphylococcus aureus*	Captive wildlife	Chimpanzee
Unwin et al. (2013)	United Kingdom	University veterinary department	*Streptococcus pneumoniae*	Captive wildlife	Chimpanzee
Mittal et al. (2014)	India	Livestock farm	*Mycobacterium tuberculosis, Mycobacterium bovis*	Livestock	Cattle
van de Vijver et al. (2014)	The Netherlands	Livestock farm	*Staphylococcus aureus*	Livestock	Swine
Franklinos et al. (2015)	England	Natural park	*Streptococcus pyogenes*	Wildlife	Hedgehog
Mätz-Rensing et al. (2015)	Germany	Zoological department	*Mycobacterium tuberculosis*	Captive wildlife	Monkey
Kandefer-Gola et al. (2016)	Poland	Household	*Mycobacterium tuberculosis*	Companion	Parrot
Loncaric et al. (2016)	Austria	Livestock farm	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Livestock; companion	Cat Horse
Nguyen Vinh et al. (2016)	Vietnam	Livestock farm	*Escherichia coli*	Livestock	Poultry
Senghore et al. (2016)	The Gambia	Natural park	*Staphylococcus aureus*	Captive wildlife	Monkey
Zachariah et al. (2017)	West Africa	National park	*Mycobacterium tuberculosis* complex	Captive wildlife	Elephant
Fernandes et al. (2018)	Brazil	Veterinary clinic	*VIM-2-producing P. aeruginosa*	Companion	Dog
Hasan et al. (2018)	Pakistan	Livestock Farm	*Enterococcus faecalis*	Livestock	Poultry

Knetsch et al. (2018)	North America, Europe, Australia, and Asia	Livestock farm	*Clostridium difficile*	Livestock	Swine
Linz et al. (2018)	Pakistan	Livestock farm	*Acinetobacter baumannii*	Livestock	Sheep
Adesokan et al. 2019	Nigeria	Livestock farm	*Mycobacterium tuberculosis*	Livestock	Cattle
Cerdà-Cuéllar et al. (2019)	Antarctica	Natural area	*Salmonella* spp.; *Campylobacter* spp.	Wildlife	Seabird
Cobo-Angel et al. (2019)	Colombia	Livestock farm	Group B streptococcus (GBS)	Livestock	Cattle
Li et al. (2019)	China	Livestock farm	Carbapenemase-producing *Escherichia coli*	Livestock	Swine
McDougall et al. (2019)	Australia	Private park	Antimicrobial resistance (AMR)	Wildlife	Flying fox
Miller et al. (2019)	South Africa	Natural park	*Mycobacterium tuberculosis, Mycobacterium bovis*	Wildlife	Elephant
On et al. (2019)	New Zealand	Private zoo	*Campylobacter* spp.	Captive wildlife	Kiwi
Post et al. (2019)	Burkina Faso	Household	*Salmonella* spp.	Livestock Companion	Various
Sorensen et al. (2019)	Denmark	Livestock farm	*Streptococcus agalactiae*	Livestock	Cattle
Ehlers et al. (2020)	Brazil	National park	*Mycobacterium tuberculosis*	Captive wildlife	Capuchin
Mbehang Nguema et al. (2020)	Gabon	Natural park	Antimicrobial resistance (AMR)	Wildlife	Bat

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Crestani et al. (2021)	Sweden	National veterinary institute	Group B streptococcus (GBS)	Livestock	Cattle
Lafleur et al. (2021)	Madagascar	Veterinary laboratory	*Mycobacterium tuberculosis*	Wildlife	Lemur
Tang et al. (2021)	China	Private zoo	*Staphylococcus aureus*	Wildlife	Monkey
Parasite					
Nasher (1988)	Saudi Arabia	Household	*Entamoeba histolytica* *Giardia intestinalis* *Hymenolepis nana*	Wildlife	Baboon
Müller-Graf et al. (1997)	Tanzania	National park	*Schistosoma mansoni*	Wildlife	Baboon
Pusey (1998)	Tanzania	National park	*Sarcoptes scabiei*	Wildlife	Chimpanzee
Nizeyi et al. (1999)	Uganda	National park	*Cryptosporidium* spp. *Giardia* spp.	Wildlife	Gorilla
Murray et al. (2000)	Tanzania	National park	*Entamoeba coli* Hookworms *Oesophagostomum* spp. *Physaloptera* spp. *Schistosoma mansoni* *Strongyloides fuelleborni* *Trichuris* spp.	Wildlife	Baboon Chimpanzee
Sleeman et al. (2000)	Rwanda	National park	*Entamoeba histolytica* *Giardia* spp. *Oesophagostomum* spp. *Strongyloides* spp. *Trichuris trichiura* *Trichostrongylus* spp.	Wildlife	Gorilla

Graczyk et al. (2001)	Uganda	National park	*Cryptosporidium parvum*	Wildlife	Gorilla
Learmonth et al. (2001)	New Zealand	Livestock farm	*Cryptosporidium parvum*	Livestock	Cattle
Graczyk et al. (2002a)	Uganda	National park	*Giardia duodenalis*	Wildlife	Gorilla
Graczyk et al. (2002b)	Uganda	National park	*Encephalitozoon intestinalis*	Wildlife	Gorilla
Kalema-Zikusoka et al. (2002)	Uganda	National park	*Sarcoptes scabiei*	Wildlife	Gorilla
Dereure et al. (2003)	Sudan	Household	Visceral leishmaniasis (VL)	Companion rodent	Dog Rodent
Guk et al. (2004)	South Korea	Laboratory	*Cryptosporidium parvum*	Livestock rodent	Cattle Mice
Noël et al. (2005)	Singapore	Various	*Blastocystis* spp.	Wildlife rodent	Lizard Python Sea snake Rat
Coklin et al. (2007)	Canada	Livestock farm	*Cryptosporidium parvum* *Giardia duodenalis*	Livestock	Cattle
Faulde et al. (2008b)	Afghanistan	Research institute; Medical institute	Zoonotic cutaneous leishmaniasis (ZCL)	Rodent	Gerbil
Adejinmi (2008)	Nigeria	Zoo	*Ascaris* spp. *Strongyloides* spp. *Trichuris* spp.	Captive wildlife	Baboon Donkey Jackal Lion Monkey Warthog

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Teichroeb et al. (2009)	Ghana	Animal sanctuary/ rehabilitation center	*Ascaris* spp. *Blastocystis* spp. *Entamoeba* spp. *Giardia duodenalis* *Isospora* spp. *Oesophagostomum* spp. *Strongyloides* spp. *Trichuris* spp.	Captive wildlife	Monkey
Ash et al. (2010)	Australia, Namibia, Zambia	Natural area; Zoo	*Giardia duodenalis*	Captive wildlife; Wildlife	African wild dog
Johnston et al. (2010)	Uganda	Natural area; National park	*Giardia duodenalis*	Wildlife Livestock	Cattle Goat Monkey Sheep
Dixon et al. (2011)	Canada	Livestock farm	*Cryptosporidium parvum* *Giardia duodenalis*	Livestock	Cattle
Sá et al. (2013)	Guinea-Bissau	National park	*Giardia intestinalis*	Wildlife	Chimpanzee
Sak et al. (2013)	Central African Republic	National park	*Enterocytozoon bieneusi* *Cryptosporidium bovis* *Encephalitozoon cuniculi* *Giardia intestinalis*	Wildlife	Gorilla

Arafa et al. (2013)	Egypt	Zoo	*Entamoeba coli* *Entamoeba histolytica* *Giardia intestinalis* *Haemonchus contortus* *Isospora felis* *Strongyloides papillosus* *Toxascaris leonina* *Trichuris* spp.	Captive wildlife	Various
Sak et al. (2014)	Rwanda	National park	*Cryptosporidium meleagridis* *Cryptosporidium muris* *Enterocytozoon* bieneusi *Encephalitozoon cuniculi*	Wildlife	Gorilla
Krawczyk et al. (2015)	The Netherlands	Animal sanctuary/ rehabilitation center	*Cryptosporidium hominis* *Cryptosporidium parvum* *Giardia duodenalis*	Wildlife	European hedgehog
Schiller et al. (2016)	Australia	Animal sanctuary/ rehabilitation center	*Cryptosporidium hominis*	Captive wildlife; Wildlife	Spectacled flying fox
Mynářová et al. (2016)	Indonesia	National park	*Cryptosporidium* spp. *Giardia intestinalis*	Wildlife	Orangutan
Li et al. (2016)	China	Multiple sites	*Pentatrichomonas hominis*	Companion; Captive wildlife; Wildlife	Dog Monkey

(continued)

Table 1 (continued)

Publications	Study location	Specimen source	Pathogen name	Animal category	Animal type
Ibrahim et al. (2016b)	Egypt	Research institute; Medical institute	*Cryptosporidium* spp.	Livestock	Buffalo Cattle
Yu et al. (2017)	China	Zoo	*Enterocytozoon bieneusi*	Captive wildlife	Monkey
Nolan et al. (2017)	Uganda	National park	*Cryptosporidium parvum* *Entamoeba coli* *Entamoeba hartmanni*	Wildlife	Gorilla
Ait Kbaich et al. (2017)	Morocco	Research institute; Medical institute	Cutaneous leishmaniasis (CL)	Companion	Dog
Tangtrongsup et al. (2019)	Thailand	Wildlife breeding center	*Giardia duodenalis* *Strongyloides* spp.	Captive wildlife	Gibbon
Mohd-Shaharuddin et al. (2019)	Malaysia	Household	*Trichuris trichiura*	Companion	Dog Cat
Shrivastava et al. (2020)	India	Household	*Cryptosporidium* spp. Adenovirus[a]	Livestock	Cattle Sheep Goat
Wang et al. (2020b)	China	Livestock farm	*Cryptosporidium* spp.	Livestock	Donkey
Fungus					
Pal et al. (1997)	India	Veterinary laboratory	*Trichophyton rubrum*	Captive wildlife	Monkey
Wrobel et al. (2008)	United States	University	*Candida albicans*	Wildlife	Various
Sharma et al. (2009)	India	Veterinary college	*Microsporum gypseum*	Companion	Dog

[a]Shrivastava et al. (2020) study included co-infection of viral and parasitic pathogens

Measles is a highly contagious virus that is endemic chiefly among humans. *Measles morbillivirus* (MeV) generally does not occur in monkeys in the wild but can become an important pathogen when nonhuman primates are held in captivity and exposed directly or indirectly with humans (i.e., tourists, visitors, caretakers, etc.) who are infected with the measles virus. Outbreaks of measles in free-living and laboratory primates are known to occur coincidentally with human outbreaks. Several measles outbreaks following shipment of monkeys or introductions into an established colony have been described (Jones-Engel et al. 2006; Levy and Mirkovic 1971; MacArthur et al. 1979; Potkay et al. 1966; Remfry 1976; Shishido 1966; Welshman 1989). The pathogenesis of MeV infection in nonhuman primates is similar to that in humans (Choi et al. 1999). It is known to induce immunosuppression in affected humans and nonhuman primates by disrupting both cellular and humoral immunity, which can result in various secondary opportunistic infections. This pathogen has resulted in significant morbidity and mortality among nonhuman primate populations. Both natural and experimental measles infection can cause disease of varying severity with pathogenesis similar to that of human MeV infection. Erythematous maculopapular skin rash and presence of multinucleated syncytial cells in the lungs are regarded as pathognomonic for measles virus infection. Endometritis, cervicitis, and abortion associated with measles virus have been described in rhesus monkeys (Choi et al. 1999; Willy et al. 1999).

Similarly, herpes virus infections are common in humans and usually result in mild disease characterized by recurrent mucocutaneous lesions. Human simplex herpesvirus (HSV) infections have also been reported in several nonhuman primates along with the domestic rabbit, chinchilla, and the African pygmy hedgehog (Allison et al. 2002; Araujo et al. 2016; Barnes et al. 2016; Bhatt et al. 1966; Bruno et al. 1997; Costa et al. 2011; Eberle and Hilliard 1989; Emmons and Lennette 1970; Gilardi et al. 2014; Gozalo et al. 2008; Heldstab et al. 1981; Huemer et al. 2002; Imura et al. 2014; Juan-Sallés et al. 1997; Kik et al. 2005; Kreutzer et al. 2011; Landolfi et al. 2005; Longa et al. 2011; Marcia Helena Braga Catroxo et al. 2020; Matushima 2014; Mätz-Rensing et al. 2003; McClure et al. 1980; Meléndez et al. 1972; Sakulwira et al. 2002; Schrenzel et al. 2003; Smith et al. 1969; Weissenböck et al. 1997; Wohlsein et al. 2002; Schmitt et al. 2014). New World monkeys are particularly susceptible and often succumb to the disease. Herpes viral interspecific transmission creates significant risk for captive primates and the human handlers working with them. Reverse zoonosis events of HSV have resulted in several outbreaks in various parts of the world. Transmission in nonhuman primates has occurred by direct contact with mucosal surfaces, wounds, and maternal milk or through contaminated feed with HSV. In contrast to infection in humans, significant and often fatal disease occurs with HSV infection in a variety of nonhuman primates and other species. Affected animals have presented with whitish vesicular lesions on the soft palate and ulcerative tongue, as well as enteritis, severe emaciation, vomiting, serous nasal and ocular discharge, dyspnea, neurological signs, prostration, and death (Landolfi et al. 2005).

While the many types of adenoviruses (AdVs) are in general thought to be host-specific, there is increasing evidence that these viruses may infrequently cross

species. In particular, there is evidence that nonhuman primates have been infected from human reservoired AdVs. This risk will likely be higher where contact between species is more prevalent, such as with captive and habituated animals (Dehghan et al. 2019; Medkour et al. 2020; Roy et al. 2009; Shrivastava et al. 2020; Pauly et al. 2015). Recently, similar AdVs in humans and gorillas have been detected and sequences derived from gorillas revealed high similarity to human sequences, suggesting the potential for zoonotic transmission (Medkour et al. 2020). Similar findings have also been shown for feline AdVs, which has been demonstrated to be genetically homologous to human AdV type 1.

Multiple types of hepatitis viruses (HV) are major threats to human and animal health. However, sources of viral infection for many animals are unknown since transmission may occur from animal to animal, human to human, animal to human, and human to animal. Although animal-to-human transmission of HV is relatively well described, pathogen transmission in the opposite direction is poorly understood. For instance, swine function as a reservoir for hepatitis E virus (HEV) infections in humans but there is no clear evidence yet of human HEV spillbacks from humans to swine or other animals. Persons with occupational contact to domestic pigs, such as slaughterhouse workers, pig farmers, or veterinarians, exhibit significantly higher anti-HV antibody prevalence than the general population. Farm animals are transported across wide geographical ranges and often interact with wild species that they would never have encountered naturally. With intensified global animal production and an increase in the movement of both animals and humans, a human-originated pathogen could easily circulate and eventually adapt in different niches/hosts/species (Messenger et al. 2014). Detection of hepatitis B virus (HBV) in old-world primates, neotropical primates, domestic animals, bats, and rodents suggests that transmission from humans to animals or animals to humans is likely to occur wherever there is habitat overlap (Vieira et al. 2019).

Multiple types of rotaviruses (RV) are a major cause of severe gastroenteritis and mortality in young children and animals. The interspecies transmission of animal RV to humans and back to animals is plausible due to the close contact between animals and humans. It may augment interspecies infections and genetic reassortment during co-infection with rotavirus strains from different host species. The reassortment may further result in the evolution of novel or atypical RV. There are several pieces of evidence that demonstrate a potential reverse zoonotic transmission cycle of human RV to animals (Abe et al. 2010; Choudhary et al. 2017; Steyer et al. 2008; Wu et al. 2017; Kumar et al. 2018; Sharma et al. 2013; Ghoneim and K.A.A.-M.a.H.S. 2019).

Human noroviruses (HuNoVs) are the predominant cause of foodborne gastroenteritis worldwide. The possibility of animal transmission of HuNoV is also supported by previous studies, which observed HuNoVs in the feces of cattle, pigs, dogs, birds, and a rat (Caddy et al. 2015; Charoenkul et al. 2020; Summa et al. 2018, 2012; Wolf et al. 2013; Mattison et al. 2007). HuNoV sequences detected in avian fecal samples were identical or nearly identical to previously published sequences from human samples and differed from sequences identified in other avian or murine samples (Summa et al. 2018).

Humans and nonhuman primates are closely related species who share a general predisposition for pathogen exchange and reverse zoonotic transmission risk. Anthroponotic respiratory viruses including human metapneumovirus (hMPV), rhinovirus, respiratory syncytial virus (RSV), and parainfluenza virus (PIV) have caused notable morbidity and mortality among wild apes including chimpanzee, gorilla, and bonobo populations. These infections have heightened concerns about the risk of human pathogen transmission to wild animals and critical effects of anthroponotic transmission on ecosystem biodiversity, conservation efforts, and the economy. HMPV and PIV have been identified as causative agents in morbidity and mortality events in other nonhuman primate populations in the wild and in captivity (Hanamura et al. 2008; Kaur et al. 2008; Köndgen et al. 2010; Patrono et al. 2018a; Scully et al. 2018; Slater et al. 2014; Szentiks et al. 2009; Unwin et al. 2013; Negrey et al. 2019; Sojka et al. 2020; Yoshida et al. 2021; Jones-Engel et al. 2001; Kilbourn et al. 2003). HMPV infections in Tanzania, hMPV and human respirovirus 3 in Uganda, rhinovirus C in Uganda, and coronavirus OC43 have been implicated to have significant mortality in wild chimpanzees (Kaur et al. 2008; Patrono et al. 2018a; Scully et al. 2018; Negrey et al. 2019). In 2009, hMPV contributed to the deaths of two gorillas during an outbreak of severe respiratory infection (Palacios et al. 2011). hRSV has been documented to simultaneously infect lowland, wild bonobos, chimpanzees, and people (Szentiks et al. 2009; Unwin et al. 2013; Grützmacher et al. 2018, 2016; Mazet et al. 2020). Co-infection with other bacterial pathogens could also worsen outcomes of anthroponotic respiratory infections in nonhuman primates. Frequent human contact predisposes wild animals to respiratory viruses of human origin, especially in zoos and wildlife parks (Kilbourn et al. 2003; Buitendijk et al. 2014; Kooriyama et al. 2013).

In addition to human respiratory viruses, Zika virus, dengue virus (DV), Ebola virus (EBOV), and human enterovirus (EV) have also been reported in animals, although routes of transmission are not clear (Kilbourn et al. 2003; Favoretto et al. 2019; Kato et al. 2013; Terzian et al. 2018; Williams et al. 2008; Barrette et al. 2009; Bermejo et al. 2006; Demetria et al. 2018; Fieldhouse et al. 2018). The H1N1pdm09 virus is anthroponotic and has demonstrated cross-species transmission in a variety of animal species, including swine, cats, ferrets, dogs, turkeys, and cheetahs. In all these reported cases, some human owners or animal caretakers were reported to have influenza-like illness and there were frequent contacts and close interaction between the infected animals and humans prior to the detection of the virus in the animal. Human-to-swine transmission of H1N1pdm09 is the most frequently reported reverse zoonoses during the last decade and detection of human-origin H1N1pdm09 in swine herds was reported in many countries (Sooksawasdi Na Ayudhya and Kuiken 2021). In Canada, the first human-to-swine transmission of H1N1pdm09 influenza was observed at a swine farm (Howden et al. 2009). Since then, the H1N1pdm09 virus has transmitted repeatedly from humans to swine spanning six continents (Forgie et al. 2011; Hofshagen et al. 2009; Moreno et al. 2010; Pereda et al. 2010; Sreta et al. 2010; Kim et al. 2011; Deng et al. 2012; Chastagner et al. 2019; Ducatez et al. 2015; Grøntvedt et al. 2013; Njabo et al. 2012; Osoro et al. 2019a; Adeola et al. 2015, 2017; Arunorat et al. 2016; Ayim-Akonor

et al. 2020; Er et al. 2016a, 2020; Rajão et al. 2013; Senthilkumar et al. 2021; Baudon et al. 2015; Meseko et al. 2014; Nagarajan et al. 2010; Ma et al. 2018; Song et al. 2010; Terebuh et al. 2010; Trevennec et al. 2012; Sabale et al. 2013; Nelson et al. 2015; Mon et al. 2020; Pippig et al. 2016; Bálint et al. 2013; McCune et al. 2012). Pigs can act as an active reservoir for H1N1pdm09 virus. Reverse zoonosis events linked to infected farmers with H1N1pdm09 virus have been supported by serological and molecular diagnostics (Forgie et al. 2011; Holyoake et al. 2011). Sequencing analysis has shown that the viruses infecting humans and pigs are highly similar; hence, H1N1pdm09 does not require major changes to adapt and cause replication in swine (Sooksawasdi Na Ayudhya and Kuiken 2021; Forgie et al. 2011; Song et al. 2010). Besides domestic turkeys, reverse zoonosis events of H1N1pdm09 virus infections have not been reported in other avian species over the years. Sporadic infections of H1N1pdm09 have been mainly reported in turkey breeder flocks and sick farm workers potentially transmitted virus to breeder turkeys during artificial insemination (Berhane et al. 2010; Mathieu et al. 2010). The farm workers were showing influenza-like illness symptoms and had antibodies against H1N1pdm09 virus in their serum samples. Viruses from infected farm workers and turkeys revealed highly similar sequences which further supported worker-to-turkey transmission (Sjurseth et al. 2017). Seroconversion against H1N1pdm09, virus isolation, and molecular detection of H1N1pdm09 virus in several organs of infected turkeys confirmed H1N1pdm09 influenza in turkey breeder flocks (Sjurseth et al. 2017; Reid et al. 2012). No mortality was reported among infected turkeys and clinical signs ranged from none to mild (Sooksawasdi Na Ayudhya and Kuiken 2021; Sjurseth et al. 2017; Reid et al. 2012).

Human influenza viruses (H3N2 and H1N1pdm09) isolated from mink strongly suggest human-to-mink virus transmission (Gagnon et al. 2009). Recently, a serological survey revealed that farmed mink were commonly infected with human H3N2 and H1N1pdm09 and transmission of human influenza viruses occurred from humans to mink (Sun et al. 2021). During the pandemic, a mink farm in Norway reported H1N1pdm09 virus infections in American mink and eight striped skunks died on a mink farm in Canada (Åkerstedt et al. 2012; Britton et al. 2010). Phylogenetic analysis revealed highly similar sequences among those derived from infected minks and humans during the same pandemic. Sequences obtained from infected mink were highly similar to human-derived isolates during the H1N1pdm09 pandemic indicating transmission from humans. The source of transmission could be sub-clinically infected humans, fomites, or virus-contaminated feed from infected pig offal. Higher mortality rates were reported among infected minks due to severe respiratory disease.

There are many reports of H1N1pdm09 virus infection in companion animals (e.g., dogs, cats) (Sooksawasdi Na Ayudhya and Kuiken 2021; Sabale et al. 2013; Ali et al. 2011; Damiani et al. 2012; Fiorentini et al. 2011; Ibrahim et al. 2016a; Kwasnik et al. 2020; Löhr et al. 2010; Pigott et al. 2014; Ramírez-Martínez et al. 2013; Said et al. 2011; Seiler et al. 2010; Yin et al. 2014; Kovalenko et al. 2021; Sponseller et al. 2010; Su et al. 2014, 2013; Zhang et al. 2015; Zhao et al. 2014; Jang et al. 2017; Lin et al. 2012; McCullers et al. 2011; Sun et al. 2014; Tangwangvivat

et al. 2019). Molecular diagnostics performed on tissue organs including tonsils, trachea, lungs, and nasal swab and pharyngeal specimens detected H1N1pdm09 viral RNA (Lin et al. 2012; Campagnolo et al. 2013). A sequence analysis revealed a close relationship to H1N1pdm09 virus from infected humans and animals during the pandemic (Fiorentini et al. 2011; Lin et al. 2014). In addition, detection of antibodies against H1N1pdm09 virus in cats and dogs during the period of virus spread in the human population strongly supports the occurrence of reverse zoonoses. Since companion animals often live together in close contact with humans, the mode of transmission could be direct contact between animals and humans during the same time period (Dundon et al. 2010; Zhao et al. 2014). A case of natural H1N1pdm09 infection in a pet ferret was also reported (Campagnolo et al. 2013; Lin et al. 2014; Swenson et al. 2010). Serological studies showed that group housing of animals likely facilitated efficient intraspecies transmission, including cat-to-cat transmission and ferret-to-ferret transmission (Fiorentini et al. 2011; Campagnolo et al. 2013). However, H1N1pdm09 virus transmission between dogs seemed to be limited. Although all of these species were susceptible to H1N1pdm09 virus infection, their clinical signs varied. Cats and ferrets often developed severe respiratory signs, including dyspnea, coughing, and sneezing, and even died from the infection (Sooksawasdi Na Ayudhya and Kuiken 2021; Sponseller et al. 2010; Campagnolo et al. 2013, 2011; Knight et al. 2016). However, dogs either showed no clinical signs or only mild respiratory signs, such as rhinorrhea and coughing (Sooksawasdi Na Ayudhya and Kuiken 2021; Lin et al. 2012).

Evidence of H1N1pdm09 virus reverse zoonoses has been shown among wild animals kept in captivity. This includes cheetah, elephants, a Bornean binturong, an American badger, a black-footed ferret, and giant panda (Crossley et al. 2012; Lin et al. 2014; Goldstein et al. 2013; Paungpin et al. 2017; Schrenzel et al. 2011; Martelli et al. 2019). All animals were housed separately from other wildlife and the source of transmission was not exactly known. It is possible that tourists, caretakers, and veterinarians might have caused a spillback of the virus to zoo animals. The infected giant panda, American badger, and the Bornean binturong showed severe signs of respiratory infection. However, the black-footed ferret did not show any clinical signs (Schrenzel et al. 2011). In addition, H1N1pdm09 has also been reported in Asian elephants. Although the source of infection could not be confirmed, animal caretakers and tourists were implicated as a possible source of transmission (Paungpin et al. 2017).

Like Asian elephants, evidence of H1N1pdm09 virus infection among nonhuman primates has been reported in some studies (Buitendijk et al. 2014; Kooriyama et al. 2013). However, there was no clear association or evidence that H1N1pdm09 was solely responsible for clinical signs of disease or mortality from H1N1pdm09 virus infection in nonhuman primates (Sooksawasdi Na Ayudhya and Kuiken 2021; Buitendijk et al. 2014). There are limited serological and epidemiological studies among captive wild animals and thus it is still largely unknown whether these animals could transmit virus to other closely associated animals or act as a new reservoir for the virus. In addition, H1N1pdm09 virus infections have also been reported in giant anteaters, seals, and sea lions (Goldstein et al. 2013; Boyce et al.

2013; Nofs et al. 2009). The only free-living wild animal species in which H1N1pdm09 virus has been reported is the striped skunk. Sequencing and phylogenetic analysis of virus isolated from affected animals were highly related to H1N1pdm09 virus circulating in humans (Britton et al. 2010, 2019). The source of infection was unclear. In one study, the skunks lived near a mink farm, suggesting that spillover of H1N1pdm09 virus from infected mink farm workers or infected mink may have occurred (Britton et al. 2010). In another study, the skunks were found in an urban park where hand feeding by park visitors normally took place (Britton et al. 2019). Clinical signs were not observed in all infected minks and only fatally infected skunks showed purulent nasal exudate.

Natural infection of SARS-CoV-2 has been reported in domesticated and wild animal species. The frequency of spillover events to other species has been relatively low, despite the high incidence of SARS-CoV-2 human infection. Some studies have reported acute SARS-CoV-2 infection in dogs and cats who had close interaction with SARS-CoV-2-infected individuals, indicating a strong possibility for human-to-pet transmission (Sailleau et al. 2020; Segalés et al. 2020; Sit et al. 2020; Ruiz-Arrondo et al. 2021; Newman et al. 2020; Musso et al. 2020; Barrs et al. 2020; Hamer et al. 2020; Gaudreault et al. 2020; Garigliany et al. 2020; Klaus et al. 2021; Pagani et al. 2021; Patterson et al. 2020). There are several reports which describe natural infection of SARS-CoV-2 infections in zoo animals involving large felids and nonhuman primates. Most zoo animals do not exhibit any clinical signs of infection and remain largely asymptomatic. Conversely, infected companion animals (e.g., dogs, cats) have usually presented with asymptomatic or mild infections involving respiratory and digestive systems. Chicken, ducks, dairy animals, and pigs have shown less susceptibility to SARS-CoV-2 infection. Camels and alpacas are known carriers of Middle East respiratory syndrome virus (MERS-CoV), but show no susceptibility to SARS-CoV-2. Naturally acquired infections of SARS-CoV-2 have been demonstrated in tigers, lions, gorillas and chimpanzees, domesticated ferrets, mink, and white-tailed deer (Kuchipudi et al. 2022; McAloose et al. 2020; Bartlett et al. 2021; Wang et al. 2020a; Mitchell 2021; Karikalan et al. 2021; Mishra et al. 2021; Fernández-Bellon et al. 2021; Glasser et al. 2021; Gortázar et al. 2021; Molenaar et al. 2020; Oreshkova et al. 2020; Oude Munnink et al. 2021; Hammer et al. 2021; Aguiló-Gisbert et al. 2021; Shriner et al. 2021). All such cases of natural infection have been associated with SARS-CoV-2-infected animal caretakers or tourists who transmitted the virus to animals by their direct or indirect contacts. Like other animals, these animals exhibited a wide range of clinical signs from mild-to-severe respiratory and gastrointestinal signs (Oreshkova et al. 2020).

Apart from direct human-to-animal transmission (e.g., by physical contact with domestic and wild animals), transmission to animals via human waste and discarded items is also likely. Contamination of aquatic systems with unmanaged feces and improperly disposed personal protective equipment from infected humans could be a route for spillover into aquatic and wild mammals. Current role of wildlife in the global epidemiology of SARS-CoV-2 seems negligible. However, wildlife could become a potential reservoir for SARS-CoV-2 over time and may further transmit

the virus to humans. Phenotypic variants could also evolve as the virus adapts to new species facilitating continued transmission to humans and other species.

2.6 Evidence of Reverse Zoonosis of Bacteria and Disease Symptoms in Animals and Humans

In recent years, reverse zoonotic transmission of *Mycobacterium tuberculosis* and *Mycobacterium bovis*, human pathogens that cause tuberculosis (TB), have been reported in a wide range of wildlife species. While the actual host range is unknown, between 1974 and 2019, *M. tuberculosis* was isolated from 22 animals. *M. tuberculosis* transmission between human and animals was reported among companion pets in households and among the livestock population (Fernandes et al. 2018; Hackendahl et al. 2004; Kandefer-Gola et al. 2016; Schmidt et al. 2008; Weese et al. 2006; Ackerman et al. 1974; Every et al. 2011; Mittal et al. 2014; Špičić et al. 2012; Thakur et al. 2012). While this finding may accentuate the importance of effective TB detection in human-habituated areas, most reverse zoonotic bacterial infections reported have been among captive wild animals. The pathogens detected in this group include *Staphylococcus aureus, Streptococcus pneumoniae,* and *Campylobacter* spp. (Szentiks et al. 2009; Nagel et al. 2013; Seguin et al. 1999; Senghore et al. 2016; Sørensen et al. 2019; Unwin et al. 2013; On et al. 2019). Close contact, common feeding and drinking sources, and exposure to infected caretakers, veterinarians, and visitors are suspected transmission routes of human-to-animal transmission, which suggests that zoos, research facilities, and wildlife sanctuaries may all serve as reservoirs of infection and spread of the disease.

An extensive study of the prevalence of reverse zoonotic transmission is usually carried out following a report of an outbreak. Prevalence studies in the livestock industry detected several cases of zoonotic bacterial species moving from a farm worker carrying the disease to susceptible livestock. *S. aureus* and *M. tuberculosis* are found in livestock animals like cattle, swine, camels, and goats (Adesokan et al. 2019; Berg et al. 2009; Cobo-Angel et al. 2019; Crestani et al. 2021; Osadebe et al. 2013; Sunde et al. 2011; van de Vijver et al. 2014; Gumi et al. 2012). Other bacterial species were found in livestock and domestic animals such as *Acinetobacter baumannii* in sheep; *Escherichia coli* in chickens, dogs, and horses; *Salmonella* in poultry; and methicillin-resistant staphylococci (MRS) in companion animals living nearby livestock farms (Linz et al. 2018; Li et al. 2019; Nguyen Vinh et al. 2016; Ewers et al. 2010; Post et al. 2019; Loncaric et al. 2016; Kottler et al. 2010). Prevalence studies found bacterial pathogens in several wild animals in zoos, in a sanctuary, and in unprotected natural areas, particularly among primate species such as apes, lemurs, baboons, gorillas, and monkeys (Schaumburg et al. 2012, 2013; Rolland et al. 1985; Nizeyi et al. 2001; Rwego et al. 2008a; Tang et al. 2021). In addition, infections were found in other terrestrial mammals such as banded mongoose and stripe-faced dunnarts (Every et al. 2011; Pesapane et al. 2013). Interestingly, reports of enteric bacteria in Antarctic wildlife suggest a spread of zoonotic agents among seabirds in Antarctica (Cerdà-Cuéllar et al. 2019).

Many studies investigate clinical, microbiological, and molecular characteristics of pathogens in order to determine the epidemiologic origin of novel antimicrobial resistant zoonotic pathogens using genomic approaches. Oftentimes, the strains are found to be of human origin. Methicillin-resistant *Staphylococcus aureus* (MRSA) are prevalent in companion animals, livestock, and wildlife, but oxacillin-resistant *S. aureus* was reported only in Taiwan (Kwon et al. 2006; Morris et al. 2006; Price et al. 2012; Rubin et al. 2011; Feßler et al. 2018; Hsieh et al. 2008). Studies suggest zoonotic pathogens harbor antimicrobial susceptibility of human origin, for example, extended-spectrum beta-lactamases (ESBL) producing enterobacteria in fruit bats, *Clostridium difficile* in farm animals, *Enterococcus faecalis* in poultry, and *E. coli* in chimpanzees (Hasan et al. 2018; Mbehang Nguema et al. 2020; Knetsch et al. 2018; Goldberg et al. 2007). Some studies of *M. tuberculosis* in wildlife unintentionally discovered a genetic similarity between human and animal strains (Goldberg et al. 2008; Michel et al. 2013; Prasad et al. 2005). Studies of human pathogens in aquatic species found the same conclusion such as *Serratia marcescens* in mammals and fish and *Streptococcus agalactiae* in elkhorn coral (Delannoy et al. 2013; Sutherland et al. 2011).

2.7 Evidence of Reverse Zoonosis of Parasites and Disease Symptoms in Animals and Humans

Zoonotic parasites can be transmitted bidirectionally from humans and animals, often via fecal-oral exposure pathways due to contaminated environmental spaces, food, or water. Poorly managed excreta from free-roaming livestock and other animals, inadequate community sanitation services, and areas with open defecation can result in fecal pollution of private and public settings which expose humans and animals to shared pathogens. Safe handling, storage, and treatment of human and animal waste are critical to the prevention of enteric parasites which often follow exposure pathways of contaminated food, water, soil, hands, personal items (i.e., fomites), and filth flies.

For instance, many *Cryptosporidium* spp. are shared between human and animal hosts with several incidents of, or potential for, reverse zoonotic transmission occurring in livestock, companion animals, and wildlife after human contact (Shrivastava et al. 2020; Wang et al. 2020b; Schiller et al. 2016; Ibrahim et al. 2016b; Learmonth et al. 2001; Coklin et al. 2007; Dixon et al. 2011; Graczyk et al. 2001; Guk et al. 2004; Krawczyk et al. 2015; Mynářová et al. 2016; Nizeyi et al. 1999; Nolan et al. 2017; Sak et al. 2014). Likewise, *Giardia duodenalis/Giardia intestinalis* has evidence of probable reverse zoonotic transmission from humans to animals (Coklin et al. 2007; Dixon et al. 2011; Krawczyk et al. 2015; Mynářová et al. 2016; Arafa et al. 2013; Graczyk et al. 2002a; Johnston et al. 2010; Nasher 1988; Sá et al. 2013; Sak et al. 2013; Sleeman et al. 2000; Tangtrongsup et al. 2019; Ash et al. 2010; Teichroeb et al. 2009). *Pentatrichomonas hominis* and *Entamoeba* sp., while considered non-pathogenic to humans, can serve as fecal indicator organisms for proximal water and/or food contamination and have the potential for reverse zoonotic transmission leading to diarrheal disease in animals (Nolan et al. 2017; Arafa et al. 2013; Li et al. 2016).

Gastrointestinal parasites *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Encephalitozoon cuniculi* can cause microsporidiosis, sometimes referred to as a fungal infection, and there is probable evidence of human-to-animal transmission (Sak et al. 2014; Yu et al. 2017; Graczyk et al. 2002b). Additionally, several soil-transmitted helminths like *Ascaris* sp., hookworm, and *Trichuris* spp. can produce infectious particles that are persistent in the environment allowing for reverse zoonoses to occur in animals that live in close proximity to humans and human waste (Arafa et al. 2013; Nasher 1988; Adejinmi 2008; Murray et al. 2000; Mohd-Shaharuddin et al. 2019; Chang et al. 2006).

Alternatively, the parasitic disease of leishmaniasis is a vector-borne zoonoses caused by the bite of an infected female sand fly. This insect vector takes blood meals from both humans and animals and can spread *Leishmania* spp. resulting in multiple disease presentations in humans such as cutaneous, visceral, and mucosal leishmaniasis. Reverse zoonoses of cutaneous and visceral leishmaniasis present a potential exposure risk for animals, particularly dogs, in the vicinity of people and the sand fly vector (Dereure et al. 2003; Faulde et al. 2008a; Ait Kbaich et al. 2017). The human skin mite vector, *Scabies scabiei*, can also be transmitted from humans to animals and cause significant morbidity and mortality among immunocompromised hosts (Kalema-Zikusoka et al. 2002; Pusey 1998).

2.8 Evidence of Reverse Zoonosis of Fungi and Disease Symptoms in Animals and Humans

Over the last 5 years, there have been an increasing number of fungal infections identified among animals and humans. Fungal spores spread in the air and soil of which some are capable of causing infection (Gnat et al. 2021). Reviewing past reports on fungal infections demonstrates cases of reverse zoonoses between a pet owner and their animal(s). One such example is a report of *Trichophyton rubrum*. This fungal species is sometimes found to be related to ringworm in baboons, cats, cattle, and dogs. In one case report, *T. rubrum* was transmitted from the owner to a pet monkey (Pal et al. 1997). In a subsequent report of human-to-animal fungal infection, *Microsporum gypseum* was found to have transmitted from a pet owner to a puppy (Sharma et al. 2009). Beyond those examples of fungal reverse zoonoses due to close contact, an analysis of clades of *Candida albicans* in humans and wildlife has suggested possible reverse zoonoses. The authors suspect that transmission may have occurred due to wildlife having close contact with poorly managed human garbage including dirty diapers or half-eaten food, which could have been the source of infection (Wrobel et al. 2008). These reports have suggested the possibility of transmission of fungal infections from humans to animals. Since a variety of fungal infections are capable of transmission to both human and animal species, more attention is needed to monitor for potential reverse zoonotic events. Despite the limited number of reported cases, this may not be a true indication of low probability for such transmissions.

2.9 The Impact of Zoonosis and Reverse Zoonosis on Animals and Humans

The expenses associated with zoonoses can be measured from related prevention efforts, such as vaccination campaigns, to the health-care costs for treating a human or animal infection (Shaw et al. 2017). However, the costs of zoonoses extend beyond a single case of disease. The impacts and consequences of zoonotic and reverse zoonotic disease encompass more than just physical, social, mental, and emotional health but also the integrity of global economies, agricultural markets, tourism, trade and transportation, gender equality, children's health, international policy, the environment, wildlife conservation, trust in public health and veterinary public health, and more (Jordan et al. 2016; McDonald 2011).

Human effects of zoonotic disease expand beyond the infected person to their household and their communities. For example, if a person is sick from a zoonosis, they may experience pain, social stigma related to the illness, high individual or institutional medical care costs, acute or long-term disability, the inability to participate in normal activities such as childcare, loss of job productivity or lost wages, disruption in education, food insecurity, a risk for additional comorbidities, and even death (Shaw et al. 2017; Jordan et al. 2016). The global socioeconomic burden of zoonotic infections disproportionately impacts lower-income countries and marginalized, vulnerable communities (Grace et al. 2012). Cases of zoonoses are often underreported and categories of neglected zoonotic diseases may not be reflected in national morbidity or mortality statistics (Grace et al. 2012). Estimating the true burden of zoonotic disease for human and animal populations within these regions is challenging.

When an animal is sick from a zoonotic disease, they may also endure pain or death in addition to weight loss, decrease in milk production, infertility, the inability to procure a high trade or sale price, and costs associated with veterinary services (Shaw et al. 2017). Besides the significant harm zoonoses can have on a household's dietary access to animal-based food items or economic opportunity through sale or barter, the region or country may experience a restriction on their ability to export or trade in agricultural goods and food products during an outbreak event (Narrod et al. 2012; Martins et al. 2015). Global livestock production and agriculture is heavily dependent upon biosecurity measures from production to consumption (Waage and Mumford 2008). Without robust sanitation and hygiene measures in place at each farm and packaging facility, the potential for zoonotic disease transmission could be markedly high (Martins et al. 2015). For example, the World Bank has estimated that the economic loss from just six major zoonotic disease outbreaks between 1997 and 2009 was at least $80 billion USD and projected the costs for the global SARS-CoV-2 pandemic to be in the trillions (World 2006). Recognizing and responding to early infections of zoonotic or reverse zoonotic disease in either the human or animal host is critical to prevent further cases or outbreaks.

Spillover events, wherein humans are exposed to zoonotic pathogens largely circulating within wild animal reservoirs, are often due to anthropogenic changes to the land that disrupt the natural wildlife host and disease cycles and create new

opportunities for animal-human contact (Plowright et al. 2020). Since early human movement of people and animals, the spread of zoonotic pathogens into, and out of, previously undisturbed natural wildlife habitats have led to outbreaks and pandemics (May et al. 2021). Reverse zoonotic transmission of virus, bacteria, and parasites to wild animals (i.e., primates) can occur from contact with humans following conflict, land-use changes, and increased housing along conservation areas and national park boundaries (Dunay et al. 2018). As a result, humans could become potential reservoirs or intermediate hosts for some pathogens. Hunters, loggers, livestock herds and herders, farmers, researchers, and visitors have been known to spread emerging and reemerging diseases to and from wild animals through zoonotic spillover and reverse zoonoses (May et al. 2021; Plowright et al. 2017). Therefore, prevention of zoonotic and reverse zoonotic disease is also crucial for the conservation and protection of global biodiversity.

2.10 How to Prevent Occurrence of Reverse Zoonosis

Conventional surveillance measures for infectious disease outbreaks include seroepidemiological studies in outbreak areas, reducing distance (quarantine and physical distancing), vaccinating humans and animals, and conducting serosurveillance (Lee et al. 2021; Gray et al. 2015; Jefferson et al. 2011; Buddle et al. 2013; Bosco-Lauth et al. 2020; Jiles et al. 2014). However, these within-outbreak approaches are principally reactive rather than preventive. As cross-species spillovers result from a multi-level interaction between hosts, pathogens, and environment, ecological interventions to eliminate the environmental conditions that favor human-to-animal transmission could prevent the occurrence of reverse zoonosis. For instance, measures for reducing prevalence in reservoir hosts (i.e., vaccination, treating infections) and manipulating reservoir-host connectivity to reduce contact and spread rates (i.e., fences and translocation) or fertility control of susceptible species could prove effective strategies (Sokolow et al. 2019). In recent years, genomic-based approaches have gained momentum in research as they allow for the accurate identification of potential hotspots and locations with a high density of susceptible groups to make ecological interventions more cost-effective and sustainable.

Geographic proximity may increase the likelihood of cross-species transmission, but successful infection is determined by the biological compatibility of hosts and recipient species, primarily immunological profiles. Phylogenetic studies found that closely related species tend to have similar immunological responses and are of greater risk of interspecies transmission (Woolhouse et al. 2001; Ricklefs and Fallon 2002). Accurate identification of vulnerable species and their habitats can inform policymakers where an escalation in public health intervention may be needed. Further, genomic surveillance data of the pathogen's dissemination patterns can broadly describe outbreak dynamics. Such insights can fill the gap in monitoring epidemics, superspreading events, and differentiating between relapse and reinfection events (Kinganda-Lusamaki et al. 2021). Recently, research focus has been on

infectious disease forecasting models for prediction of characteristics of future outbreaks. The forecasting tools can provide useful guidance for the utility, scale, and timing of prevention and mitigation strategies (Scarpino and Petri 2019; Lutz et al. 2019).

Policy legislation and reinforcement play a vital role in transforming research into practice. In livestock production and wildlife conservation where transmission is more likely, vaccination in both animals and human must be required. Further regulation on regular health checks and disease screening is subject to the geographic area and vulnerability to outbreaks. Besides monitoring the transmission among livestock and wildlife populations, One Health efforts and professionals should pay more attention to the development of education and training related to reverse zoonosis among vulnerable populations to prevent future outbreaks. Owners of companion animals are also the target of such programs to reduce transmission in households where close proximity is often a major concern of secondary infection among close contacts. Importantly, one crucial improvement with the One Health approach is the cohesive cooperation between researchers in epidemiology and public health fields. Within the included titles, there was a lack of well-roundedness and connection in fields of both animal and human disease. Therefore, an adoption of the One Health approach must underlie the importance of transdisciplinary cooperation to achieve a multifaceted understanding of the spread of infectious disease, to better protect the health of humans and animals and their shared environment.

According to the definition given by the CDC, "One Health is a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment" (Alves and Policarpo 2018). With the intent of reducing disease in both human and animals, a One Health approach seeks collaboration between experts in various areas. After reviewing reports on the topic of reverse zoonosis, several aspects of this model should be given more attention.

While human habitat has grown to have more overlap with natural habitat, leading to closer contacts between human and wild animals, the risk of disease transmission between human and domesticated animals should receive more consideration. Among recent reports on transmission of disease between animals and humans, whether zoonotic or reverse zoonotic, most of the cases were reported between farm animals and hosts (Mi et al. 2016; Europe, A.h. 2017; Kruse et al. 2004; Reperant et al. 2013; Di Marco et al. 2020; Sooksawasdi Na Ayudhya and Kuiken 2021). This grouping of animals and humans has provided an optimal environment for fostering disease transmission. However, there still lack a complete education system and preventative policy for most agriculture and food chain workers. This poses a risk not only for the transmission of disease to the host animals but would also endanger the health of the public as infectious agent could move into the larger global chain of food production and distribution.

In addition to consideration of animals within and around farms, more emphasis should be put on the threat of reverse zoonoses among companion animals. As people have close contact with their pets through sharing beds, kissing and

snuggling, and eating and drinking in proximity, the risk of disease transmission between human hosts and their pets also increases (Zhang et al. 2020; Zhao et al. 2014). For example, in recent reports on cases of SARS-CoV-2, there have been several instances of the transmission of the virus from infected people to their household pet animals (Forgie et al. 2011; Holyoake et al. 2011; Sweetline Anne et al. 2017). While early One Health work focused on researching ways to reduce disturbance of wild animals to prevent anthroponotic transmission of human pathogens, the policies and strategies relating to protecting companion animals require improvement. This is of special importance because when people are quarantined in response to a pandemic, the contact between the host and pet(s) is likely increased. In response to One Health's principle of "One Health involves everyone," we should encourage the importance of close observation of pet animals when an infected household member has been identified to prevent possible transmission in the home.

Besides seeking cooperation from stakeholders at the health professional level, we should also encourage more cooperation between researchers. Among the epidemiological reports included in this summary, the publications frequently lacked input from experts in the fields of both animal and human disease. Therefore, such reports usually suggested a possibility of transmission but did not provide tangible evidence that may have been garnered by working with a professional in the parallel areas of animal or human disease. By incorporating the principles of One Health, transdisciplinary cooperation can be enhanced that leads to a more multifaceted understanding of the spread of infectious disease that will better protect the health of all species.

2.11 Conclusions

The threat of disease derives from multiple host species and a variety of exposure pathways. The accelerating human impact on the natural world will likely introduce new opportunities for disease transmission stemming from emerging and reemerging pathogens, likely with zoonotic origins. While we must continue our efforts to discover intervention strategies to prevent, detect, and treat human infection, it is vital that we also devote new and innovative efforts towards the protection and conservation of animals. Intensified farming practices, wildlife habitat encroachment, and an increased desire for companion animals will forge even more intimate human-animal bonds in the coming years. It will take collaboration and communication between researchers and health professionals from both the human and animal fields to prepare, promote, and provide safe and healthy interactions for all.

2.12 Cross-References

- ▶ Important Zoonotic Diseases of Cattle and their Prevention Measures
- ▶ Influenza from a One Health Perspective: Infection by a Highly Versatile Virus
- ▶ Severe Acute Respiratory Syndrome Coronaviruses-2 (SARS-CoV-2)

- ▶ Small Ruminants: Zoonotic Infections
- ▶ Zoonoses Transmitted by Poultry
- ▶ Zoonoses and Poverty: The Multiple Burdens of Zoonoses in Low- and Middle-Income Countries
- ▶ Zoonotic Diseases of Swine: Food-Borne and Occupational Aspects of Infection

References

Abe M et al (2010) Molecular characterization of rotaviruses in a Japanese raccoon dog (*Nyctereutes procyonoides*) and a masked palm civet (*Paguma larvata*) in Japan. Vet Microbiol 146(3–4):253–259

Ackerman LJ, Benbrook SC, Walton BC (1974) *Mycobacterium tuberculosis* infection in a parrot (*Amazona farinosa*). Am Rev Respir Dis 109(3):388–390

Adejinmi OJ (2008) Preliminary investigation of zooanthroponosis in a Nigerian Zoological Garden. Vet Res 2:38–41

Adeola OA, Olugasa BO, Emikpe BO (2015) Detection of pandemic strain of influenza virus (A/H1N1/pdm09) in pigs, West Africa: implications and considerations for prevention of future influenza pandemics at the source. Infect Ecol Epidemiol 5:30227

Adeola OA, Olugasa BO, Emikpe BO (2017) Molecular detection of influenza A(H1N1)pdm09 viruses with M genes from human pandemic strains among Nigerian pigs, 2013-2015: implications and associated risk factors. Epidemiol Infect 145(16):3345–3360

Adesokan HK et al (2019) Reverse zoonotic tuberculosis transmission from an emerging Uganda I strain between pastoralists and cattle in South-Eastern Nigeria. BMC Vet Res 15(1):437

Aguiló-Gisbert J et al (2021) First description of SARS-CoV-2 infection in two feral American mink (Neovison vison) caught in the wild. Animals (Basel) 11(5):1422. https://doi.org/10.3390/ani11051422

Ait Kbaich M et al (2017) New epidemiological pattern of cutaneous leishmaniasis in two pre-Saharan arid provinces, southern Morocco. Acta Trop 173:11–16

Åkerstedt J et al (2012) Pneumonia caused by influenza A H1N1 2009 virus in farmed American mink (Neovison vison). Vet Rec 170(14):362

Alberts B (2015) Molecular biology of the cell. Garland Science, Taylor and Francis Group, New York

Alexander KA, Pleydell E, Williams MC, Lane EP, Nyange JF, Michel AL (2002) *Mycobacterium tuberculosis*: an emerging disease of free-ranging wildlife. Emerg Infect Dis 8(6):598–601. https://doi.org/10.3201/eid0806.010358

Ali A et al (2011) Pandemic and seasonal human influenza virus infections in domestic cats: prevalence, association with respiratory disease, and seasonality patterns. J Clin Microbiol 49 (12):4101–4105

Allison N et al (2002) Fatal herpes simplex infection in a pygmy African hedgehog (*Atelerix albiventris*). J Comp Pathol 126(1):76–78

Al-Tawfiq JA, Memish ZA (2014) Middle East respiratory syndrome coronavirus: transmission and phylogenetic evolution. Trends Microbiol 22(10):573–579

Alves RRN, Policarpo IdS (2018) Chapter 13 - Animals and human health: where do they meet?∗. In: Alves RRN, Albuquerque UP (eds) Ethnozoology. Academic Press, pp 233–259

Arafa W et al (2013) Investigation of enteric parasites of zoo animals and zookeepers in Beni-Suef Governorate, Egypt. J Vet Med Res 22(1):121–125

Araujo JL, Frade MTS, Melo CMF, Carneiro RS, Galiza GJN, Olinda RG (2016) Systemic infection due to herpes simples virus in a whitetufted-ear marmoset (*Callithrix jacchus*) in the semiarid region of Paraiba, Brasil. Vet Zootec 23(2):203–208

Arunorat J et al (2016) Determination of current reference viruses for serological study of swine influenza viruses after the introduction of pandemic 2009 H1N1 (pdmH1N1) in Thailand. J Virol Methods 236:5–9
Ash A et al (2010) Molecular epidemiology of Giardia duodenalis in an endangered carnivore–the African painted dog. Vet Parasitol 174(3–4):206–212
Ayim-Akonor M et al (2020) Exposure of domestic swine to influenza A viruses in Ghana suggests unidirectional, reverse zoonotic transmission at the human-animal interface. Zoonoses Public Health 67(6):697–707
Bálint A et al (2013) Emergence and characterisation of pandemic H1N1 influenza viruses in Hungarian swine herds. Acta Vet Hung 61(1):125–134
Barnes KJ et al (2016) Herpes simplex encephalitis in a captive black howler monkey (*Alouatta caraya*). J Vet Diagn Investig 28(1):76–78
Barrette RW et al (2009) Discovery of swine as a host for the Reston ebolavirus. Science 325(5937): 204–206
Barrs VR et al (2020) SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. Emerg Infect Dis 26(12):3071–3074
Bartlett SL et al (2021) SARS-COV-2 infection and longitudinal fecal screening in Malayan tigers (*Panthera Tigris Jacksoni*), Amur tigers (*Panthera Tigris Altaica*), and African lions (*Panthera Leo Krugeri*) at the Bronx Zoo, New York, USA. J Zoo Wildl Med 51(4):733–744
Baudon E et al (2015) Detection of novel reassortant influenza A (H3N2) and H1N1 2009 pandemic viruses in swine in Hanoi, Vietnam. Zoonoses Public Health 62(6):429–434
Berg S et al (2009) The burden of mycobacterial disease in Ethiopian cattle: implications for public health. PLoS One:**4**(4)
Berhane Y et al (2010) Molecular characterization of pandemic H1N1 influenza viruses isolated from turkeys and pathogenicity of a human pH1N1 isolate in turkeys. Avian Dis 54(4):1275–1285
Bermejo M et al (2006) Ebola outbreak killed 5000 gorillas. Science 314(5805):1564
Bhatt PN et al (1966) Viral infections of monkeys in their natural habitat in southern India. II. Serological evidence of viral infection. Am J Trop Med Hyg 15(4):561–566
Boedeker NC et al (2017) Pandemic (H1N1) 2009 influenza A virus infection associated with respiratory signs in sloth bears (*Melursus ursinus*). Zoonoses Public Health 64(7):566–571
Bosco-Lauth AM et al (2020) Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. Proc Natl Acad Sci 117(42): 26382
Bowman AS et al (2012) Molecular evidence for interspecies transmission of H3N2pM/H3N2v influenza A viruses at an Ohio agricultural fair, July 2012. Emerg Microbes Infect 1:8
Boyce WM et al (2013) Influenza A(H1N1)pdm09 virus infection in marine mammals in California. Emerg Microbes Infect 2(6):e40–e40
Britton AP et al (2010) Pandemic (H1N1) 2009 in skunks, Canada. Emerg Infect Dis 16(6):1043–1045
Britton AP et al (2019) Probable reverse zoonosis of influenza A(H1N1)pdm 09 in a striped skunk (*Mephitis mephitis*). Zoonoses Public Health 66(4):422–427
Bruno SF et al (1997) [Herpesvirus infections in free living black tufted ear marmosets (*Callithrix penicillata*, E. Geoffroyi 1812) at the State Park of Serra da Tiririca, Niterói, Rio de Janeiro, Brazil]. Berl Munch Tierarztl Wochenschr 110(11–12):427–430
Buddle BM et al (2013) Overview of vaccination trials for control of tuberculosis in cattle, wildlife and humans. Transbound Emerg Dis 60(s1):136–146
Buitendijk H et al (2014) Retrospective serology study of respiratory virus infections in captive great apes. Viruses 6(3):1442–1453
Caddy SL et al (2015) Evidence for human norovirus infection of dogs in the United kingdom. J Clin Microbiol 53(6):1873–1883
Calderón A, Guzmán C, Mattar S, Rodriguez V, Martínez C, Violet L, Martínez J, Figueiredo LTM (2019) Dengue virus in bats from Córdoba and Sucre. Colombia. Vector Borne Zoonotic Dis 19 (10):747–751. https://doi.org/10.1089/vbz.2018.2324
Calvet GA, Pereira SA, Ogrzewalska M, Pauvolid-Corrêa A, Resende PC, Tassinari WS, Costa AP, Keidel LO, da Rocha ASB, da Silva MFB, Dos Santos SA, Lima ABM, de Moraes ICV, Mendes

Junior AAV, Souza TDC, Martins EB, Ornellas RO, Corrêa ML, Antonio IMDS, Guaraldo L, Motta FDC, Brasil P, Siqueira MM, Gremião IDF, Menezes RC (2021) Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de Janeiro, Brazil. PLoS One 16(4):e0250853. https://doi.org/10.1371/journal.pone.0250853

Campagnolo ER et al (2011) Fatal pandemic (H1N1) 2009 influenza A virus infection in a Pennsylvania domestic cat. Zoonoses Public Health 58(7):500–507

Campagnolo ER et al (2013) Concurrent 2009 pandemic influenza A (H1N1) virus infection in ferrets and in a community in Pennsylvania. Zoonoses Public Health 60(2):117–124

Casagrande RA, Pannuti CS, Kanamura C, Freire WS, Grespan A, Matushima ER (2014) Fatal human herpesvirus 1 (HHV-1) infection in captive marmosets (*Callithrix jacchus* and *Callithrix penicillata*) in Brazil: clinical and pathological characterization. Pesquisa Veterinária Brasileira 34(11):1109–1114. https://doi.org/10.1590/S0100-736X2014001100013

Cerdà-Cuéllar M et al (2019) Do humans spread zoonotic enteric bacteria in Antarctica? Sci Total Environ 654:190–196

Chaintoutis SC et al (2022) Limited cross-species transmission and absence of mutations associated with SARS-CoV-2 adaptation in cats: a case study of infection in a small household setting. Transbound Emerg Dis 69(3):1606–1616

Chang LY et al (2006) Quantitative estimation of Nipah virus replication kinetics in vitro. Virol J 3:47

Charoenkul K et al (2020) Human norovirus infection in dogs, Thailand. Emerg Infect Dis 26(2):350

Charu V et al (2017) Human mobility and the spatial transmission of influenza in the United States. PLoS Comput Biol 13(2):e1005382

Chastagner A et al (2018) Spatiotemporal distribution and evolution of the A/H1N1 2009 pandemic influenza virus in pigs in France from 2009 to 2017: identification of a potential swine-specific lineage. J Virol 92(24):e00988–e00918

Chastagner A et al (2019) Bidirectional human–swine transmission of seasonal influenza A(H1N1) pdm09 virus in pig herd, France, 2018. Emerg Infect Dis 25(10):1940

Choi YK et al (1999) Fatal measles virus infection in Japanese macaques (*Macaca fuscata*). Vet Pathol 36(6):594–600

Choudhary P et al (2017) Zooanthroponotic transmission of rotavirus in Haryana State of Northern India. Acta Virol 61(1):77–85

Cleaveland S, Laurenson MK, Taylor LH (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos Trans R Soc Lond B Biol Sci 356(1411):991–999

Cobo-Angel CG et al (2019) Potential group B *Streptococcus* interspecies transmission between cattle and people in Colombian dairy farms. Sci Rep (Nature Publisher Group) 9:1–9

Coklin T et al (2007) Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada. Vet Parasitol 150(4):297–305

Coscolla M, Lewin A, Metzger S, Maetz-Rennsing K, Calvignac-Spencer S, Nitsche A, Dabrowski PW, Radonic A, Niemann S, Parkhill J, Couacy-Hymann E, Feldman J, Comas I, Boesch C, Gagneux S, Leendertz FH (2013) Novel *Mycobacterium tuberculosis* complex isolate from a wild chimpanzee. Emerg Infect Dis 19(6):969–976. https://doi.org/10.3201/eid1906.121012

Costa EA et al (2011) Outbreak of human herpesvirus type 1 infection in nonhuman primates (*Callithrix penicillata*). J Wildl Dis 47(3):690–693

Crestani C et al (2021) The fall and rise of group B Streptococcus in dairy cattle: reintroduction due to human-to-cattle host jumps? Cold Spring Harbor Laboratory Press: Cold Spring Harbor

Crossley B et al (2012) Pandemic (H1N1) 2009 in captive cheetah. Emerg Infect Dis 18(2):315–317

Damiani AM et al (2012) Serological survey in dogs and cats for influenza A(H1N1)pdm09 in Germany. Zoonoses Public Health 59(8):549–552

de Thoisy B et al (2009) Dengue infection in neotropical forest mammals. Vector Borne Zoonotic Dis 9(2):157–170

Dehghan S et al (2019) A zoonotic adenoviral human pathogen emerged through genomic recombination among human and nonhuman simian hosts. J Virol 93(18):e00564–19. https://doi.org/10.1128/JVI.00564-19. Print 2019 Sep 15.

Delannoy CM et al (2013) Human *Streptococcus agalactiae* strains in aquatic mammals and fish. BMC Microbiol 13:41

Demetria C et al (2018) Reemergence of Reston ebolavirus in cynomolgus monkeys, the Philippines, 2015. Emerg Infect Dis 24(7):1285–1291
Deng Y-M et al (2012) Transmission of influenza A(H1N1) 2009 pandemic viruses in Australian swine. Influenza Other Respir Viruses 6(3):e42–e47
Dereure J et al (2003) Visceral leishmaniasis in eastern Sudan: parasite identification in humans and dogs; host-parasite relationships. Microbes Infect 5(12):1103–1108
Despommier D, Ellis BR, Wilcox BA (2006) The role of ecotones in emerging infectious diseases. EcoHealth 3(4):281–289
Di Marco M et al (2020) Opinion: Sustainable development must account for pandemic risk. Proc Natl Acad Sci U S A 117(8):3888–3892
Dileepan M, Di D, Huang Q, Ahmed S, Heinrich D, Ly H, Liang Y (2021) Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. Virulence 12 (1):1597–1609. https://doi.org/10.1080/21505594.2021.1936433
Dixon B et al (2011) The potential for zoonotic transmission of Giardia duodenalis and *Cryptosporidium* spp. from beef and dairy cattle in Ontario, Canada. Vet Parasitol 175(1–2):20–26
dos Santos WG (2021) Impact of virus genetic variability and host immunity for the success of COVID-19 vaccines. Biomed Pharmacother 136:111272
Ducatez MF, Awoume F, Webby RJ (2015) Influenza A(H1N1)pdm09 virus in pigs, Togo, 2013. Vet Microbiol 177(1–2):201–205
Dunay E et al (2018) Pathogen transmission from humans to great apes is a growing threat to primate conservation. EcoHealth 15(1):148–162
Dundon WG et al (2010) Serologic evidence of pandemic (H1N1) 2009 infection in dogs, Italy. Emerg Infect Dis 16(12):2019–2021
Eberle R, Hilliard JK (1989) Serological evidence for variation in the incidence of herpesvirus infections in different species of apes. J Clin Microbiol 27(6):1357–1366
Ehlers LP et al (2020) Mycobacterium tuberculosis var. tuberculosis infection in two captive black capuchins (*Sapajus nigritus*) in Southern Brazil. Braz J Microbiol 51(4):2169–2173
Emmons RW, Lennette EH (1970) Natural herpesvirus hominis infection of a gibbon (Hylobates lar). Arch Gesamte Virusforsch 31(3):215–218
Er C et al (2016a) Production impact of influenza A(H1N1)pdm09 virus infection on fattening pigs in Norway. J Anim Sci 94(2):751–759
Er C et al (2016b) Occurrence and spread of influenza A(H1N1)pdm09 virus infection in Norwegian pig herds based on active serosurveillance from 2010 to 2014. Epidemiol Infect 144(15): 3148–3165
Er JC, Lium B, Framstad T (2020) Antibodies of influenza A(H1N1)pdm09 virus in pigs' sera cross-react with other influenza A virus subtypes. A retrospective epidemiological interpretation of Norway's serosurveillance data from 2009–2017. Epidemiol Infect 148:e73
Erwin PC et al (2004) *Mycobacterium tuberculosis* transmission from human to canine. Emerg Infect Dis 10(12):2258–2210
Europe, A.h. (2017) Healthy animals mean healthy people and a healthier planet. www.animalhealtheurope.eu
Every AL et al (2011) Did transmission of *Helicobacter pylori* from humans cause a disease outbreak in a colony of Stripe-faced Dunnarts (*Sminthopsis macroura*)? Vet Res 42(1):26. https://doi.org/10.1186/1297-9716-42-26
Ewers C et al (2010) Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-beta-lactamase-producing *Escherichia coli* among companion animals. J Antimicrob Chemother 65(4):651–660
Fagre C, Cohen L, Eskew EA, Farrell M, Glennon E, Joseph MB, Frank HK, Ryan S, Carlson CJ, Albery GF (2021) Spillback in the Anthropocene: the risk of human-to-wildlife pathogen transmission for conservation and public health
FAO-OIE-OMS (2019) Taking a multisectoral, one health approach: a tripartite guide to addressing zoonotic diseases in countries
Faulde M et al (2008a) Zoonotic cutaneous leishmaniasis outbreak in Mazar-e Sharif, northern Afghanistan: an epidemiological evaluation. Int J Med Microbiol 298(5–6):543–550

Faulde M et al (2008b) Differences in transmission seasons as an epidemiological tool for characterization of anthroponotic and zoonotic cutaneous leishmaniasis in northern Afghanistan. Acta Trop 105(2):131–138
Favoretto SR et al (2019) Zika virus in peridomestic neotropical primates, Northeast Brazil. Ecohealth 16(1):61–69
Fernandes MR et al (2018) Zooanthroponotic transmission of drug-resistant *Pseudomonas aeruginosa*, Brazil. Emerg Infect Dis 24(6):1160–1162
Fernández-Bellon H et al (2021) Monitoring natural SARS-CoV-2 infection in lions (*Panthera leo*) at the Barcelona Zoo: viral dynamics and host responses. Viruses 13(9):1683
Feßler AT et al (2018) Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from zoo and wild animals. Vet Microbiol 218:98–103
Fieldhouse JK, Wang X, Mallinson KA, Tsao RW, Gray GC (2018) A systematic review of evidence that enteroviruses may be zoonotic. Emerg Microbes Infect 7(1):164. https://doi.org/10.1038/s41426-018-0159-1
Fiorentini L et al (2011) Influenza A pandemic (H1N1) 2009 virus outbreak in a cat colony in Italy. Zoonoses Public Health 58(8):573–581
Forgie SE et al (2011) Swine outbreak of pandemic influenza A virus on a Canadian research farm supports human-to-swine transmission. Clin Infect Dis 52(1):10–18
Franklin AB, Bevins SN (2020) Spillover of SARS-CoV-2 into novel wild hosts in North America: a conceptual model for perpetuation of the pathogen. Sci Total Environ 733:139358
Franklinos LH et al (2015) *Streptococcus pyogenes* infection in a free-living European hedgehog (*Erinaceus europaeus*). EcoHealth 12(4):689–692
Friedmann E, Son H (2009) The human-companion animal bond: how humans benefit. Vet Clin North Am Small Anim Pract 39(2):293–326
Fritz M, Rosolen B, Krafft E, Becquart P, Elguero E, Vratskikh O, Denolly S, Boson B, Vanhomwegen J, Gouilh MA, Kodjo A, Chirouze C, Rosolen SG, Legros V, Leroy EM (2021) High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health 11:100192. https://doi.org/10.1016/j.onehlt.2020.100192
Fuentealba NA, Moré G, Bravi ME, Unzaga JM, De Felice L, Salina M, Viegas M, Nabaes Jodar MS, Valinotto LE, Rivero FD, Di Lullo D, Pecoraro M, Panei CJ (2021) First detection and molecular analysis of SARS-CoV-2 from a naturally infected cat from Argentina. Vet Microbiol 260:109179. https://doi.org/10.1016/j.vetmic.2021.109179
Gagnon CA et al (2009) Characterization of a Canadian mink H3N2 influenza A virus isolate genetically related to triple reassortant swine influenza virus. J Clin Microbiol 47(3):796–799
Garigliany M et al (2020) SARS-CoV-2 natural transmission from human to cat, Belgium, March 2020. Emerg Infect Dis 26(12):3069–3071
Gaudreault NN et al (2020) SARS-CoV-2 infection, disease and transmission in domestic cats. Emerg Microbes Infect 9(1):2322–2332
Ghoneim NH, K.A.A.-M.a.H.S (2019) The zooanthroponotic cycle of human rotavirus in rural settings and its public health burden. Biosci Res 16(1):110–117
Gilardi KVK et al (2014) Human herpes simplex virus type 1 in confiscated gorilla. Emerg Infect Dis 20(11):1883–1886
Glasser DB et al (2021) Opportunities for respiratory disease transmission from people to chimpanzees at an East African tourism site. Am J Primatol 83(2):e23228–e23228
Gnat S et al (2021) A global view on fungal infections in humans and animals: infections caused by dimorphic fungi and dermatophytoses. J Appl Microbiol 131(6):2688–2704
Goldberg TL et al (2007) Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. Biol Conserv 135(4):511–517
Goldberg TL et al (2008) Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. Emerg Infect Dis 14(9):1375–1382
Goldstein T et al (2013) Pandemic H1N1 influenza isolated from free-ranging Northern Elephant Seals in 2010 off the central California coast. PLoS One 8(5):e62259
Gortázar C et al (2021) Natural SARS-CoV-2 Infection in Kept Ferrets, Spain. Emerg Infect Dis 27 (7):1994–1996

Gozalo AS, Montoya EJ, Weller RE (2008) Dyscoria associated with herpesvirus infection in owl monkeys (*Aotus nancymae*). J Am Assoc Lab Anim Sci 47(4):68–71
Grace D et al (2012) The multiple burdens of zoonotic disease and an ecohealth approach to their assessment. Trop Anim Health Prod 44(1):67–73
Graczyk T et al (2001) Cryptosporidium parvum Genotype 2 infections in free-ranging mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. Parasitol Res 87(5):368–370
Graczyk TK et al (2002a) Anthropozoonotic *Giardia duodenalis* genotype (assemblage) A infections in habitats of free-ranging human-habituated gorillas, Uganda. J Parasitol 88(5):905–909
Graczyk TK et al (2002b) A single genotype of *Encephalitozoon intestinalis* infects free-ranging gorillas and people sharing their habitats in Uganda. Parasitol Res 88(10):926–931
Gray GC et al (2007) Swine workers and swine influenza virus infections. Emerg Infect Dis 13(12): 1871–1878
Gray GC et al (2015) Seroepidemiological study of interepidemic rift valley fever virus infection among persons with intense ruminant exposure in Madagascar and Kenya. Am Soc J Trop Med Hyg 93(6):1364–1370
Grøntvedt CA et al (2013) Influenza A(H1N1)pdm09 virus infection in Norwegian swine herds 2009/10: the risk of human to swine transmission. Prev Vet Med 110(3–4):429–434
Grützmacher KS et al (2016) Codetection of respiratory syncytial virus in habituated wild western lowland gorillas and humans during a respiratory disease outbreak. EcoHealth 13(3):499–510
Grützmacher KS et al (2018) Human respiratory syncytial virus and *Streptococcus pneumoniae* infection in wild bonobos. EcoHealth 15(2):462–466
Guk S et al (2004) Genotype and animal infectivity of a human isolate of *Cryptosporidium parvum* in the Republic of Korea. Korean J Parasitol 42(2):85–89
Gumi B et al (2012) Zoonotic transmission of tuberculosis between pastoralists and their livestock in South-East Ethiopia. EcoHealth 9(2):139–149
Hackendahl NC et al (2004) Putative transmission of *Mycobacterium tuberculosis* infection from a human to a dog. J Am Vet Med Assoc 225(10):1573–1577
Hamer SA et al (2020) Natural SARS-CoV-2 infections, including virus isolation, among serially tested cats and dogs in households with confirmed human COVID-19 cases in Texas, USA. bioRxiv: the preprint server for biology: p. 2020.12.08.416339
Hammer AS et al (2021) SARS-CoV-2 transmission between mink (*Neovison vison*) and humans, Denmark. Emerg Infect Dis 27(2):547–551
Han J, He S (2021) Urban flooding events pose risks of virus spread during the novel coronavirus (COVID-19) pandemic. Sci Total Environ 755(Pt 1):142491
Hanamura S et al (2008) Chimpanzee deaths at Mahale caused by a flu-like disease. Primates 49(1): 77–80
Hasan KA et al (2018) The unravelled Enterococcus faecalis zoonotic superbugs: emerging multiple resistant and virulent lineages isolated from poultry environment. Zoonoses Public Health 65(8):921–935
He S, Han J, Lichtfouse E (2021) Backward transmission of COVID-19 from humans to animals may propagate reinfections and induce vaccine failure. Environ Chem Lett 19(2):763–768. https://doi.org/10.1007/s10311-020-01140-4
Heldstab A et al (1981) Spontaneous generalized Herpesvirus hominis infection of a lowland gorilla (Gorilla gorilla gorilla). J Med Primatol 10(2–3):129–135
Hofshagen M et al (2009) Pandemic influenza A(H1N1)v: human to pig transmission in Norway? Euro Surveill 14(45):19406. https://doi.org/10.2807/ese.14.45.19406-en
Holyoake PK et al (2011) The first identified case of pandemic H1N1 influenza in pigs in Australia. Aust Vet J 89(11):427–431
Hosie MJ et al (2021) Detection of SARS-CoV-2 in respiratory samples from cats in the UK associated with human-to-cat transmission. Vet Rec 188(8):1
Howden KJ et al (2009) An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. Can Vet J = La revue veterinaire canadienne. 50(11):1153–1161

Hsieh JM et al (2008) Phylogenetic analysis of livestock oxacillin-resistant *Staphylococcus aureus*. Vet Microbiol 126(1–3):234–242
Huemer HP et al (2002) Fatal infection of a pet monkey with human herpesvirus. Emerg Infect Dis 8 (6):639–642
Huynh J et al (2012) Evidence supporting a zoonotic origin of human coronavirus strain NL63. J Virol 86(23):12816–12825
Ibrahim M et al (2016a) Post-pandemic seroprevalence of human influenza viruses in domestic cats. J Vet Sci 17(4):515–521
Ibrahim MA et al (2016b) Epidemiology and public health significance of *Cryptosporidium* isolated from cattle, buffaloes, and humans in Egypt. Parasitol Res 115(6):2439–2448
Imura K et al (2014) Herpes simplex virus type 1 infection in two pet marmosets in Japan. J Vet Med Sci 76(12):1667–1670
Jang H et al (2017) Seroprevalence of three influenza A viruses (H1N1, H3N2, and H3N8) in pet dogs presented to a veterinary hospital in Ohio. J Vet Sci 18(S1):291–298
Jefferson T et al (2011) Physical interventions to interrupt or reduce the spread of respiratory viruses: systematic review. (1756-1833 (Electronic)). Cochrane Database Syst Rev. 2011 Jul; 2011(7): CD006207. Published online 2011 Jul 6. https://doi.org/10.1002/14651858.CD006207.pub4
Jia P et al (2021) New approaches to anticipate the risk of reverse zoonosis. Trends Ecol Evol 36(7): 580–590
Jiles R, Klevens M, Hughes E (2014) Surveillance and seroepidemiology. In: Kaslow RA, Stanberry LR, Le Duc JW (eds) Viral infections of humans: epidemiology and control. Springer US, Boston, pp 63–79
Johnston AR et al (2010) Molecular epidemiology of cross-species *Giardia duodenalis* transmission in western Uganda. PLoS Negl Trop Dis 4(5):e683
Jones-Engel L et al (2001) Detection of antibodies to selected human pathogens among wild and pet macaques (*Macaca tonkeana*) in Sulawesi, Indonesia. Am J Primatol 54(3):171–178
Jones-Engel L et al (2006) Considering human-primate transmission of measles virus through the prism of risk analysis. Am J Primatol 68(9):868–879
Jordan H et al (2016) Costing the morbidity and mortality consequences of zoonoses using health-adjusted life years. Transbound Emerg Dis 63(5):e301–e312
Juan-Sallés C et al (1997) Spontaneous herpes simplex virus infection in common marmosets (*Callithrix jacchus*). J Vet Diagn Investig 9(3):341–345
Kalema-Zikusoka G, Kock R, Macfie E (2002) Scabies in free-ranging mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable National Park, Uganda. Vet Rec 150(1):12–15
Kandefer-Gola M et al (2016) Mycobacteriosis of the red-lored amazon parrot: a case report. Vet Med 61:233–236
Karikalan M et al (2021) Natural infection of Delta mutant of SARS-CoV-2 in Asiatic lions of India. Transbound Emerg Dis n/a(n/a)
Kato F et al (2013) Natural infection of cynomolgus monkeys with dengue virus occurs in epidemic cycles in the Philippines. J Gen Virol 94(Pt 10):2202–2207
Kaur T et al (2008) Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania. Am J Primatol 70(8):755–765
Kik MJ et al (2005) Herpes simplex infection in a juvenile orangutan (*Pongo pygmaeus pygmaeus*). J Zoo Wildl Med 36(1):131–134
Kilbourn AM et al (2003) Health evaluation of free-ranging and semi-captive orangutans (*Pongo pygmaeus pygmaeus*) in Sabah, Malaysia. J Wildl Dis 39(1):73–83
Killian ML, Swenson SL, Vincent AL, Landgraf JG, Shu B, Lindstrom S, Xu X, Klimov A, Zhang Y, Bowman AS (2013) Simultaneous infection of pigs and people with triple-reassortant swine influenza virus H1N1 at a U.S. county fair. Zoonoses Public Health 60(3):196–201. https://doi.org/10.1111/j.1863-2378.2012.01508.x
Kim SH et al (2011) Outbreak of pandemic influenza (H1N1) 2009 in pigs in Korea. Vet Rec 169 (6):155

Kinganda-Lusamaki E et al (2021) Integration of genomic sequencing into the response to the Ebola virus outbreak in Nord Kivu, Democratic Republic of the Congo. Nat Med 27(4):710–716

Klaus J et al (2021) Detection and genome sequencing of SARS-CoV-2 in a domestic cat with respiratory signs in Switzerland. Viruses 13(3):496. https://doi.org/10.3390/v13030496

Knetsch CW et al (2018) Zoonotic transfer of Clostridium difficile harboring antimicrobial resistance between farm animals and humans. J Clin Microbiol 56(3):e01384

Knight CG et al (2016) Pandemic H1N1 influenza virus infection in a Canadian cat. Can Vet J = La revue veterinaire canadienne 57(5):497–500

Köndgen S, Schenk S, Pauli G, Boesch C, Leendertz FH (2010) Noninvasive monitoring of respiratory viruses in wild chimpanzees. Ecohealth 7(3):332–341. https://doi.org/10.1007/s10393-010-0340-z

Kooriyama T et al (2013) Epidemiological study of zoonoses derived from humans in captive chimpanzees. Primates 54(1):89–98

Kottler S et al (2010) Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* carriage in three populations. J Vet Intern Med 24(1):132–139

Kovalenko G et al (2021) Serological evidence for influenza A viruses among domestic dogs and cats in Kyiv, Ukraine. Vector Borne Zoonotic Dis (Larchmont, N.Y.) 21(7):483–489

Kraemer MUG et al (2020) The effect of human mobility and control measures on the COVID-19 epidemic in China. Science (New York, N.Y.) **368**(6490):493–497

Krawczyk AI et al (2015) Presence of zoonotic agents in engorged ticks and hedgehog faeces from *Erinaceus europaeus* in (sub) urban areas. Parasit Vectors 8(1):1–6

Kreutzer R et al (2011) Systemic herpesvirus infection in an Azara's Night Monkey (*Aotus azarae*). J Med Primatol 40(3):197–199

Kruse H, Kirkemo A-M, Handeland K (2004) Wildlife as source of zoonotic infections. Emerg Infect Dis 10(12):2067–2072

Kuchipudi SV, Surendran-Nair M, Ruden RM, Yon M, Nissly RH, Vandegrift KJ, Nelli RK, Li L, Jayarao BM, Maranas CD, Levine N, Willgert K, Conlan AJK, Olsen RJ, Davis JJ, Musser JM, Hudson PJ, Kapur V (2022) Multiple spillovers from humans and onward transmission of SARS-CoV-2 in white-tailed deer. Proc Natl Acad Sci U S A 119(6):e2121644119. https://doi.org/10.1073/pnas.2121644119

Kumar N et al (2018) Molecular characterization of unusual bovine rotavirus A strains having high genetic relatedness with human rotavirus: evidence for zooanthroponotic transmission. Zoonoses Public Health 65(4):431–442

Kwasnik M et al (2020) Serologic investigation of influenza A virus infection in dogs in Poland. J Vet Diagn Investig 32(3):420–422

Kwon NH et al (2006) Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol 117(2–4):304–312

Kyriakis CS, Papatsiros VG, Athanasiou LV, Valiakos G, Brown IH, Simon G, Van Reeth K, Tsiodras S, Spyrou V, Billinis C (2016) Serological evidence of pandemic H1N1 influenza virus infections in Greek Swine. Zoonoses Public Health 63(5):370–373. https://doi.org/10.1111/zph.12235

Kyriakis CS, Zhang M, Wolf S, Jones LP, Shim BS, Chocallo AH, Hanson JM, Jia M, Liu D, Tripp RA (2017) Molecular epidemiology of swine influenza A viruses in the Southeastern United States, highlights regional differences in circulating strains. Vet Microbiol 211:174–179. https://doi.org/10.1016/j.vetmic.2017.10.016

Lafleur M et al (2021) Drug-resistant tuberculosis in pet ring-tailed lemur, Madagascar. Emerg Infect Dis 27(3):977–979

Landolfi JA et al (2005) Fatal human herpesvirus type 1 infection in a white-handed gibbon (Hylobates lar). J Vet Diagn Investig 17(4):369–371

Larsen HD, Fonager J, Lomholt FK, Dalby T, Benedetti G, Kristensen B, Urth TR, Rasmussen M, Lassaunière R, Rasmussen TB, Strandbygaard B, Lohse L, Chaine M, Møller KL, Berthelsen AN, Nørgaard SK, Sönksen UW, Boklund AE, Hammer AS, Belsham GJ, Krause TG, Mortensen S, Bøtner A, Fomsgaard A, Mølbak K (2021) Preliminary report of an outbreak of SARS-CoV-2 in

mink and mink farmers associated with community spread, Denmark, June to November 2020. Euro Surveill 26(5):2100009. https://doi.org/10.2807/1560-7917.ES.2021.26.5.210009
Learmonth J et al (2001) Seasonal shift in *Cryptosporidium parvum* transmission cycles in New Zealand. J Eukaryot Microbiol 48:34S–35S
Lee Y-H et al (2021) Retrospective seroepidemiology study of dengue virus infection in Taiwan. BMC Infect Dis 21(1):96
Leeflang M, Wanyama J, Pagani P, vant Hooft K, de Balogh K (2008) Zoonoses Diseases transmitted from animals to humans, *Agrodok-series*, pp 1–72
Lemaire D, Barbosa T, Rihet P (2012) Coping with genetic diversity: the contribution of pathogen and human genomics to modern vaccinology. Braz J Med Biol Res = Rev Bras Pesqui Med Biol 45(5):376–385
Levy BM, Mirkovic RR (1971) An epizootic of measles in a marmoset colony. Lab Anim Sci 21(1): 33–39
Li D, Zhu L, Cui H, Ling S, Fan S, Yu Z, Zhou Y, Wang T, Qian J, Xia X, Xu Z, Gao Y, Wang C (2014) Influenza A(H1N1)pdm09 virus infection in giant pandas, China. Emerg Infect Dis 20 (3):480–483. https://doi.org/10.3201/eid2003.131531
Li W et al (2016) Pentatrichomonas hominis: prevalence and molecular characterization in humans, dogs, and monkeys in northern China. Parasitol Res 115(2):569–574
Li J et al (2019) Inter-host transmission of carbapenemase-producing *Escherichia coli* among humans and backyard animals. Environ Health Perspect 127(10):EHP5251
Lin Y et al (2011) Evidence of multiple virulence subtypes in nosocomial and community-associated MRSA genotypes in companion animals from the upper midwestern and northeastern United States. Clin Med Res 9(1):7–16
Lin Y et al (2012) Genetic and pathobiologic characterization of H3N2 canine influenza viruses isolated in the Jiangsu Province of China in 2009-2010. Vet Microbiol 158(3–4):247–258
Lin HT et al (2014) Natural A(H1N1)pdm09 influenza virus infection case in a pet ferret in Taiwan. Jpn J Vet Res 62(4):181–185
Linz B et al (2018) Virulent epidemic pneumonia in sheep caused by the human pathogen *Acinetobacter baumannii*. Front Microbiol 9(November):2616
Löhr CV et al (2010) Pathology and viral antigen distribution of lethal pneumonia in domestic cats due to pandemic (H1N1) 2009 influenza A virus. Vet Pathol 47(3):378–386
Loncaric I et al (2016) Carriage of meticillin-resistant staphylococci between humans and animals on a small farm. Vet Dermatol 27(3):e48
Longa CS et al (2011) Human herpesvirus 1 in wild marmosets, Brazil, 2008. Emerg Infect Dis 17 (7):1308–1310
Lutz CS et al (2019) Applying infectious disease forecasting to public health: a path forward using influenza forecasting examples. BMC Public Health 19(1):1659
Ma MJ et al (2018) Evidence for cross-species influenza A virus transmission within swine farms, China: a one health, prospective cohort study. Clin Infect Dis 66(4):533–540
MacArthur JA et al (1979) Measles in monkeys: an epidemiological study. J Hyg 83(2):207–212
Marcia Helena Braga Catroxo AMCRPdFM, de Souza Hunold Lara M d CC, CassaroVillalobos EM, Milanelo L (2020) Outbreak of encephalitis caused by human herpesvirus type 1 (HSV-1) in non-human captive primates in São Paulo, SP, Brazil. Clinical Microbiology and Research
Martelli P et al (2019) Influenza A(H1N1)pdm09 virus infection in a Captive Giant Panda, Hong Kong. Emerg Infect Dis 25(12):2303–2306
Martins SB, Häsler B, Rushton J (2015) Economic aspects of zoonoses: impact of zoonoses on the food industry. In: Zoonoses-infections affecting humans and animals. Springer, pp 1107–1126
Mathieu C et al (2010) Pandemic (H1N1) 2009 in breeding turkeys, Valparaiso, Chile. Emerg Infect Dis 16(4):709–711
Mattison K et al (2007) Human noroviruses in swine and cattle. Emerg Infect Dis 13(8):1184–1188
Matushima RACSPKSFGR (2014) Fatal Human herpesvirus 1 (HHV-1) infection in captive marmosets (*Callithrix jacchus* and *Callithrix penicillata*) in Brazil: clinical and pathological characterization. Wildlife Medicine. Pesq. Vet. Bras. **34**(11)

Mätz-Rensing K et al (2003) Fatal Herpes simplex infection in a group of common marmosets (Callithrix jacchus). Vet Pathol 40(4):405–411
Mätz-Rensing K, Hartmann T, Wendel GM, Frick JS, Homolka S, Richter E, Munk MH, Kaup FJ (2015) Outbreak of Tuberculosis in a Colony of Rhesus Monkeys (*Macaca mulatta*) after Possible Indirect Contact with a Human TB Patient. J Comp Pathol 153(2-3):81–91. https://doi.org/10.1016/j.jcpa.2015.05.006
May PH, Gebara MF, Platais G (2021) Pandemics, conservation, and human-nature relations. Clim Change Ecol:100029
Mazet JAK et al (2020) Human respiratory syncytial virus detected in mountain gorilla respiratory outbreaks. EcoHealth 17(4):449–460
Mbehang Nguema PP et al (2020) Characterization of ESBL-producing enterobacteria from fruit bats in an unprotected area of Makokou, Gabon. Microorganisms 8(1):138. https://doi.org/10.3390/microorganisms8010138
McAloose D et al (2020) From people to panthera: natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. mBio 11(5)
McClure HM et al (1980) Natural genital herpesvirus hominis infection in chimpanzees (*Pan troglodytes* and *Pan paniscus*). Lab Anim Sci 30(5):895–901
McCullers JA et al (2011) Seroprevalence of seasonal and pandemic influenza A viruses in domestic cats. Arch Virol 156(1):117–120
McCune S et al (2012) Interspecies interactions and potential Influenza A virus risk in small swine farms in Peru. BMC Infect Dis 12(1):58
McDonald M (2011) Neglected tropical and zoonotic diseases and their impact on women's and children's health. In: The causes and impacts of neglected tropical and zoonotic diseases: Opportunities for integrated intervention strategies, pp 357–388. Institute of Medicine (US) Forum on Microbial Threats. Washington (DC): National Academies Press (US)
McDougall F et al (2019) Bats as reservoirs of antibiotic resistance determinants: A survey of class 1 integrons in Grey-headed Flying Foxes (*Pteropus poliocephalus*). Infect Genet Evol 70:107–113
Medkour H et al (2020) Adenovirus infections in African humans and wild non-human primates: great diversity and cross-species transmission. Viruses 12(6):657. https://doi.org/10.3390/v12060657
Meisner J et al (2021) Household transmission of SARS-CoV-2 from humans to dogs in Washington and Idaho: burden and risk factors. Cold Spring Harbor Laboratory Press: Cold Spring Harbor
Meléndez LV et al (1972) Two new herpesviruses from spider monkeys (*Ateles geoffroyi*)12. J Natl Cancer Inst 49(1):233–238
Meseko CA, Odaibo GN, Olaleye DO (2014) Detection and isolation of 2009 pandemic influenza A/H1N1 virus in commercial piggery, Lagos Nigeria. Vet Microbiol 168(1):197–201
Messenger AM, Barnes AN, Gray GC (2014) Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. PLoS One 9(2):e89055–e89055
Mi E, Mi E, Jeggo M (2016) Where to now for one health and ecohealth? EcoHealth 13(1):12–17
Michalak K, Austin C, Diesel S, Bacon MJ, Zimmerman P, Maslow JN (1998) *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. Emerg Infect Dis 4(2):283–287. https://doi.org/10.3201/eid0402.980217
Michel AL et al (2013) *Mycobacterium tuberculosis* at the human/wildlife interface in a high TB burden country. Transbound Emerg Dis 60(Suppl 1):46–52
Miller MA et al (2019) Fatal tuberculosis in a free-ranging African elephant and One Health implications of human pathogens in wildlife. Front Vet Sci 6(February):18
Mishra A et al (2021) SARS-CoV-2 delta variant among Asiatic lions, India. Emerg Infect Dis 27(10):2723–2725. https://doi.org/10.3201/eid2710.211500
Mitchell PK (2021) Infection with SARS-CoV-2 lineage B.1.1.7 in three Malayan tigers at the Virginia Zoological Park. Research Square
Mittal M et al (2014) Evidence of presence of *Mycobacterium tuberculosis* in bovine tissue samples by multiplex PCR: possible relevance to reverse zoonosis. Transbound Emerg Dis 61(2):97–104

Mohd-Shaharuddin N et al (2019) Molecular characterization of *Trichuris* species isolated from humans, dogs and cats in a rural community in Peninsular Malaysia. Acta Trop 190:269–272
Molenaar RJ et al (2020) Clinical and pathological findings in SARS-CoV-2 disease outbreaks in farmed mink (Neovison vison). Vet Pathol 57(5):653–657
Mon PP et al (2020) Swine influenza viruses and pandemic H1N1-2009 infection in pigs, Myanmar. Transbound Emerg Dis 67(6):2653–2666
Moreno A et al (2010) First pandemic H1N1 outbreak from a pig farm in Italy. The Open Virol 4: 52–56
Morris DO et al (2006) Clinical, microbiological, and molecular characterization of methicillin-resistant *Staphylococcus aureus* infections of cats. Am J Vet Res 67(8):1421–1425
Müller-Graf C et al (1997) *Schistosoma mansoni* infection in a natural population of olive baboons (*Papio cynocephalus anubis*) in Gombe Stream National Park, Tanzania. Parasitology 115(6): 621–627
Munir K et al (2020) Zoonotic and reverse zoonotic events of SARS-CoV-2 and their impact on global health. Emerg Microbes Infect 9(1):2222–2235
Murakami PS et al (2012) Detection of RD(Rio) strain of *Mycobacterium tuberculosis* in tapirs (*Tapirus terrestris*) from a zoo in Brazil. J Zoo Wildl Med 43(4):872–875
Murray S et al (2000) Intestinal parasites of baboons (*Papio cynocephalus anubis*) and chimpanzees (*Pan troglodytes*) in Gombe National Park. J Zoo Wildl Med 31(2):176–8. https://doi.org/10.1638/1042-7260(2000)031[0176:IPOBPC]2.0.CO;2
Musso N et al (2020) New SARS-CoV-2 infection detected in an Italian pet cat by RT-qPCR from deep pharyngeal swab. Pathogens 9(9)
Mynářová A et al (2016) Prevalence of *Cryptosporidium* spp., *Enterocytozoon bieneusi*, *Encephalitozoon* spp. and *Giardia intestinalis* in wild, semi-wild and captive orangutans (*Pongo abelii* and *Pongo pygmaeus*) on Sumatra and Borneo, Indonesia. PLoS One 11(3): e0152771. https://doi.org/10.1371/journal.pone.0152771. eCollection 2016
Nagarajan K et al (2010) Influenza A H1N1 virus in Indian pigs & its genetic relatedness with pandemic human influenza A 2009 H1N1. Indian J Med Res 132:160–167
Nagel M et al (2013) Human-associated *Staphylococcus aureus* strains within great ape populations in Central Africa (Gabon). Clin Microbiol Infect 19(11):1072–1077
Narrod C, Zinsstag J, Tiongco M (2012) A one health framework for estimating the economic costs of zoonotic diseases on society. EcoHealth 9(2):150–162
Nasher A (1988) Zoonotic parasite infections of the Arabian sacred baboon *Papio hamadryas arabicus* Thomas in Asir Province, Saudi Arabia. Ann Parasitol Hum Comp 63(6):448–454
Negrey JD et al (2019) Simultaneous outbreaks of respiratory disease in wild chimpanzees caused by distinct viruses of human origin. Emerg Microbes Infect 8(1):139–149
Neira V, Brito B, Agüero B, Berrios F, Valdés V, Gutierrez A, Ariyama N, Espinoza P, Retamal P, Holmes EC, Gonzalez-Reiche AS, Khan Z, van de Guchte A, Dutta J, Miorin L, Kehrer T, Galarce N, Almonacid LI, Levican J, van Bakel H, García-Sastre A, Medina RA (2021) A household case evidences shorter shedding of SARS-CoV-2 in naturally infected cats compared to their human owners. Emerg Microbes Infect 10(1):376–383. https://doi.org/10.1080/22221751.2020.1863132
Nelson MI, Vincent AL (2015) Reverse zoonosis of influenza to swine: new perspectives on the human–animal interface. Trends Microbiol 23(3):142–153
Nelson MI et al (2015) Influenza A viruses of human origin in swine, Brazil. Emerg Infect Dis 21 (8):1339–1347
Nelson MI et al (2019) Human-origin influenza A(H3N2) reassortant viruses in swine, Southeast Mexico. Emerg Infect Dis 25(4):691–700
Nerpel A, Yang L, Sorger J, Käsbohrer A, Walzer C, Desvars-Larrive A (2022) SARS-ANI: a global open access dataset of reported SARS-CoV-2 events in animals. Sci Data 9(1):438. https://doi.org/10.1038/s41597-022-01543-8
Newman A et al (2020) First reported cases of SARS-CoV-2 infection in companion animals - New York, March-April 2020. MMWR Morb Mortal Wkly Rep 69(23):710–713

Nguyen Vinh T et al (2016) Colonization of enteroaggregative *Escherichia coli* and Shiga toxin-producing *Escherichia coli* in chickens and humans in southern Vietnam. BMC Microbiol 16(208) 9 September 2016

Nizeyi JB et al (1999) *Cryptosporidium* sp. and *Giardia* sp. infections in mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. J Parasitol 85:1084–1088

Nizeyi JB et al (2001) Campylobacteriosis, salmonellosis, and shigellosis in free-ranging human-habituated mountain gorillas of Uganda. J Wildl Dis 37(2):239–244

Njabo KY et al (2012) Pandemic A/H1N1/2009 influenza virus in swine, Cameroon, 2010. Vet Microbiol 156(1–2):189–192

Noël C et al (2005) Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J Clin Microbiol 43(1):348–355

Nofs S et al (2009) Influenza virus A (H1N1) in giant anteaters (*Myrmecophaga tridactyla*). Emerg Infect Dis 15(7):1081–1083

Nolan MJ et al (2017) Molecular characterisation of protist parasites in human-habituated mountain gorillas (*Gorilla beringei beringei*), humans and livestock, from Bwindi impenetrable National Park, Uganda. Parasit Vectors 10(1):340

Oh P, Granich R, Scott J, Sun B, Joseph M, Stringfield C, Thisdell S, Staley J, Workman-Malcolm D, Borenstein L, Lehnkering E, Ryan P, Soukup J, Nitta A, Flood J (2002) Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a Metropolitan Zoo. Emerg Infect Dis 8(11):1290–1293. https://doi.org/10.3201/eid0811.020302

On S et al (2019) Isolation and genotyping of *Campylobacter* species from kiwi (*Apteryx spp.*) in captivity: implications for transmission to and from humans. N Z Vet J 67(3):134–137

Oreshkova N et al (2020) SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill 25(23):2001005. https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005

Osadebe LU et al (2013) Prevalence and characteristics of *Staphylococcus aureus* in Connecticut swine and swine farmers. Zoonoses Public Health 60(3):234–243

Osborne C, Cryan PM, O'Shea TJ, Oko LM, Ndaluka C, Calisher CH, Berglund AD, Klavetter ML, Bowen RA, Holmes KV, Dominguez SR (2011) Alphacoronaviruses in New World bats: prevalence, persistence, phylogeny, and potential for interaction with humans. PLoS One 6 (5):e19156. https://doi.org/10.1371/journal.pone.0019156

Osoro EM et al (2019a) Detection of pandemic influenza A/H1N1/pdm09 virus among pigs but not in humans in slaughterhouses in Kenya, 2013-2014. BMC Res Notes 12(1):628–628

Osoro EM et al (2019b) Environ Health Prev Med 24(53) (17 August 2019)

Oude Munnink BB et al (2021) Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science 371(6525):172–177

Pagani G et al (2021) Human-to-cat SARS-CoV-2 transmission: case report and full-genome sequencing from an infected pet and its owner in Northern Italy. Pathogens 10(2):252. https://doi.org/10.3390/pathogens10020252

Pal M, Matsusaka N, Chauhan P (1997) Zooanthroponotic significance of *Trichophyton rubrum* in a rhesus monkey (*Macaca mulatta*). Verh. ber. Erkeg. Zootiere 38:355–358

Palacios G et al (2011) Human metapneumovirus infection in wild mountain gorillas, Rwanda. Emerg Infect Dis 17(4):711–713

Pantin-Jackwood M et al (2010) Susceptibility of turkeys to pandemic-H1N1 virus by reproductive tract insemination. Virol J 7(1):27

Patel A, Jenkins M, Rhoden K, Barnes AN (2022) A systematic review of zoonotic enteric parasites carried by flies, cockroaches, and dung beetles. Pathogens 11(1):90. https://doi.org/10.3390/pathogens11010090

Patrono LV et al (2018a) Human coronavirus OC43 outbreak in wild chimpanzees, Côte d'Ivoire, 2016. Emerg Microbes Infect 7(1):118–118

Patrono LV et al (2018b) Human coronavirus OC43 outbreak in wild chimpanzees, Côte d'Ivoire, 2016. Emerg Microbes Infect 7(1):1–4

Patterson EI et al (2020) Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat Commun 11(1):6231

Pauly M et al (2015) Adenovirus in rural Côte D'Ivoire: high diversity and cross-species detection. EcoHealth 12(3):441–452
Paungpin W et al (2017) Serosurveillance for pandemic influenza A (H1N1) 2009 virus infection in domestic elephants, Thailand. PloS One 12(10):e0186962–e0186962
Pereda A et al (2010) Pandemic (H1N1) 2009 outbreak on pig farm, Argentina. Emerg Infect Dis 16 (2):304–307
Pesapane R, Ponder M, Alexander KA (2013) Tracking pathogen transmission at the human-wildlife interface: banded mongoose and *Escherichia coli*. EcoHealth 10(2):115–128
Pigott AM et al (2014) Acute bronchointerstitial pneumonia in two indoor cats exposed to the H1N1 influenza virus. J Vet Emerg Crit Care (San Antonio) 24(6):715–723
Pippig J et al (2016) Influenza A viruses detected in swine in Southern Germany after the H1N1 pandemic in 2009. Zoonoses Public Health 63(7):555–568
Plowright RK et al (2017) Pathways to zoonotic spillover. Nat Rev Microbiol 15(8):502–510
Plowright R et al (2020) A call to action: understanding land use-induced zoonotic spillover to protect environmental, animal, and human health
Post AS et al (2019) Supporting evidence for a human reservoir of invasive non-Typhoidal *Salmonella* from household samples in Burkina Faso. PLoS Negl Trop Dis 13(10)
Potkay S et al (1966) An epizootic of measles in a colony of rhesus monkeys (*Macaca mulatta*). Am J Vet Res 27(116):331–334
Prasad HK et al (2005) Bovine tuberculosis in India: potential basis for zoonosis. Tuberculosis (Edinb) 85(5–6):421–428
Price LB et al (2012) *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 3(1):e00305–11. https://doi.org/10.1128/mBio.00305-11. Print 2012
Pusey A (1998) Scabies in chimpanzees of Gombe National Park, Tanzania. European Association of Zoo and Wildlife Veterinarians Newsletter 1(10)
Rajão DS et al (2013) Serological evidence of swine influenza in Brazil. Influenza Other Respir Viruses 7(2):109–112
Ramírez-Martínez LA et al (2013) Evidence of transmission and risk factors for influenza A virus in household dogs and their owners. Influenza Other Respir Viruses 7(6):1292–1296
Reid SM et al (2012) First reported detection of influenza A (H1N1)pdm09 in turkeys in the United Kingdom. Avian Dis 56(4 Suppl):1062–1067
Remfry J (1976) A measles epizootic with 5 deaths in newly-imported rhesus monkeys (Macaca mulatta). Lab Anim 10(1):49–57
Reperant LA, Cornaglia G, Osterhaus ADME (2013) The importance of understanding the human-animal interface : from early hominins to global citizens. Curr Top Microbiol Immunol 365:49–81
(1999) Reversing itself, appeals court denies trial for blood recipient. AIDS Policy Law 14(14):9
Ricklefs RE, Fallon SM (2002) Diversification and host switching in avian malaria parasites. Philos Trans R Soc Lond Ser B Biol Sci 269(1494):885–892
Rith S, Netrabukkana P, Sorn S, Mumford E, Mey C, Holl D, Goutard F, Y B, Fenwick S, Robertson I, Roger F, Buchy P (2013) Serologic evidence of human influenza virus infections in swine populations, Cambodia. Influenza Other Respir Viruses 7(3):271–279. https://doi.org/10.1111/j.1750-2659.2012.00382.x
Rolland RM et al (1985) Antibiotic-resistant bacteria in wild primates: increased prevalence in baboons feeding on human refuse. Appl Environ Microbiol 49(4):791–794
Roy S et al (2009) Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog 5(7):e1000503
Rubin JE, Ball KR, Chirino-Trejo M (2011) Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. Can Vet J = La revue veterinaire canadienne 52(2):153–157
Ruiz-Arrondo I et al (2021) Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: a case of an asymptomatic cat with SARS-CoV-2 in Europe. Transbound Emerg Dis 68(2):973–976
Russell ML et al (2009) Protocol: transmission and prevention of influenza in Hutterites: zoonotic transmission of influenza A: swine & swine workers. BMC Public Health 9:420

Rwego IB et al (2008a) Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda; Transmisión de Bacterias Gastrointestinales entre Humanos, Gorilas de Montaña y Ganado en el Parque Nacional Bwindi Impenetrable, Uganda. Conserv Biol 22(6):1600–1607

Rwego IB et al (2008b) Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conserv Biol 22(6):1600–1607

Sá RM et al (2013) Gastrointestinal symbionts of chimpanzees in Cantanhez National Park, Guinea-Bissau with respect to habitat fragmentation. Am J Primatol 75(10):1032–1041

Sabale SS et al (2013) Seroprevalence of pandemic influenza H1N1 (2009) & seasonal influenza viruses in pigs in Maharashtra & Gujarat States, India, 2011. Indian J Med Res 138(2):267–269

Said AW et al (2011) A sero-survey of subtype H3 influenza A virus infection in dogs and cats in Japan. J Vet Med Sci 73(4):541–544

Sailleau C et al (2020) First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis 67(6):2324–2328

Sak B et al (2013) Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western Lowland Gorillas (*Gorilla gorilla gorilla*) at different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic. PloS one 8(8):e71840

Sak B et al (2014) Diversity of microsporidia, *Cryptosporidium* and *Giardia* in mountain gorillas (*Gorilla beringei beringei*) in Volcanoes National Park, Rwanda. PloS one 9(11):e109751

Sakulwira K et al (2002) Serological evidence of herpesvirus infection in gibbons. BMC Microbiol 2:11–11

Santini JM, Edwards SJL (2020) Host range of SARS-CoV-2 and implications for public health. Lancet Microbe 1(4):e141–e142

Scarpino SV, Petri G (2019) On the predictability of infectious disease outbreaks. Nat Commun 10 (1):898

Schaumburg F et al (2012) Drug-resistant human *Staphylococcus aureus* in sanctuary apes pose a threat to endangered wild ape populations. Am J Primatol 74(12):1071–1075

Schaumburg F et al (2013) Evaluation of non-invasive biological samples to monitor *Staphylococcus aureus* colonization in great apes and lemurs. PLoS One 8(10):e78046–e78046

Schiller SE, Webster KN, Power M (2016) Detection of *Cryptosporidium hominis* and novel *Cryptosporidium* bat genotypes in wild and captive Pteropus hosts in Australia. Infect Genet Evol 44:254–260

Schmidt V et al (2008) Transmission of tuberculosis between men and pet birds: a case report. Avian Pathol 37(6):589–592

Schmitt A et al (2014) Lethal herpesvirus simplex infection in two common marmosets (*Callithrix jacchus*). Proc Int Conf Dis Zoo Wild Anim

Schrenzel MD et al (2003) Naturally occurring fatal herpes simplex virus 1 infection in a family of white-faced saki monkeys (*Pithecia pithecia pithecia*). J Med Primatol 32(1):7–14

Schrenzel MD et al (2011) Pandemic (H1N1) 2009 virus in 3 wildlife species, San Diego, California, USA. Emerg Infect Dis 17(4):747–749

Scotch M et al (2011) Human vs. animal outbreaks of the 2009 swine-origin H1N1 influenza A epidemic. EcoHealth 8(3):376–380

Scully EJ et al (2018) Lethal Respiratory Disease Associated with Human Rhinovirus C in Wild Chimpanzees, Uganda, 2013. Emerg Infect Dis 24(2):267–274

Segalés J et al (2020) Detection of SARS-CoV-2 in a cat owned by a COVID-19-affected patient in Spain. Proc Natl Acad Sci U S A 117(40):24790–24793

Seguin JC et al (1999) Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J Clin Microbiol 37(5):1459–1463

Seiler BM et al (2010) Antibodies to influenza A virus (H1 and H3) in companion animals in Iowa, USA. Vet Rec 167(18):705–707

Senghore M et al (2016) Transmission of *Staphylococcus aureus* from humans to green monkeys in The Gambia as revealed by whole-genome sequencing. Appl Environ Microbiol 82(19):5910–5917

Senthilkumar D et al (2021) Widespread prevalence of antibodies against swine influenza A (pdm H1N1 09) virus in pigs of Eastern Uttar Pradesh, India. Curr Microbiol 78(7):2753–2761

Sharma DK, Gurudutt J, Singathia R, Lakhotia RL (2009) Zooanthroponosis of *Microsporum gypseum* infection. Haryana Vet 48:108–109
Sharma R et al (2013) Circulation of group A rotaviruses among neonates of human, cow and pig: study from Assam, a north eastern state of India. Indian J Virol 24(2):250–255
Shaw A et al (2017) DALYs, dollars and dogs: how best to analyse the economics of controlling zoonoses. Rev Sci Tech (International Office of Epizootics) 36(1):147–161
Shishido A (1966) Natural infection of measles virus in laboratory monkeys. Jpn J Med Sci Biol 19 (4):221–222
Shrikrishna D, de la Rua-Domenech R, Smith NH, Colloff A, Coutts I (2009) Human and canine pulmonary *Mycobacterium bovis* infection in the same household: re-emergence of an old zoonotic threat? Thorax 64(1):89–91. https://doi.org/10.1136/thx.2008.106302
Shriner S et al (2021) SARS-CoV-2 exposure in escaped mink, Utah, USA. Emerg Infect Dis 27(3):988
Shrivastava AK et al (2020) Major enteropathogens in humans, domestic animals, and environmental soil samples from the same locality: prevalence and transmission considerations in coastal Odisha, India. Epidemiol Health 42:e2020034–e2020034
Simon G et al (2014) European surveillance network for influenza in pigs: surveillance programs, diagnostic tools and Swine influenza virus subtypes identified in 14 European countries from 2010 to 2013. PLoS One 9(12):e115815–e115815
Sit THC et al (2020) Infection of dogs with SARS-CoV-2. Nature 586(7831):776–778
Sjurseth SK et al (2017) Human to animal transmission of influenza A(H1N1)pdm09 in a turkey breeder flock in Norway. Infect Ecol Epidemiol 7(1):1416249
Slater OM et al (2014) Human metapneumovirus infection in chimpanzees, United States. Emerg Infect Dis 20(12):2115–2118
Sleeman JM et al (2000) Gastrointestinal parasites of mountain gorillas (*Gorilla gorilla beringei*) in the Parc National des Volcans, Rwanda. J Zoo Wildl Med 31(3):322–328
Slingenbergh JI, Gilbert M, de Balogh KI, Wint W (2004) Ecological sources of zoonotic diseases. Rev Sci Tech 23(2):467–484. https://doi.org/10.20506/rst.23.2.1492
Smith PC et al (1969) The gibbon (*Hylobates lar*); a new primate host for Herpesvirus hominia. I. A natural epizootic in a laboratory colony. J Infect Dis 120(3):292–297
Smith SL, Anderson ER, Cansado-Utrilla C, Prince T, Farrell S, Brant B, Smyth S, Noble PM, Pinchbeck GL, Marshall N, Roberts L, Hughes GL, Radford AD, Patterson EI (2021) SARS-CoV-2 neutralising antibodies in dogs and cats in the United Kingdom. Curr Res Virol Sci 2:100011. https://doi.org/10.1016/j.crviro.2021.100011
Sojka PA et al (2020) Acute human orthopneumovirus infection in a captive white-handed gibbon. J Vet Diagn Invest 32(3):450–453
Sokolow SH et al (2019) Ecological interventions to prevent and manage zoonotic pathogen spillover. Philos Trans R Soc, B 374(1782):20180342
Song MS et al (2010) Evidence of human-to-swine transmission of the pandemic (H1N1) 2009 influenza virus in South Korea. J Clin Microbiol 48(9):3204–3211
Song D, Kim H, Na W, Hong M, Park SJ, Moon H, Kang B, Lyoo KS, Yeom M, Jeong DG, An DJ, Kim JK (2015) Canine susceptibility to human influenza viruses (A/pdm 09H1N1, A/H3N2 and B). J Gen Virol 96(Pt 2):254–258. https://doi.org/10.1099/vir.0.070821-0
Sooksawasdi Na Ayudhya S, Kuiken T (2021) Reverse zoonosis of COVID-19: lessons from the 2009 influenza pandemic. Vet Pathol 58(2):234–242
Sørensen UBS, Klaas IC, Boes J, Farre M (2019) The distribution of clones of *Streptococcus agalactiae* (group B streptococci) among herdspersons and dairy cows demonstrates lack of host specificity for some lineages. Vet Microbiol 235:71–79. https://doi.org/10.1016/j.vetmic.2019.06.008
Špičić S et al (2012) Molecular epidemiology of *Mycobacterium tuberculosis* transmission between cattle and man – a case report. Veterinarski Arhiv 82(3):303–310
Sponseller BA et al (2010) Influenza A pandemic (H1N1) 2009 virus infection in domestic cat. Emerg Infect Dis 16(3):534–537
Sreenivasan CC et al (2021) Susceptibility of livestock and companion animals to COVID-19. J Med Virol 93(3):1351–1360

Sreta D et al (2010) Pandemic (H1N1) 2009 virus on commercial swine farm, Thailand. Emerg Infect Dis 16(10):1587

Steele L, Orefuwa E, Dickmann P (2016) Drivers of earlier infectious disease outbreak detection: a systematic literature review. Int J Infect Dis 53:15–20

Steinmetz HW, Rutz C, Hoop RK, Grest P, Bley CR, Hatt JM (2006) Possible human-avian transmission of *Mycobacterium tuberculosis* in a green-winged macaw (*Ara chloroptera*). Avian Dis 50(4):641–645. https://doi.org/10.1637/7609-041906R.1

Stevanovic V, Vilibic-Cavlek T, Tabain I, Benvin I, Kovac S, Hruskar Z, Mauric M, Milasincic L, Antolasic L, Skrinjaric A, Staresina V, Barbic L (2021) Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. Transbound Emerg Dis 68(4):1767–1773. https://doi.org/10.1111/tbed.13924

Steyer A et al (2008) Human, porcine and bovine rotaviruses in Slovenia: evidence of interspecies transmission and genome reassortment. J Gen Virol 89(Pt 7):1690–1698

Su S et al (2013) Serologic evidence of pandemic influenza virus H1N1 2009 infection in cats in China. Clin Vaccine Immunol 20(1):115–117

Su S et al (2014) Evidence for subclinical influenza A(H1N1)pdm09 virus infection among dogs in Guangdong Province, China. J Clin Microbiol 52(5):1762–1765

Summa M, von Bonsdorff CH, Maunula L (2012) Pet dogs--a transmission route for human noroviruses? J Clin Virol 53(3):244–247

Summa M, Henttonen H, Maunula L (2018) Human noroviruses in the faeces of wild birds and rodents-new potential transmission routes. Zoonoses Public Health 65(5):512–518

Sun Y et al (2014) A serological survey of canine H3N2, pandemic H1N1/09 and human seasonal H3N2 influenza viruses in dogs in China. Vet Microbiol 168(1):193–196

Sun H et al (2021) Mink is a highly susceptible host species to circulating human and avian influenza viruses. Emerg Microbes Infect 10(1):472–480

Sunde M et al (2011) Detection of methicillin-resistant *Staphylococcus aureus* sequence type 8 in pigs, production environment, and human beings. J Vet Diagn Investig 23(2):348–350

Sutherland KP et al (2011) Human pathogen shown to cause disease in the threatened eklhorn coral Acropora palmata. PLoS One 6(8):e23468

Sweetline Anne N et al (2017) Molecular identification of *Mycobacterium tuberculosis* in cattle. Vet Microbiol 198:81–87

Swenson SL et al (2010) Natural cases of 2009 pandemic H1N1 Influenza A virus in pet ferrets. J Vet Diagn Investig 22(5):784–788

Szentiks CA et al (2009) Lethal pneumonia in a captive juvenile chimpanzee (*Pan troglodytes*) due to human-transmitted human respiratory syncytial virus (HRSV) and infection with *Streptococcus pneumoniae*. J Med Primatol 38(4):236–240

Tang Y et al (2021) The prevalence of *Staphylococcus aureus* and the occurrence of MRSA CC398 in monkey feces in a Zoo Park in Eastern China. Animals (Basel) 11(3)

Tangtrongsup S et al (2019) Intestinal parasites and the occurrence of zoonotic *Giardia duodenalis* genotype in captive gibbons at Krabokkoo wildlife breeding center, Thailand. Front Vet Sci 6:110

Tangwangvivat R et al (2019) Evidence of pandemic H1N1 influenza exposure in dogs and cats, Thailand: a serological survey. Zoonoses Public Health 66(3):349–353

Taylor LH, Latham SM, Woolhouse ME (2001) Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci 356(1411):983–989. https://doi.org/10.1098/rstb.2001.0888

Teichroeb JA et al (2009) Ecology of the gastrointestinal parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: Possible anthropozoonotic transmission. Am J Phys Anthropol 140 (3):498–507

Terebuh P et al (2010) Transmission of influenza A viruses between pigs and people, Iowa, 2002-2004. Influenza Other Respir Viruses 4(6):387–396

Terzian ACB et al (2018) Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. Sci Rep 8(1):16034

Thakur A et al (2012) Detection of *Mycobacterium bovis* and *Mycobacterium tuberculosis* from cattle: possible public health relevance. Indian J Microbiol 52(2):289–291

Thongyuan S, Kittayapong P (2017) First evidence of dengue infection in domestic dogs living in different ecological settings in Thailand. PLoS One 12(8):e0180013. https://doi.org/10.1371/journal.pone.0180013

Trevennec K et al (2012) Transmission of pandemic influenza H1N1 (2009) in Vietnamese swine in 2009-2010. Influenza Other Respir Viruses 6(5):348–357

Unwin S, Chatterton J, Chantrey J (2013) Management of severe respiratory tract disease caused by human respiratory syncytial virus and *Streptococcus pneumoniae* in captive chimpanzees (*Pan troglodytes*). J Zoo Wildl Med 44(1):105–115

van de Vijver LP et al (2014) Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in organic pig herds in the Netherlands. Zoonoses Public Health 61(5):338–345

Vieira YR et al (2019) Evaluation of HBV-Like circulation in wild and farm animals from Brazil and Uruguay. Int J Environ Res Public Health 16(15):2679

Waage J, Mumford JD (2008) Agricultural biosecurity. Philos Trans R Soc, B 363(1492):863–876

Wang L et al (2020a) Complete genome sequence of SARS-CoV-2 in a tiger from a U.S. zoological collection. Microbiol Resour Announc 9(22):e00468–20. https://doi.org/10.1128/MRA.00468-20

Wang W et al (2020b) Prevalence and genotypic identification of *Cryptosporidium* in free-ranging and farm-raised donkeys (*Equus asinus asinus*) in Xinjiang, China. Parasite 27:45

Washko RM, Hoefer H, Kiehn TE, Armstrong D, Dorsinville G, Frieden TR (1998) *Mycobacterium tuberculosis* infection in a green-winged macaw (*Ara chloroptera*): report with public health implications. J Clin Microbiol 36(4):1101–1102. https://doi.org/10.1128/JCM.36.4.1101-1102.1998

Watson SJ et al (2015) Molecular epidemiology and evolution of influenza viruses circulating within European swine between 2009 and 2013. J Virol 89(19):9920–9931

Weese JS et al (2006) Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol 115(1/3):148–155

Weingartl HM, Berhane Y, Hisanaga T, Neufeld J, Kehler H, Emburry-Hyatt C, Hooper-McGreevy K, Kasloff S, Dalman B, Bystrom J, Alexandersen S, Li Y, Pasick J (2010) Genetic and pathobiologic characterization of pandemic H1N1 2009 influenza viruses from a naturally infected swine herd. J Virol 84(5):2245–2256. https://doi.org/10.1128/JVI.02118-09

Weissenböck H et al (1997) Naturally occurring herpes simplex encephalitis in a domestic rabbit (*Oryctolagus cuniculus*). Vet Pathol 34(1):44–47

Welshman MD (1989) Measles in the cynomolgus monkey (Macaca fascicularis). Vet Rec 124(8): 184–186

Wilbur AK, Engel GA, Rompis A, A Putra IG, Lee BP, Aggimarangsee N, Chalise M, Shaw E, Oh G, Schillaci MA, Jones-Engel L (2012) From the mouths of monkeys: detection of *Mycobacterium tuberculosis* complex DNA from buccal swabs of synanthropic macaques. Am J Primatol 74(7):676–686. https://doi.org/10.1002/ajp.22022

Williams JM, Lonsdorf EV, Wilson ML, Schumacher-Stankey J, Goodall J, Pusey AE (2008) Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. Am J Primatol 70:766–777

Willy ME et al (1999) Management of a measles outbreak among Old World nonhuman primates. Lab Anim Sci 49(1):42–48

Wohlsein P et al (2002) Spontaneous human herpes virus type 1 infection in a chinchilla (*Chinchilla lanigera* f. dom.). Acta Neuropathol 104(6):674–678

Wolf S et al (2013) The simultaneous occurrence of human norovirus and hepatitis E virus in a Norway rat (*Rattus norvegicus*). Arch Virol 158(7):1575–1578

Woolhouse MEJ et al (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos Trans R Soc Lond Ser B Biol Sci 356 (1411):991–999

World B (2006) In: Jamison DT, Alleyne G (eds) Disease control priorities in developing countries, 2nd edn. World Bank Publications, Herndon, p 1448

Wrobel L et al (2008) Molecular phylogenetic analysis of a geographically and temporally matched set of *Candida albicans* isolates from humans and nonmigratory wildlife in central Illinois. Eukaryot Cell 7(9):1475–1486

Wu F-T et al (2017) Novel G9 rotavirus strains co-circulate in children and pigs, Taiwan. Sci Rep 7 (1):40731

Xiao CT et al (2013) Identification and characterization of novel porcine astroviruses (PAstVs) with high prevalence and frequent co-infection of individual pigs with multiple PAstV types. J Gen Virol 94(Pt 3):570–582

Yin X et al (2014) Serological report of pandemic and seasonal human influenza virus infection in dogs in southern China. Arch Virol 159(11):2877–2882

Yoshida T et al (2021) Prevalence of antibodies against human respiratory viruses potentially involving anthropozoonoses in wild bonobos. Primates 62:897

Yu F et al (2017) High prevalence of *Enterocytozoon bieneusi* zoonotic genotype D in captive golden snub-nosed monkey (*Rhinopithecus roxellanae*) in zoos in China. BMC Vet Res 13(1):1–7

Zachariah A et al (2017) *Mycobacterium tuberculosis* in Wild Asian Elephants, Southern India. Emerg Infect Dis 23(3):504–506

Zhang X et al (2015) Serological survey of canine H3N2, pandemic H1N1/09, and human seasonal H3N2 influenza viruses in cats in northern China, 2010–2014. Virol J 12(1):50

Zhang Q et al (2020) A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg Microbes Infect 9(1):2013–2019

Zhao F-R et al (2014) Serological report of pandemic (H1N1) 2009 infection among cats in northeastern China in 2012-02 and 2013-03. Virol J 11:49–49

Part II

Zoonoses in Food-Chain Animals with Public Health Relevance

Important Zoonotic Diseases of Cattle and Their Prevention Measures

3

Mo Salman and Katie Steneroden

Contents

Abstract

Cattle production is a vital component of the global food chain. Through meat or milk, animal protein is an essential dietary requirement for most people across the world. Increased cattle production will attempt to meet the need for more protein with both positive and negative impacts. Those impacts may include the spread of disease from livestock to humans directly or indirectly through milk, meat, hide, or manure. The following zoonotic diseases of cattle are included in this chapter due to their potential severity in human or cattle populations and/or their wide distribution or recent emergence: anthrax, bovine spongiform encephalopathy (BSE), bovine cysticercosis, bovine tuberculosis, brucellosis, cryptosporidium,

M. Salman (✉) · K. Steneroden
Colorado State University, Fort Collins, CO, USA
e-mail: mo.salman@ColoState.Edu

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_1

Escherichia coli O157:H7, leptospirosis, listeria, methicillin resistant *Staphylococcus aureus* (MRSA), Q fever, Rift Valley Fever, and *Salmonella.*

Keywords

Bovine diseases · Cattle zoonotic diseases · Veterinary public health

Cattle production is a vital component of the global food chain. Through meat or milk, animal protein is an essential dietary requirement for most people across the world. The need for animal protein is increasing. An estimated 50% increase in demand is expected by 2030 (Delgado et al. 1999; Jones and Thornton 2009). Increased cattle production will attempt to meet the need for more protein with positive and negative impacts, including the spread of diseases from livestock to people directly or indirectly through milk, meat, hide, or manure.

Threats from old and new pathogens continue to emerge, with contributions from changes in the environment, agriculture and food production systems, food processing, and the demography and connectivity of our world. At one extreme is low-intensity cattle farming, the type traditionally practiced in developing countries and rural households. The impact of disease outbreaks on the lives and livelihoods of these poor farmers is significant (Jones and Thornton 2009). In contrast, intensive farming systems in developed countries may contribute to the large-scale spread of pathogens during disease outbreaks. Zoonotic diseases can have a significant impact on national and international trade and contribute to human illness. We are faced with a changing landscape of infectious disease that affects both humans and animals. This change poses significant threats to the health and food security of the global citizenry (Atkins and Robinson 2013).

The majority of human pathogens now described are linked to animals. An average of three new infections are reported approximately every 2 years, with a new pathogen published every week (Gideon 2013). Nevertheless, good progress continues to be made in controlling several important livestock pathogens, and mechanisms are now in place to bring together the critical scientific expertise and political will to succeed.

The following zoonotic diseases of cattle are included in this chapter due to their potential severity in humans or cattle population and/or their wide distribution or recent emergence: anthrax, bovine spongiform encephalopathy (BSE), bovine cysticercosis, bovine tuberculosis, brucellosis, cryptosporidium, *Escherichia coli* O157:H7, leptospirosis, listeriosis, methicillin resistant *Staphylococcus aureus* (MRSA), Q fever, Rift Valley Fever, and *Salmonella*.

Zoonotic pathogens present considerable challenges to the health and wellbeing of cattle and humans. For some critically important diseases, the first line of defense will be implementing scientific approaches to diagnosis and control. What the future will bring with regard to zoonotic diseases is difficult to predict. A future where human and animal health practitioners work together to discover, control, and prevent zoonotic diseases will surely bring surprising and meaningful results.

Identifying and ultimately addressing emerging cross-species infections will require a "One Health" approach. Adopting and implementing appropriate intervention measures at the farm level will require behavior changes from the farmer (Ellis-Iversen et al. 2010). Some of these measures will require modification of practices and husbandry as well as policy changes. In 1989, the World Health Organization (WHO) promoted a Knowledge, Attitude, Beliefs, and Practices (KABP) framework that was later modified to Knowledge, Attitude, and Practices (KAP) as a tool to improve disease prevention strategies in different cultures around the world. In a recent example, a socio-ecological model that included dairy workers and an external agency in Colorado, USA, was presented by Palomares Velosa et al. (2020).

We attempted in this chapter to identify prevention measures for the underlying diseases with the hope that further assessment of these measures using the KAP approach under the One Health Concept can be applied.

3.1 Anthrax

Bacillus anthracis, the causative agent of anthrax, has a worldwide distribution in animal and human populations. In developing countries, anthrax is a significant problem in livestock and wildlife and among occupationally exposed individuals, including veterinarians, agricultural workers, and butchers (WHO 2013a). Anthrax is no longer a significant livestock disease in developed countries due to appropriate control measures, including prophylactic vaccination. While anthrax does occur sporadically in developed countries, its primary significance lies in its potential use as a bioterrorism agent.

Bacillus anthracis is a Gram-positive bacterium that forms spores when exposed to oxygen, which are highly resistant and long lasting in the environment. Human anthrax cases are associated with infection in livestock or exposure to contaminated products such as carcasses, hides, or wool. Instances of animal anthrax are associated with spore-contaminated pastures. The incidence of anthrax varies with the soil type, climate, animal husbandry, industrial hygiene, and disease reporting status of the country. Globally, anthrax is underreported in both humans and animal populations due to under-diagnosis and lack of internal and international reporting.

Infection can enter the body by ingestion, inhalation, or direct contact. It is generally considered that animals are infected by ingestion of contaminated food or water. In humans, infection mainly occurs by direct contact through a break in the skin. Biting flies and other insects have the ability to transmit the disease mechanically.

In cattle, anthrax usually manifests as peracute or acute disease. The peracute form is most common at the beginning of an outbreak, and animals are found dead without premonitory signs. After death, discharge of blood from the nostrils, mouth, anus, and vulva are common. The acute form runs a course of about 48 h with severe depression, lethargy, abortion, and fever. Necropsy findings include the absence of rigor mortis and gross enlargement of the spleen with natural orifices exuding dark, tarry unclotted blood. If anthrax is suspected, the carcass should not be opened, as

exposure to oxygen will cause spores to form, which may infect individuals and contaminate the environment.

In humans, the three main forms of disease are cutaneous, gastrointestinal, and inhalation anthrax. Cutaneous anthrax is most common and accounts for the vast majority of cases. The gastrointestinal form occurs from eating contaminated meat. Inhalation anthrax occurs through inhalation of the spores and is the most severe form (Decker 2003).

There are different assays for screening and diagnosis of anthrax in cattle. A stained smear of peripheral blood is usually considered the primary screening test to determine the presence of the bacilli in the blood. Confirmation is done by blood culture to identify the bacterial colonies. Fluorescent antibody techniques may also be used to confirm the infection. Animal passage assay may be necessary if antibiotic therapy is used (Dragon et al. 1999).

Two types of vaccines are currently used in cattle. The most known vaccine is the live attenuated strain of *B. anthracis*, resulting in long-term immunity (26 months), but there is a risk of causing the disease. The second vaccine is the cell-free filtrate of a culture of *B. anthracis,* which is incapable of causing anthrax, but it has only a short-term immunity (3–6 months) (WHO 2013a).

Treatment in animals and humans is mainly through the application of antibiotics. In animals, penicillin, streptomycin, and oxytetracycline are used. Anti-anthrax serum may be used in animals during the early stages of the disease, but severely ill animals are unlikely to recover. Human treatment is by penicillin and other antibiotics (Dragon et al. 1999).

Control measures are wide range and include vaccination, appropriate carcass disposal methods and decontamination, quarantine, and movement restrictions on milk and meat.

3.2 Bovine Spongiform Encephalopathy (BSE)

Bovine spongiform encephalopathy (BSE), also known as "mad cow disease," is a degenerative neurological disease of cattle. BSE is caused by misfolded proteins (prions) in the host cell that build up in the central nervous system (CNS) and eventually kill nerve cells. The nature of the transmissible agent is not well understood. The most accepted theory is that the agent is a modified form of a normal protein known as prion protein. For not yet understood reasons, the normal prion protein changes into a pathogenic (harmful) form that then damages the central nervous system.

BSE is one of several rare neurological diseases called transmissible spongiform encephalopathy (TSE). The other TSE diseases include scrapie, which affects sheep and goats, transmissible mink encephalopathy, feline spongiform encephalopathy, and chronic wasting disease of deer and elk. There are six TSE diseases that affect humans: kuru, classical Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, and sporadic fatal insomnia.

Variant Creutzfeldt-Jakob disease (vCJD) is a rare human TSE that research from the United Kingdom has associated with consuming products contaminated with CNS tissue from BSE-infected cattle. There have been about 200 cases in the world (most of these in the United Kingdom). Human TSEs also include sporadic Creutzfeldt-Jakob disease (sCJD or CJD), which is not related to BSE. About 85% of CJD cases are sporadic, with an annual incidence of about one case per 1 million people worldwide. The new variant or variant form (vCJD) affects younger people (average age at onset is 26 years) and has different clinical features from CJD.

There is strong epidemiologic and laboratory evidence suggesting that the same infectious agent causes vCJD and BSE. All cases of confirmed vCJD have occurred in people who have lived in geographic areas with BSE cases; the majority occurred in the United Kingdom, which has had the largest number of cases of BSE in cattle. The specific foods, if any, that may be associated with the transmission of this agent from cattle to humans are unknown. However, milk and milk products are unlikely to pose any risk for human exposure to the BSE agent.

Research indicates that the first probable infections of BSE in cows occurred during the 1970s, with the first two cases of BSE being identified in 1986. BSE may have originated from feeding cattle meat-and-bone meal (MBM) that contained BSE-infected products from a spontaneously occurring case of BSE or scrapie-infected sheep products. There is strong evidence and general agreement that the outbreak was amplified and spread throughout the United Kingdom cattle industry by feeding rendered, prion-infected, bovine meat-and-bone meal to young calves.

There is increasing evidence that there are different strains of BSE, the typical BSE strain responsible for the outbreak in the United Kingdom and two atypical strains (H and L strains). The typical BSE strain is responsible for most of the BSE cases in the world. In cattle naturally infected with BSE, the BSE agent has been found in brain tissue, in the spinal cord, and the retina of the eye. Additional experimental studies suggest that the BSE agent may also be present in the small intestine, tonsil, bone marrow, and dorsal root ganglia (lying along the vertebral column).

In response to the BSE epidemic, several countries instituted a series of measures to minimize the risk of disease transmission among both animals and humans. These included a ban on feeding ruminant protein to ruminants and removal of some “high risk” materials (such as brain, spinal cord, and intestines) from cattle at slaughter. Following the institution of these measures, the number of BSE cases has been decreased significantly (USDA-APHIS 2006, 2007).

To prevent BSE from entering the country, several countries prohibited the importation of live ruminants from countries where BSE is known to exist in native cattle. Some countries eliminated the importation of live ruminants and most ruminant products, including meat, meat-and-bone meal, offal, glands, etc., from all of Europe. Most of these countries also prohibited the use of mammalian protein in the manufacture of animal feeds given to ruminants. Testing for BSE under a national surveillance program among slaughtered cattle was implemented in several developed countries. Due to these safeguard measures, the risk of transmitting the BSE agent to humans has become negligible (Salman et al. 2012).

3.3 Bovine Cysticercosis –Taeniasis

Although bovine cysticercosis does not in itself represent an exceptionally serious human health risk, it is a signal of much more serious food safety and public health concerns. A finding of bovine cysticercosis is a signal that the animal feed system is contaminated and that cows are consuming human feces. Aside from *Taenia saginata*, other contaminants that pose threats to bovine and human health would also be expected to be present in human feces. These contaminants include but are not limited to drug-resistant bacteria, such as *E. coli* and *Salmonella*, *Taenia solium* (the pork tapeworm), drug residues, pain killers, hormones, other prescription drugs, illicit drugs, heavy metals, solvents, and other toxicants.

Taenia saginata (T. saginata) is a cestode tapeworm that causes bovine cysticercosis in cattle and taeniasis in humans. *Taenia saginata* is found worldwide, and human disease is highly endemic in Latin America, Africa, Asia, and some Mediterranean countries. Bovine cysticercosis occurs in areas where poor sanitation, poor food inspection, and close contact between humans and livestock are common.

Taenia saginata infection cycles between humans (primary host) and cattle (reservoir host). Humans infected with the tapeworm pass the eggs in their feces. Cattle become infected by ingesting materials contaminated with tapeworm eggs. Larvae form cysticerci in the animal's muscle tissue, humans ingest cysticerci in raw or undercooked beef, and the cycle continues. Tapeworms cannot be passed from person to person or spread between cattle. Clinical signs of cysticercosis in cattle and humans are mild to nonexistent. The most visible sign of tapeworm infection in humans is the active passing of tapeworm segments through the anus and in the feces.

Diagnosis of bovine cysticercosis is largely made during visual inspection of the carcass at slaughter. Serological tests, including ELISA, have been used in epidemiological studies for individual and herd diagnosis (WHO 2005). Taeniasis in humans is diagnosed by finding eggs or cestode segments on the human body or in the feces. Feces microscopy, ELISA, and molecular tests such as PCR may also be used (WHO 2005).

Infection in humans can be prevented by proper meat inspection and handling of meat at slaughter. When the disease is found in cattle, the meat may be condemned or temperature treated by freezing or heating to kill the parasite. Preventing and treating disease in people will prevent disease in cattle. Tapeworm eggs can survive in the environment for many months, depending on humidity and temperature. Infected people can shed hundreds of thousands of eggs each day, so people need to seek treatment to break the cycle.

3.4 Bovine Tuberculosis

Bovine tuberculosis (BTB) is a zoonotic and economically important disease of livestock. The disease was described over 2000 years ago and is responsible for devastating illness and death in humans and animals. Bovine tuberculosis has been

largely controlled in developing countries through government control programs and milk pasteurization. In developing nations where surveillance and control measures are inadequate, humans continue to become infected with BTB through animal contact and ingesting unpasteurized dairy products. Few developing countries have BTB control programs and immune system compromising disease conditions such as HIV allow for co-infection and increased morbidity and mortality (Miller and Sweeney 2013).

Most warm-blooded vertebrates, including humans, are susceptible to the disease-causing agents. Although the principle reservoir of *Mycobacterium bovis* (*M. bovis*) is cattle, this organism has a wide host range capable of producing progressive disease. Ungulates differ somewhat in resistance to *M. bovis* but have similar immune responses and pathological conditions. They all exhibit the classical lesions of tuberculosis.

The infection is caused by the bacterial genus *Mycobacterium.* Mycobacteria are acid-fast, aerobic, non-spore-forming, nonmotile, gram-positive rods containing high lipid content. Some of the lipids possess virulent and immunologic properties. The possible pathogenic role and the effect on the immune response of components of the complex mycobacterial cell wall are the subject of much attention and controversy (Behr 2013).

Bovine tuberculosis occurs throughout the world. The prevalence of *M. bovis* in cattle is low in developed countries due to successful eradication programs. Other countries have experienced increases in the rate of infection due to relaxation in surveillance activities.

Risk factors for cattle include overcrowding, the introduction of tuberculous animals, soil type, wildlife contact in specific geographical regions (UK, Ireland: badger, New Zealand: possum), the purpose of the cattle: dairy vs. beef, and type of management and husbandry – specifically in the type of disposal of the manure.

The most common mode of transmission of BTB is the aerogenous route. Infection can occur by ingestion and other less likely modes such as milk-borne, congenital, or sexually transmitted. Bacteria are excreted in exhaled air, sputum, feces, urine, milk, and discharges from the uterus, vagina, and draining peripheral lymph nodes. Cattle can develop bovine tuberculosis through exposure to other *M. bovis* infected species such as humans, deer, and elk (Bovine TB Advisory Group 2009).

Clinical signs of disease in cattle are variable depending on the location and extent of the lesions. Even with advanced disease, visible signs are frequently absent. If superficial lymph nodes are involved, they may be visibly enlarged and can rupture and drain through the skin. Enlarged internal nodes can cause signs of obstruction. With pulmonary involvement, a chronic cough can develop due to bronchopneumonia. In advanced lung disease, dyspnea occurs with increased respiratory rate and depth. Tuberculosis mastitis causes a marked induration and hypertrophy of the udder. General findings include anorexia, dyspnea, weight loss, weakness, and low-grade fluctuating fever. Often the main sign of tuberculosis is emaciation, despite adequate nutrition and care.

A definitive diagnosis for mycobacterial infection can be made by bacterial isolation and identification, which can be difficult and time-consuming. For example, in M. *bovis*

cultures, visible growth arises following 3–8 weeks of incubation. Conventional mycobacteriological identification procedures on culture media rely on differences in culture growth times, colony morphology, cellular morphology, antimicrobial sensitivity, and various biochemical test reactions. More recent radiometric procedures can expedite mycobacterial detection times, whereas gas-liquid chromatography and DNA probes can accelerate mycobacterial identification from cultures. Research on the use of DNA probes, specifically polymerase chain reaction (PCR), is currently in progress for molecular epidemiology of the disease in livestock species.

The tuberculin skin test is an in vivo diagnostic test that evaluates the cell-mediated immune response to mycobacteria exposure. The test is unable to differentiate between disease and immunity. To determine whether or not an animal is infected with *M. bovis*, tuberculin made from either the human or bovine bacilli (the mammalian tuberculins) is injected intradermally into the animal. Reactivity to tuberculin made from either of these bacilli is similar and is usually the greatest in animals sensitized specifically to these bacilli. The inflammatory response to the injection peaks 24–72 h after tuberculin injection and can linger for several weeks before diminishing. Failure of an animal with observable evidence of tuberculosis to show a palpable skin response to tuberculin at the time of test reading has been defined as anergy. Anergy indicates deficient T lymphocyte function.

Vaccines against *M. bovis* stimulate cell-mediated immunity. BCG (Bacillus of Calmette-Guerin, the modified *M. bovis* vaccine strain named after its two developers) is an attenuated strain of *M. bovis* used in human vaccination. BCG has also been utilized extensively to vaccinate cattle in numerous countries for many years. Protection produced by BCG vaccination of cattle is poor and causes tuberculin sensitivity in the animals, interfering with control and eradication programs based on tuberculin skin testing. By 1968, none of the national control programs for bovine tuberculosis included vaccination.

Treatment of tuberculosis in animals, in general, is discouraged due to possible public health hazards in retaining tuberculous animals. However, numerous procedures have been tried throughout the years without success to treat tuberculous cattle, including injection of live or dead bacilli, specific diets, fresh air, change of climatic conditions, x-ray therapy, serotherapy pneumothorax, and pneumoperitoneum. Chemotherapeutic drugs, including isoniazid, have been used in cattle and were found to only suppress the bacilli during the duration of drug therapy, with shedding of the organism possible after treatment.

Control measures include test and slaughter, active detection of lesion in cattle in slaughterhouses followed by traceback systems, and control of the disease in wildlife populations.

3.5 Brucellosis

Brucellosis is a zoonotic disease of major social and economic importance in most countries of the world. It is caused by several species of *Brucella* bacteria and affects several livestock species – mainly cattle, sheep, and goats. The economic importance

of the disease in cattle is due to a loss of production, primarily decreased milk production, abortion, and infertility. Brucellosis is found worldwide; however, it is limited to a specific *Brucella* species and host species in some geographical areas. Several countries have succeeded in eradicating the disease from specific host species; other countries are engaged in eradication programs. The growing phenomenon of international migration and tourism renews our concern with the prevalence and persistence of human brucellosis.

The *Brucella* spp. have a wide host range; however, they are not readily transmitted from preferential to dissimilar hosts. Nonpreferential hosts may harbor the bacteria, but it is considered an incidental infection. This incidental infection is usually localized and/or shows different clinical and pathological manifestations from those observed in the specific host. The host preferences of this bacterial agent are *Brucella abortus* in cattle, *Brucella melitensis* in sheep and goats, *Brucella suis* in swine, and *Brucella ovis* in sheep (Moreno et al. 2002).

The bacteria is an intracellular organism which is an important factor in its survival in the host and may explain both the transitory titers occurring in some hosts following isolated episodes of bacteremia and the disappearance of titers in hosts with latent infection. The bacteria can survive on grass for variable periods depending on environmental conditions. In temperate climates, infectivity may persist for 100 days in winter and 30 days in summer. The organism is susceptible to heat, sunlight, and standard disinfectants, but freezing is conducive to almost indefinite survival (Blasco and Molina-Flores 2011).

Risk factors associated with infection and the diseases in cattle population include: (1) contact with infected materials – aborted fetus, placenta, semen, secretion, etc.; (2) direct contact with infected animals – including wildlife species; (3) high population density, particularly in dairy farming systems; (4) breeding management and husbandry such as contaminated maternity pens, unregulated breeding time; and (5) poor hygiene/husbandry – particularly during calving seasons.

The infection in humans is nonspecific and manifests as fluctuating fever, joint pain, sweating, and weakness. Transmission to humans occurs through contact with contaminated materials from infected animals, particularly as an occupational hazard, consumption of infected milk and dairy products, nonintentional injection of live animal vaccine, and inhalation of large amounts of bacteria-contaminated aerosols. Human brucellosis is most serious when it results from exposure to *B. melitensis*, which is usually linked to exposure to infected goats and sheep (Corbel 2006).

The disease in animals is transmitted through ingestion of contaminated materials, penetration of intact skin and conjunctiva, and contamination of the udder during milking. Intra-herd spread occurs by both vertical and horizontal transmission. Congenital infection due to in utero infection does occur, but its importance has not been defined. Horizontal transmission can occur both directly and indirectly. Flies, dogs, rats, ticks, contaminated boots, fodder, and other inanimate objects are possible ways for indirect transmission. Preventive measures in cattle populations

are mainly related to early detection of infected cattle with the removal of serologically positive animals (test and culling) and vaccine application.

No reliable vaccine is available for human use. Humans are usually treated prophylactically with antibiotics if exposure is suspected. Preventive measures for human infection include precaution in handling contaminated materials from infected animals and precautions during the use of the vaccine in animals and avoiding consumption of unpasteurized milk or dairy products.

3.6 *Cryptosporidium parvum*

Cryptosporidium parvum is a coccidian protozoan that is an important cause of diarrhea in cattle and humans worldwide. It has emerged since the 1970s as a major cause of calf-hood diarrhea. It is one of the top four agents responsible for moderate to severe gastrointestinal illness in children in developing countries and can be a fatal complication of AIDS (Kotloff et al. 2013; Mosier and Oberst 2000). Cryptosporidiosis is one of the most common causes of waterborne disease among humans in the United States (CDC 2013a).

Cryptosporidium parvum resides in the host's small intestine, where it forms oocysts, which are shed in great numbers in the feces. Transmission occurs through ingestion of food and water contaminated with fecal matter from infected animals or humans, direct contact with infected feces, or ingestion of contaminated water. Large outbreaks have been associated with drinking water, food, swimming pools, and lakes.

Community-wide outbreaks of cryptosporidiosis have been linked to drinking municipal water or recreational water contaminated with *Cryptosporidium*. One large-scale outbreak occurred in Wisconsin, USA, in 1993 when more than 400,000 people became ill from a malfunctioning municipal water filtration system. The total cost of outbreak-associated illness was USD 92 million (Corso et al. 2003). The source of the *Cryptosporidium* oocysts in this outbreak, whether from cattle, slaughterhouse runoff, or human sewage, remains speculative (Mac Kenzie et al. 1994).

In healthy humans, infection is usually asymptomatic and self-limiting. The disease can be severe in immunodeficient people with profuse watery diarrhea and substantial fluid loss. Most animals can become infected with *Cryptosporidium* spp., but clinical signs of diarrhea, tenesmus, anorexia, and weight loss are most commonly observed in calves less than one-month-old.

Cryptosporidiosis is diagnosed by examining fecal samples using acid-fast staining, direct fluorescent antibody, and/or enzyme immunoassays (CDC 2013a). The oocysts are not shed continuously, and repeated sampling may be necessary. Cryptosporidiosis can also be diagnosed in stained biopsy/necropsy specimens or fresh intestinal scrapings. Molecular methods, which can detect *Cryptosporidium* species, are increasingly being used in diagnostic laboratories.

There is no specific treatment available for cryptosporidiosis. Supportive therapy is usually effective. Prevention efforts focus on handwashing, especially after handling or being around animals and before eating or handling food.

3.7 *E. coli* O157:H7

Escherichia coli is in the family *Enterobacteriaceae* and is a normal component of the flora in the large intestine of humans and warm-blooded animals. *E. coli* O157:H7 is a specific pathogenic subset of *E. coli* found worldwide that produces watery diarrhea, hemorrhagic colitis, and rarely, hemolytic-uremia syndrome (HUS) in children.

Cattle are reservoir hosts, harbor the bacteria asymptomatically, and are an important source of infection for humans. Prevalence estimates vary, and it appears that while a large percentage of cattle herds may have infected animals, the actual number of individual infected animals at any one time is relatively low (USDA 2003). The costs associated with attempts to control prevalence in cattle, contaminated food recall, and human healthcare costs make the economic and social burden of *E. coli* O157:H7 high (Callaway 2010).

Transmission of *E. coli* O157:H7 occurs through consumption of contaminated food or water, direct contact with infected animals, their feces, or contaminated soil. Primary sources of *E. coli* O157:H7 outbreaks are raw or undercooked ground meat products, raw milk, and fecal contamination of vegetables. Person-to-person spread can occur during outbreaks (Spickler 2009). Visiting farms and other venues where the general public might directly contact farm animals, particularly calves, has been identified as an important risk factor for *E. coli* O157:H7 infection (WHO 2011a). A low dose of bacteria is sufficient for infection.

E. coli O157:H7 occurs asymptomatically in cattle and is shed intermittently. In humans, illness can range from mild diarrhea to severe hemorrhagic colitis. In most cases, the illness is self-limiting. Hemolytic uremic syndrome, a particularly severe complication, can occur in a small percentage of cases leading to renal failure and death in children and the elderly. Selective and differential culture media have been developed to diagnose *E. coli* O157:H7 in human and bovine fecal samples.

Measures to prevent and control *E. coli* O157:H7 in cattle include management changes (biosecurity, housing, transport, and stress reduction), water and feed management, including additives and probiotics, bacteriophages, and vaccines (Callaway 2010). Preharvest strategies are important but do not eliminate the need for good sanitation in processing plants and households. Good hygienic slaughtering practices reduce contamination of carcasses. Education on hygienic handling of foods is essential for farm workers, abattoir, and food production workers to reduce contamination. Household preventive measures are similar to those recommended for other foodborne diseases (WHO 2011a).

3.8 Leptospirosis

Leptospirosis is a zoonotic disease of worldwide importance. Also a neglected tropical disease, leptospirosis largely affects vulnerable rural and semi-urban populations. The global annual incidence of endemic human leptospirosis is grossly underestimated due to lack of awareness, underdiagnosis, misdiagnosis, and difficulty with diagnostic testing. Efforts to determine the burden of disease are ongoing (WHO 2011b). Leptospirosis is endemic in countries with humid subtropical and tropical climates, and epidemics often occur due to flooding. Individuals at greatest risk include farmers, ranchers, slaughterhouse workers, trappers, loggers, veterinarians, sewer workers, rice field workers, and military personnel.

Leptospirosis is caused by a variety of species of *Leptospira*, a spirochete with more than 250 pathogenic serovars that are adapted to different wild or domestic reservoir hosts. The classification system for *Leptospira* changed in 1989, leading to some confusion, as pathogenic and nonpathogenic serovars are now included in the same species. Serovars vary by geographic region (Spickler 2005a). Host adaptation is not a static situation as serovars adapt to new hosts, vaccine pressures alter serovars in different species, and climate change may alter hosts and serovars. These facts lead to difficulties in the prediction, prevention, and use of vaccines. Reservoir hosts include wild mammals (rats and rodents are the most common) as well as domestic cattle, pigs, pigs, and sheep and dogs. Reservoir hosts experience asymptomatic, mild, or chronic disease and can shed for months to years.

Leptospires reside in the kidneys of infected reservoir hosts and are shed in urine into the environment where they can live for long periods of time, depending on environmental conditions. Freshwater ponds, streams, runoff, and groundwater are common water sources of leptospires. *Leptospira* spp. can also be excreted in vaginal secretions and with aborted fetuses after calving (Spickler 2005a). *Leptospira* spp. can be spread directly between individuals, through skin contact with contaminated water or urine, ingested in contaminated food or water, or spread via aerosol.

At least 13 serovars of *Leptospira* spp. have been isolated from cattle. Clinical signs vary with the serovar and in acutely affected calves include fever, anorexia, conjunctivitis, and diarrhea. In adult cattle, clinical signs may be mild and go undetected. More severe infection may result in abortions, decreased fertility, or decreased milk yields (Spickler 2005a). Clinical signs are associated with kidney disease, liver disease, or reproductive dysfunction; younger animals suffer more severe disease. Differential diagnosis includes brucellosis, neosporosis, bovine viral diarrhea (BVD), and infectious bovine rhinotracheitis (IBR).

In humans, disease ranges from mild to severe depending on the serovar and immune status of the patient. Clinical signs mimic other infectious diseases, including influenza, hepatitis, dengue, hantavirus, yellow fever, malaria, brucellosis, borreliosis, typhoid fever, other enteric diseases, and pneumonia (Spickler 2005a).

Rapid screening tests are available for presumptive diagnosis in humans, but require confirmatory diagnosis by culture, PCR, or microagglutination test (MAT).

The most commonly used test for diagnosis in animals is the MAT test. ELISA tests are also used.

Human vaccines against leptospirosis are available in some countries. Animal vaccines are in use and must contain serovars present in the local environment; most of them require a yearly booster shot. In developed countries, cattle, pigs, and dogs are routinely immunized. In developing countries, vaccines with locally relevant serovars are not as available (Hartskeerl et al. 2011). Prevention programs must be tailor-made and based on predominant serovar and local reservoir hosts. Public health prevention measures include reservoir control through rodent control and vaccination of livestock and dogs, improved sanitation, improvement of water sources that may be contaminated, and outreach and education for high-risk individuals and high-risk areas.

3.9 Listeriosis

Listeriosis, also called Circling disease or Silage sickness, is a disease with worldwide distribution that can affect all ruminants. The etiological agent is usually *Listeria monocytogenes*; however, sheep can also get listeriosis from *Listeria ivanovii* infection (Todar 2003).

The etiological agent *Listeria monocytogenes* is a ubiquitous bacteria characterized as a small, gram-positive, non-spore-forming, catalase-positive, facultative anaerobic, motile rod sometimes arranged in short chains with the ability to produce flagella at room temperatures, but not at 37 °C (Todar 2003). Infection occurs mainly through ingestion of *L. monocytogenes* contaminated soil, vegetation, and silage.

Age and sex are not considered risk factors; however, cattle under 3 years of age are more prone to developing clinical signs of disease. The disease clinically manifests in cattle in one of four forms and is more common during cold weather. These forms are encephalitic, visceral/septicemic, abortion, and ophthalmitic. The encephalitic form is most common in adult cattle, while neonates often suffer from the septicemic or visceral forms. Abortion occurs due to placentitis resulting in fetal death if there is intrauterine infection. The ophthalmitic form is associated with bacterial contamination of the cornea from a feed source. Lactating cows may also contract clinical mastitis associated with listeriosis.

Most animals infected with *L. monocytogenes* show no clinical signs, but can still spread bacteria in the environment and to other animals. Listeriosis is diagnosed by the history of previous cases in the area, accompanied by some clinical signs. A confirmed diagnosis can be made by postmortem examination with histopathology of the pontomedullary region of the brainstem or bacterial culture. Usually, there are no gross lesions seen in the brain at necropsy. Microscopic lesions can include multifocal asymmetrical micro-abscesses and mononuclear cell meningoencephalitis in the brainstem, anterior spinal cord, and, occasionally, cerebellum (George 2002). Treatment of confirmed cases is with either oxytetracycline or penicillin G. Antibiotic therapy works best in animals treated in the early stages of the disease.

Animal to human transmission of *L. monocytogenes* occurs either directly through contact with infected animals or indirectly through ingestion of milk, cheese, meat, eggs, or vegetables. The bacterium is inactivated by pasteurization; however, contamination of pasteurized products with the raw product has been reported as a source of infection. Listeriosis in humans occurs primarily in pregnant women, newborns, the elderly, and immunosuppressed (e.g., transplant recipients or AIDS patients). It has been reported that as many as 5–10% of humans may be asymptomatic carriers of *Listeria* spp. in their feces or vagina. Disease in adult humans is commonly the encephalitic or septicemic/visceral form (Rebhun 1995).

Preventive measures can reduce the spread of infection. In dairy cattle, feeding spoiled silage and other rotting vegetation should be avoided. All cattle showing any signs of disease should be isolated from healthy animals. Good hygiene and sanitation on the farm are essential. Other preventive measures include thoroughly washing raw vegetables, cooking raw meats, proper hygiene during food preparation, and consuming only pasteurized dairy products. Humans should avoid contact with animals that have suffered from abortions as well as with the aborted materials (placenta and fetuses). Livestock and crop producers can help control the spread of *L. monocytogenes* by avoiding the use of untreated manure on vegetable crops. It is almost impossible to produce listeria-free products because infected animals do not always show signs of disease. As a result, people at high risk should avoid exposure to food items commonly associated with listeriosis.

3.10 Methicillin-Resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) are Gram-positive bacteria that are resistant to methicillin and other beta-lactams in this large group of antibiotics that are widely used in veterinary and human medicine. MRSA is found worldwide in humans and animals.

MRSA was first isolated from cattle with mastitis in 1972, which was the first recognition of this emerging disease in animals (Devriese et al. 1972). Since that time, MRSA has been found in many species of animals, including pigs, horses, dogs, cats, pet birds, zoo animals, and marine mammals (Spickler 2011). Most of the strains isolated from animals have been of human origin; this changed, however, in 2003–2005 with the emergence of a new type of MRSA, CC398, isolated from humans and pigs in the Netherlands. This livestock-associated strain appears to be less host-specific than other MRSA strains and has spread to other livestock, including cattle (Vanderhaeghen et al. 2010). The livestock-associated MRSA can cause disease in animals as well as in humans in close contact with them (Vanderhaeghen et al. 2010), and there is evidence of limited human-to-human spread of this strain as well (Voss et al. 2005). The data on this new type of livestock-associated MRSA is limited, and the burden of CC398 in cattle is unclear (Vanderhaeghen et al. 2010).

MRSA is transmitted most commonly through direct contact with colonized or infected individuals (animals or humans) (Spickler 2011). Contaminated

environments, including air in confinement operations, are other potential routes (Gibbs et al. 2006). Human and livestock-associated strains of MRSA can be found in contaminated food (Jones et al. 2002), meat (van Loo et al. 2007; de Boer et al. 2009), and raw milk products (Normanno et al. 2007).

Cattle colonized or infected with MRSA most commonly present with clinical or subclinical mastitis. MRSA colonization has been associated with veal calves (Graveland et al. 2010) and beef calves (Mooij et al. 2007). MRSA can cause a wide variety of infections in humans, including skin and soft tissue infections as well as more invasive infections including pneumonia, endocarditis, septic arthritis, and septicemia; MRSA is one of the most prevalent causes of nosocomial infections worldwide (Spickler 2011).

Diagnosis of infection or colonization with *S. aureus* can be accomplished through culture of the organism. Methicillin-resistant strains can be identified through antibiotic susceptibility or genetic testing. Genetic testing can identify the various human and animal-associated strains.

In general, prevention and control of MRSA include good biosecurity and infection control practices, including hand washing, barrier precautions, and environmental disinfection (Spickler 2011). MRSA is not particularly hardy and can be inactivated by sodium hypochlorite, alcohols, and quaternary ammonium compounds (Spickler 2011). The emerging livestock-associated MRSA urgently requires more research to determine the risk factors and transmission routes (Vanderhaeghen et al. 2010).

3.11 Q Fever

Q fever is a highly contagious zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular bacterium. Livestock are the major source of infection in humans worldwide. Q fever can infect a wide range of hosts, including pets, wildlife, birds, reptiles, and ticks. Because illness can be mild and go undetected, Q fever is under-diagnosed and under-reported globally, and the true burden of disease is unknown. However, a large outbreak with approximately 4000 human cases occurred in the Netherlands during 2007–2010. Dairy goat farms near densely populated areas were the source of the outbreak, which was spread via a windborne route (Schimmer et al. 2009).

Animals that carry this organism usually do not show any signs of disease, but abortions and stillbirths can occur with great quantities of bacteria shed. Both symptomatic and asymptomatic animals shed *C. burnetii* in large quantities at parturition. The bacteria can also be shed in feces, urine, and milk. The organisms persist in the environment for long periods, are highly resistant to disinfectants, and can be spread long distances by the wind (Spickler 2007).

Human infection usually occurs from the inhalation of bacteria from air that is contaminated by the feces of infected animals. Q fever is also rarely transmitted to humans by tick bites and ingestion of unpasteurized milk or milk products (CDC 2013b). Most often, sporadic cases occur in occupationally exposed people, such as

biomedical research facility workers, farmers, ranch-hands, veterinarians, and slaughterhouse workers (CDC 2013b). These cases tend to result from exposure to parturient ruminants; however, cats, dogs, rabbits, and other species have also been implicated. Although Q fever is usually asymptomatic or mild, a small percentage of people develop serious disease. Pneumonia or hepatitis may occur in acute cases, and chronic infections can result in endocarditis or a wide variety of other diseases (Spickler 2007).

In humans, Q fever is usually diagnosed by serology or PCR. Diagnosis of Q fever in aborting animals involves testing of the fetuses and placentas. Veterinary diagnosticians typically identify the organism using special stains applied to microscopic sections of these tissues and/or PCR.

Q fever can be prevented in humans by limiting exposure to livestock during birthing, personal hygiene measures and wearing personal protective equipment, and only eating and drinking pasteurized milk and milk products. In animals, prevention of Q fever is based on herd management and prevention of contact with wildlife and tick vectors. Isolating infected pregnant animals and disposing of reproductive tissues can decrease transmission (Spickler 2007). Prevention in humans and animals can be difficult because Q fever can be transmitted on fomites or in aerosols over great distances. Effective vaccines are available in some countries for both humans and animals.

3.12 Rift Valley Fever

Rift valley fever (RVF) is a zoonotic disease that primarily affects ruminants (cattle, sheep, goats, and camels) and can also infect humans. The disease can be severe in both humans and animals and may cause severe economic losses because of livestock death and abortion. Infection with RVF is caused by a virus in the family Bunyaviridae and is primarily transmitted by mosquitoes. RVF has recently received more attention as a potential agricultural and zoonotic disease threat in Europe and North America due to the increasing numbers of competent vector species in those regions (Salman 2013).

RVF is endemic in much of Africa, with occasional spread to countries in the Arabian Peninsula. Epidemics occur sporadically when climate conditions support breeding of mosquitoes. Rift Valley Fever virus (RVFV) was first isolated from lambs in the Rift Valley of Kenya in the 1930s. Major outbreaks have been recorded in many parts of Africa since that time, and the virus was first detected outside of the African continent in Saudi Arabia and Yemen in 2000. The first report of RVF outside of Africa was attributed to the importation of cattle and small ruminants from the Horn of Africa (Pepin et al. 2010).

Transmission of infection in cattle is mainly via the bites of infected mosquitoes. As an epidemic progress, direct contact transmission by infectious animals or contaminated tissues, including aborted fetuses may occur. Transmission via infected mosquitoes is important for disseminating RVFV between herds over

short distances and over long distances through the movement of infected animals or translocation of infected mosquitoes (Abdo-Salem et al. 2011).

Disease, especially in young animals, may be severe and includes fever, depression, and anorexia. The classic clinical sign of RVF in a herd of cattle is a large number of nearly simultaneous abortions among pregnant animals, regardless of the stage of pregnancy. This abortion storm differentiates RVF from other common infectious causes of abortion in cattle, such as Q fever, chlamydiosis, brucellosis, salmonellosis, listeriosis, or toxoplasmosis. RVF may also cause sudden death in cattle. Aborted fetal materials and placental membranes contain large numbers of virus particles, which can either contaminate the local environment directly or infect animals or humans in close contact. The RVFV may persist for relatively long periods in the environment.

Direct contact and aerosol exposure to infected tissues or bodily fluids constitute the main routes of infection for humans. Certain groups are at increased risk due to occupation, such as herders, farmers, slaughterhouse workers, and veterinarians. There is evidence for virus shedding into milk, so that consumption of unpasteurized milk has major consequences for disease transmission and public health. Most human infections are inapparent or demonstrate mild flu-like symptoms (fever, headache, and myalgia). The infection progresses with severe complications in some cases, including hemorrhagic fever, encephalitis, and acute hepatitis.

RVFV can be diagnosed using several methods, including virus isolation from blood and other tissues and by using serological tests such as ELISA.

There is presently no vaccine licensed for human use, although inactivated vaccines have been in development. Both live attenuated virus vaccines and inactivated virus vaccines are available for use in livestock. The live vaccine produces better immunity and requires only one dose but may induce abortions and congenital defects in pregnant animals. Inactivated vaccines require multiple doses to provide protection making their use problematic in endemic areas.

In endemic areas, sustained animal vaccination programs can help to prevent outbreaks.

To slow the expansion of RVF, livestock movement restrictions may prevent the disease from entering new areas. Outbreaks of RVF in animals precede outbreaks in humans, so sustained surveillance and monitoring systems in animals can act as an early warning system to public health authorities. Raising human awareness of protective measures for mosquito bites and safe handling practices during slaughter, appropriate barrier precautions, and proper pasteurization of milk to prevent spread from animals may prevent human infection. Vector control, RVF forecasting, and climatic models to predict when climate conditions are favorable for RVF outbreaks can also help guide prevention efforts.

3.13 *Salmonella*

Salmonella is a major cause of foodborne disease globally. The global burden of zoonotic disease from *Salmonella* is high. An estimated 93.8 million illnesses and 155,000 deaths result each year from nontyphoidal *Salmonella,* the vast majority of

which are foodborne (Majowicz et al. 2010). Over 100,000 human cases are reported each year in the EU alone, with an estimated overall economic burden as high as EUR 3 billion a year (EFSA 2013). *Salmonella* strains resistant to a range of antimicrobials have emerged since the 1990s and are now a serious public health concern (WHO 2013b). Salmonellosis has a worldwide distribution, but serovars vary geographically. *Salmonella* is most prevalent where livestock are farmed intensively (Spickler 2005a).

Salmonella bacteria are classified into over 2500 different serovars based on surface proteins. *Salmonella* is shed in the feces of various infected animals, including cattle, which are infected by ingestion of contaminated feed, water, or grass. The bacteria are hardy and can survive for months to years in the environment (Spickler 2005a).

Transmission is generally through the fecal-oral route and humans most commonly contract salmonellosis through consumption of contaminated food, including meat, eggs, poultry, and unpasteurized milk products. Less often, *Salmonella* is transmitted through green vegetables contaminated by manure. Person-to-person transmission through the fecal-oral route can also occur. Human cases may also occur through contact with infected livestock, which often do not show signs of disease. Most cases of salmonellosis in humans are mild but can result in severe disease and death depending on host facts and the strain of *Salmonella*. Humans may develop diarrhea, abdominal cramping, and fever, which can be very severe.

Salmonella is often carried asymptomatically in cattle, but young, stressed, or pregnant animals are the most susceptible to infection, which may result in enteritis and septicemia (Spickler 2005a). *Salmonella* infection is diagnosed by isolating the organism from feces. In cases of disseminated disease, bacteria can be isolated from the blood.

To reduce the risk of foodborne transmission, basic food hygiene practices and adequate cooking should be used. To prevent transmission from animals to humans, hand hygiene after touching or working with animals is critical. To reduce the risk of *Salmonella* in cattle, herd management strategies and proactive biosecurity, rodent control, and *Salmonella*-free feed and water sources should be utilized. Fecal contamination of water supplies and feed should be prevented. Vaccines are available in some countries for some serovars and can reduce colonization, shedding, and clinical disease (Spickler 2005a).

3.14 Summary

Zoonotic diseases originating from cattle can cause mild or asymptomatic human infection or severe disease and death. A number of zoonotic diseases were not covered in this chapter but might be considered to depending on geographic location and local circumstances, for example, rabies, ringworm, and Human African Trypanosomiasis. While some diseases are rare, the potential for serious outcomes makes it critical for veterinarians and public health practitioners to provide outreach to those individuals at greatest risk, including farmers – small scale and large.

3.15 Cross-References

- ▶ A Review of Hendra Virus and Nipah Virus Infections in Man and Other Animals
- ▶ Bacterial Pathogens Associated with Aquaculture Products
- ▶ *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control
- ▶ Cysticercosis
- ▶ Enteropathogenic *Yersinia* spp.
- ▶ Glanders and Melioidosis: A Zoonosis and a Sapronosis
- ▶ Important Zoonoses in Animals: Parapoxviruses (PPV)
- ▶ Q Fever (*Coxiella burnetii*)
- ▶ The Zoonotic Agent Salmonella
- ▶ Zoonoses Transmitted by Poultry

References

Abdo-Salem S, Waret-Szkuta A, Roger F (2011) Risk assessment of the introduction of Rift Valley fever from the Horn of Africa to Yemen via legal trade of small ruminants. Trop Anim Health Prod 43:471–480

Atkins PJ, Robinson PA (2013) Coalition culls and zoonotic ontologies. Environ Plan 45(6): 1372–1386

Behr MA (2013) Evolution of mycobacterium tuberculosis. In: Divangahi (ed) The new paradigm of immunity to tuberculosis (advances in experimental medicine and biology), vol 783. Springer, New York, pp 81–91

Blasco JM, Molina-Flores B (2011) Control and eradication of brucellosis. Vet Clin North Am Food Anim Pract 27:95–104

Bovine TB Advisory Group – Bovine tuberculosis in England: towards eradication. Final report of the Bovine TB Advisory Group (2009). http://www.bovinetb.info/docs/bovine-tuberculosis-in-england-towards-eradication.pdf. Accessed 14 Aug 2013

Callaway T (2010) Pre-harvest control of *E. coli* O157:H7. Pre-harvest management controls and intervention options for reducing *Escherichia coli* O157:H7 shedding in cattle. White paper of the Cattlemen's Beef Board and National Cattlemen's Beef Association. http://www.beefresearch.org/CMDocs/BeefResearch/Safety_Fact_Sheets/Pre_Harvest_Control_of%20E_coli.pdf. Accessed 11 Sept 2013

Centers for Disease Control and Prevention (CDC) (2013a) Parasites – *Cryptosporidium*. http://www.cdc.gov/parasites/crypto/. Accessed 14 Aug 2013

Centers for Disease Control and Prevention (CDC) (2013b) Diagnosis and management of Q fever – United States, 2013 recommendations from CDC and the Q Fever Working Group. MMWR 62(3):1–29

Corbel M (2006) Brucellosis in humans and animals. World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health, Geneva. http://www.who.int/csr/resources/publications/Brucellosis.pdf. Accessed 14 Aug 2013

Corso PS, Kramer MH, Blair KA, Addiss DG, Davis JP, Haddix AC (2003) Cost of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. Emerg Infect Dis [serial online]. http://wwwnc.cdc.gov/eid/article/9/4/02-0417.htm. Accessed 14 Aug 2013

de Boer E et al (2009) Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int J Food Microbiol 134(1–2):52–56. https://doi.org/10.1016/j.ijfoodmicro.2008.12.007

Decker J (2003) Deadly diseases and epidemics, anthrax. Chelesa House Publishers, Pennsylvania, pp 27–28. ISBN 0-7910-7302-5

Delgado CL, Rosegrant MW et al (1999) The coming livestock revolution, background paper no. 6. Food and Agriculture Organization of the United Nations. http://www.un.org/esa/sustdev/csd/ecn172000-bp6.pdf. Accessed 22 Aug 2013

Devriese LA, Van Damme LR, Fameree L (1972) Methicillin- (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. Zentralbl Veterinarmed Reihe 19:598–605

Dragon DC, Elkin BT, Nishi JS et al (1999) A review of anthrax in Canada and implications for research on the disease in northern bison. J Appl Microbiol 87(2):208–213. https://doi.org/10.1046/j.1365-2672.1999.00872.x. PMID 10475950

Ellis-Iversen J, Cook AJC, Watson E, Nielen M, Larkin L, Wooldridge M, Hogeveen H (2010) Perceptions, circumstances and motivators that influence implementation of zoonotic control programs on cattle farm. Prev Vet Med 93(4):276–285

European Food Safety Authority (EFSA) (2013). http://www.efsa.europa.eu/en/topics/topic/salmonella.htm. Accessed 14 Aug 2013

George LW (2002) Listeriosis. In: Smith, Bradford P (eds) Large animal internal medicine, 3rd edn. Mosby, Philadelphia, pp 946–949

Gibbs SG et al (2006) Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. Environ Health Perspect 114: 1032–1037

Gideon Informatics (2013). http://www.gideononline.com. Accessed 14 Aug 2013

Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, Heederik D (2010) Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One 5(6):e10990

Hartskeerl RA, Collares-Pereira M, Ellis WA (2011) Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin Microbiol Infect 17:494–501

Jones PG, Thornton PK (2009) Croppers to livestock keepers: livelihood transitions to 2050 in Africa due to climate change. Environ Sci Pol 12:427–437

Jones T, Kellum M, Porter S, Bell M, Schaffner W (2002) An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis. 2002 8:82–84

Kotloff K, Nataro J, Blackwelder W et al (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet, Early Online Publication, 14 May 2013. https://doi.org/10.1016/S0140-6736(13)60844-2

Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP (1994) A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N Engl J Med 331:161–167

Majowicz S, Musto J, Scallan E et al (2010) The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50:882–889

McDaniel CJ, Cardwell DM, Moeller RB Jr, Gray GC (2014) Humans and cattle: a review of bovine zoonoses. Vector Borne Zoonotic Dis 14(1):1–19. https://doi.org/10.1089/vbz.2012.1164

Miller RS, Sweeney SJ (2013) Mycobacterium bovis (bovine tuberculosis) infection in North American wildlife: current status and opportunities for mitigation of risks of further infection in wildlife populations. Epidemiol Infect 141(7):1357–1370

Mooij R et al (2007) MRSA in calves [English abstract]. Infectieziekten Bulletin 18:234–236

Moreno E, Cloeckaert A, Moriyon I (2002) *Brucella* evolution and taxonomy. Vet Microbiol 90: 209–227

Mosier DA, Oberst RD (2000) Cryptosporidiosis: a global challenge. Ann N Y Acad Sci 916: 102–111

Normanno G et al (2007) Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. Int J Food Microbiol 117:219–222

Palomares Velosa JE, Salman MD, Roman-Muniz IN, Reynolds S, Linke L, Magnuson R, McConnel CS, Rao S (2020) Socio-ecological factors of zoonotic diseases exposure in Colorado dairy workers. J Agromedicine:1–11. https://doi.org/10.1080/1059924X.2020.1725700

Pepin M, Bouloy M, Bird B et al (2010) Rift Valley fever virus (*Bunyaviridae: Phlebovirus*): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet Res 201(41):61

Rebhun WC (1995) Listeriosis. In: Diseases of dairy cattle, vol 121. Lippincott Williams Wilkins, Philadelphia, pp 410–413

Salman M (2013) Is the United State really at risk for introduction of Rift Valley fever virus? J Am Vet Med Assoc. Mar 1 242(5):606–608

Salman M, Silano V, Heim D, Kreysa J (2012) Geographical BSE risk assessment and its impact on disease detection and dissemination. Prev Vet Med 105(4):255–264

Schimmer B, Dijkstra F, Velllema P et al (2009) Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. Euro Surveill 14:3

Spickler AR (2003) Taenia infections. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php. Accessed 10 Aug 2013

Spickler AR (2005a) Cryptosporidiosis. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php. Accessed 14 Aug 2013

Spickler AR (2005b) Leptospirosis. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php. Accessed 14 Aug 2013

Spickler AR (2005c) Salmonellosis. At http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php

Spickler AR (2007) Q fever. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php. Accessed 10 Aug 2013

Spickler AR (2009) Enterohemorrhagic *Escherichia coli* infections. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php. Accessed 14 Aug 2013

Spickler AR (2011) Methicillin resistant *Staphylococcus aureus*. http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php. Accessed 14 Aug 2013

Todar K (2003) *Listeria monocytogenes* and Listeriosis. Todar's online textbook of bacteriology. University of Wisconsin-Madison, Department of Bacteriology. http://textbookofbacteriology.net/Listeria.html

USDA-APHIS (2003) Escherichia coli O157 on U.S. Dairy Operations.http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy02/Dairy02_is_Ecoli.pdf Accessed 14 Aug 2013

USDA-APHIS (2006) An estimate of the prevalence of BSE in the United States, July 20, 2006. http://www.aphis.usda.gov/animal_health/animal_diseases/bse/downloads/bseprev-est_final_7-20-06.pdf. Accessed 19 Aug 2013

USDA-APHIS (2007). http://www.aphis.usda.gov/peer_review/downloads/risk_assessment_%20final9-2007.pdf. Attachment 1 from Revised assessment of Bovine Spongiform Encephalopathy (BSE) Risks associated with the importation of certain commodities from BSE minimal risk regions (Canada). Accessed 14 Aug 2013

van Loo IHM et al (2007) Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. Emerg Infect Dis 13:1753–1755

Vanderhaeghen W, Hermans K, Haesebrouck F et al (2010) Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. Epidemiol Infect 138:606–625

Voss A et al (2005) Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965–1966

World Health Organization (2005) WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniosis/cysticercosis. Editor: KD Murrell. www.oie.int/doc/ged/d11245.pdf. Accessed 30 Apr 2013

World Health Organization (WHO) (2011a) Enterohaemorrhagic *Escherichia coli* (EHEC). http://www.who.int/mediacentre/factsheets/fs125/en/. Accessed 14 Aug 2013

World Health Organization (WHO) (2011b) Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group. http://libdoc.who.int/publications/2011/9789241501521_eng.pdf. Accessed 09 Aug 2013

World Health Organization (WHO) (2013a) Zoonoses: Anthrax. http://www.who.int/vaccine_research/diseases/zoonotic/en/index1.html. Accessed 19 Aug 2013

World Health Organization (WHO) (2013b) *Salmonella* (non-typhoidal) fact sheet N°139 Updated August 2013. http://www.who.int/mediacentre/factsheets/fs139/en/#. Accessed 14 Aug 2013

Zoonotic Diseases of Swine: Food-Borne and Occupational Aspects of Infection

4

Dipendra Thapaliya, Blake M. Hanson, Ashley Kates, Cassandra A. Klostermann, Rajeshwari Nair, Shylo E. Wardyn, and Tara C. Smith

Contents

D. Thapaliya · A. Kates · C. A. Klostermann · R. Nair · S. E. Wardyn
Department of Epidemiology, University of Iowa, Lowa, IA, USA

B. M. Hanson
Department of Epidemiology, Human Genetics and Environmental Sciences, University of Texas Health Sciences Center, Houston, TX, USA

T. C. Smith (✉)
Department of Epidemiology, University of Iowa, Lowa, IA, USA

Kent State University College of Public Health, Kent, OH, USA
e-mail: tsmit176@kent.edu

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_2

Abstract

Swine and their products have become a central part of food systems around the world. Global pork production has rapidly increased over the past 30 years, leading to the intensification of the swine industry: though there are fewer farms now, those farms that do persist raise ever-larger numbers of animals. This increases the transmission of pathogens both among animal herds, and between animals and their human caretakers. Furthermore, increased stress to animals and the potential for amplification of pathogens in the farming environment can lead to a higher burden of disease-causing organisms in and on meat products, which then make their way to consumers worldwide. As such, swine and their meat products have the potential to introduce new zoonotic diseases into populations via multiple routes of transmission. Here we discuss several examples of zoonotic diseases of swine origin, reviewing diseases with bacterial, viral, or parasitic causes.

Keywords

Swine · Zoonoses · Bacteria · Viruses · Parasites · Epidemiology · Microbiology · Food-borne pathogens

4.1 Background and Introduction

Pork is rapidly becoming the world's source of protein, accounting for approximately 35% of all meat production (FAO 2017). Global pork production increased more than 80% between 1985 and 2010 (Fournie et al. 2012), and this trend has led to the intensification of swine husbandry, with fewer and fewer facilities present, but each raising larger numbers of individual animals. China has been a driver of this market, accounting for approximately 50% of total global pig production (Fournie et al. 2012). As swine production has intensified, so has concern over how these modifications in husbandry may affect the transmission of disease among pigs as well as to human caretakers. It has been estimated that more than 60 % of emerging diseases are zoonotic (Jones et al. 2008). A review (Fournie et al. 2012) identified 77 pathogens that had not been described in swine prior to 1985, including 39 viruses and 32 bacterial species. Not surprisingly, the top 20% of pork-producing countries accounted for 82% of these emerging pathogens. Of these 77 novel species found to infect swine, 30 (39%) are zoonotic, and 26% of these were identified in the context of an outbreak investigation (Fournie et al. 2012). Densely populated South East Asia is the epicenter of emergence of novel zoonotic diseases due to inter-species transmission. However, outbreaks of host specific lethal zoonoses have occurred in industrialized nations as well (Davies 2012). It is plausible that a dramatic change in swine industry demographics in recent decades without adequate biosecurity may have served as a tonic for the emergence of swine zoonosis (Davies 2012). Zoonotic diseases impose significant economic burden with increased morbidity and mortality

globally. A change in ecological niche, climatic change, rapid growth in human population, and socio-economic factors are among the major contributing factors for the emergence of zoonoses (Jones et al. 2008).

Outbreaks of human disease related to swine-origin pathogens, including *Streptococcus suis* in China (Lun et al. 2007), Nipah virus in Malaysia (Chua 2012), and the novel H1N1 variant influenza virus have gained significant media attention in the past decades; along with Hepatitis E virus, these also have shown an increased interest in the scientific literature (VanderWaal and Deen 2018). Here we discuss several examples of zoonotic diseases of swine origin, reviewing diseases with bacterial, viral, or parasitic causes.

4.1.1 Yersinia enterocolitica

Yersinia enterocolitica is a Gram negative bacterium in the family *Enterobacteriaceae. Y. enterocolitica* is widely distributed throughout nature, having many animal and aquatic reservoirs; however, swine are considered the main reservoir for strains that are pathogenic to humans. It is the main causative agent of yersiniosis, a disease that affects animals and humans worldwide (Holt et al. 2000).

Yersinia enterocolitica can be classified into distinct subgroups based on biochemical characteristics (biotypes) and O-antigen specificity (serotypes). There are six biotypes (1A, 1B, 2, 3, 4, and 5) and 60 serotypes, 11 of which are associated with human illness (Nesbakken et al. 2006; Bottone 1997). Biotype 1B is considered the only highly pathogenic strain, while the others are considered moderately pathogenic, except for biotype 1A, which is considered nonpathogenic although this has recently become a contentious topic due to recent reports of 1A infections (Stephan et al. 2013). Biotype 1B is mainly found in North America and Japan and is different from other biotypes in that it can be found in water and other environmental sources, and can also be carried by swine and rodents. Biotypes 2 and 4 are associated with human infections in Europe; their main reservoirs are pigs and cattle. Biotypes 3 and 5 are uncommon, but are also associated with animal reservoirs (EFSA 2009; Fredriksson-Ahomaa et al. 2006a).

Yersiniosis is a gastrointestinal disease, causing fever and watery, occasionally bloody, diarrhea. Rarely, *Y. enterocolitica* can cause septicemia, and in some cases, long-term sequelae can occur. Symptoms generally occur 4–7 days after exposure and may last for up to a month (Bottone 1997; Huovinen et al. 2010), but can be non-specific likely leading to under- or misdiagnosis (Chlebicz and Slizewska 2018). Approximately 16.5 cases per 1000,000 persons occur each year in Europe (EFSA 2009), while in the United States, approximately 3.5 cases per 1000,000 are seen each year (Long et al. 2010). In many developing countries, no sufficient diagnostics are available so less is known about the number of cases in many areas (Carniel and Hinnebusch 2012). Children are infected more frequently than adults, and infections occur most commonly in temperate locations during colder months (Bottone 1997).

Pigs are commonly asymptomatic carriers of pathogenic strains of *Y. enterocolitica*. The bacteria typically reside in the gastrointestinal tract, especially the tonsils, lymph

nodes, intestines, and feces (Fredriksson-Ahomaa et al. 2007; Bhaduri et al. 2005). Cattle and goats have also been found to be carriers (Lanada et al. 2005a, b), and milk products from these animals have been the source of numerous outbreaks in human populations (Black et al. 1978; Shayegani et al. 1983; Morse et al. 1984; Tacket et al. 1984; Ackers et al. 2000). Deer, rabbits, rodents (Quan et al. 1974), dogs (Byun et al. 2011), and cats (Fredriksson-Ahomaa et al. 2001) have also been found to carry as well as to be infected with *Y. enterocolitica*. In addition to livestock, water sources including wells, rivers, and lakes can serve as reservoirs for the bacteria as a result of contamination by feces of carriers or leakage from latrines.

The major risk factors for developing yersiniosis include eating raw or undercooked pork (Boqvist et al. 2009; Fredriksson-Ahomaa et al. 2006b), drinking contaminated milk (Black et al. 1978; Tacket et al. 1984; Ackers et al. 2000), and consuming contaminated drinking water (Thompson and Gravel 1986; Christensen 1979). Porcine sources are frequently associated with the pathogenic serotypes O:3, O:9, and O:5, 27 and sometimes with the highly virulent serotype O:8. Outbreaks in 2006 in Norway were identified as biotype 2 and 4 and indicated a processed pork product to be the likely source (Grahek-Ogden et al. 2007; Stenstad et al. 2007). In the United States, raw pork intestines were found to be the source of an outbreak among infants (Lee et al. 1990; Jones 2003). The occurrence of pathogenic *Y. enterocolitica* in pigs and pork has been established by PCR in several studies (Korte et al. 2003; Fredriksson-Ahomaa et al. 2003). The *ail* gene located within the genome of pathogenic *Y. enterocolitica* strains is the most frequently used target of amplification for positive identification. In Switzerland, the prevalence of *ail*-positive *Y. enterocolitica* in tonsils of slaughter pigs was shown to be 88% by PCR and 34% by culture methods (Fredriksson-Ahomaa et al. 2007). In the USA, *ail*-positive *Y. enterocolitica* were detected in 12% of pig feces sampled by PCR, and in 4% of them using culture methods. Similarly, 40% of the pig lymph nodes were positive by PCR, but none by culturing (Boyapalle et al. 2001). These results indicate that PCR based assays are the most sensitive and accurate means to detect *Y. enterocolitica* colonization.

Clinical presentations of yersiniosis are typical of enteric illness. Infants and children often present with fever, vomiting, and bloody diarrhea that can last from 3–28 days (Metchock et al. 1991; Lee et al. 1991). Adults generally have 1 to 2 weeks of fever, diarrhea, and abdominal pain that can mimic appendicitis. In more severe cases of gastroenteritis, necrotizing enterocolitis, and ulceration may occur. *Y. enterocolitica* can also cause septicemia, leading to abscesses in the liver and spleen, pneumonia, septic arthritis, meningitis, cellulitis, empyema, osteomyelitis, and may evolve into endocarditis. Post-infection sequelae may also occur, particularly after infections with biotype 4, serotype O:3 (Bottone 1999). Reactive arthritis and erythema nodosum are the most common sequelae, but glomerulonephritis and myocarditis have also been reported (Bottone 1997).

Yersiniosis is diagnosed by positive identification of *Y. enterocolitica* in stool samples, although it is not routinely tested for. It can also be recovered from the throat, lymph nodes, joint fluid, urine, bile, or blood. Most cases resolve on their own, although it may take up to 3 weeks to recover. In severe cases, antibiotics such as aminoglycosides, doxycycline, trimethoprim-sulfamethoxazole, or fluoroquinolones

may be prescribed. Prevention is key in avoiding infection. Raw or undercooked pork and unpasteurized milk or milk products should be avoided, as should drinking untreated water. Good hand hygiene when preparing food and after contact with animals should also be practiced to avoid infection.

4.1.2 Staphylococcus aureus

Staphylococcus aureus is a nonmotile, nonspore-forming, Gram positive coccus that occurs singly, in pairs, or in clusters. *S. aureus* produces protein A (*spa*), which is used in molecular testing for strain typing purposes, as well as several other toxins and superantigens (De Vos et al. 2009).

S. aureus is often isolated from the nasal membranes and skin of warm-blooded animals. Approximately 20–30% of the human population is colonized with *S. aureus* in the nose, throat, or both (Smith et al. 2012; Gorwitz et al. 2008; Graham 3rd et al. 2006). The most important site for colonization are the anterior nares (Wertheim et al. 2005). Colonization itself is not harmful; however, it is a risk factor for developing subsequent infections (Graham 3rd et al. 2006; Fritz et al. 2009). Both asymptomatic carriers and infected individuals may transmit the bacterium to others through close contact. *S. aureus* may also be acquired via contact with fomites contaminated with the organism, as well as with animals that are colonized or infected with *S. aureus.*

Skin infections including furuncles, carbuncles, impetigo, and scalded skin syndrome, as well as more severe infections like pneumonia, osteomyelitis, endocarditis, myocarditis, pericarditis, enterocolitis, mastitis, cystitis, prostatitis, cervicitis, cerebritis, meningitis, bacteremia, toxic shock syndrome, and abscesses of muscles, skin, and organs can occur as a result of *S. aureus* infection. Other mammals and birds are also susceptible to infections, including mastitis, synovitis, arthritis, endometritis, furuncles, suppurative dermatitis, pyemia, and septicemia (De Vos et al. 2009). Pigs are common carriers of *S. aureus*; one study in the USA found overall MRSA prevalence was 70% (147/209) from 7 farms in the Midwest (Smith et al. 2009). In the Netherlands, surveillance for MRSA on hog farms has shown that isolates obtained from swine and their human caretakers are frequently indistinguishable, suggesting that the organism is transmitted between the two species (Smith et al. 2009; Huijsdens et al. 2006; Khanna et al. 2007).

S. aureus infections are often resistant to many antibiotics. Approximately 1.5% of the US population carries methicillin-resistant *S. aureus* (MRSA) (Gorwitz et al. 2008). Resistance to methicillin developed within 6 months of the first clinical use and has become a major cause of morbidity and mortality around the world. In the USA in 2011, there were 80,461 invasive MRSA infections, an incidence rate of 25.82 cases per 100,000 persons. In 2017, looking at *Staphyloccocus aureus* more broadly, it was estimated that this organism caused almost 120,000 bloodstream infections and close to 20,000 deaths (Kourtis et al. 2019). Further, many animals, including cows, goats, sheep, rabbits, and poultry, can be infected by *S. aureus,* and these infections can have large economic costs (Fitzgerald 2012).

The epidemiology of MRSA has changed rapidly in the past few decades. After developing resistance in the 1960s following methicillin introduction, MRSA became a superbug that primarily affected hospitalized patients. Due to association with the healthcare environment, these infections were called healthcare-associated MRSA (HA-MRSA). More recently, cases of MRSA infection have been detected in people without prior hospitalization and with no underlying illnesses or healthcare related risk factors; these are referred to as community-associated MRSA (CA-MRSA) infections. Cases of HA-MRSA are usually resistant to several classes of antibiotics and tend to carry the methicillin-resistance gene, *mecA*, on the Staphylococcal Chromosome Cassette (SCC) of type II (SCC*mec* type II). They are often associated with *spa* type t002 and multi-locus sequence type (MLST) ST5. Contrastingly, CA-MRSA infections tend to be resistant to fewer classes of antibiotics, carry the Panton-Valentine leukocidin (PVL) encoding gene, and carry SCC*mec* type IV, *spa* type t008, and MLST ST8. A third group of infections, livestock-associated MRSA (LA-MRSA), has recently been identified (Wulf and Voss 2008) and has typically been associated with swine or cattle. LA-MRSA include strains such as ST398 and ST9, often carry SCC*mec* type V, are typically PVL negative, and (like HA-MRSA) tend to be resistant to multiple classes of antibiotics. However, both CA-MRSA and LA-MRSA have caused nosocomial infections in hospitals (Jenkins et al. 2009; Fanoy et al. 2009; van Rijen et al. 2008, 2009; Wulf et al. 2008; Kourbatova et al. 2005; Seybold et al. 2006; Tattevin et al. 2009).

Livestock-associated MRSA first came to attention in 2005 after its identification in pigs in France (Armand-Lefevre et al. 2005) and in swine farmers in the Netherlands (Wulf and Voss 2008). Dutch researchers found that swine farmers were colonized with MRSA at a rate of 760 times higher than that of the general population (Voss et al. 2005). Since then, LA-MRSA has been found in a number of countries in Europe, Asia, and the Americas (Smith and Pearson 2011; Graveland et al. 2011; Fluit 2012).

Recent reports from Germany and the Netherlands have found a high proportion of ST398 carriage in areas that have a high density of livestock (Kock et al. 2009; Kock et al. 2011; Wulf et al. 2012). While originally thought not to cause severe infections, there have been increasing reports of invasive disease caused by ST398 (Hartmeyer et al. 2010; Mammina et al. 2010; Potel et al. 2010; Aspiroz et al. 2010). Methicillin-sensitive *S. aureus* (MSSA) ST398 isolates have also caused invasive disease in the eastern USA (Mediavilla et al. 2012), Europe (Witte et al. 2007; Declercq et al. 2008; van Belkum et al. 2008), South America (Jimenez et al. 2011), and Canada (Golding et al. 2010), and at least one death in France (Laurent 2009) (reviewed in (Smith and Wardyn 2015).

While the majority of individuals colonized or infected with LA-MRSA have had contact with swine, colonization with ST398 has also occurred in individuals lacking any identified contact with a livestock reservoir (Bhat et al. 2009; Aires-de-Sousa et al. 2006). Genomic analyses of ST398 (Price et al. 2012) and ST9 (Yu et al. 2021) suggest a similar pattern of evolution, from a human origin isolate to animal-adapted strains that have become increasingly antibiotic-resistant.

It has been suggested that one mode of transmission into the community is via contaminated food. Numerous studies in the USA have found MRSA in 5% of 120 meat samples (Pu et al. 2008), MSSA in 16.4% and MRSA in 1.2% of 125 meat samples (Hanson et al. 2011), MSSA in 64.8% and MRSA in 6.6% of 256 pork samples (O'Brien et al. 2012), and multi-drug resistant *S. aureus* in 52% of 136 meat and poultry samples (Waters et al. 2011). Additionally, two studies in the Netherlands found rates of 2.5% of 79 pork and beef samples (van Loo et al. 2007) and 11.9% of 2217 meat and poultry samples, respectively (de Boer et al. 2009). However, to date there have not been any confirmed infections with ST398 caused by contaminated food.

Most MRSA skin infections appear as pustules or boils which often are red, swollen, painful, and have pus or other drainage. They often are mistaken for spider or insect bites. These skin infections commonly occur at sites of visible skin trauma, such as cuts and abrasions, and areas of the body covered by hair. Health professionals may provide antibiotics and drainage if necessary to treat such infections. More severe infections may require hospitalization and intravenous antibiotic therapy. Good hygiene is the key to prevention of MRSA infections.

4.1.3 Salmonella

Salmonella is a genus of Gram-negative, rod shaped, non-spore forming enterobacteria with peritrichous flagella. Originally, classified utilizing serotyping of the somatic lipopolysaccharide (O) and flagellar protein (H) antigens, each serological variant (serovar), was considered its own species under the *Salmonella* genus (White 1926; Kauffmann 1978) as reviewed in (Beltran et al. 1988). This methodology led to misclassifications due to horizontal transfer of cell surface antigens, leading to classification of genetically distinct strains within the same serovar (Beltran et al. 1988; Selander et al. 1990).

In 2005, the Judicial Commission of the International Committee for Systematics and Prokaryotes (JICSP) decided to change the type species of the *Salmonella* genus to *enterica* with subspecies and serovars. (Prokaryotes JCotICoSo 2005). The JICSP indicated *Salmonella enterica* had seven subspecies, *enterica* (type I), *salamae* (type II), *arizonae* (type IIIa), *diarizonae* (type IVb), *bongori* (type V), *houtenae* (type IV), and *indica* (type VI). Subspecies *bongori* was shortly after promoted to species status (Grimont and Weill 2007). *S. bongori* and all subspecies of *S. enterica* besides *S. enterica* subsp. *enterica* are associated mainly with cold-blooded animals (Aleksic et al. 1996; Woodward et al. 1997), but can rarely cause human infection (CDC 2008, 2012a). The primary cause of human infection is *S. enterica* subsp. *enterica* (CDC 2008), as referenced in (Desai et al. 2013).

The CDC defines salmonellosis as an infection with a *Salmonella* spp. bacterium. These infections can often manifest with diarrhea (potentially bloody), fever, and abdominal cramps between 12 and 72 h post infection (CDC 2009). The illness often lasts between 4 and 7 days and is usually self-limiting. *Salmonella* infection can

necessitate hospitalization in a small number of individuals (Mead et al. 1999) and is estimated to cause 150,000 deaths per year globally, most commonly in children infected with serotypes Enteritidis or Typhimurium (Whiley and Ross 2015). Each year, *Salmonella* spp. cause roughly 1.3 billion cases of nontyphoidal salmonellosis worldwide (Chimalizeni et al. 2010). Within the United States, there are estimated to be over one million cases per year, with 95% of these estimated to be caused by foodborne exposure to *Salmonella* (Mead et al. 1999; Jiang et al. 2015; Anderson et al. 2016). The burden on the United States economy from these cases was estimated to be between $0.5 billion and $2.3 billion (Frenzen et al. 2002). These estimates are likely underestimates due to the omission of secondary complications due to *Salmonella* infections. The estimates fail to include complications such as reactive arthritis or costs such as pain and suffering, or travel to obtain medical care.

The most important zoonotic reservoir for *Salmonella* are food animals, with the most important food product being eggs (Ebel and Schlosser 2000). Egg consumption has been shown to be the largest risk factor associated with *Salmonella enterica* infection (Hope et al. 2002). Pork contamination is also a possible source of human infection. In swine, *Salmonella* infection is mainly subclinical, with rare cases manifesting as enterocolitis or septicemia (Barker and Van Dreumel 1985), as referenced in (Fosse et al. 2009). In the United States, the percentage of farms positive for *Salmonella* are estimated to range between 38.2% and 83% with the number of positive pigs in the USA from 6% to 24.6% (Oosterom and Notermans 1983; Davies et al. 1997). Transmission from pig to pig is often due to fecal shedding of the bacteria. Within swine herds, sows were observed to have an increase in *Salmonella* shedding at weaning (Nollet et al. 2005) as well as in their weaned piglets (Kranker et al. 2003). While *Salmonella* is considered primarily fecal borne, swine feed has also been shown to be a potential source of *Salmonella* infection for swine (Harris et al. 1997) with experimental data showing animals may become infected through the consumption of contaminated feed (Smith 1960). Additional risk factors for transmission between herds of swine are: contact with humans, contaminated equipment, or contaminated slurry (Langvad et al. 2006).

Individual outbreaks of *Salmonella* spp. have also been attributed to pork products. In 1989, a small northern England town experienced an outbreak where 206 individuals were infected with serovar Typhimurium (Maguire et al. 1993). Serotyping and antibiotic resistance profiles matched the infective strain to that found in cold cuts of pork purchased from a local butcher shop. In a study by Davies et al., several of the most prevalent serotypes found in swine were also among the most common causes of human infection (Davies et al. 1997).

Attempts to control *Salmonella* spp. prevalence on farms have had mixed outcomes. The use of all-in/all-out systems with multiple sites handling different stages of the rearing process have been shown to have no benefit in reducing *Salmonella* prevalence when compared to farrow-to-finish systems (Davies et al. 1997). These all-in/all-out systems may actually have a greater prevalence of *Salmonella* in finishing pigs than farrow-to-finish systems and fecal shedding of *Salmonella* was higher than observed in farrow-to-finish (Davies et al. 1997). Number of pigs per pen

was also observed to be a risk factor for fecal shedding of *Salmonella* (Linton et al. 1970). Acidification or fermentation of feed is postulated to be protective against *Salmonella* contamination as dry feed and trough feeding have been shown to have an increased contamination risk (Lo Fo Wong et al. 2004; van der Wolf et al. 1999, 2001), but this has not been studied extensively using experimental designs.

In North America, *Salmonella* control programs have been implemented at slaughter to decrease human exposure to *Salmonella* (Funk and Gebreyes 2004). This Pathogen Reduction: Hazard Analysis and Critical Control Point (HACCP) system established slaughter point performance standards for processing plants and has been shown to decrease contamination of pork products with *Salmonella* (Agriculture FSaISUDo 2004). In European Union countries, a farm-to-slaughter program has been implemented to reduce *Salmonella* (Lo Fo Wong et al. 2002). This plan calls for control measures at all production levels and focuses specifically on transportation and handling of the swine to limit the transmission between herds. In addition to prevention methods within the production system, consumer prevention is recommended by the CDC (2010). In addition to recommendations dealing with protecting infants from *Salmonella* exposure, the CDC suggests cooking meat and poultry thoroughly, washing hands, utensils, and kitchen surfaces following contact with raw meat or poultry.

4.1.4 Campylobacter

Campylobacter is a genus of Gram-negative, spiral-spiral shaped bacteria that causes disease in both humans and animals (CDC 2010). *Campylobacter* is the most common cause of gastroenteritis in many developed (Nichols et al. 2012) and developing countries, causing more diarrhea than *Salmonella* globally (WHO 2011) and thought to be the most common food-borne bacterial zoonosis globally, causing up to 500 million yearly infections (Kashoma et al. 2015). In developing countries, infections of those under the age of two are most frequent (WHO 2011). While there are 17 species in the *Campylobacter* genus, *C. jejuni* and *C. coli* are the most frequent causes of infection (WHO 2011). Most cases are sporadic events and not part of outbreaks (CDC 2010). The main route of transmission from animals to humans is through undercooked meat and meat products, contaminated milk, or contaminated water (WHO 2011).

Disease in humans usually occurs 2 to 5 days after infection (WHO 2011) and presents with diarrhea, cramping, abdominal pain, and fever. Most infected individuals recover within 5 to 10 days. In some severe cases, a small amount of people may develop Guillian-Barré syndrome. *Campylobacter* is thought to be responsible for between 20% (Tam et al. 2007) to 40% of cases of Guillian-Barré syndrome (CDC 2010). *Campylobacter* infections tend to be higher in males across all age groups, which suggests a higher susceptibility in males and not participation in at-risk behaviors (Nichols et al. 2012; Louis et al. 2005). In recent years, infections in those over 50 years of age have become more common, especially in men, as has

infection in those between 20 and 32 years (Nichols et al. 2012). The increase in infections in those over 50 may be due to use of proton pump inhibitors (PPI's) (Nichols et al. 2012; Leonard et al. 2007). Seasonality of the infection has been noted, with the greatest impact of seasonality being in young children (Nichols et al. 2012). *Campylobacter* infection rates begin to rise in May and peak between mid-June and mid-July (Nichols et al. 2012; Louis et al. 2005). This seasonality has been observed in many temperate countries (Nylen et al. 2002). Infection rates also tend to be higher in rural compared to urban regions (Strachan et al. 2009; Sibbald and Sharp 1985). This could be reflective of proximity to livestock or differences in access to healthcare (Nichols et al. 2012). Since 1989, there has been a steady increase in the presence of antimicrobial resistant *Campylobacter* isolates. Full and intermediate resistance to ampicillin, ciprofloxacin, nalidixic acid, tetracycline, and erythromycin has been shown (Nichols et al. 2012).

Campylobacter species can be isolated from poultry, cattle, sheep, and goats as well as pigs (Chlebicz and Slizewska 2018); bacteria may be spread to humans via meat products or dairy, especially raw milk products. When swine are infected with *Campylobacter*, it is frequently *C. coli*; however, *C. jejuni* has been seen recently as well (Jensen et al. 2006). *Campylobacter* infections can cause diarrhea in pigs, and often colonizes the intestinal tract. Both *C. jejuni* and *C. coli* have been found in the intestinal tract of pigs and are known to be excreted in their feces (Jensen et al. 2006); studies have suggested that approximately 38–63% of pigs may carry *Campylobacter* in their alimentary tract (Chlebicz and Slizewska 2018). *Campylobacter* has also been identified in the stomach, tonsils, liver, and carcass surfaces of swine. High colonization rates may represent an occupational health hazard, since a low dose of bacteria can cause infection (Nesbakken et al. 2003). Antimicrobial susceptibility to ciprofloxacin and nalidxic acid has been reported in swine strains. It has also been shown that *C. coli* has higher levels of quinolone resistance than *C. jejuni* in swine (von Altrock et al. 2013). However, it is unlikely that swine are a major source of foodborne *Campylobacter*iosis, as *Campylobacter* is rarely detected in retail pork, but may be a source of occupational exposure (Nesbakken et al. 2003). It has also been shown that while there is contamination of pigs in slaughter houses, *Campylobacter* spp. do not spread throughout the operation (von Altrock et al. 2013).

Campylobacter infections do not generally require treatment and are self-limiting (CDC 2010). When disease is severe, electrolyte and fluid replacement may be necessary. Antimicrobials (erythromycin, tetracycline, and quinolones) can be used to treat severe disease or to eliminate carriage (WHO 2011). Several steps can be taken to prevent *Campylobacter* infection. Proper food handling and hand hygiene can help prevent infection. All meats should be thoroughly cooked and measures should be taken to prevent cross contamination. Hands should be washed thoroughly before handling food and persons with diarrhea should wash their hands frequently to reduce the spread of infection (CDC 2010). Improved biosecurity measures and hygienic slaughtering practices will reduce the fecal contamination of carcasses (WHO 2011). Cooling meat with CO_2 has also been shown to kill the bacteria (Nesbakken et al. 2003). Adequate disposal of feces and decontamination of fecal contaminated articles will also help reduce transmission (WHO 2011).

4.1.5 Streptococcus suis

Streptococcus suis (*S. suis*) is a Gram-positive facultative anaerobe bacterium reported to colonize and cause infections primarily in the swine population worldwide (Fulde and Valentin-Weigand 2013; Wertheim et al. 2009). In conjunction with *Actinobacillus suis* and *Haemophilus parasuis*, *S. suis* completes the triad of the "Suis-ide" disease agents given its association with a wide range of severe clinical conditions in the swine population (MacInnes and Desrosiers 1999). *S. suis* causes severe infections in pigs resulting in major economic losses to the porcine industry worldwide (Fittipaldi et al. 2012). Zoonotic infections due to *S. suis* have been reported in countries with a high density of pigs and intensive swine production (Lun et al. 2007; Wertheim et al. 2009). The increasing prevalence of infections due to *S. suis* both in swine and humans over the last few years have urged investigators to better understand the epidemiology and zoonotic potential of this primarily "pig pathogen."

S. suis isolates are verified by serotyping using slide agglutination test, capsular reaction, capillary precipitation or a co-agglutination test (Staats et al. 1997). Serotyping is based on polysaccharide capsular antigen detection . Thirty-five serotypes (1–34 and 1/2) have been identified using these tests (Lun et al. 2007; Higgins and Gottschalk 1990; Gottschalk et al. 1989, 1991a, b, 1999; Higgins et al. 1995). Serotypes 32 and 34 are observed to be closely related to *S. orisratti* (Hill et al. 2005). Serotype 2 is the most frequently reported serotype worldwide and is considered the most pathogenic both in pigs and humans. Other serotypes implicated in diseases are types 1–9 and 14 (Gottschalk et al. 2007). However the presence of "*S. suis*-like strains" which are biochemically similar but genetically distinct have been identified (reviewed in (Hlebowicz et al. 2019)). Therefore, molecular methods including multilocus sequence typing have been increasingly used for typing, with at least 16 sequence types identified to date (Goyette-Desjardins et al. 2014).

Pigs colonized with *S. suis* typically harbor the organism in their tonsils and may never exhibit clinical signs or symptoms (carriers). Some carrier piglets eventually develop bacteremia, septicemia or meningitis due to dissemination of *S. suis* from tonsils and other mucosal surfaces (Fittipaldi et al. 2012; Staats et al. 1997). Disease syndromes in swine also include arthritis, pneumonia, endocarditis, encephalitis, polyserositis, abscesses, and abortion (Wertheim et al. 2009). Death occurs within hours of the onset of clinical signs in pigs with peracute, i.e., very violent or acute forms of infection. Acute disease typically characterized by fever (up to 42 °C), depression, anorexia and lassitude may result in deaths, chronicity, or healthy carriers. In its chronic form, lameness and/or residual central nervous system signs may be apparent (Fulde and Valentin-Weigand 2013). Clinical manifestations of *S. suis* are observed to vary by geographical location (Wangkaew et al. 2006; Yu et al. 2006; Tang et al. 2006). There have been varying reports on the incubation period of *S. suis* ranging from 3 h to 14 days (Yu et al. 2006), and 60 h to 1 week (Mai et al. 2008). Short incubation periods are found to be consistent with direct entry of *S. suis* into the blood stream through skin wounds. There have been no consistent findings in seasonal variation of *S. suis* infection (Wangkaew et al. 2006; Mai et al. 2008; Huang et al. 2005).

S. suis infection is reported in domesticated pigs (Staats et al. 1997). In addition, the organism has been isolated from the intestinal flora of wild boars, dogs, cats, horses, deer and ruminants (Staats et al. 1997; Devriese et al. 1992; Baums et al. 2007; Devriese and Haesebrouck 1992). The rate of asymptomatic carriage in pigs is estimated to be around 80%, representing a potential source of infection to other animals and humans (Lun et al. 2007; Staats et al. 1997; Arends et al. 1984; Ngo et al. 2011). Pigs acquire *S. suis* via vertical and horizontal transmission as the sow is capable of harboring *S. suis* in the genital tract (Fulde and Valentin-Weigand 2013; Fittipaldi et al. 2012; Gottschalk 2011). Carrier rates are highest in pigs between 4 and 10 weeks of age, but infection can occur at any age (Staats et al. 1997; Clifton-Hadley et al. 1984). Environmental contaminants such as feces, dust, water, and feed are considered to be secondary sources of infection (Staats et al. 1997). Vectors such as houseflies (Fulde and Valentin-Weigand 2013; Staats et al. 1997; Enright et al. 1987) and mice (Fulde and Valentin-Weigand 2013; Staats et al. 1997; Williams et al. 1988) are also considered to play a role in disease transmission to pigs. Factors such as stress, crowding, poor ventilation, and concurrent disease could potentially predispose herds to an outbreak of *S. suis* infection (Fulde and Valentin-Weigand 2013; Staats et al. 1997). Morbidity rate in pigs ranges from $<1\%$ to $>50\%$, rarely exceeding 5% (Wertheim et al. 2009). Nevertheless, research has demonstrated that morbidity due to *S. suis* is severely enhanced in the presence of other bacterial and viral infectious agents suggesting the importance of surveillance for *S. suis* (Staats et al. 1997).

Human *S. suis* infection is considered an emerging zoonosis (Lun et al. 2007; Wertheim et al. 2009). Studies observed that longer duration of exposure to pigs and pork affects *S. suis* carriage in the population (Elbers et al. 1999; Smith et al. 2008; Strangmann et al. 2002). Infection rate in individuals with high-risk exposures is estimated to be 1500 times higher than that of the general population (Lun et al. 2007; Arends and Zanen 1988). Pig farmers (Smith et al. 2008; Bartelink and van Kregten 1995; Breton et al. 1986; Sriskandan and Slater 2006; Fowler et al. 2013), abattoir-workers (Arends and Zanen 1988; Bartelink and van Kregten 1995; Breton et al. 1986), veterinarians (Elbers et al. 1999), hunters (Baums et al. 2007; Halaby et al. 2000), and meat-processing workers (Tramontana et al. 2008; Yu et al. 2005) are observed to have a higher risk of *S. suis* infection . Consumption of uncooked or partially cooked pork products is also considered a potential risk factor for *S. suis* infection (Wertheim et al. 2009; Wangsomboonsiri et al. 2008). A mortality rate of 17% was observed in the population and about 2/3 of deaths occurred in the first 24 h after admission (Wangsomboonsiri et al. 2008). Human infections are typically reported as sporadic cases with an exception of two large outbreaks resulting in 25 and 204 cases, and 14 and 38 deaths, respectively (Yu et al. 2006; Tang et al. 2006). Person-to-person transmission is unlikely to occur without very close contact such as with infected blood. Nevertheless, there is strong evidence of *S. suis* transmission from pigs to humans and a great potential for reverse zoonoses, i.e., transmission from humans to animals.

S. suis is sensitive to antibiotics such as penicillin, ampicillin, amoxicillin, ceftriaxone, and cephalosporin (Lun et al. 2007). Clinical disease is known to be suppressed by fortifying feed with antibiotics at therapeutic levels (Staats et al. 1997). However, it

does not eliminate carriers thus negatively impacting transmission of *S. suis.* One of the major drawbacks is the development of antimicrobial resistant *S. suis* isolated from both pigs and humans (Mai et al. 2008; Gottschalk et al. 1991c; Prieto et al. 1994; Aarestrup et al. 1998; Marie et al. 2002; Shneerson et al. 1980; Wisselink et al. 2006; Vela et al. 2005). Vaccines currently in use prevent outbreak in pig herds, but are observed to have varying efficacy (Lun et al. 2007; Haesebrouck et al. 2004). A human vaccine for *S. suis* is not available (Lun et al. 2007; Wertheim et al. 2009).

Prevention of *S. suis* transmission in both humans and pigs depends on control of contact with sick animals. Improving pig-raising and breeding conditions and vaccination of pigs could ensure reduction in *S. suis* infection outbreaks and prevent transmission to humans (Lun et al. 2007). In addition, the potential risk of transmission via contact or consumption of contaminated pork products can be diminished by education and increasing awareness on preventative measures to eliminate this mode of transmission (Lun et al. 2007). World Health Organization (WHO) recommends cooking pork to an internal temperature of 70 °C or until juices appear clear rather than pink (Lun et al. 2007). Use of clean gloves and hand hygiene should also be encouraged when handling raw or undercooked pork products. Review of the current literature exposed a knowledge gap on differences in the virulence capacity and geographical variation of *S. suis* strains. Addition of this information to other available epidemiological data on *S. suis* is warranted to prevent further propagation and losses worldwide due to this pathogen.

4.1.6 Shiga-Toxin Producing Escherchia coli (STEC)

Escherchia coli, a short, rod shaped, Gram-negative, non-sporing, facultative anaerobic bacterium belongs to the family *Enterobacteriacae* (Sussman 1985; Mainil 2013). The gastro-intestinal tract of humans and other warm blooded animals are the primary hosts of this organism (Cheleste et al. 2002; Bell 2002). Although most *E. coli* strains are non-pathogenic, and part of normal microflora, some strains have evolved as pathogenic (Mainil 2013; Bell 2002; CDC 2012b). Pathogenic strains of *E. coli* acquire mobile virulence gene located on pathogenicity islands, integrated bacteriophages, or on plasmids (Bell 2002; FAO/WHO 2011), and are able to cause wide spectrum of diseases in many species including pigs, cattle, rabbits and humans (Mainil 2013; Jay et al. 2007). On the basis of their virulence traits, pathogenic strains of *E.coli* are categorized into at least six groups: entero-pathogenic *E.coli* (EPEC), entero-toxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), entero-haemorrhagic *E. coli* (EHEC), entero-aggregative *E. coli* (EaggEC), and diffusely adherent *E. coli* (DAEC) (Bell 2002; CDC 2012b; Catalina Lopez-Saucedo et al. 2003).

Shiga toxin-producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC), are a diverse group of pathogens that has become of significant health concern. These strains of *E. coli* are able to cause disease in both humans and animals. Although EHEC 0157:H7 is recognized as the most prominent STEC, over 200 non-O157:H7 STEC serotypes have been identified, and over 100 strains can cause disease in humans (Bell 2002; Rangel et al. 2005; Fratamico et al. 2004;

Patricia and Griffin 1991). In the United States, most EHEC strains are serotype O157:H7 that accounts for 30–50% of EHEC strains (Johnson and Sears 2006). Infection with non-O157:H7 serotype is more common in other nations including Australia, Argentina, and many European countries, and may account for the majority of haemolytic uremic syndrome (HUS) infections in these countries (Fratamico et al. 2004). Serotypes 026, 045, 0103, 0111, 0121, and 0145 have been associated with human disease and may account for approximately 70% of Non-O157:H7 STEC human infections in the United States (Wells et al. 2012). *E. coli* O157:H7 was first identified as pathogenic strain following two outbreaks of hemorrhagic colitis by consuming undercooked ground beef in 1982 in the United States (Rangel et al. 2005; Patricia and Griffin 1991; Pennington 2010; Beilei Ge et al. 2002; Phillip Tarr and Chandler 2005). Since the discovery of *E. coli* O157:H7, large foodborne outbreaks and sporadic incidence have been documented in the United States and many parts of the world (FAO/WHO 2011; Phillip Tarr and Chandler 2005; Tiiomas et al. 1995). Annually, EHEC O157:H7 and other serotypes of STEC accounts approximately 110,000 cases of illness in the United States (Cornick and Helgerson 2004).

STEC is a worldwide public health threat. Over 100 different serotypes can cause human illness (Acheson 1999). The exact global prevalence of STEC infection is unknown since there is no uniform surveillance and reporting system. Annually, an estimated 73,000 cases are caused by *E. coli* O157:H7 in the United States leading to estimated 2,168 hospitalizations and 61 deaths (Rangel et al. 2005; Beilei Ge et al. 2002). Non-O157:H7 accounts for 37,740 cases and 30 deaths annually in the United States (Beilei Ge et al. 2002). Studies have indicated that STEC infection is more prevalent in the northern regions of the United States, and is more common in summer season (Phillip Tarr and Chandler 2005; Tiiomas et al. 1995). *E. coli* O157:H7 can infect people of any age. However, children and elderly are more prone to develop severe illness and HUS compared to any other age groups (Phillip Tarr and Chandler 2005; FAO/WHO 2011). Various studies have suggested that animals including cattle, sheep, goats, and pigs are reservoirs for different STEC strains (Cheleste et al. 2002; Bell 2002; Fratamico et al. 2004). Although cattle are considered to be the primary reservoir of *E. coli* O157:H7, it is implicated in fecal shedding of other domestic livestock and wildlife (Jay et al. 2007). Evidence from epidemiological studies suggests that domestic pigs are potential reservoirs and biologically competent hosts of *E.coli* O157:H7 (Jay et al. 2007; Fratamico et al. 2004; Cornick and Helgerson 2004). In 2006, a spinach associated outbreak of *E. coli* in the United States caused 205 cases of illness and six deaths. A successful isolation of the outbreak strain from feral swine living close to spinach field provides insight on swine-to-swine transmission and transmission between cattle and swine. A study conducted by Jay et al. was able to recover related *E. coli* O157: H7 subtypes from feral swine, cattle, surface water, soil, and sediment that were contaminated with spinach causing the outbreak (Jay et al. 2007). *E. coli* O157: H7-infected swine can shed the bacteria in feces for about 2 months thus serving as a reservoir host (Cornick and Helgerson 2004). Rios et al. isolated enterohemorrhagic STEC subgroup 026 and 0111 from the intestinal content of pigs. These strains had

virulence genes (*stx1, stx2*) suggesting they were potential human pathogens (Fratamico et al. 2004; Maritza Rios et al. 1999). Fratamico et al. isolated STEC serogroup O2, O5, O7, O8, O9, O15, O65, O91, O101, O120, O121, O163, and several others from fecal samples of pigs (Fratamico et al. 2004). Other studies have indicated that STEC strains can be isolated from both healthy pigs and pigs with diarrhea and edema disease (Fratamico et al. 2004; Cornick and Helgerson 2004).

Various O, H, and K antigens of *E.coli* are identified (Kauffmann 1947). Virulent strains have gens for fimbriae, adhesions, and wide varieties of exotoxins that help pathogenic *E. coli* to colonize human tissues (Mainil 2013). *E. coli* O157:H7 produces a type III secretion system that injects two types of proteins, which disrupt the cells metabolism and provide surface for attachment (Mainil 2013; Pennington 2010). Shiga toxin is the key virulence factor of STEC (Patricia and Griffin 1991; Werner Brunder and Karch 1997), and it causes necrosis of host cells and tissues (Pennington 2010). Although several virulence factors encoded by a 60-MDa plasmid such as a bifuctional catalase-peroxidase, secreted serine protease (EspP), α-hemolysin (EHEC-Hly), and chromosomally encoded enterotoxin EAST1 have been found, their role in pathogenicity still remains unclear (Cheleste et al. 2002; Werner Brunder and Karch 1997; Paul and Mead 1998). All *E. coli* belonging to STEC strains can produce Shiga toxin1 (*Stx1*) and/or Shiga toxin 2 (*Stx2*) or variants of *Stx1 or Stx2*. *Stx2e* variant strain of STEC cause edema disease in swine (Fratamico et al. 2004; Patricia and Griffin 1991).

The incubation period of STEC infection is 2–4 days, but may vary from 1–5 days (Acheson 1999). Many people infected with STEC remain asymptomatic (Pennington 2010); others suffer from mild to severe gastro-intestinal symptoms. STEC infection ranges from mild to life threatening. Symptoms include watery diarrhea which can be bloody as the disease progresses), severe abdominal pain, low to mild-grade fever and nausea and vomiting. Fecal and peripheral leukocytosis is often present. Most people recover within 5–7 days of the onset of infection (Cheleste et al. 2002; Bell 2002; Patricia and Griffin 1991; Acheson 1999). Hemolytic uremic syndrome (HUS) is developed in 5–10% of STEC cases (Acheson 1999). HUS is a serious complication characterized by hemolytic anemia, thrombocytopenia, fever, and kidney damage (Cheleste et al. 2002; Rangel et al. 2005; Acheson 1999; Frederick Koster et al. 1978). HUS often develops in children below age 5 as a complication of *E. coli* infection. HUS accounts 15% of EHEC infection in children below 10 years old. HUS is seen as a complication in 6–9% of overall infections (Bell 2002; Phillip Tarr and Chandler 2005; Tiiomas et al. 1995). 5–10% of HUS patient may die or develop further complications (stroke) (Cheleste et al. 2002). An estimated 50% of HUS patients may have permanent kidney damage. Since patients with HUS are in risk of renal failure, they should be hospitalized (Cheleste et al. 2002; Acheson 1999). The mortality of HUS is approximately 5% (Acheson 1999), and the case fatality rate of HUS is approximately 10% (Bell 2002).

The use of antibiotics could aid in Shiga toxin production thus exacerbating the disease; as such, this treatment is not recommended in the United States. Symptomatic treatment along with maintaining hydration is very important to prevent further complications. Prevention is the most important aspect of STEC infection (Acheson

1999; Paul and Mead 1998). Frequent hand-washing is the most effective tools to avoid person-to-person transmission. Proper handling of foods, preventing temperature abuse and cross-contamination of foods, as well as maintaining a proper storage temperature is essential. Boiling water before drinking can help to stop waterborne transmission in developing countries where drinking water system is poor. The practice of using animal fecal as manure for crops used for human consumption should be stopped. Foods should be cooked to the optimum temperature. Undercooked meat and unpasteurized milk should not be consumed (Bell 2002; CDC 2012b; Acheson 1999).

4.2 Colistin-Resistant Organisms

While not a single species, the emergence of transmissible colistin resistance (a polymixin antibiotic) in important food-borne organisms has been a key concern in recent years. A novel plasmid-mediated colistin resistance gene, *mcr-1*, was reported in animals and humans in China (Liu et al. 2016), potentially emergent due to use of polymixins in livestock farming in the country. The initial report examined *E. coli* previously collected from pigs at slaughter and retail meat products, and *E. coli* and *Klebsiella pneumoniae* obtained from human patients; isolates were collected between 2011 and 2014. *mcr-1* was detected in a portion of all samples tested, ranging from 0.7% (human samples of *K. pneumoniae,* 2014) to 28.0% (*E. coli* in retail chicken meat samples, 2014). Pigs at slaughter and pork product positivity ranged from 6.3% positive (2011, retail pork) to 25.4% (pigs at slaughter, 2013). Since this time, *mcr-1* has been found in samples dating back to at least 2002 and on 5 continents (Wang et al. 2018); while most were in *E. coli*, other food-borne pathogens including *Salmonella enterica* were also positive. Related colistin genes *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* have also been identified (Rebelo et al. 2018).

Colistin is an antibiotic of last resort for multi-drug resistant infections by Gram-negative bacteria; as such, transmissible resistance to this drug is concerning. Colistin was used in animal production for both treatment and growth promotion purposes in some countries in Europe and Asia (Rhouma et al. 2016). However, this does not appear to be the only potential reason for emergence, as *mcr-1*-positive organisms have been identified in countries even where colistin has not been used in animal populations (such as the United States).

4.3 Hepatitis E Virus (HEV)

Hepatitis E virus (HEV) belongs to the *Hepeviridae* family, and is the most common cause of acute viral hepatitis globally. A single-stranded positive-sense RNA virus, it was first visualized in 1983 (Balayan et al. 1983).

There are four genotypes of HEV that appear to infect humans: HEV1, HEV2, HEV3, and HEV4. While HEV1 and HEV2 do not appear to be spread between humans and animals, zoonotic transmission is common with HEV3 and HEV4. Pigs

are the main reservoir, but infection has also been documented in wild boars (De Sabato et al. 2020) and a variety of other wild and domestic species, including cats, dogs, horses, deer, sheep, cattle, and rabbits (Izopet et al. 2012; Schlosser et al. 2014; Doceul et al. 2016; Yan et al. 2016).

While large epidemics of HEV1 and HEV2 can occur among human populations due to fecal contamination of water supplies (especially during flooding and heavy rainfall), transmission of HEV3/HEV4 from animals to humans seem to occur more commonly due to close contact with infected animals or animal products. This can include contact with animal feces or other secretions (including milk of infected animals), or handling/consumption of under-cooked meat products. As such, farmers, veterinarians, and slaughterhouse workers are more prone to zoonotic HEV infections than the general population (Aslan and Balaban 2020).

Symptoms of HEV infection are generally mild; infections may be asymptomatic. Symptoms may include nausea, vomiting, malaise, and fever, which in some patients continue to jaundice and other hepatic symptoms (Lhomme et al. 2020), which tend to be more severe in those infected with HEV1/HEV2 than the zoonotic HEV strains (Pischke and Wedemeyer 2013). Infection is generally cleared without treatment, but in severe cases, therapy with the antiviral ribavirin may be helpful (Peron et al. 2011). There is currently no vaccine to prevent infection, although vaccines are in development (Zhu et al. 2010); as such, safety during animal contact and consumption of animal products are the primary ways to prevent zoonotic HEV infections at this time.

4.4 Japanese Encephalitis Virus (JEV)

Japanese encephalitis virus (JEV) belongs to the genus *Flavivirus*, family *Flaviviridae* (Weaver and Barrett 2004; van den Hurk et al. 2009; Solomon 2004). This virus was first isolated from a fatal human encephalitis case in Japan in 1935 (Weaver and Barrett 2004) and from *Culex tritaeniorhynchus* mosquitoes in 1938 (van den Hurk et al. 2009). This arbovirus (arthropod-transmitted) (Weaver and Barrett 2004; Igarashi 2002) is the leading cause of worldwide epidemics of viral encephalitis (Weaver and Barrett 2004; Tom Solomon et al. 2000). This single stranded positive sense RNA virus with a genome length of 11 kilobases (Weaver and Barrett 2004; Solomon 2004) consists of a spherical virion with a 30 nm core that is surrounded by a lipid envelop. The RNA genome of JEV encodes a single polypeptide that is cleaved into non-structural proteins such as NSI, 2A, 2B, 3, 4A, 4B, and 5, and structural capsid, member (M) and envelope (E) proteins (Tiroumourougane et al. 2002; Spickler 2007). The E protein plays vital antigenic role as it is important for viral attachment and entry into host cells (Solomon 2004; Mouhamadou Diagana and Dumas 2007). This virus has only one serotype and two subtypes, and is closely related to St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, and dengue fever virus (Solomon 2004; Tiroumourougane et al. 2002; Spickler 2007). On the basis of nucleotide sequencing of the viral pre-membrane (prM), JEV can be categorized into four different genotypes. Moreover, the phylogenic analysis of the viral envelop 'E' gene has classified JEV strains into five genotypes (Health WOfA 2009). A wide

range of host species might be infected by JEV including cattle, snakes, birds, pigs, horses, and other farm animals (Weaver and Barrett 2004; van den Hurk et al. 2009; Spickler 2007). High heat (56 °C for 30 min), acidic environment (pH 1–3), and various chemicals and disinfectants such as iodine, phenol, and formaldehyde also inactivate the virus. JEV is quite sensitive to ultraviolet light and gamma irradiation (Health WOfA 2009).

JEV is transmitted between wild and domestic birds and pigs by *Culex* species mosquitoes (Tom Solomon et al. 2000; van-den-Hurk et al. 2008). *Culex tritaeniorhynchus* plays a major role, because many animals such as horses, swine, humans, and birds are susceptible hosts. It is also the most important vector for human infections (Weaver and Barrett 2004; Tom Solomon et al. 2000). These mosquitoes particularly breed in pools of stagnant water, especially in flooded rice fields (Tom Solomon et al. 2000; Erlanger et al. 2009). JEV has also been isolated from other species of mosquitoes (Tiroumourougane et al. 2002). Ardeid or wading birds (herons and egrets) are considered as the primary maintenance hosts (Igarashi 2002; van-den-Hurk et al. 2008; Erlanger et al. 2009) and pigs are the main amplifying hosts (Weaver and Barrett 2004; van-den-Hurk et al. 2008, 2009; Tom Solomon et al. 2000; Spickler 2007; Erlanger et al. 2009), which are necessary for pre-epizootic amplification of the virus. Pigs can act as maintenance hosts in endemic regions (van den Hurk et al. 2009). Pigs in close proximity to humans are the most important natural hosts for transmission of JEV to humans (Weaver and Barrett 2004; Solomon 2004; Tom Solomon et al. 2000; Tiroumourougane et al. 2002). Pigs have a prolonged and high viraemia and a high natural infection rate of 98–100% (van den Hurk et al. 2009). Domestic pig rearing aids in the transmission to humans (Erlanger et al. 2009). Humans and horses are dead-end or incidental hosts (van den Hurk et al. 2009; Tiroumourougane et al. 2002). Human-to-human transmission of JEV has not been reported yet (Tiroumourougane et al. 2002).

JEV remains the major cause of viral encephalitis in Southeast Asia (van-den-Hurk et al. 2008), but it is widely spread in eastern and south-eastern Asian countries, the Pacific Rim, and in Northern Australia. However, related neurotropic flaviviruses are found worldwide (Tom Solomon et al. 2000; Erlanger et al. 2009). Japanese encephalitis claims about 50,000 human cases and 15,000 deaths annually (Weaver and Barrett 2004; Tom Solomon et al. 2000). Due to lack of surveillance and inadequate data collection, the actual incidence rate might be a lot higher. It is estimated that 175,000 cases of Japanese encephalitis occurs annually worldwide. 11,000 cases and more than 2000 deaths resulted from JEV outbreaks in Nepal and Northern India between 2005 and 2007 (van den Hurk et al. 2009). Children under 15 years of age are mainly affected by JEV in endemic areas (Tiroumourougane et al. 2002). Pediatric encephalitis is caused by this virus in many Asian countries including India, Korea, and China. More than one third of world populations are at risk of infection of JEV. The epidemiological patterns of JEV involve endemic and epidemic activities in tropical regions and temperate and subtropical areas, respectively. There is no seasonal pattern in endemic areas, but epidemic activity is observed in summer and autumn months in temperate and subtropical areas. Migratory birds help the virus to travel large distances (Weaver and Barrett 2004).

Japanese encephalitis is mainly a disease of rural areas. It is endemic in tropical regions and often associated with irrigated rice agriculture (van den Hurk et al. 2009). The annual incidence of Japanese encephalitis is between 10 and 100 per 100,000 population in endemic areas (Tiroumourougane et al. 2002).

The incubation period of Japanese encephalitis in man is not exactly known. It varies from 1–6 days, and can be as long as 14 days (Tiroumourougane et al. 2002). Incubation period in horses is 8–10 days (Spickler 2007). Most infections of Japanese encephalitis are asymptomatic. Clinical features are developed only in 1 in 50 to 1 in 1000 infections. The clinical manifestations range from mild flu-like illness to severe and lethal meningoencephalomyelitis (van den Hurk et al. 2009; Tom Solomon et al. 2000). High grade of fever with or without rigors, headache, general malaise, and vomiting are present in the prodromal stage. It is followed by the encephalitis stage which is characterized by abnormal movements, muscular rigidity, neck stiffness, convulsions, altered neurological functions, and other CNS signs (Tiroumourougane et al. 2002). Convulsion often occurs and it is reported in about 85% of children and 10% of adults (Tom Solomon et al. 2000). The recovery stage may be accompanied by signs of CNS injury. Thick, slow speech, aphasia, and paresis are seen as residual neurological impairments. Complications include secondary bacterial infection, urinary tract infection, and stasis ulcers (Tiroumourougane et al. 2002). Abnormal mental behaviours may exist in some patients leading to misdiagnosis of mental illness (Tom Solomon et al. 2000). Poliomyelitis-like acute flaccid paralysis and "fever associated seizure disorder" has also been identified in some cases (Tiroumourougane et al. 2002). Almost one third of patients admitted to hospital with Japanese encephalitis die (Tom Solomon et al. 2000), and 45% to 70% of survivors suffer from neurological sequelae that last for months. The case fatality rate of Japanese encephalitis can be as high as 67%. Higher fatality rates are seen in children and the elderly population (van den Hurk et al. 2009). The mortality rate varies in a range of 8.5–72% (Tiroumourougane et al. 2002). Pigs with Japanese encephalitis exhibit reproductive disease with stillbirth as the most common symptom (van den Hurk et al. 2009).

JEV can be detected using reverse transcriptase polymerase chain reaction in human CSF samples. But the reliability of this test still remains unconfirmed. Serological tests such as IgM and IgG ELISA are widely used for the detection of antibodies to JEV in human and swine (CDC 2021). However, this type of testing requires complex equipment and it is not feasible in the rural areas of developing countries. A recent modification of this test to a simple nitrocellulose membrane based format is more useful in rural areas, since it does not require any sophisticated equipment and can be interpreted by eye vision (Tom Solomon et al. 2000). Virus neutralization and epitope blocking ELISAs can help to differentiate if cross-reactions have been occurred in serological tests due to the presence of other viruses of the JEV serogroup (Kitai et al. 2007; Jacobson et al. 2007). Various other serological tests such as haemagglutination inhibition, the complement fixation test, single radial hemolysis, and neutralisation are still in practice in some laboratories. Other antigen detection techniques in CSF include reverse passive

hemagglutination, immunofluorescence, and staphylococcal coagglutination tests using polyclonal or monoclonal antibodies (Tom Solomon et al. 2000).

Treatment of Japanese encephalitis involves supportive and symptomatic care. Although isoquinolone compounds and monoclonal antibodies are effective in vitro and animal models, respectively, there is no specific treatment available for this disease. Controlling convulsion and raised intracranial pressure are crucial. Physical therapy and excellent nursing care are important to prevent further complications (Solomon 2004; Tom Solomon et al. 2000; Spickler 2007).

Prevention strategies should be focused on control of mosquito vectors, improvement in animal husbandry practices, changing agricultural practices, preventing amplification of the virus in pigs and birds, measures against reservoirs, and immunizing of species at risk (van-den-Hurk et al. 2008, 2009; Tom Solomon et al. 2000; Tiroumourougane et al. 2002; Spickler 2007; Erlanger et al. 2009). Prevention of mosquitoes from biting human is essential (Tiroumourougane et al. 2002). Use of bed nets, wearing long sleeved shirts and long trousers, and application of insect repellent on exposed body surfaces, avoidance of outdoor sleeping, and decreasing outdoor activity during twilight and dawn would be beneficial (Spickler 2007; Mackenzie and Smith 2006). Relocation of piggeries away from residential areas and making them mosquito proof could decrease the risk of JEV. Vaccine is recommended to people, especially for high-risk group such as laboratory workers with potential risk of exposure to JEV, travelers who spend more than a month in endemic areas, and residents of endemic areas. Travelers who spend less than 30 days during epidemics and carry out extensive outdoor activity should also get vaccinated. Vaccinations of pigs also yield positive results (Tiroumourougane et al. 2002). Vero cell-derived JEV vaccine is currently used in the United States (Fischer et al. 2010).

4.5 Nipah virus

The first cases of Nipah virus (NiV) infection were identified during an outbreak in Malaysia in September 1998 and involved pig farmers who had contact with pigs on a regular basis (Luby and Gurley 2012). JEV was initially suspected due to the similar clinical presentation in humans and because it is endemic in the outbreak area in Malaysia (Chua 2012). Although known measures to control JEV were taken, such as vaccination, the number of cases did not decrease (Chua 2012). The cause of the outbreak was eventually discovered to be a novel paramyxovirus (Chua et al. 1999, 2000). Humans with direct contact with pigs were at an increased risk of contracting the virus (Chua 2003). During March 1999 in Singapore, there was an outbreak of NiV-associated encephalitis and/or pneumonia in abattoir workers (Paton et al. 1999) who were more likely to have exposures to the urine or feces of imported Malaysian pigs during the corresponding outbreak (Luby and Gurley 2012). An isolate obtained from a deceased abattoir worker in Singapore was identified as having the identical nucleotide sequence as the isolates collected from human and pig cases in Malaysia (Paton et al. 1999). This provides evidence that the

same strain infected human and pig cases in Malaysia and that the outbreak had spread to a different country.

Isolates collected from the lungs and respiratory secretions from infected pigs revealed the presence of NiV and this taken in conjunction with the observation that many of the NiV human cases were in contact with pigs has led to the suspicion that NiV may be transmitted from pigs to humans through infected saliva and potentially infected urine (Uppal 2000).

When officials could no longer ignore the potential of a novel agent as the culprit, they began further diagnostic tests that identified a novel paramyxovirus (Chua et al. 1999, 2000, 2007) that was closely related to Hendra virus (Ksiazek et al. 2011). This novel virus was named Nipah virus after Kampung Sungei Nipah, the residential village of the patient from whom the first virus was isolated (Ksiazek et al. 2011). Since this initial outbreak in 1998–1999, the exact geographical distribution of NiV remains unknown, but it appears to occur in Asia Pacific and South East Asia (Young et al. 1996) with Singapore, Bangladesh, and India as countries specifically encountering NiV outbreaks (Luby and Gurley 2012). Nipah virus outbreaks have been reported nearly every year beginning in 2001 in the western and northwestern regions of Bangladesh and in West Bengal, India (Luby and Gurley 2012).

Through surveillance efforts, fruit bats, from the genus *Pteropus*, have been found to be the reservoir of NiV (Arif et al. 2012). This virus can then be spread to humans through pigs acting as an intermediate host (Arif et al. 2012). The distribution of this genus of fruit bats encompasses areas from the eastern coast of Africa to the South East of Asia, to the Philippines and Pacific Islands, and as far south as Australia (Young et al. 1996). The distribution of this genus of bats suggests that it may be possible for NiV to be spread across the regions inhabited by these fruit bats; public health officials need to be aware of the potential for NiV to spread to these susceptible areas that may not have seen this disease before. A study performed in Goalondo of Rajbari district in central Bangladesh during a 2004 outbreak found that NiV was found in 14% of blood samples contained antibodies from clinically healthy bats (Arif et al. 2012).

It is suspected that pigs may contract the virus from ingesting fruits that have been nibbled on by NiV infected fruit bats living near pig farms (Arif et al. 2012). Moreover, the virus has been isolated from fruits that have been half eaten by bats in Malaysia (Chua et al. 2000). The majority of NiV-infected pigs develop mild illness (Parashar et al. 2000), while some pigs never develop clinical signs of disease. In infected adult pigs, the case fatality rate is less than 1–5% (Mohd Nor et al. 2000). Some mathematical models suggest that in order for NiV to be sustained at epidemic levels in pigs, multiple spillover events are necessary for the development of a dynamic population with numbers of susceptible pigs above the needed threshold level to maintain transmission within pigs for months (Pulliam et al. 2012).

There is evidence that various husbandry practices may play a role in determining how long an epidemic will last and in influencing the characteristics of an outbreak (Luby and Gurley 2012). In large factory farms, thousands of pigs are raised together in more compact conditions and have an increased risk of interacting with pigs from other farms, so longer transmission chains in pigs will be more likely to occur (Luby

and Gurley 2012). However, in rural areas with fewer pigs kept on individual farms that may have limited contact with pigs from other farms, a shorter transmission chain may be seen, because the number of susceptible pigs will decrease more slowly overtime since fewer will be in contact with each other. This second type of husbandry practice has not been linked to an outbreak to date; however, human cases have been linked to animal infections with this form of husbandry (Luby and Gurley 2012).

The majority of infected pigs present with a mild illness, if an apparent illness is detected at all. Those which develop clinical symptoms present most often with fever, agitation, trembling, and twitching (Mohd Nor et al. 2000). These symptoms appear along with labored and rapid respirations, increased drooling, and a loud, nonproductive barking cough (Mohd Nor et al. 2000). Almost all of the pigs with symptoms are often diagnosed with acute respiratory syndrome (Kay-Sin Tan and Goh 1999; Chua 2003). Necropsies revealed that pigs with severe disease have had extensive lung damage with giant cell pneumonia; NiV antigen can be detected in lung tissue as well as in the epithelial cells lining the upper airways, respiratory secretions, and in renal tubular epithelial cells (Chua et al. 2000; Middleton et al. 2002).

Symptoms in humans may vary from severe to mild to asymptomatic (O'Sullivan et al. 1997), may lead to debilitating chronic neurologic conditions, and can be fatal (Arif et al. 2012). The incubation period has been reported to be between 4 and 18 days, but occasionally clinical symptoms may not develop until an average of 8 months after an exposure (Holmes 2001). Clinical symptoms in humans usually present as severe acute encephalitis with individual symptoms that include fever, headache, vomiting, breathing difficulty, seizures, and progression into coma (Luby and Gurley 2012; Arif et al. 2012). Patients may also develop pneumonia from an accumulation of respiratory secretions in the lungs, which has been noted to occur in up to 25% of cases (Chua et al. 1999; Chadha et al. 2006).

Cases with NiV infection may also develop chronic illness leading to severe neurologic conditions later in life, even if the case did not present with acute symptoms shortly after exposure (Luby and Gurley 2012; Tan et al. 2002). Patients who had developed acute encephalitis and appeared to have recovered may also develop neurologic conditions and relapses of encephalitis months to years after their initial infection, with NiV antigen being found in the neurons of those who died after a case of late-onset encephalitis (Tan et al. 2002).

In many situations, patients are more likely to be in direct contact with pigs that appeared to be sick when compared to farm controls (Parashar et al. 2000). Direct contact with pigs involves activities such as feeding pigs, processing piglets, aiding in breeding and birthing, injecting and medicating pigs, and handling dead pigs (Parashar et al. 2000). The case fatality rate may be high for humans. During the 1998–1999 Malaysia outbreak, of the 283 reported cases, 39% or 109 cases died (Chua 2003). Although contact with pigs increases the risk of contracting NiV, there have been reported cases of disease in humans without pig contact or without direct pig contact such as cleaning a crate that was used to house infected pigs (Parashar et al. 2000; Kay-Sin Tan and Goh 1999). This suggests that it may be possible for pig secretions or excretions to be infectious for hours, if not days (Kay-Sin Tan and Goh 1999).

Human-to-human transmission has been reported, but the rates vary geographically (Luby and Gurley 2012). Numerous outbreaks involving human-to-human transmission have been recognized in Bangladesh and India, while only very few reports on human-to-human transmission exist from Malaysia (Luby and Gurley 2012) and the Philippines (Ching et al. 2015), where horses rather than pigs were implicated. In a 2004 Bangladesh outbreak, a chain of NiV transmission was sustained through five generations, which is the longest reported chain of human-to-human transmission (Gurley et al. 2007). Through reviewing human NiV cases from 2001–2007 in Bangladesh, Luby et al. (2009) found that 51%, or 63 out of 122 cases, contracted NiV after being in close contact with a patient. Various investigations in Bangladesh suggest that the primary mode of transmission between humans is through respiratory secretions and that those who did have difficulty breathing as one of their symptoms were more likely to spread the virus than those who did not (12% compared to 0%, $p = 0.03$) (Luby et al. 2009). During the 2004 Bangladesh outbreak referred to earlier, people caring for human cases who shared utensils, ate the patients' leftover food, slept in the same bed with a coughing patient, and fed and/or hugged dying patients were more likely to contract NiV (Blum et al. 2009).

Since direct contact with pigs has shown to increase the risk of contracting NiV, the use of personal protective equipment (PPE) is imperative in reducing the risk of contracting the virus (Uppal 2000). General safety measures that should be taken when contact with suspected NiV infected pigs occurs is changing needles between every pig, using soap or detergent to wash the hands and body, disinfecting abattoir and veterinary equipment, spraying disinfectant on trucks every time they leave a farm, disinfecting dead pigs before burying them, and avoiding contact with blood, urine, and feces (Uppal 2000). Recommendations for PPE to protect individuals are goggles and face masks for eye protection, masks that cover the nose and mouth, rubber gloves, a long sleeved shirt, a long apron, long pants, and rubber boots (Uppal 2000). Measures in addition to PPE use have been implemented to control the spread of the disease. In Malaysia, when PPE was employed to those who are in direct contact with pigs, especially sick pigs, along with the implementation of livestock transportation restrictions, and culling of a large number of pigs (over 900 thousand), the amount of human cases drastically declined (Uppal 2000). The importance of control must be stressed because during an outbreak there is a large social impact due to the potential closure of schools, loss of human life leading to fewer community members able to work and earn money, loss of pig populations (especially when culling is implemented) which minimizes farmer's wages (even if marginal compensation occurs for their lost revenue), and decreases a community's moral while increasing a sense of panic (Uppal 2000).

4.6 Swine Influenza virus

Influenza is an acute respiratory disease caused by viruses of the family *Orthomyxoviridae* (Capua and Munoz 2013; Thacker and Janke 2008). The virus was first isolated in 1930 in the United States (Kothalawala et al. 2006). Estimated 36,000

human deaths and 200,000 hospitalizations are due to influenza virus infections in the United States per year (Ramirez et al. 2006). The genome of the virus is composed of eight negative-strand RNA segments. This allows both for a high mutation rate (as an RNA virus) leading to antigenic drift and for mixing of genomic segments via recombination causing antigenic shift of the virus. Two major surface glycoprotein, hemagglutin (HA), and neuraminidase (N) are important tools for subtype classification, and determination of antigenicity and pathogenicity (Thacker and Janke 2008; Kothalawala et al. 2006). With the recent discovery of a new influenza viral subtypes in bats (Tong et al. 2012, 2013; Campos et al. 2019), influenza viruses are now grouped according to the expression of 18 HA (H1-H18) and 10 NA (N1-N10) subtypes (Capua and Munoz 2013).

Influenza viruses may be subject to antigenic drift and antigenic shift, which impose major challenges in vaccine development. Antigenic drift is a minor genetic variation within subtypes due to a series of point mutations. Antigenic shift is caused by reassortment of genes from two different viruses that result in a new combination of H or N segments (Kothalawala et al. 2006). For example, the 2009 pandemic H1N1 virus (H1N1pdm09) was the result of a virus reassortment event in swine (Skowronski et al. 2013). The H1N1 virus contains segments from North American-like triple reassortant swine H1 viruses (6 genes) and Eurasian avian-like swine viruses (neuraminidase and matrix genes) (Pascua et al. 2012).

The ultimate host of all influenza viruses appears to be wild birds, specifically waterfowl (Thacker and Janke 2008), where the viruses replicate in the respiratory tract and intestine typically without any signs and symptoms of disease. Although mammalian species are all derived from birds, studies have shown interspecies transmission of influenza virus (e.g., H3N8 from horses to dogs) (Kothalawala et al. 2006; Pascua et al. 2012; Myers et al. 2006). Interspecies transmission of influenza virus A is the principal mechanism of emergence of novel strains, and pigs likely play an important role in such transmission (Myers et al. 2006; Bowman et al. 2012).

Viral attachment on host cells was thought to depend on the specificity of binding to particular sialic acids (SAs). Prior research suggested that avian influenza viruses bind to α2–3 SAs, while influenza viruses infecting mammals preferentially attach to α2–6 SAs. However, recent research (reviewed in (Capua and Munoz 2013)) suggests that it is more complicated than that. Nevertheless, the cells of pigs can bind both "avian" and "human" types of influenza viruses suggesting that they may be important in the generation of novel variants that could go on to infect humans and spread zoonotically (Scholtissek 1990).

Influenza viruses become a pandemic threat when they become capable of being transmitted efficiently from human to human and if limited protective immunity exists in the human population. There are three major interfaces for human-to-pig contact: commercial swine production, abattoirs, and agricultural fairs (in the United States). Agricultural fairs provide common ground for the transmission of influenza viruses between humans and pigs. Fairs are unique because "they facilitate prolonged comingling of pigs from numerous sources raised under varied management programs with millions of persons who have widely disparate histories of exposure to various influenza viruses" (Bowman et al. 2012).

Prior to the 2009 pandemic, a review article identified 37 civilian and 13 military cases of swine influenza in humans in the literature from 1958–2005 (Myers et al. 2007), of which 19 cases were reported in the United States, 6 in Czechoslovakia, 4 in the Netherlands, 3 in Russia, 3 in Switzerland, 1 in Canada, and 1 in Hong Kong. The majority of the cases had some kind of exposure to live swine. There were no unique clinical features to distinguish swine from human influenza; healthy people and those with underlying conditions were both at risk (Myers et al. 2007). In a 2006 study of farmers, meat processing workers, veterinarians, and individuals lacking swine exposure, elevated titers to swine viruses were found in individuals with occupational exposure (Myers et al. 2006). Thus, it appears that even prior to 2009, transmission of swine influenza viruses to humans was not a rare event. In one paper examining the 2009 pandemic, evidence was found of at least 49 discrete introductions of H1N1pdm09 influenza virus from humans into swine in 2009 (Nelson et al. 2012), suggesting bidirectional spread of these viruses from swine to humans and backwards.

"Classic" swine influenza A (H1N1) virus (cH1N1) was the predominant subtype of swine influenza viruses in North America for nearly 80 years. The entry of the H3N2 virus in 1998 that was composed of avian, human, and swine influenza genes into the US swine population resulted in the emergence of multiple reassortment influenza viruses (Thacker and Janke 2008). The true incidence of swine-to-human transmission of influenza virus A is unknown. During the period of December 2005 to April 2012, 36 human infections with variant influenza virus A were reported by the Centers for Disease Control and Prevention in the United States. There might be far more cases than what is actually reported (CDC 2013a). The US national swine influenza virus surveillance program is passive and focuses on swine showing signs of influenza-like illness and on reacting to reports of variant influenza A cases in humans. Thus, subclinical infections are unlikely to be reported. Surveillance should instead be carried out in both healthy and sick animals (Bowman et al. 2012), but this is a costly proposal.

4.7 H1N1 2009 (Influenza A H1N1pdm09)

The earliest reports of H1N1 swine infections in the USA occurred at state fairs in Minnesota and South Dakota. An estimated 150 million people attend at fairs in North America (Bowman et al. 2012) which can be a conduit to introduce influenza viruses into swine herds or vice versa (Bowman et al. 2012; Gray et al. 2012). Eleven of fifty-seven (19%) of swine tested in Minnesota in 2009 were found to be influenza positive by rRT-PCR; four of them harbored influenza viruses similar to H1N1pdm09. This occurred during the second wave of the 2009 pandemic. It is possible that these show pigs were infected by their owners or others prior to arrival at the fair. Notably, all pigs found to be positive exhibited infections which were subclinical in nature (Gray et al. 2012), again emphasizing the need for surveillance even of animals which appear to be healthy.

4.8 H3N2 variant

H3N2 variant viruses contain the matrix gene from the 2009 H1N1 pandemic virus. These triple-reassortant swine viruses (A/Sw/OH/511445/2007, A/Ohio/01/2007, and A/Ohio/02/2007) were found both in pigs and humans in a 2007 fair in Ohio (Killian et al. 2013). In 2011, 12 cases of H3N2v were found in Indiana, Iowa, Maine, Pennsylvania, and West Virginia. In 2012, 309 cases H3N2v infections were found across 12 states. This virus had been seen in human since July 2011 and in swine in "many states." Fifteen of the sixteen cases had recent contact with pigs at a fair, and person-to-person spread was seen in at least three cases. Most cases were mild (CDC 2013b, c). Another outbreak of H3N2v occurred at a Pennsylvania fair in August of 2011. In this outbreak, one confirmed infection in a child was identified; serological studies determined that 82 additional suspected, 4 probable, and 3 confirmed cases also had attended the fair. The highest risk of transmission was in those who touched swine during their fair visit. Some reports of symptomatic pigs were noted, but all of these animals were sold or slaughtered before they could be tested (Wong et al. 2012). In 2012, 11 patients of H3N2v were hospitalized in Ohio; sporadic infections have been detected since that time (CDC 2013). Of the reported 11 cases hospitalized, 10 had direct or indirect contact with pigs. One patient, a 61-year-old woman with type 2 diabetes, died as a result of H3N2v infection (Centers for Disease C, Prevention 2012). Additional novel H3N2 viruses were detected in swine in Oklahoma in 2017, likely transmitted to pigs from humans (Zeller et al. 2018). Compared to many of the common H1 lineages, swine H3 lineages tend to be more geographically restricted (Anderson et al. 2021).

A recent study using a ferret model found that the zoonotic potential of four representative triple-reassortant swine influenza viruses caused mild disease and were inefficiently transferred via air, but one (an H1N2 virus) replicated well the upper and lower respiratory tract of ferrets, was efficiently transmitted via respiratory droplets, and showed high lethality. As such, some field isolates from swine may show zoonotic potential (Pascua et al. 2012). Although there have been multiple outbreaks of H3N2v in recent years, a recent analysis suggests that the current pandemic potential of H3N2v is low (Skowronski et al. 2013).

Individuals working with swine or visiting swine fairs are urged to practice good hygiene and the use of proper personal protective equipment (PPE) while working with pig or pig facilities. Isolation of sick or infected pigs, partial depopulation and segregation of early weaned piglets would help to prevent the transmission of swine influenza. Inactivated, whole virus and subunit influenza virus vaccines are available for humans, horses, birds, and pigs (Kothalawala et al. 2006). However, due to ongoing mutations in the HA and NA genes via antigenic drift, these vaccines need to be revised annually. Due to its ability to elicit both humoral and cellular immune responses, a live attenuated influenza virus vaccine is considered relatively better compared with an inactivated vaccine; it is currently licensed in the United States (Schnitzler and Schnitzler 2009). Researchers have been studying DNA vaccines as a novel alternative to the conventional vaccines using chicken, mouse, ferret, and

primate models. Nonetheless, so far it has not been successful in pigs (Thacker and Janke 2008; Kothalawala et al. 2006).

4.9 Swine Parasitic Zoonoses: *Trichinella* and *Taenia*

Parasites with zoonotic potential such as *Trichinella* spp. and *Taenia* spp. are public health hazards affecting both animals and humans. Global eradication of these pathogens is a challenge due to limitations in implementation of strict regulations and policies, methodological issues in early diagnosis and treatment, and above all varying cultural and social practices that govern the propagation of these parasites.

Trichinella spp. are nematode worms that are one of the most widespread food-borne zoonotic pathogens in the world (Pozio 2007). Strains of *Trichinella* have been isolated from domestic and wild animals in 66 countries (Pozio 2007). This parasite was first discovered in 1835, but was linked to food-consumption and disease only in 1860 (Dupouy-Camet 2000). The main source of human infection is pork and pork products, game meat, and horse meat. Nevertheless, *Trichinella* spp. has been reported to infect other animals such as wild boars, rats, cats, dogs, bear, walrus, jackals, raccoon, foxes, warthogs, crocodiles, lizards, and even birds (Pozio et al. 2009). Due to its vast host range and difficulties in identification of the pathogen or establishing an early diagnosis, *Trichinella* is one of the most resilient and persistent zoonotic parasites.

Taeniasis caused by *Taenia* spp. (tapeworm) is a food-borne infection commonly observed in developing or less developed countries and adds to the global public health burden of parasitic infections. To meet the scope of this chapter, we will discuss only *Taenia solium* (*T. solium*), since this parasite is commonly observed in pigs. *T. solium* (pork tapeworm) infections are commonly referred to as the taeniasis/cysticercosis complex (Garcia et al. 2003a). Cysticercosis is endemic in Central and South America, sub-Saharan Africa, most of Asia, and parts of Oceania (Garcia et al. 2003b). Neurocysticercosis is the most common cause of late adult-onset seizures in the developing world (Epilepsy CoTDotILA 1994). More than 1000 new cases of neurocysticercosis (Hawk et al. 2005) are diagnosed in the United States each year, and it is the most prevalent infection of the brain, worldwide (Garcia et al. 2003b; Shandera et al. 1994).

4.9.1 Trichinella

The genus *Trichinella* has a broad range of host species. Nevertheless, clinical infection is apparent only in humans (Gottstein et al. 2009). *Trichinella* spp. are found in animals all over the world, except Antarctica possibly due to absence of surveillance (Pozio 2007). Parasites in the genus *Trichinella* are classified by the presence of a collagen capsule: encapsulated and nonencapsulated. Within the genus, *T. spiralis* is the species most adapted to domestic and wild pigs and is also most

commonly isolated from human infections (Pozio and Darwin Murrell 2006). Other *Trichinella* spp. found in pigs are *T. britovi* (wild boar), *T. nelsoni* (bush pigs), *T. pseudospiralis* (domestic and wild pigs). Horses fattened with pork scraps are known to be infected with *Trichinella*.

Prevalence estimates depend on the geographical region and the detection methods used: *Trichinella* antibodies were found in 0.35% of pigs in the Netherlands (van der Giessen et al. 2007), 0.2% of wild boar muscle samples contained *Trichinella* in Spain (Boadella et al. 2012), a 0.37% prevalence was estimated in pigs in the northeastern United States (Gamble et al. 1999), in China prevalence data ranged from 0.01% to 29.95% by serological testing to 0–5.75% in pigs slaughtered at abattoirs, respectively (Cui and Wang 2011), 19.9% Vietnam pigs tested positive by E/S ELISA (Vu Thi et al. 2010), antibody prevalence was 0.0002–0.0003% in wild boars in France (Pozio et al. 1996), a prevalence of 1.3% was established for wild boars in Finland (Oivanen et al. 2002), and of 11.4% wild boars in Argentina by artificial digestion (Cohen et al. 2010). Despite a broad spectrum of hosts, *Trichinella* spp. are most common in porcine omnivores, mainly in domestic pigs, different races of wild pigs, wild boars, bush pigs, and warthogs (Pozio 2005).

Human trichinellosis has been documented in 55 countries and is most often linked to established food-consumption behaviors including consumption of raw or undercooked pork or pork products. A 2011 study observed about 261 reports of trichinellosis outbreaks worldwide (Murrell and Pozio 2011). This study estimated 65,818 cases and 42 deaths reported from 41 countries between 1989 and 2009 (Murrell and Pozio 2011). About 87% of the cases were reported from the WHO-European region, of these 50% were reported from Romania alone (Murrell and Pozio 2011).

Trichinellosis is the infection caused by *Trichinella* spp. in humans. Severity of trichinellosis depends on the load of ingested parasite, frequency of consumption of infected meat, method of cooking and treating meat before consumption, species involved in reproduction of larvae, and the amount of alcohol consumed at the time of meat consumption, with alcohol acting as a protective factor (Murrell and Pozio 2011; Xu et al. 1995). The estimated minimum dose necessary for causing symptomatic trichinellosis ranges from 70 to 150 larvae (Murrell and Bruschi 1994; Dupouy-Camet et al. 2002). The major signs of classical trichinellosis are myalgia, diarrhea, fever, periorbital and facial edema, and headaches as per an algorithm used to diagnose acute trichinellosis (Murrell and Pozio 2011). Complications usually develop within 2 weeks of infection (Ancelle et al. 2005; Lachkar et al. 2008; Dupouy-Camet 2007; Bessoudo et al. 1981; Compton et al. 1993; Ellrodt et al. 1987; Fourestie et al. 1993). Mortality due to trichinellosis is very rare and is estimated to be around 0.4% (Murrell and Pozio 2011; Dupouy-Camet 2007; Kociecka 2000; Ancelle et al. 1988). Humans are observed to be asymptomatic while being chronically infected with the larvae (Dupouy-Camet et al. 2002). Children are found to be more resilient to *Trichinella* infection, while it may result in abortion or premature delivery in pregnant women (Dupouy-Camet et al. 2002).

Globalization and migration of population with culturally unique food practices and illegal introduction of *Trichinella* infected meat from endemic to non-endemic

countries are some of the risk factors for human trichinellosis particularly in countries with low-incidence (Pozio and Marucci 2003; Gallardo et al. 2007; Nockler et al. 2007; Stensvold et al. 2007). Impact of food-consumption practices are reflected by the low incidence of trichinellosis in the Muslim population worldwide (Pozio 2007; Haim et al. 1997; Marva et al. 2005). International travelers and hunters are two high-risk groups observed to acquire *Trichinella* infection from endemic countries and exposure to *Trichinella* infected animals, respectively (Ancelle et al. 2005; Moller et al. 2005a; Wang et al. 2006; Shiota et al. 1999; Nakamura et al. 2003; Centers for Disease Control & Prevention 2004). This population also plays a major role in propagation of *Trichinella* to a naïve population.

Diagnosis of *Trichinella* infection in animals is conducted by direct methods such as meat inspection using conventional trichinoscopy (Epizooties OId 2004; Kapel 2005; Nockler and CMO 2007). Other methods used in animal detection are artificial digestion using the magnetic stirrer method (Kapel et al. 2005), multiplex PCR (Pozio and La Rosa 2003), and serological tests for IgG antibodies (Nockler et al. 2005). Enzyme-linked immunosorbent assay (ELISA) using metabolic E/S antigens or tyvelose ELISA are the most commonly used methods for the detection and confirmation of *Trichinella* infection (Moller et al. 2005b; Gamble and Graham 1984; Gamble et al. 1983). Diagnosis in humans is based on clinical, epidemiological and laboratory criteria, as outlined by the European Center for Disease Control (Dupouy-Camet 2007).

Initiation of anthelminthic therapy such as albendazole and mebendazole early in the infection is recommended and observed to be beneficial for the cure of trichinellosis (Dupouy-Camet et al. 2002). Other treatment options are available based on the severity of infection (Gottstein et al. 2009; Dupouy-Camet et al. 2002; Dupouy-Camet 2007). Prognosis is reported to be poor for severe cases with cardiac or cerebral complications and fatality was found to be about 5% in severe infections despite therapy (Gottstein et al. 2009).

4.10 Taenia (*T. solium*)

The life cycle of *T. solium* is divided between two hosts. Humans are the definitive host for the adult *T. solium*, while both pigs and humans may harbor the larvae or cysticerci with pigs being the typical intermediate host. Human infection due to *T. solium* occurs when larvae are consumed by eating poorly cooked or raw pork products. Larvae attach to the mucosa of the human small intestine using their scolex (head) and grow into adult tapeworms (Flisser 1994). Eggs from these adult worms are shed in human feces. Contamination of pig feed with such human feces results in ingestion of eggs by the pigs. Ingested eggs develop into a larval stage, travel through the intestinal wall, enter the bloodstream, and lodge in various pig tissues eventually forming cysts (porcine cysticercosis) (Garcia et al. 2003a). Ingestion of such infected pig or pork products by humans result in intestinal infection or taeniasis. Humans can also ingest *T. solium* eggs through the fecal-oral route or by auto-infection (Hawk et al. 2005). Autoinfection is the retrograde transmission of

taenia segments from human intestine back into the stomach, followed by release of eggs into the gut (Garcia et al. 2003b; Hawk et al. 2005; Flisser et al. 2006). Fecal-oral contamination occurs in infected food handlers who do not practice good hand hygiene. Even vegetarians who do not consume pork may acquire cysticercosis via wind, water, flies, and other indirect means of transmission (Martinez et al. 2000).

Clinical manifestation of cysticercosis depends on the organ affected (Garcia et al. 2003b). The most common clinical manifestation is neurocysticercosis that develops once the viable cysticerci enter the central nervous system. Epileptic seizures are the most common presentation of neurocysticercosis. Neurocysticercosis and ocular cysticercosis are associated with significant morbidity (Garcia et al. 2005). Other types of extraneural cysticercosis are subcutaneous, muscular, cardiac, and – in rare cases – limb enlargement due to massive parasite burdens (muscular pseudohypertrophy) (Garcia et al. 2003a).

T. solium is one of the major public health hazards and causes of economic problems in pig husbandry (Flisser et al. 2005). Transmission of adult tapeworms and larvae are associated with poor hygiene and sanitation, low living standards, lack of meat inspection and control, and lack of education and awareness (Flisser et al. 2006). In addition, immigrants, overseas domestic workers, international travelers, and transport of infected pigs have spread the disease to non-endemic areas (Hira et al. 2004; Rajshekhar et al. 2003). *T. solium* is widely prevalent in regions where pigs are reared in free ranging systems and raw or undercooked pork is consumed. In most endemic villages, more than 10% of the population is observed to be seropositive for *T. solium* with an observed maximum of 25% (Garcia et al. 2003b). Studies have found that up to 6% of the general population in endemic countries may harbor adult tapeworms (Allan et al. 1996a). A recent meta-analysis on the prevalence of neurocysticercosis in people with epilepsy including studies from Latin America, India, and sub-Saharan Africa found that neurocysticercosis was the cause of epilepsy in 30% of the population with epilepsy (Ndimubanzi et al. 2010). Cases of intestinal taeniasis are consistently observed to cluster in families possibly due to food consumption habits (Allan et al. 1996b). Rate of porcine infection varies and seropositivity has been observed in 30–60% pigs (Garcia et al. 1999; Gonzalez et al. 1990).

Diagnosis of cysticercosis is based on a set of criteria developed using clinical, radiologic, histologic, and epidemiologic findings (Garcia and Del Brutto 2003; Kraft 2007). Neurocysticercosis is diagnosed using a computed tomography (CT) scan and performing a cerebrospinal fluid (CSF) examination (Garcia et al. 2005; Garcia and Del Brutto 2003; Kraft 2007). The MRI is a better tool in detecting certain pathological changes (Garcia et al. 2003a). Antibodies against *T. solium* generally persist even after the cyst dies, hence serology should be used as a confirmatory test secondary to clinical signs and imaging studies (Garcia et al. 2005). Frequency of stool examination for *T. solium* eggs vary among patients and may be related to the severity of infection (Garcia and Del Brutto 1999; Gilman et al. 2000). In addition, ELISA and PCR tests for coproantigen detection may be useful in screening for *T. solium* carriers in endemic regions (Mahanty et al. 2010).

Antiparasitic treatment such as albendazole and praziquantel in conjunction with steroids are the treatment of choice in most types of cysticercosis (Baranwal et al.

1998, 2001; Carpio et al. 1995; Padma et al. 1994; Padma et al. 1995; Corona et al. 1996; Pretell et al. 2001). Studies have shown 100% effectiveness using the TSOL18 vaccine developed against *T. solium* in pigs (Gonzalez et al. 2005; Flisser et al. 2004; Lightowlers 2004). The vaccine requires at least two doses to be effective.

Some preventative strategies for parasitic infections such as *Trichinella* and *T. solium* are improved living conditions by better sanitation, education and commercial pig production, regular monitoring and mandatory reporting for the pathogen (Nockler and Kapel 2007; Community E 2005), strict regulations in pig-farms and slaughterhouses (Gottstein et al. 2009), adequate vaccination of pigs, health education and human mass chemotherapy (Gottstein et al. 2009), pig corralling, and regulations for processing pork and pork products (Garcia et al. 2007; Hill et al. 2010). However, these preventative practices are not implemented in high-risk countries such as Eastern Europe, Asia, parts of South America, and in the African subcontinent. These regions have the potential for exponential increase in transmission of pathogens due to the dense pig population, interaction between humans and pigs, and the vast reservoir for *Trichinella* in regional wildlife. Human cultural and social practices are one of the greatest challenges in prevention of transmission of food-borne parasites such as *Trichinella* and *T. solium* on a global basis.

4.11 Discussion

In recent decades, the global emergence of infectious diseases in human, domestic animals, and wildlife have attracted a greater attention of researchers and agencies. As a result, studies have now demonstrated zoonotic pathogens by quantitative analysis as risk factors for emergence in humans (Cleaveland et al. 2001). Rapid growth of human population and globalization of trade are considered to be main factors responsible for emergence of zoonotic diseases. However, several other direct and indirect factors such as ecological disruption, increasing movement of animal species, uncultivatable organisms, and terrorism play potential role in disease expansion (Brown 2004). A study by Jones *et al.* found a significant correlation between emerging infectious disease origin and socio-economic, environmental, and ecological factors (Jones et al. 2008).

Zoonotic diseases account for 75% of all emerging diseases affecting the human population in the last two decades (Brown 2004). Almost two-thirds (61%) of human infectious diseases are zoonotic in nature. A database of disease causing pathogens of humans and domestic mammals constructed by Cleaveland *et al.* showed that 61.6% of 1415 pathogens able to cause human diseases have an animal origin. A high prevalence of multi-host pathogens were observed in both human and domestic mammal pathogens, suggesting cross-species transmission (Cleaveland et al. 2001). The COVID-19 pandemic demonstrates once again how vulnerable we are to such emerging zoonotic pathogens, and susceptibility of pigs to the virus seems to be low but with mixed results to date (Pickering et al. 2021; Vergara-Alert et al. 2021).

Recent decades have witnessed the rapid growth of pork industry (Pappas 2013). In fact, pork is the most widely eaten meat in the world, leading to the production of 1.3

billion pigs each year globally (Health CfFSaP 2013). Global increase in pork production and predicted increase in annual pork demand have reached 80% and 7%, respectively (Pappas 2013). However, the projected human population growth still outcompetes the meat supply. This increase in demand of pork production has led to intensification and industrialization of production systems. Production modifications with larger herds in a contained area have the potential to make pigs and their caretakers increasingly vulnerable to inter-species pathogen transmission (Pappas 2013).

A recent review of emerging pig pathogens revealed at least 77 novel emerging species, of which 35 were pig-specific zoonotic (Pappas 2013) suggesting an urgent need of considering pigs as a potential vehicle for zoonotic disease transmission. The pig may also serve as an amplifying host, as is the case for both Japanese encephalitis virus and Nipah viruses discussed above (Weaver and Barrett 2004; Pappas 2013).

The economic burden imposed by zoonotic diseases is paramount. It has been estimated that the zoonotic epidemics between the period of 1995 and 2008 caused more than 120 billion dollars in economic loss. Major recent infectious disease outbreaks have been zoonotic, leading to unprecedented human morbidity and mortality causing greater human productivity loss (Cascio et al. 2011). For example, cysticercosis alone, a parasitic infection that is caused by uncooked or undercooked pork, imposes an estimated economic burden between US $18.6 million and US$34.2 million in the eastern cape of South Africa (Carabin et al. 2006).

As zoonotic infections go beyond the individual and affect the household, especially in agricultural settings, the economic impact is even more pronounced. Animal loss due to disease or mandatory regulations adds more to the economic burden of zoonoses. For example, the slaughter of more than one million pigs in Malaysia in 1999 in the wake of Nipah virus outbreak caused destruction of the local swine industry. It is predicted that the majority of future infectious disease outbreaks will be zoonotic in origin (Cascio et al. 2011), potentially amplifying this cycle.

4.12 Conclusions/Recommendations

Zoonotic diseases directly impact human morbidity and mortality. An indirect impact of zoonotic diseases is via disruption of the food chain, thus causing grave economic loss. The globe is under the threat of emergence of new species of zoonotic pathogens and their pandemic potential, such as the H1N1 pandemic of 2009 and the COVID-19 pandemic. Public health intervention programs to tackle the rapid emergence of zoonoses are essential, but underfunded and understaffed. Public health interventions should be designed in the light of evidence-based practices to address the zoonotic diseases that are often neglected and associated with poverty such as zoonotic helminthes, protozoan, viral and bacterial infections. Disease-specific research priorities to support zoonotic disease control are crucial to combat the epidemics and pandemics of zoonoses (World Health Organization 2012). What was effective in the mitigation of zoonotic diseases epidemics or pandemics a decade ago could be antediluvian in the current era largely due to rapid human growth, transportation, movement, and human migration. Early detection, rapid and enhanced surveillance,

and ongoing research for the discovery of new knowledge are warranted for the effective mitigation of emerging zoonotic infectious diseases. A long-term socio-political and economic commitment by government and private agencies across the globe is essential to combat the global threat of zoonotic diseases. Policy-makers should consider prioritizing for improved surveillance, multi-sectorial interactions between public health, livestock, agriculture, natural resource and wildlife, and a measure to precisely assess the burden of zoonoses (World Health Organization 2012).

Practice of good personal hygiene such as proper hand washing after working with animal and animal products, use of proper personal protective equipment (PPE) while working with animal or animal facilities, isolation of sick or infected animal, and good practice of cleaning and disinfection would help to prevent the zoonotic disease infection and transmission. High-risk-groups should get vaccinated if one is available.

References

Aarestrup FM, Jorsal SE, Jensen NE (1998) Serological characterization and antimicrobial susceptibility of *Streptococcus suis* isolates from diagnostic samples in Denmark during 1995 and 1996. Vet Microbiol 60:59–66

Acheson DWK (1999) Shiga toxin-producing *Escherichia coli*. Clin Microbiol Newsl 21:183–188

Ackers ML, Schoenfeld S, Markman J, Smith MG, Nicholson MA et al (2000) An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk. J Infect Dis 181: 1834–1837

Agriculture FSaISUDo (2004) Progress report on salmonella testing of raw meat and poulty products, 1998–2001

Aires-de-Sousa M, Conceicao T, de Lencastre H (2006) Unusually high prevalence of nosocomial Panton-Valentine leukocidin-positive *Staphylococcus aureus* isolates in Cape Verde Islands. J Clin Microbiol 44:3790–3793

Aleksic S, Heinzerling F, Bockemuhl J (1996) Human infection caused by Salmonellae of subspecies II to VI in Germany, 1977-1992. Zentralbl Bakteriol 283:391–398

Allan JC, Velasquez-Tohom M, Torres-Alvarez R, Yurrita P, Garcia-Noval J (1996a) Field trial of the coproantigen-based diagnosis of *Taenia solium* taeniasis by enzyme-linked immunosorbent assay. Am J Trop Med Hyg 54:352–356

Allan JC, Velasquez-Tohom M, Garcia-Noval J, Torres-Alvarez R, Yurrita P et al (1996b) Epidemiology of intestinal taeniasis in four, rural, Guatemalan communities. Ann Trop Med Parasitol 90:157–165

Ancelle T, Dupouy-Camet J, Bougnoux ME, Fourestie V, Petit H et al (1988) Two outbreaks of trichinosis caused by horsemeat in France in 1985. Am J Epidemiol 127:1302–1311

Ancelle T, De Bruyne A, Poisson D, Dupouy-Camet J (2005) Outbreak of trichinellosis due to consumption of bear meat from Canada, France, September 2005. Euro Surveill 10 (E051013):051013

Anderson TC, Nguyen TA, Adams JK, Garrett NM, Bopp CA, Baker JB, McNeil C, Torres P, Ettestad PJ, Erdman MM, Brinson DL, Gomez TM, Barton Behravesh C (2016) Multistate outbreak of human *Salmonella* Typhimurium infections linked to live poultry from agricultural feed stores and mail-order hatcheries, United States 2013. One Health 2:144–149

Anderson TK, Chang J, Arendsee ZW, Venkatesh D, Souza CK, Kimble JB, Lewis NS, Davis CT, Vincent AL (2021) Swine influenza A viruses and the tangled relationship with humans. Cold Spring Harb Perspect Med 11(3):a038737

Arends JP, Zanen HC (1988) Meningitis caused by *Streptococcus suis* in humans. Rev Infect Dis 10:131–137

Arends JP, Hartwig N, Rudolphy M, Zanen HC (1984) Carrier rate of *Streptococcus suis* capsular type 2 in palatine tonsils of slaughtered pigs. J Clin Microbiol 20:945–947

Arif SM, Basher A, Quddus MR, Faiz MA (2012) Re-emergence Nipah - a review. Mymensingh Med J 21:772–779

Armand-Lefevre L, Ruimy R, Andremont A (2005) Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11:711–714

Aslan AT, Balaban HY (2020) Hepatitis E virus: epidemiology, diagnosis, clinical manifestations, and treatment. World J Gastroenterol 26(37):5543–5560

Aspiroz C, Lozano C, Vindel A, Lasarte JJ, Zarazaga M et al (2010) Skin lesion caused by ST398 and ST1 MRSA, Spain. Emerg Infect Dis 16:157–159

Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschuk VF (1983) Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 20(1):23–31

Baranwal AK, Singhi PD, Khandelwal N, Singhi SC (1998) Albendazole therapy in children with focal seizures and single small enhancing computerized tomographic lesions: a randomized, placebo-controlled, double blind trial. Pediatr Infect Dis J 17:696–700

Baranwal AK, Singhi PD, Singhi SC, Khandelwal N (2001) Seizure recurrence in children with focal seizures and single small enhancing computed tomographic lesions: prognostic factors on long-term follow-up. J Child Neurol 16:443–445

Barker IK, Van Dreumel AA (1985) Pathology of domestic animals. In: Jubb KVF, Kennedy PC, Palmer N (eds) Pathology of domestic animals. Academic Press, Orlando, pp 1–239

Bartelink AK, van Kregten E (1995) *Streptococcus suis* as threat to pig-farmers and abattoir workers. Lancet 346:1707

Baums CG, Verkuhlen GJ, Rehm T, Silva LM, Beyerbach M et al (2007) Prevalence of *Streptococcus suis* genotypes in wild boars of Northwestern Germany. Appl Environ Microbiol 73:711–717

Beilei Ge SZ, Hall R, Meng J (2002) A PCR-ELISA for detecting Shiga toxin-producing *Escherichia coli*. Microb Infect 4:285–290

Bell C (2002) Approach to the control of entero-haemorrhagic *Escherichia coli* (EHEC). Int J Food Microbiol 78:197–216

Beltran P, Musser JM, Helmuth R, Farmer JJ 3rd, Frerichs WM et al (1988) Toward a population genetic analysis of *Salmonella*: genetic diversity and relationships among strains of serotypes S. choleraesuis, S. derby, S. dublin, S. enteritidis, S. heidelberg, S. infantis, S. newport, and S. typhimurium. Proc Natl Acad Sci U S A 85:7753–7757

Bessoudo R, Marrie TJ, Smith ER (1981) Cardiac involvement in trichinosis. Chest 79:698–699

Bhaduri S, Wesley IV, Bush EJ (2005) Prevalence of pathogenic *Yersinia enterocolitica* strains in pigs in the United States. Appl Environ Microbiol 71:7117–7121

Bhat M, Dumortier C, Taylor BS, Miller M, Vasquez G et al (2009) *Staphylococcus aureus* ST398, New York City and Dominican Republic. Emerg Infect Dis 15:285–287

Black RE, Jackson RJ, Tsai T, Medvesky M, Shayegani M et al (1978) Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. N Engl J Med 298:76–79

Blum LS, Khan R, Nahar N, Breiman RF (2009) In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. Am J Trop Med Hyg 80:96–102

Boadella M, Barasona JA, Pozio E, Montoro V, Vicente J et al (2012) Spatio-temporal trends and risk factors for *Trichinella* species infection in wild boar (Sus scrofa) populations of Central Spain: a long-term study. Int J Parasitol 42:739–745

Boqvist S, Pettersson H, Svensson A, Andersson Y (2009) Sources of sporadic *Yersinia enterocolitica* infection in children in Sweden, 2004: a case-control study. Epidemiol Infect 137:897–905

Bottone EJ (1997) *Yersinia enterocolitica*: the charisma continues. Clin Microbiol Rev 10:257–276

Bottone EJ (1999) *Yersinia enterocolitica*: overview and epidemiologic correlates. Microbes Infect 1:323–333

Bowman AS, Nolting JM, Nelson SW, Slemons RD (2012) Subclinical influenza virus A infections in pigs exhibited at agricultural fairs, Ohio, USA, 2009-2011. Emerg Infect Dis 18:1945–1950

Boyapalle S, Wesley IV, Hurd HS, Reddy PG (2001) Comparison of culture, multiplex, and 5′ nuclease polymerase chain reaction assays for the rapid detection of *Yersinia enterocolitica* in swine and pork products. J Food Prot 64:1352–1361

Breton J, Mitchell WR, Rosendal S (1986) *Streptococcus suis* in slaughter pigs and abattoir workers. Can J Vet Res 50:338–341

Brown C (2004) Emerging zoonoses and pathogens of public health significance – an overview. Rev Sci Tech 23:435–442

Byun JW, Yoon SS, Lim SK, Lee OS, Jung BY (2011) Hepatic yersiniosis caused by *Yersinia enterocolitica* 4:O3 in an adult dog. J Vet Diagn Investig 23:376–378

Campos ACA, Goes LGB, Moreira-Soto A, de Carvalho C, Ambar G, Sander AL, Fischer C, Ruckert da Rosa A, Cardoso de Oliveira D, Kataoka APG, Pedro WA, Martorelli LFA, Queiroz LH, Cruz-Neto AP, Durigon EL, Drexler JF (2019) Bat Influenza A(HL18NL11) Virus in Fruit Bats, Brazil. Emerg Infect Dis 25(2):333–337

Capua I, Munoz O (2013) Emergence of influenza viruses with zoonotic potential: open issues which need to be addressed. A review. Vet Microbiol 165:7–12

Carabin H, Krecek RC, Cowan LD, Michael L, Foyaca-Sibat H et al (2006) Estimation of the cost of *Taenia solium* cysticercosis in eastern Cape Province, South Africa. Tropical Med Int Health 11:906–916

Carniel E, Hinnebusch BJ (2012) *Yersinia*: systems biology and control. Caister Academic Press, Norfolk

Carpio A, Santillan F, Leon P, Flores C, Hauser WA (1995) Is the course of neurocysticercosis modified by treatment with antihelminthic agents? Arch Intern Med 155:1982–1988

Cascio A, Bosilkovski M, Rodriguez-Morales AJ, Pappas G (2011) The socio-ecology of zoonotic infections. Clin Microbiol Infect 17:336–342

Catalina Lopez-Saucedo JFC, Villegas-Sepulveda N, Rocio T, Raul Velazquez F, Torres J, Tarr PI, Estrada-Gracia T (2003) Single multiplex polymerase chain reaction to detect diverse loci associated with diarrhegenic *Escherichia coli*. Emerg Infect Dis 9:127–131

CDC (2008) Salmonella surveillance: annual summary, 2006. US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta

CDC (2009) Salmonellosis. Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID). Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)

CDC (2010) Prevention

CDC (2012a) *Salmonella* surveillance: annual summary, 2009. US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta

CDC (2012b) *Escherichia coli*. Centers for Disease Control and Prevention, Atlanta

CDC (2013a) Increase in influenza A H3N2v virus infections in three U.S. states. Retrieved 13 Oct 2013, from http://www.bt.cdc.gov/han/han00325.asp

CDC (2013b) Influenza A (H3N2) variant virus. http://www.cdc.gov/flu/swineflu/h3n2v-cases.htm

CDC (2013c) Increase in influenza A H3N2v virus infections in three U.S. states

CDC (2021) Japanese encephalitis: diagnostic testing. Retrieved 4 Jan 2021, from https://www.cdc.gov/japaneseencephalitis/healthcareproviders/healthcareproviders-diagnostic.html

Centers for Disease C, Prevention (2004) Trichinellosis associated with bear meat–New York and Tennessee, 2003. MMWR Morb Mortal Wkly Rep 53:606–610

Centers for Disease C, Prevention (2012) Influenza A (H3N2) variant virus-related hospitalizations: Ohio, 2012. MMWR Morb Mortal Wkly Rep 61:764–767

Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE et al (2006) Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 12:235–240

Cheleste M, Thorpe JMR, Acheson DWK (2002) Enterohemorrhagic and other Shiga toxin-producing *Escherichia coli*. In: Donnenberg MS (ed) *Escherichia coli*: virulence mechanism of a versatile pathogen. Academic Press, San Diego, pp 119–154

Chimalizeni Y, Kawaza K, Molyneux E (2010) The epidemiology and management of non typhoidal *Salmonella* infections. Adv Exp Med Biol 659:33–46

Ching PK, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF Jr, Bolo GC Jr, Sejvar JJ, Eagles D, Playford G, Dueger E, Kaku Y, Morikawa S, Kuroda M, Marsh GA, McCullough S, Foxwell AR (2015) Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 21(2):328–331

Chlebicz A, Slizewska K (2018) Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. Int J Environ Res Public Health 15(5):863

Christensen SG (1979) Isolation of *Yersinia enterocolitica* O:3 from a well suspected as the source of yersiniosis in a baby. Acta Vet Scand 20:154–156

Chua KB (2003) Nipah virus outbreak in Malaysia. J Clin Virol 26:265–275

Chua KB (2012) Introduction: Nipah virus–discovery and origin. Curr Top Microbiol Immunol 359:1–9

Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS et al (1999) Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 354:1257–1259

Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A et al (2000) Nipah virus: a recently emergent deadly paramyxovirus. Science 288:1432–1435

Chua KB, Wong EM, Cropp BC, Hyatt AD (2007) Role of electron microscopy in Nipah virus outbreak investigation and control. Med J Malaysia 62:139–142

Cleaveland S, Laurenson MK, Taylor LH (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos Trans R Soc Lond Ser B Biol Sci 356:991–999

Clifton-Hadley FA, Alexander TJ, Upton I, Duffus WP (1984) Further studies on the subclinical carrier state of *Streptococcus suis* type 2 in pigs. Vet Rec 114:513–518

Cohen M, Costantino SN, Calcagno MA, Blanco GA, Pozio E et al (2010) *Trichinella* infection in wild boars (*Sus scrofa*) from a protected area of Argentina and its relationship with the presence of humans. Vet Parasitol 169:362–366

Community E (2005) Regulation (EC) no. 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for Trichinella in meat. Off J Eur Commun 330:60–82

Compton SJ, Celum CL, Lee C, Thompson D, Sumi SM et al (1993) Trichinosis with ventilatory failure and persistent myocarditis. Clin Infect Dis 16:500–504

Cornick NA, Helgerson AF (2004) Transmission and infectious dose of *Escherichia coli* O157:H7 in swine. Appl Environ Microbiol 70:5331–5335

Corona T, Lugo R, Medina R, Sotelo J (1996) Single-day praziquantel therapy for neurocysticercosis. N Engl J Med 334:125

Cui J, Wang ZQ (2011) An epidemiological overview of swine trichinellosis in China. Vet J 190: 323–328

Davies PR (2012) One world, one health: the threat of emerging swine diseases. A North American perspective. Transbound Emerg Dis 59:18–26

Davies PR, Morrow WE, Jones FT, Deen J, Fedorka-Cray PJ et al (1997) Prevalence of *Salmonella* in finishing swine raised in different production systems in North Carolina, USA. Epidemiol Infect 119:237–244

de Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, de Neeling AJ et al (2009) Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int J Food Microbiol 134:52–56

De Sabato L, Amoroso MG, Ianiro G, Esposito C, De Grossi L, Fusco G, Barone A, Martini E, Ostanello F, Di Bartolo I (2020) Detection of hepatitis E virus in livers and muscle tissues of wild boars in Italy. Food Environ Virol 12(1):1–8

De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W et al (2009) Bergey's manual of systematic bacteriology. Springer, New York. <v. 1-2, 4-5 in 7> p

Declercq P, Petre D, Gordts B, Voss A (2008) Complicated community-acquired soft tissue infection by MRSA from porcine origin. Infection 36:590–592

Desai PT, Porwollik S, Long F, Cheng P, Wollam A et al (2013) Evolutionary genomics of *Salmonella* enterica subspecies. MBio 4:501

Devriese LA, Haesebrouck F (1992) *Streptococcus suis* infections in horses and cats. Vet Rec 130:380

Devriese LA, Cruz Colque JI, De Herdt P, Haesebrouck F (1992) Identification and composition of the tonsillar and anal enterococcal and streptococcal flora of dogs and cats. J Appl Bacteriol 73:421–425

Doceul V, Bagdassarian E, Demange A, Pavio N (2016) Zoonotic hepatitis E virus: classification, animal reservoirs and transmission routes. Viruses 8(10):270

Dupouy-Camet J (2000) Trichinellosis: a worldwide zoonosis. Vet Parasitol 93:191–200

Dupouy-Camet JB (2007) Management and diagnosis of human trichinellosis. In: Dupouy-Camet JaM KD (ed) FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO) and World Organization for Animal Health (OIE), Paris, pp 37–68

Dupouy-Camet J, Kociecka W, Bruschi F, Bolas-Fernandez F, Pozio E (2002) Opinion on the diagnosis and treatment of human trichinellosis. Expert Opin Pharmacother 3:1117–1130

Ebel E, Schlosser W (2000) Estimating the annual fraction of eggs contaminated with *Salmonella* enteritidis in the United States. Int J Food Microbiol 61:51–62

EFSA (2009) The European Union summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in 2009. EFSA J 9. Electronic document, https://www.efsa.europa.eu/en/efsajournal/pub/2090

Elbers AR, Vecht U, Osterhaus AD, Groen J, Wisselink HJ et al (1999) Low prevalence of antibodies against the zoonotic agents *Brucella abortus*, *Leptospira* spp., *Streptococcus suis* serotype II, hantavirus, and lymphocytic choriomeningitis virus among veterinarians and pig farmers in the southern part of The Netherlands. Vet Q 21:50–54

Ellrodt A, Halfon P, Le Bras P, Halimi P, Bouree P et al (1987) Multifocal central nervous system lesions in three patients with trichinosis. Arch Neurol 44:432–434

Enright MR, Alexander TJ, Clifton-Hadley FA (1987) Role of houseflies (Musca domestica) in the epidemiology of *Streptococcus suis* type 2. Vet Rec 121:132–133

Epilepsy CoTDotILA (1994) Relationship between epilepsy and tropical diseases. Epilepsia 35: 89–93

Epizooties OId (2004) Trichinellosis, chapter 2.2.9. In manual of standards for diagnostic tests and vaccines, 5th edn. Office International des Epizooties, Paris

Erlanger TE, Weiss S, Keiser J, Utzinger J, Wiedenmayer K (2009) Past, present, and future of Japanese encephalitis. Emerg Infect Dis 15:1–7

Fanoy E, Helmhout LC, van der Vaart WL, Weijdema K, van Santen-Verheuvel MG et al (2009) An outbreak of non-typeable MRSA within a residential care facility. Euro Surveill 14:1

FAO (2017) Food outlook: biannual reports on global food markets

FAO/WHO (2011) Enterohaemorrhagic *Escherichia coli* in raw beef and beef products: approaches for the provision of scientific advice: meeting report. FAO/WHO [Food and Agriculture Organization of the United Nations/World Health Organization], 126 p

Fischer ML, Staples JE, Hills S (2010) Japanese encephalitis vaccines: recommendation of the Advisory Committee on Immunization Practices (ACIP). Centers for Disease Control. 1–27 p

Fittipaldi N, Segura M, Grenier D, Gottschalk M (2012) Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. Future Microbiol 7:259–279

Fitzgerald JR (2012) Livestock-associated *Staphylococcus* aureus: origin, evolution and public health threat. Trends Microbiol 20:192–198

Flisser A (1994) Taeniasis and cysticercosis due to *Taenia solium*. Prog Clin Parasitol 4:77–116

Flisser A, Gauci CG, Zoli A, Martinez-Ocana J, Garza-Rodriguez A et al (2004) Induction of protection against porcine cysticercosis by vaccination with recombinant oncosphere antigens. Infect Immun 72:5292–5297

Flisser A, Vazquez-Mendoza A, Martinez-Ocana J, Gomez-Colin E, Leyva RS et al (2005) Short report: evaluation of a self-detection tool for tapeworm carriers for use in public health. Am J Trop Med Hyg 72:510–512

Flisser A, Rodriguez-Canul R, Willingham AL 3rd (2006) Control of the taeniosis/cysticercosis complex: future developments. Vet Parasitol 139:283–292

Fluit AC (2012) Livestock-associated *Staphylococcus aureus*. Clin Microbiol Infect 18:735–744

Fosse J, Seegers H, Magras C (2009) Prevalence and risk factors for bacterial food-borne zoonotic hazards in slaughter pigs: a review. Zoonoses Public Health 56:429–454

Fourestie V, Douceron H, Brugieres P, Ancelle T, Lejonc JL et al (1993) Neurotrichinosis. A cerebrovascular disease associated with myocardial injury and hypereosinophilia. Brain 116 (Pt 3):603–616

Fournie G, Kearsley-Fleet L, Otte J, Pfeiffer D (2012) Trends in the emergence of swine pathogens. Food and Agriculture Organization of the United Nations, Thailand

Fowler HN, Brown P, Rovira A, Shade B, Klammer K et al (2013) *Streptococcus suis* meningitis in swine worker, Minnesota, USA. Emerg Infect Dis 19:330–331

Fratamico PM, Bagi LK, Bush EJ, Solow BT (2004) Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in swine feces recovered in the National Animal Health Monitoring System's Swine 2000 study. Appl Environ Microbiol 70:7173–7178

Frederick Koster JL, Walker L, Tung KSK, Gilman RH, Rahaman M, Majid MA, Islam S, Williams RC (1978) Hemolytic-uremic syndrome after shigellosis. N Engl J Med 298:927–933

Fredriksson-Ahomaa M, Korte T, Korkeala H (2001) Transmission of *Yersinia enterocolitica* 4/O:3 to pets via contaminated pork. Lett Appl Microbiol 32:375–378

Fredriksson-Ahomaa M, Niskanen T, Bucher M, Korte T, Stolle A et al (2003) Different *Yersinia enterocolitica* 4:O3 genotypes found in pig tonsils in Southern Germany and Finland. Syst Appl Microbiol 26:132–137

Fredriksson-Ahomaa M, Stolle A, Korkeala H (2006a) Molecular epidemiology of *Yersinia enterocolitica* infections. FEMS Immunol Med Microbiol 47:315–329

Fredriksson-Ahomaa M, Stolle A, Siitonen A, Korkeala H (2006b) Sporadic human *Yersinia enterocolitica* infections caused by bioserotype 4/O: 3 originate mainly from pigs. J Med Microbiol 55:747–749

Fredriksson-Ahomaa M, Stolle A, Stephan R (2007) Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. Int J Food Microbiol 119:207–212

Frenzen PD, Riggs TL, Buzby JC, Breuer T, Roberts T et al (2002) *Salmonella* cost estimate updated using foodnet data. J Food Saf 22:6

Fritz SA, Epplin EK, Garbutt J, Storch GA (2009) Skin infection in children colonized with community-associated methicillin-resistant *Staphylococcus aureus*. J Infect 59:394

Fulde M, Valentin-Weigand P (2013) Epidemiology and pathogenicity of zoonotic streptococci. Curr Top Microbiol Immunol 368:49–81

Funk J, Gebreyes WA (2004) Risk factors associated with *Salmonella* prevalence on swine farms. J Swine Health Product 12:246–251

Gallardo MT, Mateos L, Artieda J, Wesslen L, Ruiz C et al (2007) Outbreak of trichinellosis in Spain and Sweden due to consumption of wild boar meat contaminated with *Trichinella britovi*. Euro Surveill 12:E070315. 070311

Gamble HR, Graham CE (1984) Monoclonal antibody-purified antigen for the immunodiagnosis of trichinosis. Am J Vet Res 45:67–74

Gamble HR, Anderson WR, Graham CE, Murrell KD (1983) Diagnosis of swine trichinosis by enzyme-linked immunosorbent assay (ELISA) using an excretory–secretory antigen. Vet Parasitol 13:349–361

Gamble HR, Brady RC, Bulaga LL, Berthoud CL, Smith WG et al (1999) Prevalence and risk association for *Trichinella* infection in domestic pigs in the northeastern United States. Vet Parasitol 82:59–69

Garcia HH, Del Brutto OH (1999) Heavy nonencephalitic cerebral cysticercosis in tapeworm carriers. The cysticercosis working group in Peru. Neurology 53:1582–1584

Garcia HH, Del Brutto OH (2003) Imaging findings in neurocysticercosis. Acta Trop 87:71–78

Garcia HH, Gilman RH, Gonzalez AE, Pacheco R, Verastegui M et al (1999) Human and porcine *Taenia solium* infection in a village in the highlands of Cusco, Peru. The Cysticercosis Working Group in Peru. Acta Trop 73:31–36

Garcia HH, Gonzalez AE, Gilman RH, Cysticerosis Working Group in P (2003a) Diagnosis, treatment and control of Taenia solium cysticercosis. Curr Opin Infect Dis 16:411–419
Garcia HH, Gonzalez AE, Evans CA, Gilman RH, Cysticercosis Working Group in P (2003b) Taenia solium cysticercosis. Lancet 362:547–556
Garcia HH, Del Brutto OH, Nash TE, White AC Jr, Tsang VC et al (2005) New concepts in the diagnosis and management of neurocysticercosis (Taenia solium). Am J Trop Med Hyg 72:3–9
Garcia HH, Gonzalez AE, Del Brutto OH, Tsang VC, Llanos-Zavalaga F et al (2007) Strategies for the elimination of taeniasis/cysticercosis. J Neurol Sci 262:153–157
Gilman RH, Del Brutto OH, Garcia HH, Martinez M (2000) Prevalence of taeniosis among patients with neurocysticercosis is related to severity of infection. The Cysticercosis Working Group in Peru. Neurology 55:1062
Golding GR, Bryden L, Levett PN, McDonald RR, Wong A et al (2010) Livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 398 in humans, Canada. Emerg Infect Dis 16:587–594
Gonzalez AE, Cama V, Gilman RH, Tsang VC, Pilcher JB et al (1990) Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. Am J Trop Med Hyg 43:194–199
Gonzalez AE, Gauci CG, Barber D, Gilman RH, Tsang VC et al (2005) Vaccination of pigs to control human neurocysticercosis. Am J Trop Med Hyg 72:837–839
Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK et al (2008) Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis 197:1226–1234
Gottschalk M (2011) Streptococcosis. In: Karriker L, Ramirez A, Schwartz KJ, Stevenson G, Zimmerman J (eds) Diseases of Swine. Wiley, New Jersey
Gottschalk M, Higgins R, Jacques M, Mittal KR, Henrichsen J (1989) Description of 14 new capsular types of *Streptococcus suis*. J Clin Microbiol 27:2633–2636
Gottschalk M, Higgins R, Jacques M, Beaudoin M, Henrichsen J (1991a) Characterization of six new capsular types (23 through 28) of *Streptococcus suis*. J Clin Microbiol 29:2590–2594
Gottschalk M, Higgins R, Jacques M, Beaudoin M, Henrichsen J (1991b) Isolation and characterization of *Streptococcus suis* capsular types 9-22. J Vet Diagn Investig 3:60–65
Gottschalk M, Turgeon P, Higgins R, Beaudoin M, Bourgault AM (1991c) Susceptibility of *Streptococcus suis* to penicillin. J Vet Diagn Investig 3:170–172
Gottschalk M, Lacouture S, Odierno L (1999) Immunomagnetic isolation of *Streptococcus suis* serotypes 2 and 1/2 from swine tonsils. J Clin Microbiol 37:2877–2881
Gottschalk M, Segura M, Xu J (2007) *Streptococcus suis* infections in humans: the Chinese experience and the situation in North America. Anim Health Res Rev 8:29–45
Gottstein B, Pozio E, Nockler K (2009) Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin Microbiol Rev 22:127–145, Table of Contents
Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M (2014) *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. Emerg Microbes Infect 3(6):e45
Graham PL 3rd, Lin SX, Larson EL (2006) A U.S. population-based survey of *Staphylococcus aureus* colonization. Ann Intern Med 144:318–325
Grahek-Ogden D, Schimmer B, Cudjoe KS, Nygard K, Kapperud G (2007) Outbreak of *Yersinia enterocolitica* serogroup O:9 infection and processed pork, Norway. Emerg Infect Dis 13:754–756
Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA (2011) Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. Int J Med Microbiol 301: 630–634
Gray GC, Bender JB, Bridges CB, Daly RF, Krueger WS et al (2012) Influenza A(H1N1)pdm09 virus among healthy show pigs, United States. Emerg Infect Dis 18:1519–1521
Grimont PA, Weill F-X (2007) Antigenic formulae of the *Salmonella* serovars. WHO collaborating Centre for reference and research on Salmonella. Institut Pasteur, Paris
Gurley ES, Montgomery JM, Hossain MJ, Bell M, Azad AK et al (2007) Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infect Dis 13:1031–1037

Haesebrouck F, Pasmans F, Chiers K, Maes D, Ducatelle R et al (2004) Efficacy of vaccines against bacterial diseases in swine: what can we expect? Vet Microbiol 100:255–268
Haim M, Efrat M, Wilson M, Schantz PM, Cohen D et al (1997) An outbreak of *Trichinella spiralis* infection in southern Lebanon. Epidemiol Infect 119:357–362
Halaby T, Hoitsma E, Hupperts R, Spanjaard L, Luirink M et al (2000) *Streptococcus suis* meningitis, a poacher's risk. Eur J Clin Microbiol Infect Dis 19:943–945
Hanson BM, Dressler AE, Harper AL, Scheibel RP, Wardyn SE et al (2011) Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J Infect Public Health 4:169–174
Harris IT, Fedorka-Cray PJ, Gray JT, Thomas LA, Ferris K (1997) Prevalence of *Salmonella* organisms in swine feed. J Am Vet Med Assoc 210:382–385
Hartmeyer GN, Gahrn-Hansen B, Skov RL, Kolmos HJ (2010) Pig-associated methicillin-resistant *Staphylococcus aureus*: family transmission and severe pneumonia in a newborn. Scand J Infect Dis 42:318–320
Hawk MW, Shahlaie K, Kim KD, Theis JH (2005) Neurocysticercosis: a review. Surg Neurol 63: 123–132. discussion 132
Health CfFSaP (2013) Swine diseases and resources
Health WOfA (2009) Japanese encephalitis. World Organization for Animal Health
Higgins R, Gottschalk M (1990) An update on *Streptococcus suis* identification. J Vet Diagn Investig 2:249–252
Higgins R, Gottschalk M, Boudreau M, Lebrun A, Henrichsen J (1995) Description of six new capsular types (29-34) of *Streptococcus suis*. J Vet Diagn Investig 7:405–406
Hill JE, Gottschalk M, Brousseau R, Harel J, Hemmingsen SM et al (2005) Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*. Vet Microbiol 107:63–69
Hill DE, Pierce V, Darwin Murrell K, Ratliffe N, Rupp B et al (2010) Cessation of *Trichinella spiralis* transmission among scavenging mammals after the removal of infected pigs from a poorly managed farm: implications for trichinae transmission in the US. Zoonoses Public Health 57:e116–e123
Hira PR, Francis I, Abdella NA, Gupta R, Ai-Ali FM et al (2004) Cysticercosis: imported and autochthonous infections in Kuwait. Trans R Soc Trop Med Hyg 98:233–239
Hlebowicz M, Jakubowski P, Smiatacz T (2019) *Streptococcus suis* meningitis: epidemiology, clinical presentation and treatment. Vector Borne Zoonotic Dis 19(8):557–562
Holmes EC (2001) On the origin and evolution of the human immunodeficiency virus (HIV). Biol Rev Camb Philos Soc 76:239–254
Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams SP (2000) Bergey's manual of determinative bacteriology. Lippincott Williams & Wilkins, Philadelphia. Hensyl WR, editor
Hope BK, Baker AR, Edel ED, Hogue AT, Schlosser WD et al (2002) An overview of the salmonella enteritidis risk assessment for shell eggs and egg products. Risk Anal 22:203–218
Huang YT, Teng LJ, Ho SW, Hsueh PR (2005) *Streptococcus suis* infection. J Microbiol Immunol Infect 38:306–313
Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME et al (2006) Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob 5:26
Huovinen E, Sihvonen LM, Virtanen MJ, Haukka K, Siitonen A et al (2010) Symptoms and sources of *Yersinia enterocolitica*-infection: a case-control study. BMC Infect Dis 10:122
Igarashi A (2002) Control of Japanese encephalitis in Japan: immunization of humans and animals, and vector control. Curr Top Microbiol Immunol 267:139–152
Izopet J, Dubois M, Bertagnoli S, Lhomme S, Marchandeau S, Boucher S, Kamar N, Abravanel F, Guerin JL (2012) Hepatitis E virus strains in rabbits and evidence of a closely related strain in humans, France. Emerg Infect Dis 18(8):1274–1281
Jacobson JAH, Hills SL, Winkler JL, Mammen M, Thaisomboonsuk B, Marfin AA et al (2007) Evaluation of three immunoglobulin M antibody capture enzyme-linked immunosorbent assays for diagnosis of Japanese encephalitis. Am J Trop Med Hygiene 77:164–168

Jay MT, Cooley M, Carychao D, Wiscomb GW, Sweitzer RA, Crawford-Miksza L, Farrar JA, Lau DK, O'Connell J, Millington A, Asmundson RV, Atwill ER, Mandrell RE (2007) *Escherichia coli* O157:H7in feral swine near spinach fields and cattle, Central California Coast. Emerg Infect Dis 13(12):1908–1911. Centers for Disease Control and Prevention

Jenkins TC, McCollister BD, Sharma R, McFann KK, Madinger NE et al (2009) Epidemiology of healthcare-associated bloodstream infection caused by USA300 strains of methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals. Infect Control Hosp Epidemiol 30:233–241

Jensen AN, Dalsgaard A, Baggesen DL, Nielsen EM (2006) The occurrence and characterization of *Campylobacter jejuni* and *C. coli* in organic pigs and their outdoor environment. Vet Microbiol 116:96–105

Jiang C, Shaw KS, Upperman CR, Blythe D, Mitchell C, Murtugudde R, Sapkota AR, Sapkota A (2015) Climate change, extreme events and increased risk of salmonellosis in Maryland, USA: evidence for coastal vulnerability. Environ Int 83:58–62

Jimenez JN, Velez LA, Mediavilla JR, Ocampo AM, Vanegas JM et al (2011) Livestock-associated methicillin-susceptible *Staphylococcus aureus* ST398 infection in woman, Colombia. Emerg Infect Dis 17:1970–1971

Johnson KETC, Sears CL (2006) The emerging clinical importance of non-O157:H7 Shiga toxin-producing *Escherichia coli*. Clin Infect Dis 43:1587–1595

Jones TF (2003) From pig to pacifier: chitterling-associated yersiniosis outbreak among black infants. Emerg Infect Dis 9:1007–1009

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D et al (2008) Global trends in emerging infectious diseases. Nature 451:990–993

Kapel CM (2005) Changes in the EU legislation on *Trichinella* inspection–new challenges in the epidemiology. Vet Parasitol 132:189–194

Kapel CM, Webster P, Gamble HR (2005) Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Vet Parasitol 132:101–105

Kashoma IP, Kassem A II, Kumar BM, Kessy W, Gebreyes RR, Kazwala, Rajashekara G (2015) Antimicrobial resistance and genotypic diversity of campylobacter isolated from pigs, dairy, and beef cattle in Tanzania. Front Microbiol 6:1240

Kauffmann F (1947) The serology of the coli group. J Immunol 57:71–100

Kauffmann F (1978) Das Fundament. Munksgaard, Copenhagen

Kay-Sin Tan C-TT, Goh K-J (1999) Epidemiological aspects of Nipah virus infection. Neurol J South East Asia 4:77–81

Khanna T, Friendship R, Dewey C, Weese JS (2007) Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol 128:298

Killian ML, Swenson SL, Vincent AL, Landgraf JG, Shu B et al (2013) Simultaneous infection of pigs and people with triple-reassortant swine influenza virus H1N1 at a U.S. county fair. Zoonoses Public Health 60:196–201

Kitai Y, Shoda M, Kondo T, Konishi E (2007) Epitope-blocking enzyme-linked immunosorbent assay to differentiate west nile virus from Japanese encephalitis virus infections in equine sera. Clin Vaccine Immunol 14:1024–1031

Kociecka W (2000) Trichinellosis: human disease, diagnosis and treatment. Vet Parasitol 93: 365–383

Kock R, Harlizius J, Bressan N, Laerberg R, Wieler LH et al (2009) Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. Eur J Clin Microbiol Infect Dis 28: 1375–1382

Kock R, Siam K, Al-Malat S, Christmann J, Schaumburg F et al (2011) Characteristics of hospital patients colonized with livestock-associated meticillin-resistant *Staphylococcus aureus* (MRSA) CC398 versus other MRSA clones. J Hosp Infect 79:292–296

Korte T, Fredriksson-Ahomaa M, Korkeala H (2003) Prevalence and characterisation of *yadA*-positive *Yersinia enterocolitica* in pig tonsils in 1995 and 1999. Adv Exp Med Biol 529:367–369

Kothalawala H, Toussaint MJ, Gruys E (2006) An overview of swine influenza. Vet Q 28:46–53

Kourbatova EV, Halvosa JS, King MD, Ray SM, White N et al (2005) Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 clone as a cause of health care-associated infections among patients with prosthetic joint infections. Am J Infect Control 33: 385–391

Kourtis AP, Hatfield K, Baggs J, Mu Y, See I, Epson E, Nadle J, Kainer MA, Dumyati G, Petit S, Ray SM, M. a. g. Emerging Infections Program, Ham D, Capers C, Ewing H, Coffin N, McDonald LC, Jernigan J, Cardo D (2019) Vital signs: epidemiology and recent trends in methicillin-resistant and in methicillin-susceptible *Staphylococcus aureus* bloodstream infections - United States. MMWR Morb Mortal Wkly Rep 68(9):214–219

Kraft R (2007) Cysticercosis: an emerging parasitic disease. Am Fam Physician 76:91–96

Kranker S, Alban L, Boes J, Dahl J (2003) Longitudinal study of *Salmonella enterica* serotype Typhimurium infection in three Danish farrow-to-finish swine herds. J Clin Microbiol 41: 2282–2288

Ksiazek TG, Rota PA, Rollin PE (2011) A review of Nipah and Hendra viruses with an historical aside. Virus Res 162:173–183

Lachkar S, Abboud P, Gargala G, Etienne M, Gauliard E et al (2008) Troponin dosage in a patient with asymptomatic myocarditis due to trichinellosis. Rev Med Interne 29:246–248

Lanada EB, Morris RS, Jackson R, Fenwick SG (2005a) A cohort study of *Yersinia* infection in goats. Aust Vet J 83:567–571

Lanada EB, Morris RS, Jackson R, Fenwick SG (2005b) Prevalence of *Yersinia* species in goat flocks. Aust Vet J 83:563–566

Langvad B, Skov MN, Rattenborg E, Olsen JE, Baggesen DL (2006) Transmission routes of *Salmonella* Typhimurium DT 104 between 14 cattle and pig herds in Denmark demonstrated by molecular fingerprinting. J Appl Microbiol 101:883–890

Laurent F (2009) Molecular Characterization of antimicrobial resistance genes and virulence genes by using microarrays in representative ST398 MRSA isolates from pigs in France. London

Lee LA, Gerber AR, Lonsway DR, Smith JD, Carter GP et al (1990) *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. N Engl J Med 322:984–987

Lee LA, Taylor J, Carter GP, Quinn B, Farmer JJ 3rd et al (1991) *Yersinia enterocolitica* O:3: an emerging cause of pediatric gastroenteritis in the United States. The *Yersinia enterocolitica* collaborative study group. J Infect Dis 163:660–663

Leonard J, Marshall JK, Moayyedi P (2007) Systematic review of the risk of enteric infection in patients taking acid suppression. Am J Gastroenterol 102:2047–2056. quiz 2057

Lhomme S, Marion O, Abravanel F, Izopet J, Kamar N (2020) Clinical manifestations, pathogenesis and treatment of hepatitis E virus infections. J Clin Med 9(2):331

Lightowlers MW (2004) Vaccination for the prevention of cysticercosis. Dev Biol (Basel) 119: 361–368

Linton AH, Heard TW, Grimshaw JJ, Pollard P (1970) Computer-based analysis of epidemiological data arising from salmonellosis in pigs. Res Vet Sci 11:523–532

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16(2):161–168

Lo Fo Wong DMA, Hald T, Van Der Wolf PJ, Swanenburg M (2002) Epidemiology and control measures for *Salmonella* in pigs and pork. Livest Prod Sci 76:215–222

Lo Fo Wong DM, Dahl J, Stege H, van der Wolf PJ, Leontides L et al (2004) Herd-level risk factors for subclinical *Salmonella* infection in European finishing-pig herds. Prev Vet Med 62:253–266

Long C, Jones TF, Vugia DJ, Scheftel J, Strockbine N et al (2010) *Yersinia pseudotuberculosis* and *Y. enterocolitica* infections, food net, 1996-2007. Emerg Infect Dis 16:566–567

Louis VR, Gillespie IA, O'Brien SJ, Russek-Cohen E, Pearson AD et al (2005) Temperature-driven campylobacter seasonality in England and Wales. Appl Environ Microbiol 71:85–92

Luby SP, Gurley ES (2012) Epidemiology of henipavirus disease in humans. Curr Top Microbiol Immunol 359:25–40

Luby SP, Hossain MJ, Gurley ES, Ahmed BN, Banu S et al (2009) Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. Emerg Infect Dis 15:1229–1235

Lun ZR, Wang QP, Chen XG, Li AX, Zhu XQ (2007) *Streptococcus suis*: an emerging zoonotic pathogen. Lancet Infect Dis 7:201–209

Marva E, Markovics A, Gdalevich M, Asor N, Sadik C et al (2005) Trichinellosis outbreak. Emerg Infect Dis 11:1979–1981

Martinez MJ, de Aluja AS, Gemmell M (2000) Failure to incriminate domestic flies (*Diptera: Muscidae*) as mechanical vectors of *Taenia* eggs (*Cyclophyllidea: Taeniidae*) in rural Mexico. J Med Entomol 37:489–491

MacInnes JI, Desrosiers R (1999) Agents of the "suis-ide diseases" of swine: *Actinobacillus suis*, *Haemophilus parasuis*, and *Streptococcus suis*. Can J Vet Res 63:83–89

Mackenzie JW, Smith D (2006) Japanese encephalitis virus: the geographic distribution, incidence, and spread of a virus with a propensity to emerge in new areas. Prospect Med Virol 16:201–268

Maguire HCF, Codd AA, Mackay VE, Rowe B, Mitchell E (1993) A large outbreak of human salmonellosis traced to a local pig farm. Epidemiol Infect 110:239–246

Mahanty S, Garcia HH, Cysticercosis Working Group in P (2010) Cysticercosis and neurocysticercosis as pathogens affecting the nervous system. Prog Neurobiol 91:172–184

Mai NT, Hoa NT, Nga TV, Linh le D, Chau TT et al (2008) *Streptococcus suis* meningitis in adults in Vietnam. Clin Infect Dis 46:659–667

Mainil J (2013) *Escherichia coli* virulence factors. Vet Immunol Immunopathol 152:2–12

Mammina C, Cala C, Plano MR, Bonura C, Vella A et al (2010) Ventilator-associated pneumonia and MRSA ST398, Italy. Emerg Infect Dis 16:730–731

Marie J, Morvan H, Berthelot-Herault F, Sanders P, Kempf I et al (2002) Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. J Antimicrob Chemother 50:201–209

Maritza Rios VP, Trucksis M, Arellano C, Borie C, Alexandre M, Fica A, Levine MM (1999) Clinical diversity of chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic-uremic syndrome, asymptomatic subjects, animal reservoirs, and food products. J Clin Microbiol 37:778–781

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS et al (1999) Food-related illness and death in the United States. Emerg Infect Dis 5:607–625

Mediavilla JR, Chen L, Uhlemann AC, Hanson BM, Rosenthal M et al (2012) Methicillin-susceptible *Staphylococcus aureus* ST398, New York and New Jersey, USA. Emerg Infect Dis 18:700–702

Metchock B, Lonsway DR, Carter GP, Lee LA, McGowan JE Jr (1991) *Yersinia enterocolitica*: a frequent seasonal stool isolate from children at an urban hospital in the Southeast United States. J Clin Microbiol 29:2868–2869

Middleton DJ, Westbury HA, Morrissy CJ, van der Heide BM, Russell GM et al (2002) Experimental Nipah virus infection in pigs and cats. J Comp Pathol 126:124–136

Mohd Nor MN, Gan CH, Ong BL (2000) Nipah virus infection of pigs in peninsular Malaysia. Rev Sci Tech 19:160–165

Moller LN, Petersen E, Gamble HR, Kapel CM (2005b) Comparison of two antigens for demonstration of *Trichinella* spp. antibodies in blood and muscle fluid of foxes, pigs and wild boars. Vet Parasitol 132:81–84

Moller LN, Petersen E, Kapel CM, Melbye M, Koch A (2005a) Outbreak of trichinellosis associated with consumption of game meat in West Greenland. Vet Parasitol 132:131–136

Mouhamadou Diagana P-MP, Dumas M (2007) Japanese encephalitis revisited. J Neurol Sci 262: 165–170

Morse DL, Shayegani M, Gallo RJ (1984) Epidemiologic investigation of a *Yersinia* camp outbreak linked to a food handler. Am J Public Health 74:589–592

Murrell KD, Pozio E (2011) Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerg Infect Dis 17:2194–2202

Murrell KD, Bruschi F (1994) Clinical trichinellosis. Prog Clin Parasitol 4:117–150

Myers KP, Olsen CW, Gray GC (2007) Cases of swine influenza in humans: a review of the literature. Clin Infect Dis 44:1084–1088

Myers KP, Olsen CW, Setterquist SF, Capuano AW, Donham KJ et al (2006) Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? Clin Infect Dis 42: 14–20

Ndimubanzi PC, Carabin H, Budke CM, Nguyen H, Qian YJ et al (2010) A systematic review of the frequency of neurocyticercosis with a focus on people with epilepsy. PLoS Negl Trop Dis 4:e870

Nesbakken T, Iversen T, Eckner K, Lium B (2006) Testing of pathogenic *Yersinia enterocolitica* in pig herds based on the natural dynamic of infection. Int J Food Microbiol 111:99–104

Nesbakken T, Eckner K, Hoidal HK, Rotterud O (2003) Occurence of *Yersina enterocolitica* and *campylobacter* spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. Int J Food Microbiol 80:231–249

Nockler K, Kapel CMO (2007) Detection and surveillance for *Trichinella*: meat inspection and hygiene, and legislation. In: Dupouy-Camet JaM KD (ed) FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO) and World Organization for Animal Health (OIE), Paris, pp 69–97

Nakamura T, Miura T, Nakaoka T, Nagano I, Takahashi Y et al (2003) A case of trichinellosis with spontaneous remission. Kansenshogaku Zasshi 77:839–843

Nelson MI, Detmer SE, Wentworth DE, Tan Y, Schwartzbard A et al (2012) Genomic reassortment of influenza A virus in North American swine, 1998-2011. J Gen Virol 93:2584–2589

Ngo TH, Tran TB, Tran TT, Nguyen VD, Campbell J et al (2011) Slaughterhouse pigs are a major reservoir of *Streptococcus suis* serotype 2 capable of causing human infection in southern Vietnam. PLoS One 6:e17943

Nichols GL, Richardson JF, Sheppard SK, Lane C, Sarran C (2012) *Campylobacter* epidemiology: a descriptive study reviewing 1 million cases in England and Wales between 1989 and 2011. BMJ Open 2:e001179

Nollet N, Houf K, Dewulf J, De Kruif A, De Zutter L et al (2005) *Salmonella* in sows: a longitudinal study in farrow-to-finish pig herds. Vet Res 36:645–656

Nockler K, Wichmann-Schauer H, Hiller P, Muller A, Bogner K (2007) Trichinellosis outbreak in Bavaria caused by cured sausage from Romania, January 2007. Euro Surveill 12:E070823. 070822

Nockler KK, CMO (2007) Detection and surveillance for *Trichinella*: meat inspection and hygiene, and legislation. In: Dupouy-Camet JaM KD (ed) FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO) and World Organization for Animal Health (OIE), Paris, pp 69–97

Nockler K, Serrano FJ, Boireau P, Kapel CM, Pozio E (2005) Experimental studies in pigs on *Trichinella* detection in different diagnostic matrices. Vet Parasitol 132:85–90

Nylen G, Dunstan F, Palmer SR, Andersson Y, Bager F et al (2002) The seasonal distribution of campylobacter infection in nine European countries and New Zealand. Epidemiol Infect 128:601

O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE et al (2012) MRSA in conventional and alternative retail pork products. PLoS One 7:e30092

Oivanen L, Kapel CM, Pozio E, La Rosa G, Mikkonen T et al (2002) Associations between Trichinella species and host species in Finland. J Parasitol 88:84–88

Oosterom J, Notermans S (1983) Further research into the possibility of salmonella-free fattening and slaughter of pigs. J Hyg (Lond) 91:59–69

O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R et al (1997) Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet 349:93–95

Padma MV, Behari M, Misra NK, Ahuja GK (1995) Albendazole in neurocysticercosis. Natl Med J India 8:255–258

Pascua PN, Song MS, Lee JH, Baek YH, Kwon HI et al (2012) Virulence and transmissibility of H1N2 influenza virus in ferrets imply the continuing threat of triple-reassortant swine viruses. Proc Natl Acad Sci U S A 109:15900–15905

Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE et al (1999) Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 354:1253–1256

Paul S, Mead PMG (1998) *Escherchia coli* O157:H7. Lancet 352:1207–1212

Patricia M, Griffin RVT (1991) The epidemiology of infections caused by *Escherichia coli* 0157: H7, other Enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 13:60–98

Parashar UD, Sunn LM, Ong F, Mounts AW, Arif MT et al (2000) Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 181:1755–1759

Padma MV, Behari M, Misra NK, Ahuja GK (1994) Albendazole in single CT ring lesions in epilepsy. Neurology 44:1344–1346

Pennington H (2010) *Escherichia coli* 0157. Lancet 376:1428–1435

Peron JM, Dalton H, Izopet J, Kamar N (2011) Acute autochthonous hepatitis E in western patients with underlying chronic liver disease: a role for ribavirin? J Hepatol 54(6):1323–1324. author reply 1324-1325

Pickering BS, Smith G, Pinette MM, Embury-Hyatt C, Moffat E, Marszal P, Lewis CE (2021) Susceptibility of domestic swine to experimental infection with severe acute respiratory syndrome Coronavirus 2. Emerg Infect Dis 27(1):104–112

Pischke S, Wedemeyer H (2013) Hepatitis E virus infection: multiple faces of an underestimated problem. J Hepatol 58(5):1045–1046

Phillip Tarr CAG, Chandler WL (2005) Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 365:1073–1086

Potel C, Alvarez-Fernandez M, Constenla L, Alvarez P, Perez S (2010) First human isolates of methicillin-resistant *Staphylococcus aureus* sequence type 398 in Spain. Eur J Clin Microbiol Infect Dis 29:351–352

Pozio E, La Rosa G (2003) PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. Methods Mol Biol 216:299–309

Pozio E, Marucci G (2003) Trichinella-infected pork products: a dangerous gift. Trends Parasitol 19:338

Pozio E (2007) World distribution of *Trichinella* spp. infections in animals and humans. Vet Parasitol 149:3–21

Pozio E, Rinaldi L, Marucci G, Musella V, Galati F et al (2009) Hosts and habitats of *Trichinella spiralis* and *Trichinella britovi* in Europe. Int J Parasitol 39:71–79

Pozio E, Darwin Murrell K (2006) Systematics and epidemiology of *Trichinella*. Adv Parasitol 63: 367–439

Pappas G (2013) Socio-economic, industrial and cultural parameters of pig-borne infections. Clin Microbiol Infect 19:605–610

Pretell EJ, Garcia HH, Gilman RH, Saavedra H, Martinez M et al (2001) Failure of one-day praziquantel treatment in patients with multiple neurocysticercosis lesions. Clin Neurol Neurosurg 103:175–177

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM (2012) *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio 3(1): e00305–e00311

Prieto C, Garcia FJ, Suarez P, Imaz M, Castro JM (1994) Biochemical traits and antimicrobial susceptibility of *Streptococcus suis* isolated from slaughtered pigs. Zentralbl Veterinarmed B 41: 608–617

Pozio E, La Rosa G, Serrano FJ, Barrat J, Rossi L (1996) Environmental and human influence on the ecology of *Trichinella spiralis* and *Trichinella britovi* in Western Europe. Parasitology 113 (Pt 6):527–533

Pozio E (2005) The broad spectrum of *Trichinella* hosts: from cold- to warm-blooded animals. Vet Parasitol 132:3–11

Prokaryotes JCotICoSo (2005) The type species of the genus *Salmonella* Lignieres 1900 is *Salmonella* enterica (ex Kauffmann and Edwards 1952) Le minor and Popoff 1987, with the type strain LT2T, and conservation of the epithet enterica in *Salmonella* enterica over all earlier epithets that may be applied to this species. Opinion 80. Int J Syst Evol Microbiol 55:519–520

Pulliam JR, Epstein JH, Dushoff J, Rahman SA, Bunning M et al (2012) Agricultural intensification, priming for persistence and the emergence of Nipah virus: a lethal bat-borne zoonosis. J R Soc Interface 9:89–101

Pu S, Han F, Ge B (2008) Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from Louisiana retail meats. Appl Environ Microbiol 75:265

Quan TJ, Meek JL, Tsuchiya KR, Hudson BW, Barnes AM (1974) Experimental pathogenicity of recent North American isolates of Yersinia enterocolitica. J Infect Dis 129:341–344

Rajshekhar V, Joshi DD, Doanh NQ, van De N, Xiaonong Z (2003) *Taenia* solium taeniosis/cysticercosis in Asia: epidemiology, impact and issues. Acta Trop 87:53–60

Ramirez A, Capuano AW, Wellman DA, Lesher KA, Setterquist SF et al (2006) Preventing zoonotic influenza virus infection. Emerg Infect Dis 12:996–1000

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL (2005) Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerg Infect Dis 11(4):603–609

Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS (2018) Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 for surveillance purposes. Euro Surveill 23(6):17–00672

Rhouma M, Beaudry F, Letellier A (2016) Resistance to colistin: what is the fate for this antibiotic in pig production? Int J Antimicrob Agents 48(2):119–126

Scholtissek C (1990) Pigs as "mixing vessels" for the creation of new pandemic influenza A viruses. Med Princ Pract 2:65–71

Schlosser J, Eiden M, Vina-Rodriguez A, Fast C, Dremsek P, Lange E, Ulrich RG, Groschup MH (2014) Natural and experimental hepatitis E virus genotype 3-infection in European wild boar is transmissible to domestic pigs. Vet Res 45:121

Schnitzler SU, Schnitzler P (2009) An update on swine-origin influenza virus A/H1N1: a review. Virus Genes 39:279–292

Selander RK, Beltran P, Smith NH, Helmuth R, Rubin FA et al (1990) Evolutionary genetic relationships of clones of *Salmonella* serovars that cause human typhoid and other enteric fevers. Infect Immun 58:2262–2275

Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF et al (2006) Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis 42:647–656

Shandera WX, White AC Jr, Chen JC, Diaz P, Armstrong R (1994) Neurocysticercosis in Houston, Texas. A report of 112 cases. Medicine (Baltimore) 73:37–52

Shayegani M, Morse D, DeForge I, Root T, Parsons LM et al (1983) Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* serogroup O:8. J Clin Microbiol 17:35–40

Shiota T, Arizono N, Yoshioka T, Ishikawa Y, Fujitake J et al (1999) Imported trichinellosis with severe myositis–report of a case. Kansenshogaku Zasshi 73:76–82

Shneerson JM, Chattopadhyay B, Murphy MF, Fawcett IW (1980) Permanent perceptive deafness due to *Streptococcus suis* type II infection. J Laryngol Otol 94:425–427

Sibbald CJ, Sharp JCM (1985) *Campylobacter* infection in urban and rural populations in Scotland. J Hyg Camb 95:87–93

Skowronski DM, Moser FS, Janjua NZ, Davoudi B, English KM et al (2013) H3N2v and other influenza epidemic risk based on age-specific estimates of sero-protection and contact network interactions. PLoS One 8:e54015

Staats JJ, Feder I, Okwumabua O, Chengappa MM (1997) *Streptococcus suis*: past and present. Vet 2018 Res Commun 21:381–407

Stensvold CR, Nielsen HV, Molbak K (2007) A case of trichinellosis in Denmark, imported from Poland, June 2007. Euro Surveill 12:E070809. 070803

Stephan R, Joutsen S, Hofer E, Sade E, Bjorkroth J et al (2013) Characteristics of *Yersinia enterocolitica* biotype 1A strains isolated from patients and asymptomatic carriers. Eur J Clin Microbiol Infect Dis 32:869

Stenstad T, Grahek-Ogden D, Nilsen M, Skaare D, Martinsen TA et al (2007) An outbreak of *Yersinia enterocolitica* O:9-infection. Tidsskr Nor Laegeforen 127:586–589

Smith TC, Wardyn SE (2015) Human infections with *Staphylococcus aureus* CC398. Curr Environ Health Rep 2(1):41–51

Smith HW (1960) The effect of feeding pigs on food naturally contaminated with salmonellae. J Hyg (Lond) 58:381–389

Smith TC, Forshey BM, Hanson BM, Wardyn SE, Moritz ED (2012) Molecular and epidemiologic predictors of *Staphylococcus aureus* colonization site in a population with limited nosocomial exposure. Am J Infect Control 40:992–996

Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP et al (2009) Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One 4:e4258

Smith TC, Pearson N (2011) The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoonotic Dis 11:327–339

Smith TC, Capuano AW, Boese B, Myers KP, Gray GC (2008) Exposure to *Streptococcus suis* among US swine workers. Emerg Infect Dis 14:1925–1927

Solomon T (2004) Flavivirus encephalitis. N Engl J Med 351:370–378

Spickler AR (2007) Japanese encephalitis

Sriskandan S, Slater JD (2006) Invasive disease and toxic shock due to zoonotic *Streptococcus suis*: an emerging infection in the East? PLoS Med 3:e187

Strangmann E, Froleke H, Kohse KP (2002) Septic shock caused by *Streptococcus suis*: case report and investigation of a risk group. Int J Hyg Environ Health 205:385–392

Strachan NJ, Gormley FJ, Rotariu O, Ogden ID, Miller G et al (2009) Attribution of campylobacter infections in Northeast Scotland to specific sources by use of multilocus sequence typing. J Infect Dis 199:1205–1208

Sussman M (1985) *Escherichia coli* in human and animal disease. In: Sussman M (ed) The virulence of *Escherichia coli*. Academic Press, San Diego, pp 2–45

Tattevin P, Diep BA, Jula M, Perdreau-Remington F (2009) Methicillin-resistant *Staphylococcus aureus* USA300 clone in long-term care facility. Emerg Infect Dis 15:953–955

Tacket CO, Narain JP, Sattin R, Lofgren JP, Konigsberg C Jr et al (1984) A multistate outbreak of infections caused by *Yersinia enterocolitica* transmitted by pasteurized milk. JAMA 251: 483–486

Tam CC, O'Brien SJ, Petersen I, Islam A, Hayward A et al (2007) Guillain-Barre syndrome and preceding infection with *Campylobacter*, influenza and Epstein-Barr virus in the general practice research database. PLoS One 2:e344

Tan CT, Goh KJ, Wong KT, Sarji SA, Chua KB et al (2002) Relapsed and late-onset Nipah encephalitis. Ann Neurol 51:703–708

Tang J, Wang C, Feng Y, Yang W, Song H et al (2006) Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. PLoS Med 3:e151

Tiiomas G, Boyce DLS, Griffin PM (1995) *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. N Engl J Med 333:364–368

Tiroumourougane SV, Raghava P, Srinivasan S (2002) Japanese viral encephalitis. Postgrad Med J 78:205–215

Thacker E, Janke B (2008) Swine influenza virus: zoonotic potential and vaccination strategies for the control of avian and swine influenzas. J Infect Dis 197(Suppl 1):S19–S24

Thompson JS, Gravel MJ (1986) Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype O:3 from well water. Can J Microbiol 32:700–701

Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA et al (2012) A distinct lineage of influenza A virus from bats. Proc Natl Acad Sci U S A 109:4269–4274

Tom Solomon NMD, Kneen R, Gainsborough M, Vaughn DW, Khanh VT (2000) Japanese encephalitis. J Neurol Neurosurg Psychiatry 68:405–415

Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J, Chen LM, Johnson A, Tao Y, Dreyfus C, Yu W, McBride R, Carney PJ, Gilbert AT, Chang J, Guo Z, Davis CT, Paulson JC, Stevens J, Rupprecht CE, Holmes EC, Wilson IA, Donis RO (2013) New world bats harbor diverse influenza A viruses. PLoS Pathog 9(10):e1003657

Tramontana AR, Graham M, Sinickas V, Bak N (2008) An Australian case of *Streptococcus suis* toxic shock syndrome associated with occupational exposure to animal carcasses. Med J Aust 188:538–539

Uppal PK (2000) Emergence of Nipah virus in Malaysia. Ann N Y Acad Sci 916:354–357

van Belkum A, Melles DC, Peeters JK, van Leeuwen WB, van Duijkeren E et al (2008) Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and humans. Emerg Infect Dis 14:479–483

van der Wolf PJ, Wolbers WB, Elbers AR, van der Heijden HM, Koppen JM et al (2001) Herd level husbandry factors associated with the serological *Salmonella* prevalence in finishing pig herds in The Netherlands. Vet Microbiol 78:205–219

van der Wolf PJ, Bongers JH, Elbers AR, Franssen FM, Hunneman WA et al (1999) *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. Vet Microbiol 67:263–275

van-den-Hurk AF, Ritchie SA, Johansen CA, Mackenzie JS, Smith GA (2008) Domestic pigs and Japanese encephalitis virus infection, Australia. Emerg Infect Dis 14:1736–1738

van den Hurk AF, Ritchie SA, Mackenzie JS (2009) Ecology and geographical expansion of Japanese Encephalitis virus. Ann Rev Entomol 54:17–35

van Loo IH, Diederen BM, Savelkoul PH, Woudenberg JH, Roosendaal R et al (2007) Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. Emerg Infect Dis 13: 1753–1755

van Rijen MM, Bosch T, Heck ME, Kluytmans JA (2009) Meticillin-resistant *Staphylococcus aureus* epidemiology and transmission in a Dutch hospital. J Hosp Infect 72:299–306

van Rijen MM, Van Keulen PH, Kluytmans JA (2008) Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. Clin Infect Dis 46:261–263

van der Giessen J, Fonville M, Bouwknegt M, Langelaar M, Vollema A (2007) Seroprevalence of *Trichinella* spiralis and *Toxoplasma gondii* in pigs from different housing systems in The Netherlands. Vet Parasitol 148:371–374

VanderWaal K, Deen J (2018) Global trends in infectious diseases of swine. Proc Natl Acad Sci U S A 115(45):11495–11500

Vergara-Alert J, Rodon J, Carrillo J, Te N, Izquierdo-Useros N, Rodriguez de la Concepcion ML, Avila-Nieto C, Guallar V, Valencia A, Cantero G, Blanco J, Clotet B, Bensaid A, Segales J (2021) Pigs are not susceptible to SARS-CoV-2 infection but are a model for viral immunogenicity studies. Transbound Emerg Dis 68(4):1721–1725

Vela AI, Moreno MA, Cebolla JA, Gonzalez S, Latre MV et al (2005) Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. Vet Microbiol 105:143–147

von Altrock A, Hamedy A, Merle R, Waldmann KH (2013) Campylobacter spp. – prevalence on pig livers and antimicrobial susceptibility. Prev Vet Med 109:152–157

Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M (2005) Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965–1966

Vu Thi N, Dorny P, La Rosa G, To Long T, Nguyen Van C et al (2010) High prevalence of anti-Trichinella IgG in domestic pigs of the Son La province, Vietnam. Vet Parasitol 168:136–140

Wang ZQ, Cui J, Xu BL (2006) The epidemiology of human trichinellosis in China during 2000-2003. Acta Trop 97:247–251

Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, Jin L, Zhang Q, Liu Y, Rieux A, Dorai-Schneiders T, Weinert LA, Iqbal Z, Didelot X, Wang H, Balloux F (2018) The global distribution and spread of the mobilized colistin resistance gene *mcr*-1. Nat Commun 9(1):1179

Wangsomboonsiri W, Luksananun T, Saksornchai S, Ketwong K, Sungkanuparph S (2008) *Streptococcus suis* infection and risk factors for mortality. J Infect 57:392–396

Wangkaew S, Chaiwarith R, Tharavichitkul P, Supparatpinyo K (2006) *Streptococcus suis* infection: a series of 41 cases from Chiang Mai University Hospital. J Infect 52:455–460

Waters AE, Contente-Cuomo T, Buchhagen J, Liu CM, Watson L et al (2011) Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin Infect Dis 52:1227–1230

Wells JE, Kalchayanand N, Berry ED, Oliver WT (2012) Effects of antimicrobials fed as dietary growth promoters on fecal shedding of *Campylobacter*, *Salmonella* and shiga-toxin producing *Escherichia coli* in Swine. J Appl Microbiol 114:318–328

White PB (1926) Great Britain medical research council special report: no. 103. Her Majesty's Stationery Office, London

WHO (2011) Campylobacter

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A et al (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–762

Wertheim HF, Nghia HD, Taylor W, Schultsz C (2009) *Streptococcus suis*: an emerging human pathogen. Clin Infect Dis 48:617–625

Werner Brunder HS, Karch H (1997) ESpP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. Mol Microbiol 24: 767–778

Weaver SC, Barrett AD (2004) Transmission cycles, host range, evolution and emergence of arboviral disease. Nat Rev Microbiol 2:789–801

Whiley H, Ross K (2015) *Salmonella* and eggs: from production to plate. Int J Environ Res Public Health 12(3):2543–2556

Witte W, Strommenger B, Stanek C, Cuny C (2007) Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg Infect Dis 13:255–258

Williams AE, Blakemore WF, Alexander TJ (1988) A murine model of Streptococcus suis type 2 meningitis in the pig. Res Vet Sci 45:394–399

Wisselink HJ, Veldman KT, Van den Eede C, Salmon SA, Mevius DJ (2006) Quantitative susceptibility of *Streptococcus suis* strains isolated from diseased pigs in seven European countries to antimicrobial agents licensed in veterinary medicine. Vet Microbiol 113:73–82

World Health Organization (2012) Research priorities for zoonoses and marginalized infections. World Health Organ Tech Rep Ser ix–xi:1–119, 112 p following 119

Wong KK, Greenbaum A, Moll ME, Lando J, Moore EL et al (2012) Outbreak of influenza A (H3N2) variant virus infection among attendees of an agricultural fair, Pennsylvania, USA, 2011. Emerg Infect Dis 18:1937–1944

Woodward DL, Khakhria R, Johnson WM (1997) Human salmonellosis associated with exotic pets. J Clin Microbiol 35:2786–2790

Wulf M, Voss A (2008) MRSA in livestock animals-an epidemic waiting to happen? Clin Microbiol Infect 14:519–521

Wulf MW, Markestein A, van der Linden FT, Voss A, Klaassen C et al (2008) First outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch hospital, June 2007. Euro Surveill 13:8051

Wulf MW, Verduin CM, van Nes A, Huijsdens X, Voss A (2012) Infection and colonization with methicillin resistant *Staphylococcus aureus* ST398 versus other MRSA in an area with a high density of pig farms. Eur J Clin Microbiol Infect Dis 31:61–65

Xu B, Chang J, Gao G, Tang B, Hong J et al (1995) Four outbreaks of human trichinellosis in Henan Province. Chin Med J 108:872–874

Yan B, Zhang L, Gong L, Lv J, Feng Y, Liu J, Song L, Xu Q, Jiang M, Xu A (2016) Hepatitis E virus in yellow cattle, Shandong, Eastern China. Emerg Infect Dis 22(12):2211–2212

Young PL, Halpin K, Selleck PW, Field H, Gravel JL et al (1996) Serologic evidence for the presence in *Pteropus* bats of a paramyxovirus related to equine morbillivirus. Emerg Infect Dis 2:239–240

Yu H, Jing H, Chen Z, Zheng H, Zhu X et al (2006) Human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis 12:914–920

Yu HJ, Liu XC, Wang SW, Liu LG, Zu RQ et al (2005) Matched case-control study for risk factors of human *Streptococcus suis* infection in Sichuan Province, China. Zhonghua Liu Xing Bing Xue Za Zhi 26:636–639

Yu F, Cienfuegos-Gallet AV, Cunningham MH, Jin Y, Wang B, Kreiswirth BN, Chen L (2021) Molecular evolution and adaptation of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) sequence type 9. mSystems 6(3):e0049221

Zeller MA, Li G, Harmon KM, Zhang J, Vincent AL, Anderson TK, Gauger PC (2018) Complete genome sequences of two novel human-like H3N2 influenza A viruses, A/swine/Oklahoma/65980/2017 (H3N2) and A/swine/Oklahoma/65260/2017 (H3N2), detected in swine in the United States. Microbiol Resour Announc 7(20):e01203–e01218

Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wang H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Yan Q, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS (2010) Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 376(9744):895–902

Small Ruminants: Zoonotic Infections

5

Live or Dead – Direct or Indirect – New or Old

Snorre Stuen

Contents

S. Stuen (✉)
Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Sandnes, Norway
e-mail: snorre.stuen@nmbu.no

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_3

Abstract

Sheep and goat can be infected with several pathogens. While some may have a great impact on the small ruminant industry, others are more important as zoonotic agents. Human infections can occur from contact with both live and dead animals, animal products, and wastes, whereas some are well known while others have only recently been discovered. Several microorganisms may cause severe infection if transmitted to vulnerable people, such as pregnant and immune-comprised persons. Climate change, increased globalization, loss of natural habitats, improved diagnostics, and an active surveillance will increase records of zoonotic infections. The present chapter will briefly deal with a selected number of zoonotic microorganisms where small ruminants may play an important role as hosts for human infection.

Keywords

Sheep · Goats · Zoonosis · Review

5.1 Introduction

Sheep and goats, which were domesticated by humans as early as 9.000 years ago, are distributed worldwide and their products, such as milk, meat, wool, and skin are used extensively. Microorganisms have therefore been shared between humans and small ruminants for a long period of time. In this context, small ruminants may carry zoonotic pathogens, either transiently or permanently. The risk of transmission to humans varies considerably due to geographical area, seasons, climatic conditions, and management systems. The impact on human health will also depend on pathogen species/subspecies, virulence of the pathogen, level of exposure, presence of co-infections, susceptibility of the host, the host immune status, and the transmission route.

In general, microorganisms can be transmitted from small ruminants to humans by direct contact, aerosols, milk, meat, or indirectly by manure, feces, urine, and wool. For instance, the development of zoonotic infections through exposure to manure, either directly or indirectly, constitutes a real and significant risk of human health. Contamination of ground, irrigation or drinking water provides not only a source of infection, but also a means to spread the pathogens (Milinovich and Klieve 2011).

Water and foodborne zoonotic pathogens, such as *Campylobacter jejuni*, *Cryptosporidium parvum*, *Escherichia coli* (EHEC), *Giardia duodenalis*, and *Salmonella* spp. are widespread and have a wide host range. *Salmonella* spp., for instance, are ubiquitous in nature and have been isolated from a wide variety of vertebrate hosts. *Salmonella* is also commonly found in farm animals, their environments, and is one of the most important foodborne zoonotic microorganisms (Milinovich and Klieve 2011). Infections from goat products due to consumption of raw or uncooked goat meat, milk, and cheese have been documented. Severe gastroenteritis and even fatalities may occur (Desenclos et al.1996; Espié and Vaillant 2005). However, human salmonellosis may not normally arise as a result of contact with small ruminants, since this transmission pathway seems to be less frequent than that from other animals such as cattle and poultry (Kirby 1985; Rabinowitz and Conti 2010). Although *S. enterica* subsp. *diarizonae* is commonly found in sheep, the impact on human health seems to be limited (Sören et al. 2015). Similarly, all the mentioned pathogens are important zoonotic agents, but small ruminants play normally a less important role as reservoir for human infection compared to other species (Palmer et al. 1998). These microorganisms will therefore not be described further in this condensed review.

Some links between diseases in humans and animals are still debated. For instance, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) causing Johne´s disease in ruminants has been isolated from some humans with Crohn´s disease, a chronic granulomatous infection of the human intestine (Sharp 2007; Smith and Sherman 2009). The association between this bacterium and the disease is still unclear (Agrawal et al. 2020). The presence of MAP in a percentage of Crohn´s disease patients is either associated with the pathogenesis of the disease or these patients may be more likely to be colonized by these organisms. The unanswered question raises the issue of meat, milk, and water contamination by MAP and human health (West et al. 2009; Singh and Gopinath 2011). Transmissible spongiform encephalopathies such as classical scrapie is an old, widespread, and well-known disease in small ruminants, but its zoonotic potential has not yet been verified, although bovine spongiform encephalopathies, another prion disease, may cause human disease (Ganter 2015). It has also been questioned if Borna disease virus infection in sheep, one of the principle species affected could be transmitted to humans (Chalmers et al. 2005; Dürrwald et al. 2007). However, recent investigation indicates that sheep only represent an accidental dead-end host, with no contribution to the spread of the virus (Rubbenstroth et al. 2019). The issues mentioned above will not be discussed further in this chapter.

5.2 Specific Infections

Zoonotic pathogens detected in small ruminants of which several may cause severe infection in humans, are listed in Tables 1, 2 and 3. These lists, however, are not complete. Microorganisms may, for instance, be transmitted to humans due to the lack of normal hygiene procedures when handling infectious material. Common pathogens, such as *Staphylococus aureus* and *Trueperella pyogenes*, which regularly cause infections in small ruminants, are not covered by this chapter. In this short

Table 1 Zoonotic bacteria, rickettsia, and chlamydia detected in small ruminants

Pathogen	Host	Distribution	Transmission	Clinical symptoms (small ruminants)	Clinical symptoms (human)	References
Anaplasma capra	Wild deer, small ruminants	Asia, Europe	Ticks	Mild - subclinical	Flu-like CNS-symptoms	Peng et al. (2021)
Anaplamsa ovis	Small ruminants	Worldwide	Ticks	Mild- severe, Anemia	Flu-like (only one case)	Chochlakis et al. (2010) Stuen (2020)
Anaplasma phagocytophilum (several variants)	Several mammals	Northern Hemisphere (Ixodes-tick)	Ticks	Mild - subclinical Mild- severe, Anemia Fever, abortion (secondary infections)	Variable, flu-like – severe infection	Woldehiwet (2010) Stuen (2020)
Bacillus anthracis	Several mammals	Worldwide	Aerosols, cutaneous, oral (spores)	Found dead	Variable, cutaneous, pulmonary, and intestinal form	Turnbull (1998)
Borrelia burgdorferi sensu lato	Several, incl. small rodents, birds	Northern Hemisphere (Ixodes-tick)	Ticks	Subclinical, arthritis	Variable Acute - subacute -chronic form	Stanek et al. (2002)
Brucella melitensis (*B. abortus*)	Several, mainly small ruminants	Widespread, especially Mediterranean, Middle East	Oral (aerosols, cutaneous)	Abortion, arthritis	Variable, undulating fever chronic	See text
Burkholderia pseudomallei	Several	Widespread, mainly tropical areas	Oral, insects, vertical transmission	Abscesses, weight loss, polyarthritis meningoencephalitis	Pneumonia, septis, genitourinary infection, abscesses, suppurative parotitis, encephalomyelitis	Cheng and Currie (2005) Smith and Sherman (2009)

(continued)

Table 1 (continued)

Pathogen	Host	Distribution	Transmission	Clinical symptoms (small ruminants)	Clinical symptoms (human)	References
Camphylobacter jejuni	Several, esp. poultry	Widespread	Oral	Abortion, watery diarrhea	Flu-like, Diarrhea	Skirrow (1998)
Chlamydopila abortus	Several, mainly small ruminants	Widespread	Aerosols	Abortion	Abortion, stillbirth, puerperal sepsis, renal failure, hepatic dysfunction, DIC	See text
Corynebacterium pseudotuberculosis	Several, incl. domestic animals	Widespread	Cutaneous, oral	Caseous lymphadenitis	Suppurative granulomatous lymphadenitis	Thomas (1998) Smith and Sherman (2009)
Coxiella burnetii	Several, incl. livestock	Widespread	Aerosols, oral (cutaneous, ticks)	Abortion, stillbirth, weak offspring	Flu-like, pneumonia, endocarditis, hepatitis	See text
Dermatophilus congolensis	Several species	Worldwide	Cutaneous	Dermatitis (exudate)	Dermatitis	Stewart (1972a, b) Hyslop (1980)
Ehrlichia ruminantium	Ruminants	Africa, Caribbean	Ticks	Heartwater	Severe, encephalitis, vasculitis	Allsopp (2010)
Escherichia coli (EHEC)	Several	Worldwide	Oral	Heartwater	Variable Diarrhea, hemorrahagic colitis HUS	Nelson et al. (1998) Smith and Sherman (2009)

Francisella tularensis	Several hosts, esp. rodents	Worldwide	Aerosols, oral, cutaneous, ticks	Sepsis	Variable Bubonic - intestinal - pneumonic form	Pearson (1998) Genchi et al. (2015)
Leptospira spp. serovar Pomona (serovar Hardjo-bovis)	Several mammals, incl. cattle, pig	Unknown	Oral, cutaneous	Fever, depression, dyspnea,weakness, anemia, icterus, hemoglobinuria	Flu-like, encephalitis	See text
Listeria monocytogenes (L. ivanovii)	Several	Worldwide	Oral, cutaneous	Abortion, encephalitis, septicemia, mastitis, diarrhea, ocular disease	Meningitis, encephalitis, septicemia	See text
Mycobacterium avium subspecies paratuberculosis (Johnés disease)	Several, esp. ruminants and rabbits	Widespread	Oral	Progressive weight loss, diarrhea, intermandibular edema	Chronic bronchitis, cervical lymphadenopathy, disseminated disease (Crohn´s disease?)	Gallagher and Jenkins (1998) Smith and Sherman (2009)
Panola Mountain Ehrlichia *Salmonella* spp.	Small runinants Several	USA, Africa Worldwide	Ticks Oral	Unknown Gastroenteritis, septicemia, abortion	Mild, sore neck Enteric fever, gastroenteritis, diarrhea, septocemia	Stuen (2020) Humphrey et al. (1998) Mearns (2007) Smith and Sherman (2009)
Yersinia enterocolitica *Y. paratuberculosis*	Several	Widespread	Oral	Enteritis, mastitis, abortion, ill-thrift	Enterocolitis, polyarthritis, erythema nodosum, exudative pharyngitis, sepsis	Butler (1998) Smith and Sherman (2009)

Table 2 Zoonotic virus detected in small ruminants

Pathogen	Host	Distribution	Transmission	Clinical symptoms (small ruminants)	Clinical symptoms (human)	References
Crimean-Congo hemorragic fever (genus *Nairovirus*)	Several, including wild and domestic animals	Asia, Africa, southeastern Europe	Ticks, especially *Hyalomma* spp.	Unknown	Variable, high fever, hemorrhages, multiorgan failure, high fatality rate	Bente et al. (2014)
Louping-ill (genus *Flavivirus*)	Several, especially sheep and grouse	Scandinavia, United Kingdom	Ticks, (*Ixodes ricinus*) (Aerosols, oral, cutaneous)	Variable, subclinical - incoordination - paralysis	Tick-borne encephalitis	Reid and Chianini (2007)
Nairobi sheep disease (genus *Nairovirus*)	Small ruminants	Africa	Ticks (mainly *Rhipicephalus appendiculatus*)	Fever, diarrhea, gastroenteritis, death	Rare.benign illness	Swanepoel (1998) Smith and Sherman (2009)
Orf (genus *Parapoxvirus*)	Several, mainly small ruminants	Worldwide	Cutaneous (oral)	Wart-like outgrows	Skin lesions	See text
Rabies (genus *Lyssavirus*)	Several	Widespread	Bite, (Aerosols, oral)	Behavior changes, paralysis, paralytic/ furious condition, death	Nonspecific (prodromal period) Paralysis, aggression, unconsciousness, paralysis	King (1998)
Rift Valley Fever (genus *Phlebovirus*)	Ungulates	Africa, Arabian Peninsula	Mosquitoes (Aedes spp.) . Other insects	Abortion, fever, listlessness, recumbency	Flu-like Menigoencephalitis, hemorrhagic fever, photophobia, retinitis	See text

Severe fever with thrombocytopenia syndrome (SFTS)	Several species	Asia, especially China	Ticks, especially *Hemophysalis longicornis*	Unknown	Fever, gastrointestinal symptoms, thrombocytopenia, leukocytopenia, lymphadenopathy	Chen et al. (2019)
Tick-borne encephalitis	Several, mainly small rodents, ticks	Asia, Europe	Ticks, oral	Mild, subclinical	Biphasic fever, tick-borne encephalitis Fever, rash, arthralgia	Ruzek et al. (2019)
Wesselsbron disease (genus *Flavivirus*)	Several, incl. domestic ruminants and rodents	South Africa	Mosquitos (Aedes), (Aerosols, oral)	Abortion, sudden death	Fever, rash, arthralgia	Leake (1998) Smith and Sherman (2009)

Table 3 Zoonotic parasites and fungi detected in small ruminants

Pathogen	Host	Distribution	Transmission	Clinical symptoms (small ruminants)	Clinical symptoms (human)	References
Babesia venatorum	Wild deer, especially roe deer	Europe	Ticks Oral	Unknown	Anemia, fever, dark urine	Azagi et al. (2020) Gray et al. (2019)
Cryptosporidium parvum	Several, esp. cattle	Worldwide	Oral	Subclinical (adults) Watery diarrhea (young animals)	Diarrhea	Wright and Coop (2007) Smith and Sherman (2009)
Dicrocoelium dendriticum	Several, esp. domestic ruminants	Widespread	Oral	Subclinical Weight loss, Anemia	Subclinical Constipation / diarrhea, hepatomegaly	Smith and Sherman (2009)
Echinococcus granulosus	Several (intermediate hosts) Canids (end host)	Widespread	Oral	Mainly subclinical	Variable (localization/size) Shock/pulmonary edema	See text

Eurytrema pancreaticum	Several domestic animals, esp. ruminants	Asia, South America	Oral	Subclinical, ill-thrift, weight loss emaciation	Nonspecific	Lloyd and Soulsby (1998) Taylor et al. (2007) Smith and Sherman (2009)
Fasciola hepatica	Several ruminant Snail: (intermediate hosts)	Worldwide	Oral	Acute, subacute, chronic (anemia, icterus, submandibular edema, death)	Variable Acute (hepatic) - chronic (biliary) phase	Mas-Coma (2005) Smith and Sherman (2009) Fried and Abruzzi (2010)
Giardia duodenalis	Several species	Worldwide	Oral	Subclinical, enteritis, diarrhea	Variable, diarrhea, chronic syndrome	Thompson (1998) Taylor et al. (2007)
Oestrus ovis	Small ruminants	Unknown	Flies	Nasal discharge, sneezing, rubbing (noses) (unthriftiness/incoordination),	Catarrhal conjunctivitis, stomatitis	Beesley (1998) Taylor et al. (2007)

(continued)

Table 3 (continued)

Pathogen	Host	Distribution	Transmission	Clinical symptoms (small ruminants)	Clinical symptoms (human)	References
Schistosoma spp.	Several	Widespread, esp. tropics, subtropics)	Cutaneous	Rhinitis, enteritis, hepatitis, pneumonia	Multisystemic Nonspecific Dermatitis	Taylor (1998) Smith and Sherman (2009)
Taenia multiceps (*Coenurosis cerebralis*)	Sheep	Unknown,	Oral	Depression, blindness, convulsions	Variable, (localization)	Lloyd (1998)
Toxoplama gondii	Multiple intermediate hosts, Felid family (end host)	Worldwide	Oral (tissue cysts)	Abortion, stillborn, weak offspring	Mild – transient - serious Abortion, congenital lesions	See text
Tricophyton verrucosum	Several, mainly cattle	Worldwide	Cutaneous	Alopecia, scaling, crusting, folliculitis	Dermatophytosis	Sparkes (1998)

review, the focus is on the distribution, hosts, disease manifestations, transmission, diagnosis, treatment, and control measures on a selected number of zoonotic micro-organisms where small ruminants may play an important role as reservoir hosts for human infection. Focus will be on the following pathogens: *Brucella melitensis*, *Chlamydophila abortus*, *Coxiella burnetii*, *Echinococcus granulosus*, *Leptospira interrogans*, *Listeria monocytogenes*, Orf-virus, Rift Valley fever virus, tick-borne pathogens (several), and *Toxoplasma gondii*.

5.3 Brucellosis

5.3.1 The Pathogen

Bacteria of the genus *Brucella* are Gram-negative coccobacilli. There are four important species that may cause infection in humans, whereas *B. melitensis* is considered as the most invasive producing the most severe disease in humans (Godfroid et al. 2005; El-Koumi et al. 2013). *B. melitensis* is associated with small ruminants, although *B. abortus* may occasionally cause infection in sheep and goats.

5.3.2 Occurrence

Worldwide, particularly in the Mediterranean region, Middle East, parts of Asia and Africa, and Central and South America (Corbel 1997; Castrucci 2007).

5.3.3 Hosts

B. melitensis is primarily found in sheep, goats, and camels, but cattle, dogs, and rats can also acquire the infection (Castrucci 2007).

5.3.4 Disease in Small Ruminants

B. melitensis may cause abortion, and occasionally orchitis and arthritis. Usually, abortion occurs from mid to late pregnancy. The infection may persist in the udder to the following pregnancies. Excretion of bacteria may last for 2 months in vaginal discharges and up to 180 days in milk after delivery or abortion (Castrucci 2007; Scott 2007; Smith and Sherman 2009).

5.3.5 Disease in Humans

It has been estimated that around 500.000 cases of human brucellosis occur annually (Franco et al. 2007). The incubation period varies from 1 week to several months.

Human brucellosis can be both an acute and a chronic febrile illness with a variety of clinical manifestations. The patient may show fever, chills, headache, muscle and joint pains, malaise, nausea, night sweats, and lack of appetite for 3–6 weeks. The condition may also show a variety of nonspecific hematological changes, such as anemia and leucopenia (Plommet et al. 1998; El-Koumi et al. 2013).

5.3.6 Transmission (Small Ruminants-Human)

The main route of entry is via the nasopharynx, although a cutaneous route of infection does also exist. Material from abortions represents the main source of transmission in ruminants, with the excretion of enormous numbers of bacteria in the placenta, fetal fluids, and fetus (Castrucci 2007). Humans, however, are mainly infected through ingestion of fresh (unpasteurized) milk, cheese, and meat, but also through direct contact with infected animals, semen, vaginal fluids, or infectious aerosols (Castrucci 2007; Smith and Sherman 2009). The environmental resistance of the pathogens varies; the organisms can, for instance, survive in dust for 3–44 days, in tap water for 30 days, on pasture between 15 and 35 days, and in liquid manure at 15 °C or below for up to 8 months (Plommet et al. 1998; Castrucci 2007).

5.3.7 Diagnosis in Small Ruminants

If abortion occurs, *B. meliensis* infection can be confirmed by bacteriological methods (aborted fetus and placenta) or serology (aborted ewe/doe). The diagnosis in the chronic stage of the infection is difficult, since the infection may become non-apparent. In nonpregnant ewes, the bacterium is not excreted from the vagina. However, during pregnancy, excretion starts at the time of delivery or abortion and could continue for several months (Castrucci 2007). There are several serological tests available, such as the standard agglutination test (SAT), Rose Bengal test, complement fixation test, and ELISA.

Although all organs may be infected, microscopic examination should focus on material with suspected large amounts of bacteria, such as placenta, fetus, and vaginal discharges in case of abortion. Stained tissue smears, bacterial culture, or PCR can be used for identification (Plommet et al. 1998; Redkar et al. 2001).

5.3.8 Treatment and Control

Brucellosis has been controlled in many countries, however it remains an important health issue in many developing countries. *B. melitensis* is considered as an important food safety concern in human because it may be present in dairy products made from milk of infected small ruminants. The bacteria survive for days in fresh milk,

weeks in ice cream, and months in butter, although the bacteria are killed by pasteurization and are sensitive to common disinfectants (Godfroid et al. 2005).

Chemotherapy is not 100 % effective, so little is accomplished with the control and eradication of brucellosis in small ruminants. The best scheme is to identify and cull the infected animals (Castrucci 2007). Vaccination of sheep and goats with an attenuated strain of *B. melitensis* is considered to be the main control strategy. Vaccination prevents abortion and reduces pathogen shedding from immunized animals, although the vaccine may retain some degree of virulence, which may result in abortion and excretion in milk. The vaccine may also interfere with serological testing (Godfroid et al. 2005). In addition, vaccination of replacement animals is not sufficient to control the disease, especially in countries with high prevalence, uncontrolled animal movements, nomadic and low socioeconomic conditions, and illegal import of animals (Ebrahimi et al. 2012).

Surveillance, testing, and massive immunization of animals, and national brucellosis control are necessary to eradicate the disease (El-Koumi et al. 2013). For human consumption, unpasteurized milk and milk products should be avoided. No human vaccine exists, however recent results are promising in developing a recombinant vaccine against *B. melitensis* (Gomez et al. 2013).

5.4 Chlamydophilosis (Ovine Enzootic Abortion (OEA)

5.4.1 The Pathogen

Ovine enzootic abortion (OEA) is caused by the obligate intracellular Gram-negative bacterium *Chlamydophila (*former *Chlamydia) abortus*. The organism belongs to the family *Chlamydiaceae* and genus *Chlamydophila*, which comprise two distinct developmental forms, a small extracellular infectious elementary body (EB) and a larger intracellular noninfectious, metabolically active reticulate body (Longbottom and Coulter 2003).

5.4.2 Occurrence

C. abortus is recognized as a major cause of reproductive loss in sheep and goats worldwide, although the disease does not appear to be a problem in either Australia or New Zealand (Aitken and Longbottom 2007).

5.4.3 Hosts

Main hosts are small ruminants, but the organism can also infect cattle, pigs, horses, and deer, although such infections are thought to be less common (Aitken and Longbottom 2007).

5.4.4 Disease in Small Ruminants

Chlamydophilosis is an important cause of abortion worldwide. In previously uninfected farms, up to 60% abortion rate of pregnant ewes or does has been reported (Ganter 2015). Infection in animals is usually asymptomatic, except for abortion, although some behavioral changes or a vaginal discharge may be observed. Ewes/does may deliver stillborn or weakly offspring that fail to survive. The majority of infected placentas will have thickened red intercotyledonary membranes, dark red cotyledons, and a creamy-yellow colored exudate on the surface. An infectious vaginal discharge may be observed for several days following abortion, but otherwise the ewes/does are clinically normal and are considered immune to further disease (Longbottom and Coulter 2003; Aitken and Longbottom 2007; Smith and Sherman 2009).

5.4.5 Disease in Humans

Human infections with *C. abortus* are rarely reported or remain underdiagnosed, as the organism normally induces asymptomatic or mild flu-like illness (Ganter 2015). The greatest threat of human infection seems to exist for pregnant women, where the outcome of infection in the first trimester of pregnancy is likely spontaneous abortion, while later infection causes stillbirths or preterm labor (Hyde and Benirschke 1997). Several cases of abortion, puerperal sepsis, and shock, including renal failure, hepatic dysfunction, and disseminated intravascular coagulation, as well as death have been reported (Buxton 1986; Bloodworth et al. 1987).

5.4.6 Transmission (Small Ruminants-Human)

Most cases in humans infection are associated with direct exposure to infected sheep or goats via inhalation of dust and aerosols during abortion or normal parturition. The major sources of infection are contact with placental membranes, dead fetuses, live lambs/kids born to infected mothers, and vaginal discharges (Aitken and Longbottom 2007; Smith and Sherman 2009).

5.4.7 Diagnosis in Small Ruminants

A presumptive diagnosis of infection can be made based on abortion in the last 2–3 weeks of gestation and examination of the placenta. Pathological changes involve both the intercotyledonary membranes and the cotyledons. This is usually confirmed by the identification of large numbers of EBs in stained smears prepared from the placental membranes and cotyledons using, for instance, a modified Ziehl-Nielsen procedure. Other methods of antigen detection include immunohistochemical staining of tissue sections, immunoassays, DNA amplification methods, and isolation in cell-culture. Serological testing is normally performed by the complement fixation test on paired blood samples. However, none of the current serological tests

have been proven to be suitable for detecting infection prior to abortion and are not able to differentiate vaccinated animals from those infected with wild-type strains (Longbottom 2008; Sachse et al. 2009).

5.4.8 Treatment and Control

If OEA is suspected to be present in a flock or herd, the administration of long-acting oxytetracyclines will reduce the severity of infection and losses resulting from abortion. Although such treatment will reduce losses and limit the shedding of infectious organisms, it does not eliminate the infection nor reverse any pathological placental damage already done; thus abortions or the delivery of stillborn or weakly lambs can still occur and the shed organisms are a source of infection for naïve animals (Longbottom and Coulter 2003; Aitken and Longbottom 2007). Animals that have aborted are considered immune to further disease. Ewes, however, may become persistently infected carriers and continue to excrete infectious organisms at the next oestrus (Papp et al. 1994; Papp and Shewen 1996).

In humans, early therapeutic intervention is important, whereas tetracycline, erythromycin, and clarithromycin should be used. Severely ill patients require supportive therapy (Sillis and Longbottom 2010).

During an OEA outbreak the primary aim is to limit the spread of infection to other naïve animals. Affected animals should be identified and isolated as quickly as possible. All dead fetuses, placental membranes, and bedding should be carefully destroyed and lambing pens cleaned and disinfected. Pregnant women and immune-compromised individuals are advised not to work with sheep, particularly during the lambing period and should avoid all contact with possible sources of infection. Basic hygiene procedures, including thorough washing of hands and the use of disposable gloves are essential when handling potentially infected materials (Winter and Charnley 1999; Longbottom and Coulter 2003).

Live-attenuated vaccines based on a temperature-sensitive mutant *C. abortus* strain have been used for several years. These vaccines must be administered at least 4 weeks prior to mating and cannot be used in combination with antibiotic treatment. Good protection from abortion is obtained, but does not completely eradicate the shedding of infectious organisms at parturition. Moreover, some vaccinated animals still abort as a result of wild-type infections. Vaccine development to produce the next generation OEA vaccine continues to progress. This is likely to be a subunit vaccine based on protective recombinant antigens identified through comparative genomic and proteomic approaches (Longbottom et al. 2013; Entrican et al. 2012).

5.5 Contagious Ecthyma (orf)

5.5.1 The Pathogen

Contagious ecthyma is caused by orf-virus, a DNA- and poxvirus belonging to the genus *Parapoxvirus*.

5.5.2 Occurrence

Orf-virus is distributed worldwide.

5.5.3 Hosts

Several ruminants may be affected by orf-virus, especially small ruminants.

5.5.4 Disease in Small Ruminants

Orf-virus affects the skin primarily around the mouth and udder. There is considerable heterogenicity between virus isolates, but it is still not confirmed if different virulence exists. A correlation between genetic variability and virulence has to be further elucidated. Genetic differences in orf virus strains seem to be due to geographic locations and animal hosts involved (Reid and Rodger 2007; Li et al. 2012).

The clinical manifestation is variable. Symptoms are seen most frequently in young lambs, normally in two peaks, first in spring shortly after lambing and then 3–4 months later. Morbidity usually approaches 100%, while in most outbreaks the mortality is low. However, occasionally up to 80% mortality has been recorded. Severity of outbreaks seems to be attributed to environmental factors (Reid and Rodger 2007).

The lesions usually develop at sites where the skin or the mucous membranes are traumatized. The first clinical signs are local erythema, followed by formation of papules, vesicles, and pustules ending in scab formation. Without secondary infections the lesions resolve within approximately four weeks. In natural cases, proliferation often gives rise to wart-like outgrows, which may develop into extensive cauliflower-like structures that persist for a long period. Lesions are normally found around the mouth and nostrils, but may also develop on the buccal cavity, esophagus, ears, axilla, poll, lower limbs, and coronet. The infection can also spread to the udder thus increasing the risk of mastitis (Reid and Rodger 2007; Smith and Sherman 2009; Li et al. 2012).

5.5.5 Disease in Humans

In humans, after an incubation period of 3–7 days, a macropustular reaction occurs, most commonly found on one finger. As in small ruminants, the development stages comprise erythema, papules, vesicles, pustules, and scabs. Several lesions may be present on hand and arm, but single lesions are more common. These are usually raised, circular, or oval and about 0.5–1.5 cm in diameter, often with central vesiculation and pustulation. The lesions will normally heal and detach after 6–8 weeks without leaving a scar. However, secondary bacterial infection can cause complications, especially lymphangitis and lymphadenitis of the draining lymph nodes, which may be associated with flu-like symptoms. Infection may in some cases develop into a generalized reaction, including widespread maculopapular

eruption and Erythema multiforme. Extensive lesions have especially been seen in immunosuppressed people (Martin 1991a; Reid and Rodger 2007).

5.5.6 Transmission (Small Ruminants-Human)

Humans are mainly infected by direct contact with lesions from live animals. Infection can also be transmitted by fomites. Persons directly handling infected animals, particularly when bottle-feeding lambs, shearing, and slaughtering sheep are especially at risk (Reid and Rodger 2007).

5.5.7 Diagnosis in Small Ruminants

Diagnosis is mainly based on clinical signs, such as papillomatous lesions around the lips and nostrils. However, the clinical picture may be atypical and laboratory confirmation is necessary. Electron microscopy has earlier been used to verify the diagnosis, but PCR-methods are now available (Reid and Rodger 2007).

5.5.8 Treatment and Control

Outbreaks spread rapidly in a flock, with most animals becoming affected within a few weeks. Such outbreaks will last for 6–8 weeks. No specific treatment is available. The main treatment is to avoid secondary infections. A live vaccine is available in some countries. If vaccination during an outbreak is considered necessary, an autogenous vaccine can also be prepared (Reid and Rodger 2007). Vaccine development using a DNA-vaccine has showed promising results (Zhao et al. 2011).

Persistently infected animals with no clinical symptoms have been described. The importance of these animals in the epidemiology of the infection is unknown. The virus may survive in buildings and handling facilities between epidemics. Orf-virus is known to survive in dry scabs for a long period, up to 23 years at 7 °C, but the infectivity is lost more rapidly at higher temperature and at more moist conditions. Disinfection of the actual pens should be performed. Infection in humans can normally be avoided through good hygienic procedures. Protective gloves should be used when handling infectious animals or infective material (Reid and Rodger 2007; Smith and Sherman 2009).

5.6 Echinococcosis (hydatidosis)

5.6.1 The Pathogen

E. granulosus is a tapeworm that belongs to the class *Cestoda* and the family *Taeniidae*. Several species of genus *Echinococcus* exist, but it is mainly *E. granulosus* that involves small ruminants as intermediate hosts. Ten genotypes (G1-G10) including five species

have been characterized, of which three (G1 to G3) are "sheep" strains belonging to *E. granulosus* sensu stricto (Moro and Schantz 2009), while G1 and G6 (*E. canadensis*) affect goats (Smith and Sherman 2009; Moro and Schantz 2009; Wen et al. 2019).

5.6.2 Occurrence

E. granulosus is widespread in areas where sheep are reared (Brunetti and White 2012).

5.6.3 Hosts

The definite host are domestic dogs and some wild canids. There are several intermediate hosts such as sheep, goats, cattle, swine, camelids, cervids, lagomorphs, and humans. The sheep strain G1 is most commonly associated with human infection (Moro and Schantz 2009).

5.6.4 Disease in Small Ruminants

Cestode eggs, which contain oncospheres must be ingested in order to continue the life cycle of the parasite. After ingestion, the larval stage will develop to cysts (hydatid cyst) in different organs, most commonly in liver and lungs. No definite clinical symptoms have been observed in small ruminants, even in cases with multiple cysts in either liver or lungs (Taylor et al. 2007).

5.6.5 Disease in Humans

E. granulosus cysts in humans may take years to develop and produce clinical symptoms. Many cysts remain asymptomatic throughout life and are only discovered by accident. However, the infection can result in respiratory distress and abdominal enlargement depending on which organ is affected. Clinical symptoms depend on the location and size of the *E. granulosus* cyst, and are mainly due to the pressure on the actual organ and on surrounding tissues. In man, the hydatid cysts may be 5–10 cm in diameter or even larger (Martin 1991b). The most common localization is the liver (70%), followed by the lungs. Rupture of the cyst is often fatal, due to anaphylactic shock or pulmonary edema (Moro and Schantz 2009; Brunetti and White 2012).

5.6.6 Transmission (Small Ruminants-Human)

The dog-sheep-dog cycle is the most important cycle in several endemic areas. Small ruminants normally contract *E. granulococus* by gracing on pasture contaminated by dog feces containing cestode eggs. The dogs are again infected by ingestion of viscera with fertile cysts (Moro and Schantz 2009).

Man can be infected by direct contact to dogs or indirectly through contaminated food, water, and infected objects. Dogs may carry eggs on the body surface and a person can become infected by touching the animal. Close contact with dogs and lack of hygiene are important factors for transmission. Another important source of human infection is through vegetables and water contaminated with eggs. Ingestion of infected flies may also transmit the infection (Lawson and Gemmell 1990). However, direct transmission from small ruminants to man has not been observed (Moro and Schantz 2009; Smith and Sherman 2009).

5.6.7 Diagnosis in Small Ruminants

Numerous tests have been developed for the diagnosis in humans, although few reliable serological tests are available for small ruminants. Various imaging techniques can be used to identify the hydatid cysts, but postmortem examination is still the most reliable method for diagnosis in intermediate hosts (Moro and Schantz 2009; Smith and Sherman 2009), although molecular diagnosis may also be used to identify infected animals (Wen et al. 2019).

5.6.8 Treatment and Control

The main control measurement is to interrupt the transmission cycle from the intermediate to the definite host. The infection cycle would be halted if dogs lack access to the viscera of intermediate hosts. In addition, the number of dogs might be reduced or treated with efficient anthelmintics. Treatment of infected sheep/goat in order to stop the infectivity of the cysts is not yet possible. Recombinant vaccines have been developed both for sheep and dogs with promising results (Lightowlers et al. 1999; Zhang and McManus 2008).

Oncospheres have little resistance to desiccation and high temperature; however they may survive in water/damp sand for 225 days at 6 °C (Lawson and Gemmell 1983). Hygiene is important to prevent human infection, as eggs may be swallowed with uncooked vegetables contaminated with dog feces or from fingers contaminated from soil or the fur of an infected dog. Close contact with possibly infected dogs should therefore be avoided. Early diagnosis in human is important to avoid complications and rupture of the cysts. Surgery was earlier the traditional approach for treatment in humans, but anthelmintics, percutaneous procedures, and a watch-and-wait approach are now more commonly used (Brunetti and White 2012).

5.7 Leptospirosis

5.7.1 The Pathogen

Leptospirosis is caused by helical Gram-negative organisms of the family *Leptospiracea* and the genus *Leptospira*. More than 250 serovariants have been detected (Cerqueira and Picardeau 2009). The main serovariants infecting small

ruminants seem to be *L. borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona (West et al. 2009).

5.7.2 Occurrence

Leptospira involving small ruminants have a worldwide distribution.

5.7.3 Hosts

Several hosts are involved, including cattle and swine.

5.7.4 Disease in Small Ruminants

Small ruminants have been found infected with the three species of the genus *Leptospira*, namely, *L interrogans*, *L. borgpetersenii*, and *L. kirschneri* (Levett 2001; West et al. 2009; Smith and Sherman 2009). There are several serovariants within each species, such as *L. interrogans* serovar Pomona. Virulence of these strains varies, whereas the majority of leptospiral infections in small ruminants are subclinical and disease seems to be uncommon. However, septicemia, depression, anorexia, and in some cases hematuria may occur. Severe illness is characterized by jaundice, hematuria, and hemoglobinuria, which may progress to a fatal outcome. Abortion has also been reported (Smith and Sherman 2009; West et al. 2009).

Sheep could be infected with *L. interrogans* Hardjobovis, but are usually asymptomatic and studies indicate that sheep are only transiently infected with this serovariant (West et al. 2009). In addition, serovar *L. grippotyphosa*, *L. icterohemorrhagiae*, and *L. serjoe* have been involved in clinical leptospirosis in goats (Smith and Sherman 2009).

5.7.5 Disease in Humans

Human disease varies widely according to the serovar of *Leptospira* involved. The incubation period varies from 2 to 30 days. In the acute febrile stage, the clinical symptoms are related to a generalized vasculitis, such as severe headache, muscle pain, conjunctival suffusion, rash, and photophobia. Intrauterine infection and fetal death may occur in pregnant women. The infection may proceed to aseptic meningitis and renal failure (Ellis 1998).

5.7.6 Transmission (Small Ruminants-Human)

Human infection may be acquired through occupational, recreational, or avocational exposures. Leptospires persist in the kidney and genital tracks of carrier animals and

are excreted in the urine and genital fluids. Survival outside the host is favored by warm and moist conditions. Transmission is mainly due to direct or indirect contact with persistently infected animals and occurs through contact with infected urine, products of abortion, handling of infected kidneys, and ingestion of infected milk. However, a recent study excludes raw milk as a source of human infection (Fratini et al. 2016). Leptospires gain access to the host mainly through mucous membranes and abraded and water-softened skin (Ellis 1998).

5.7.7 Diagnosis in Small Ruminants

The diagnosis is based on laboratory confirmation, such as PCR analyses of blood, CSF or tissue biopsy, and serology (such as Microscopic agglutination test (MAT) and ELISA). Leptospires in the urine is, however, not a common feature of serovar Pomona infection in sheep (West et al. 2009). No reliable method exists for detection of carrier animals.

5.7.8 Treatment and Control

Leptospira are important pathogens in developing countries, where poor work and living conditions increase the opportunity for transmission from animals to man. The infection often occurs after heavy rainfall when surface water accumulates in the paddocks. Clinical cases should be treated with antibiotics (West et al. 2009). Vaccines based on killed whole leptospiral cells have been available for several years. Recent vaccine developments based on recombinant proteins showed promising results (Yan et al. 2010; Félix et al. 2011).

To avoid spread of the infection, infected animals should be identified and contact with carrier animals should be minimized. A potential for venereal transmission of *Leptospira* strains in small ruminants have been reported (Lilenbaum et al. 2008; Arent et al. 2013). Prevention should be based on environmental control, such as rodent control, elimination of standing water, and avoidance of damp beddings. In addition, contact with infected herds and import of infected animals should be avoided. In order to prevent the human infections, common water sources or potentially contaminated water supplies should be restricted. Farmers, milkers, slaughterhouse, and meat-processing workers as well as veterinarians have an increased risk for exposure (Dorjee et al. 2008; Smith and Sherman 2009).

5.8 Listeriosis

5.8.1 The Pathogen

Listeria monocytogenes is a Gram-positive coccobacillus within the genus *Listeria*. At least 16 serotypes with numerous subtypes of *L. monocytogenes* exist. *L. ivanovii* may occasionally cause abortion in small ruminants, but this bacterium rarely infects

humans causing bacteremia, fetal loss, or gastroenteritis (Smith and Sherman 2009; Guillet et al. 2010).

5.8.2 Occurrence

L. monocytogenes is ubiquitious in the environment.

5.8.3 Hosts

Several animals including small ruminants can be infected with *L. monocytogenes*. The natural reservoir appears to be the mammalian gastrointestinal tract. Grazing animals may ingest the bacteria and further contaminate vegetation and soil (Scott 2007).

5.8.4 Disease in Small Ruminants

There are mainly six manifestations of the disease: abortion, septicemia, encephalitis, diarrhea, mastitis, and ocular infections. Clinical manifestations vary according to the route of infection. *L. monocytogenes* often affects the pregnant uterus and the central nervous system. During pregnancy, infection spreads to the fetus, which will either be born severely ill or die *in utero* (Scott 2007).

Listeriosis is one of the most common neurological diseases in adult sheep. Sheep aged 18–24 months are often affected due to molar teeth eruption, which may facilitate infection. Lesions are normally localized in the brainstem and clinical signs indicate unilateral dysfunction of the third to seventh cranial nerves. Facial nerve paralysis with dropping ear, muzzle pulled to one side, and lowered upper eyelids are typical symptoms. Profuse salivation and retained food material in the cheek is also typical. Keratoconjunctivitis and iritis may occur, in addition to partial paralysis of the pharynx. The clinical course in sheep and goats is often rapid, and death may occur 4–48 h after onset of clinical symptoms (Scott 2007; Smith and Sherman 2009).

5.8.5 Disease in Humans

Systemic *L. monocytogenes* infection is a serious, but usually sporadic, invasive disease that primarily affects pregnant women, neonates, and immune-compromised persons (Cork and Checkley 2011). Infections can be treated successfully with antibiotics, but 20–40% of human cases are fatal (McLauchlin and Van der Mee-Marquit 1998).

The infective dose of *L. monocytogenes* is not known. The incubation period from foodborne infection varies widely from 3 up to 70 days, with a medium

incubation period estimated to be around 3 weeks. There may be strain variation in pathogenicity, but this has to be unraveled more closely. Outbreaks of listeriosis are usually spread via the fecal-oral route, resulting in a self-limiting gastroenteritis in healthy persons. However, cutaneous infection has also been observed in people during deliveries of listeria-infected animals. During pregnancy, infection spreads to the fetus. In nonpregnant humans, listeriosis usually presents as meningitis, encephalitis, or septicemia in the immune-compromised and elderly (McLauchlin and Van der Mee-Marquit 1998; Swaminathan and Gerner-Smidt 2007; Cork 2011).

5.8.6 Transmission (Small Ruminants-Human)

Foodborne transmission of *L. monocytogenes* is the main route of infection, whereas unpasteurized dairy products are the main source of human infection. Other sources include uncooked food of animal origin and contaminated raw vegetables. *L. monocytogenes* may also be transmitted by direct contact with infected animals or animal products. In such cases, the disease occurs principally as papular or cutaneous lesions, usually on the arms or the wrist 1–4 days after attending a listeria-abortion. This manifestation, however, has mainly been seen after contact with cattle (McLauchlin and Van der Mee-Marquit 1998; Smith and Sherman 2009).

5.8.7 Diagnosis in Small Ruminants

Unilateral cranial nerve paralysis affecting the eye, eyelid, ear, and lips with ataxia are typical for listeriosis. Samples from cerebrospinal fluid can support the diagnosis. At postmortem examination, histological lesions such as microabscesses and perivascular cuffing in the brainstem and medulla are pathognomonic of listeriosis. Aborted fetuses due to *L. monocytogenes* are usually autolytic with miliary necrotic foci scattered throughout the liver and spleen, while listeria-septicemia is often accomplished by focal hepatic necrosis. Listeriosis, however, can only be confirmed by isolation or identification of *L. monocytogenes* (Low and Donachie 1991; Scott 2007).

5.8.8 Treatment and Control

Infection can be treated with antibiotics. The drug of choice is high-dosed penicillin. Supportive therapy including fluids and electrolytes are required for animals having difficulty eating and drinking (Scott 2007).

In an outbreak, affected animals should be segregated. In silage-fed ruminants, listeriosis is mainly a winter-spring disease and is normally seen in animals fed with poorly conserved silage. Outbreaks may occur within 10 days of feeding poor silage. Use of the particular roughage should be discontinued. However, due to an incubation period of 1–3 weeks, most of the *Listeria*-infected silage may not be

longer available. Animal to animal transmission may occur via the fecal-oral route. A live attenuated vaccine for use in sheep has been developed, but the results from field trial vaccinations are equivocal (Scott 2007). However, new vaccine technologies seem promising in developing a protective immune response against *L. monocytogenes* (Carrasco-Marin et al. 2012; Kim et al. 2012; Luo and Cai 2012; Mohamed et al. 2012).

To avoid infection in humans, hygiene during food preparation and storage as well as avoidance of unpasteurized dairy products are preventive measures. However, *L. monocytogenes* can survive in soil or silage for more than 2 years. It is also found in excreta from apparently healthy animals, although carriage in the gut is likely to be transitory. Control measures should be focused on avoiding *Listeria*-contaminated food, especially since the bacteria maintain to grow during refrigeration. Pregnant women and immune-compromised individuals are at increased risk for developing listeriosis (McLauchlin and Van der Mee-Marquet 1998).

5.9 Q-Fever (Coxiella burnetii)

5.9.1 The Pathogen

Q-fever is caused by the intracellular organism *Coxiella burnetii* within the genus *Coxiella* and the order *Legionellales* (Seshadri et al. 2003). The organism exists in two different antigenic phases. In nature, *C. burnetii* exists in phase 1 form, which is virulent. However, when cultivated in cell cultures or hen eggs the organism mutates irreversibly to the phase II form, which is less virulent (Quevedo Diaz and Lukacova 1998). *C. burnetii* has mainly two different morphologic forms, a large and a small form. In addition, an endospore-like structure is observed in the large form, which is highly resistant to environmental degradation, such as high temperatures, ultraviolet light, and osmotic shock (Mearns 2007).

5.9.2 Occurrence

Q fever is a worldwide zoonosis that occurs in all geographic and climate zones, with the exception of Antarctica and possibly New Zealand (Hilbink et al. 1993; West et al. 2009). However, Q fever is not a reportable disease in many countries, so it is difficult to know exactly where it occurs.

5.9.3 Hosts

C. burnetii is able to infect many animal species including mammals, birds and several arthropods. However, cattle, sheep, and goat seem to be the primary animal reservoirs for human infection (Maurin and Raoult 1999).

5.9.4 Disease in Small Ruminants

In animals, *C. burnetii* infections are generally asymptomatic, except for abortion, stillbirth, and the delivery of weak offspring. However, *C. burnetii* may induce pneumonia, conjunctivitis, and hepatitis (Arricau-Bouvery and Rodolakis 2005). High abortion rates are rarely observed, although abortion storms in some caprine herds have been described (Sanford et al. 1994). In the outbreak of Q-fever in the Netherlands (2007–2009), abortion rate up to 60%, mainly in the final month of pregnancy, was reported in goats (Roest et al. 2011). Aborted fetuses appear normal, but infected placentas exhibit intercotyledonary fibrous thickening and discolored exudates that may be mineralized (Moore et al. 1991).

5.9.5 Disease in Humans

In humans, acute Q fever is rarely diagnosed, because of nonspecific initial clinical signs, such as fever, pneumonia, headache, and weakness. However, 1–2% of infected individuals can develop chronic infection that may result in severe granulomatous hepatitis, osteomyelitis and valvular endocarditis. Chronic infection can manifest itself within a few months or even years after the acute infection (Fournier et al. 1998; Ganter 2015).

5.9.6 Transmission (Small Ruminants-Human)

Contaminated aerosols generated from desiccation of infected placentas, body fluids, or dust from contaminated manure are the main sources of both animal and human infection, and the control of fecal excretion and placental bacterial discharge is essential (Arricau-Bouvery and Rodolakis 2005). Grazing contaminated pasture and tick bites are other modes of transmission. Single animals may shed large amount of *C. burnetii*, the so-called super-spreaders, within infected flocks (Bauer et al. 2020). The organism is also highly infectious, with an infective dose of 1–10 bacteria (Tigertt et al. 1961). Because *C. burnetii* is extremely resistant to desiccation and to physical and chemical agents, it survives in the environment for long periods. The endospore-like form survives in dust for 120 days, in tick feces for 568 days and in wool for 12–16 months at 4–6 °C (Mearns 2007).

5.9.7 Diagnosis in Small Ruminants

Current alternatives to diagnose *C. burnetii* infection in ruminants include serological analysis, isolation by cell culture, live animal inoculation, immunohistochemical, and PCR-based detection. In the acute phase of the infection, *C. burnetii* can be detected in lungs, spleen, liver, and blood (Fournier et al. 1998; Maurin and Raoult 1999).

Placental smear or impression of placentas can be stained, for instance, by using a modified Ziehl-Nielsen procedure (Mearns 2007). Several serologic tests are available, such as complement fixation test, ELISA, and a fluorescent antibody test. However, carrier animals may also have an antibody titer increase in late pregnancy (Kovacova et al. 1998; Smith and Sherman 2009). For Q fever diagnosis, it has been recommended to use PCR and immunofluorescence tests of *Coxiella* on parturition products and vaginal secretions at abortion (Arricau Bouvery et al. 2003).

5.9.8 Treatment and Control

If Q fever is suspected, aborting animals and animals in late pregnancy should be treated with tetracycline, although this treatment does not totally suppress the abortions and shedding of *C. burnetii* at lambing (Berri et al. 2005). Placentas and aborted fetuses should be destroyed properly and aborted animals should be isolated. In addition, materials such as bedding and straw contaminated with birth fluids and other secretions from affected animals should be destroyed (Smith and Sherman 2009).

The spread of *C. burnetii* infection in domestic animals depends on many factors, such as population density of animals, the system of rearing and management at parturition. Because the environment can remain infected for a long time and many species can be carriers, test and cull strategies are not appropriate for infected herds (Smith and Sherman 2009). However, during the recent outbreak of Q fever in humans in the Netherlands, the Dutch Government decided to cull more than 50.000 pregnant ewes and goats in order to halter the worst outbreak of Q fever ever known where more than 4000 human cases have been recorded from 2007–2010. The reason for this strategy was that dairy goats were believed to be the main source of human infection (van der Hoek et al. 2012).

In animals, the uterus and mammary gland of females are sites for persistent *C. burnetii* infection. Reactivation of the bacterium during pregnancy results in shedding of a great amount of infectious agent into the environment during abortion or via birth fluids, placenta, and fetal membranes (Sawyer et al. 1987). Over 10^9 bacteria per gram of placenta may be released at the time of delivery (Babudieri 1959). Studies indicate that ewes shed the bacterium mostly in feces and vaginal mucus, while in goats shedding in milk seems to be the most frequent route (Rodolakis et al. 2007; Rodolakis 2009).

In animals, the most effective vaccines are those composed of inactivated whole phase I bacteria. Bacterial shedding in placentas and milk was strongly reduced in experimental infection or in natural Q fever infection in ewes vaccinated with phase I vaccines (Sampere et al. 2003). Since phase I vaccine are dangerous to produce, a subunit vaccine has been investigated (Arricau-Bouvery and Rodolakis 2005).

To prevent human infection, drinking raw milk or consumption of raw milk products should be restricted. For inactivation, pasteurization of milk at 62.8 °C for

30 min or at 71.7 °C for 15 s is required (Kazar 1999). Q fever often occurs as an occupational disease. Persons at particular risk are livestock handlers, processors of animal products, abattoir workers, those in contact with dairy products, veterinarians, and laboratory personnel working with *C. burnetii*-infected animals (Maurin and Raoult 1999). In addition, it is necessary to inform vulnerable persons such as immunosuppressed patients or those suffering from cardiac valvopathy and pregnant women that they must avoid contact with animals during lambing and kidding (Arricau-Bouvery and Rodolakis 2005).

5.10 Rift Valley Fever (RVF)

5.10.1 The Pathogen

RVFV (Rift Valley fever virus) is a single-stranded RNA-virus in the genus *Phlebovirus* of the family *Bunyaviridae*.

5.10.2 Distribution

RVFV is mainly distributed in sub-Saharan Africa, but has also been identified in Northern Africa and on the Arabian Peninsula (Bath 2007).

5.10.3 Hosts

RVFV may cause infection in several ungulate species, although their importance as reservoir host has to be unraveled. Mosquito vectors, such as in the genus *Aedes*, may maintain the virus in endemic areas by transovarial transmission. Other insects, such as *Culex* species, may also be involved in epidemics (Bath 2007). Vertical transmission occurs in all livestock species, even in pregnant ewes with no detectable viremia (Antonis et al. 2013).

5.10.4 Disease in Small Ruminants

RVFV can infect a wide variety of tissues, such as liver, lymphoid, and nervous tissue. The incubation period is short, as little as 12 h in young lambs and up to 72 h in adult sheep. High fever, anorexia, listlessness, and recumbency are common in young lambs. However, clinical signs are not always observed, since young animals may die rapidly. Mortality rate may exceed 90% in lambs under 2 weeks old. Abortion is a common sign in adult animals, and this may occur at any time during pregnancy and reach up to 100%. Infection in older animals is often subclinical (Bath 2007).

5.10.5 Disease in Humans

Human infection can result in a variety of clinical outcome, while most cases induce a self-limited febrile illness (Wright et al. 2019). Fatality rate is normally less than 1%, but the death toll can mount to several hundreds in severe outbreaks (Swanepoel 1998; Bath 2007). The largest recorded outbreak in humans was in 1997–1998 in East Africa where approximately 89.000 human cases and 478 fatalities were recorded (CDCP 1998). Typical symptoms in humans are flu-like illness after a short incubation period of 2–6 days. Other symptoms are photophobia, retinitis, meningoencephalitis, and hemorrhagic fever. The symptoms may be severe in patients with a preexisting liver disease. Sequelae may include widespread hemorrhages, jaundice, shock, liver, and kidney failure and death.

5.10.6 Transmission (Small Ruminants-Human)

The route of transmission in animals is via different mosquitoes. The virus has been isolated from more than 30 mosquito species. In addition, RVFV has also been isolated from flies and midges (*Culicoides*). Both biological and mechanical vector transmission may occur. In Sub-Saharan areas the main vector seems to be mosquitoes within the genus *Aedes* (Bath 2007; Smith and Sherman 2009). Heavy rainfall often precedes a RVF outbreak, where an increase in vector population may increase transmission potential (Wright et al. 2019).

The main transmission route in humans is via direct or indirect exposure to infected blood, tissues, or body fluids of infected animals, for instance, in connection with slaughtering, butchering, obstetrical procedures, or treatment of infected animals. Infection may also occur via vectors, aerosols, and consumption of unpasteurized milk. Direct person-to-person transmission has not been reported. Persons at risk are veterinarians, farmers, shepherds and abattoir workers (Swanepoel 1998; Smith and Sherman 2009).

5.10.7 Diagnosis in Small Ruminants

The diagnosis is based on clinical symptoms and postmortem examination. In young lambs, widespread hemorrhages and liver necrosis are often recorded. Disseminated intravascular coagulopathy may occur in several internal organs. Samples from spleen, liver, and brain should be used for histological examination. The diagnosis, however, has to be verified by PCR, virus isolation, and serological investigations (such as ELISA and hemagglutination-inhibition test) (Bath 2007).

5.10.8 Treatment and Control

Outbreak of RVF occurs at irregular intervals. The virus may persist in a vector/natural host cycle and low level of virus activity is found between outbreaks.

Infected eggs from *Aedes* species may survive in the soil for years. The single most important responsible factor for an outbreak of RVF is heavy rainfall and widespread flooding, which favors multiplication of the vectors. Movement of infected animals or winds that transport infected mosquitoes over long distances may spread the disease to non-endemic areas (Sellers 1980). The development of early warning systems and surveillance in and around endemic areas in order to recognize animal and human cases as early as possible are crucial in order to control the infection.

There is no treatment available for infected animals, since the disease is usually very acute and the lesions too severe. Control measures rely on the use of efficient vaccines. A live attenuated vaccine is available for nonpregnant animals, while an inactivated whole virus vaccine can be used for pregnant animals. The last vaccine requires a booster and annual revaccination. A recombinant vaccine has recently been developed, but it must be tested in appropriate animal models before being used as a livestock and human vaccine (Indran and Ikegami 2012; Morrill et al. 2013). When handling suspicious animals, wearing of eye protection, protective clothing, gloves, and masks should be mandatory (Swanepoel 1998). Only general supportive treatment is available for human cases of RVF and no licensed vaccine is yet available (Wright et al. 2019).

5.11 Tick-Borne Pathogens

5.11.1 The Pathogens

Around 900 species of ticks have been described (Guglielmone et al. 2014), of which several are associated to small ruminants especially in the genera *Amblyomma*, *Haemophysalis*, *Hyalomma*, and *Rhipicephalus*, although *Dermacentor* and *Ixodes* ticks may also infest sheep and goats (Stuen 2020). Tick-borne infections detected in small ruminants may again affect humans, such as *Anaplasma phagocytophilum*, *A. ovis*, *A. capra*, *Borrelia burgdorferi* sensu lato, Crimean-Congo hemorrhagic fever (CCHF)-virus, *Ehrlichia ruminantium,* Panola Mountain *Ehrlichia* (PME), tick-borne encephalitis (TBE)-virus, Louping ill (LI)-virus, and *Babesia venatorium* (Bente et al. 2014; Böhm et al. 2017; Gray et al. 2019; Stuen 2020).

In addition, a recently described serious virus infection in humans caused by severe fever with thrombocytopenia syndrome virus (SFTSV) has been detected in China, were *Haemaphysalis longicornis* seems to be the main vector species. A lot of animal species may be involved as potential hosts, including small ruminants. However, whether SFTSV causes clinical symptoms in sheep and goats or undergoes animal to human transmission requires further studies (Chen et al. 2019).

Only a few human cases have been reported from *A. ovis*, *E. ruminantium*, and PME, and *B. venatorum* infection in sheep has so far only been reported once (Allsopp 2010; Chochlakis et al. 2010; Böhm et al. 2017; Stuen 2020). *Coxiella burnetii*, and *Francisella tularensis* may also be transmitted by ticks, although other transmission routes are normally more important (Arricau-Bouvery and Rodolakis 2005; Mailles and Vaillant 2014; Borde et al. 2017). In addition, *B. burgdorferi* s.l. infection in sheep seems to be rare (Stanek et al. 2002).

Although small ruminants can be infected with different zoonotic pathogens, small ruminants are seldom important hosts for these infections, although they could be important accidental hosts. The most important zoonotic tick-borne pathogens in this context seem to be *A. capra, A. phagocytophilum,* CCHF-, TBE-, and LI-*virus* (Stuen 2020).

5.11.2 Occurrence

Tick-borne pathogens related to small ruminants may occur on all continents. *A. phagoctophilum*, known as a sheep infection for more than two centuries, is reported worldwide, especially in Europe (Stuen 2020). Only scattered information from Asia and Europe is available concerning the distribution of *A. capra*, since it was first described in 2015 (Peng et al. 2021). Human CCHF is widespread in Asia, Africa, and south-eastern Europe (Bente et al. 2014), while TBE-virus is spread in Europe and Asia with mainly three genetically distinguishable subtypes within partly overlapping geographical areas: Western European TBEV (mainly transmitted by *I. ricinus*), and Siberian and Far Eastern TBEV (predominantly transmitted by *I. persulcatus*) (Ruzek et al. 2019). In contrast, LI virus is localized only in north-western Europe, especially in the UK (Jeffries et al. 2014).

However, distribution of these infections changes continuously due to migration and transportation of vectors and animals, and an increased globalization of animals and their products, driven directly or indirectly by climate changes. These changes will have a huge effect on the distribution and establishment of both hosts, pathogens and vectors (Shope 1991).

5.11.3 Hosts

Small ruminants are important hosts for *A. phagocytophilum*, and LI-virus, while only accidental hosts for CCHM- and TBE-virus. Several enzootic cycles of *A. phagocytophilum* occur in nature, whereas variants with a more important zoonotic potential seem mainly to involve small rodents, hedgehogs (*Erinaceus europaeus*), and wild boar (*Sus scrofa*) (Stuen 2020). *A. carpa* seems to be related to domestic and wild ruminants, but the importance of small ruminants as hosts has still to be unraveled (Peng et al. 2021). CCHM-virus is maintained in several genera of ixodid ticks, especially *Hyalomma* ticks, and through transient viremia in a variety of wild and domestic mammals (Bente et al. 2014). Small rodents and *Ixodes* spp. ticks are important reservoir hosts for TBE-virus, while red grouse (*Lagopus lagopus scotica*), mountain hare (*Lepus timidus*), and sheep are the main reservoirs for LI virus (Reid and Chianini 2007; Salat and Ruzek 2020).

5.11.4 Disease in Small Ruminants

The tick-borne pathogens mentioned above may cause a variable degree of clinical signs in small ruminants, although only *A. phagocytophilum* and LI-virus seem to introduce severe clinical illness. *A. phagocytophilum* causes tick-borne fever (TBF) in ruminants, a febrile disease. TBF by itself is seldom fatal, but the infection induces immunosuppression that makes affected animals vulnerable to secondary infections, such as pyemia and septicemia. TBF may therefore cause severe economic and welfare challenges in the sheep industry (Stuen and Longbottom 2011). LI virus may also cause severe fever illness in sheep, with CNS-symptoms that may progress into fatal encephalitis. High death rate occurs by simultaneous infection with *A. phagocytophilum* (Jeffries et al. 2014).

In contrast, *A. capra,* CCHF- and TBE-virus may cause mild or subclinical symptoms in small ruminants (Bente et al. 2014; Salat and Ruzek 2020; Stuen 2020), although there has been a report on clinical symptoms in sheep after natural infection with TBE-virus (Böhm et al. 2017).

5.11.5 Disease in Humans

Several tick-borne infections cause severe disease in human, such as anaplasmosis, CCHF, and TBE. *A. phagocytophilum* causes flu-like symptoms, but severe complications have been reported involving septic shock like syndrome and acute respiratory distress symptoms. The mortality rate is around 1% (Bakken and Dumler 2015). More than 15.000 human cases have so far been reported, mainly in the USA (Stuen 2020). However, phylogentic studies show that strains/variants of *A. phagocytophilum* isolated from sheep differ from isolates normally affecting humans, indicating that sheep are an uncommon reservoir host for clinical cases in humans (Scharf et al. 2011; Jahfari et al. 2014).

A. capra may cause flu-like symptoms, but also rash, eschar, and gastrointestinal symptoms that may progress to central nervous involvement. Several hundred cases have so far been reported, mainly from China (Peng et al. 2021).

CCHF show a variety of symptoms, from a mild, unspecific febrile syndrome to a multiorgan failure, shock, and hemorrhages with up to 20–30% or even higher fatality rates. Several thousand cases have so far been reported, with an increasing number in several countries (Bente et al. 2014).

Signs of TBE are typically divided into two phases, first a viremic phase with flu-like symptoms that may progress into a neurological (second) phase, with meningitis, meningoencephalitis, and meningoencephalomyelitis (Ruzek et al. 2019). The western and Sibirian subtypes usually result in a rather mild form of TBE with a morality rate less than 2%, while the Far-Eastern subtype is associated with higher fatality rates, although viral subtypes are not the sole determinant of TBE

severity (Ruzek et al. 2019). Between 10.000 and 15.000 cases of TBF are reported in Europe and Asia each year (Salat and Ruzek 2020).

LI-virus may cause the same symptoms, although milder, as TBE-virus. Several LI-cases were diagnosed earlier, but no human patients suffering from LI-virus encephalitis have been definitively diagnosed over the past 20 years (Jeffries et al. 2014).

5.11.6 Transmission (Small Ruminants-Human)

A. phagocytophilum and LI-virus are mainly transmitted to humans by tick bites, although other routes of transmission have been reported, such as through contact with infected animals (Jeffries et al. 2014; Stuen 2020).

CCHF infections occur via tick bites or exposure to blood or body fluids of an infected animal through occupational exposure to infected livestock, such as farmers, abattoir workers, butchers, veterinarians, and laboratory scientists. However, infected animals seem only to develop a transient viremia (Bente et al. 2014).

TBE-virus is mainly transmitted from small ruminants to humans by consumption of unpasteurized milk or milk products from infected animals (Salat and Ruzek 2020). It has been estimated that around 1% of all TBE cases are caused by foodborne infections (Ruzek et al. 2019).

5.11.7 Diagnosis (Small Ruminants)

Diagnosis of tick-borne infections may be based on clinics, stained blood smear (especially *A. phagocytophilum*), serology, or molecular methods. Postmortem analysis may support the diagnosis. However, clinical symptoms or pathological changes may not be observed or may even be absent. Serological tests are available for most of the pathogens mentioned above. Although in order to verify the diagnosis, PCR-methods are often necessary, the infection can be difficult to detect in persistently infected animals (Reid and Chianini 2007; Stuen and Longbottom 2011; Bente et al. 2014; Ruzek et al. 2019).

5.11.8 Treatment and Control

Tick control measures are necessary in order to reduce tick exposure and to limit tick distribution and expansion. Different methods are available, such as draining, fencing, mechanical clearing of bushes, control burning, removal of leaf litter, partial removal of forest canopy, reduction of hosts, and use of herbicides and acaricides. Current control strategies are mainly based on the reduction of tick infestation by application of chemical acaricides, mostly done by dipping or pour on-application of pyrethroids. However, several of these treatments are not environmentally friendly and may cause chemical residues in

animal products (Samish et al. 2004; Stuen and Longbottom 2011). Anti-tick vaccine development is a solution, but still not available, except for vaccine against *Rhipicephalus microplus* (Stuen and Longbottom 2011).

Infection may occur through occupational exposure to infected livestock. Infected animals should therefore be handled with care. The drug of choice in treatment against *A. phagocytophilum* infection is tetracycline. Fluoroquinolone antibiotics and rifampin may be alternative drugs, especially in humans, in patients with intolerance to tetracycline. No vaccine is yet available against *A. phagocytophilum,* mainly due to the challenge in choosing conserved antigens, especially since antigenic variation of surface proteins occurs during the infection (Stuen and Longbottom 2011).

A formalin-inactivated CCHF vaccine is available for humans, although lack of proper animal models has hampered the effort to develop a more efficient vaccine. Several TBE-vaccines are also available for humans, which are widely used in TBE endemic areas. However, there is no specific treatment against these virus infections; supportive and symptomatic therapies are therefore the mainstay of TBE and CCHF management (Bente et al. 2014; Ruzek et al. 2019).

There is no specific treatment for LI in animals, although an inactivated virus vaccine is currently available for animals. There is no commercial LI vaccine licensed for human use, although neutralization antibodies from commercial TBE vaccines may provide some cross-protection against LI-virus infection (Jeffries et al. 2014).

5.12 Toxoplasmosis

5.12.1 The Pathogen

Toxoplasma gondii is a protozooan parasite within the family *Sarcocystiidae* and genus *Toxoplasma*. The life cycle can be divided into two parts, a sexual cycle, restricted to enteroepithelial cells in cats and the production of oocysts, and an asexual cycle (forming tissue cysts), which occurs in a wide range of warm-blooded intermediate hosts. Six major clades of *T. gondii* have been characterized (Buxton and Rodger 2007; Su et al. 2012).

5.12.2 Occurrence

T. gondii has a worldwide distribution.

5.12.3 Hosts

Multiple intermediate hosts seem to exist, but the most important domestic hosts are pigs, sheep, and goats. The final host is in the felid family.

5.12.4 Disease in Small Ruminants

Clinical toxoplasmosis causes abortion and neonatal mortality in small ruminants, especially in sheep. Mummified fetuses, stillborn or weak offspring are common features. However, infection in early pregnancy (< 55 days) may result in death or expulsion of a small fetus. Clinical signs in aborting animals are usually not observed. Abortion is associated with primary infection during pregnancy in non-immune animals and is most commonly seen in young animals. A long-lasting immunity develops following primary exposure and animals are unlikely to abort again due to toxoplasmosis (Buxton and Rodger 2007; Smith and Sherman 2009).

5.12.5 Disease in Humans

In most cases, toxoplasmosis in human is a disease with relatively mild and transient symptoms. However, primary infection during pregnancy may lead to intrauterine infection, and result in abortion or congenital lesions in the fetus. In addition, in patients with impaired immunity, *T. gondii* may lead to serious and even fatal infection (Dubey and Beattie 1988).

5.12.6 Transmission (Small Ruminants-Human)

The proportion of the human population infected with *T. gondii* depends on the age, area, and environment. Most human infection appears to result either from exposure to oocysts from a contaminated environment or from ingestion of raw or lightly cooked meat containing tissue cysts. The most common way for infection from small ruminants to humans is by ingestion of tissue cysts. In addition, human infection through drinking of unpasteurized goat milk has been reported. A low risk may also apply when assisting infected animals at lambing or kidding. However, both these last modes of transmission are probably of low significance (Dubey and Beattie 1988; Smith 1991).

5.12.7 Diagnosis in Small Ruminants

Abortion due to *T. gondii* occurs mainly in young animals. Typical clinical signs of abortion result following infection in mid-gestation, with ewes and does producing stillborn and/or weekly offspring often accompanied by a mummified fetus. Cotyledons will also show characteristic lesions, such as white foci of necrosis 2–3mm in diameter, which may become mineralized. Diagnosis may include serology (such as Sabin dye test, IFAT, MAT, and ELISA), histology, immune-histochemistry, and PCR methods (Buxton and Rodger 2007; Taylor et al. 2007; Smith and Sherman 2009).

5.12.8 Treatment and Control

Susceptible animal gets infected by ingestion feed or water contaminated with oocysts. The oocysts are highly resistant and survive for a long period (> 500 days) at room temperature in moist conditions. The main source of *Toxoplasma* infection in small ruminants are oocysts excreted from cats. Susceptible cats become infected with *T. gondii* after ingestion of tissue cysts from, for instance, small rodents and may excrete a large numbers of oocysts, which then sporulate and become infective within a few days and remain so for several months. Infected feces will then contaminate beddings, stores of hay, concentrates, water supplies, and pasture. It has been estimated that although < 1% of the cat population may excrete oocysts at any time, contamination of the environment is readily maintained (Dubey and Beattie 1988; Buxton and Rodger 2007).

During an outbreak of toxoplasma-caused abortion little can be done. Infected placentas and dead lambs or kids should be buried or disposed to prevent their ingestion by other animals. Animal to animal transmission during lambing or kidding does not appear to occur to any significant extent. More direct preventive measures include chemoprophylaxis, chemotherapy, and vaccination. A live vaccine based on an attenuated strain of *T. gondii* has been developed for sheep (Buxton and Rodger 2007; Smith and Sherman 2009).

In humans, as already mentioned, the most common way for infection from small ruminants is by ingestion of raw or lightly cooked meat. Tissue cysts may be viable for the lifetime of infected sheep (Dubey and Beattie 1988). Treatment of meat by curing, smoking, freezing at -20 °C is usually sufficient to kill the encysted *T. gondii*. However, cysts can survive insufficient microwave cooking (Lundén and Uggla 1992). Treatment of tissue cysts in infected sheep to prevent human exposure to meat-borne toxoplasmosis has shown promising results (Kul et al. 2013). Shepherds, veterinary surgeons, slaughterhouse staff, and butchers are especially at risk for contracting infection from small ruminants.

5.13 Concluding Remarks

Only a limited number of topics are covered by this brief review and important issues such as differential diagnoses are not included or discussed. A correct and swift diagnosis is a prerequisite for proper treatment and control. This may not always be available due to long incubation periods, unspecific clinical symptoms, and imprecise diagnostic tests. Some pathogens may survive unnoticed in animals or animal products for a long period of time. Anthrax in humans, for instance, has occurred when handling imported goat skins for drum making, skins contaminated with spores of *B. anthracis* (Anaraki et al. 2008).

Microbial transmission will always occur between species, but the risk of transmission can be reduced with proper hygiene, management, husbandry, and prophylactic treatment. Climate change, increased population, and globalization will have a

huge impact on the occurrence and distribution of these infections. In this context, recent vaccine developments against several zoonotic pathogens through genomic, transcriptomic, and proteomic approaches are promising.

Conflict of Interest Statement The author has nothing to disclose.

References

Agrawal G, Aitken J, Hamblin H et al (2020) Putting Crohn's on the MAP: Five common questions on the contribution of *Mycobacterium avium* subspecies *paratuberculosis* to the pathophysiology of Crohn's disease. Dig Dis Sci 06653-0

Aitken ID, Longbottom D (2007) Chlamydial abortion. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 105–112

Allsopp BA (2010) Natural history of *Ehrlichia ruminantium*. Vet Parasitol 167:123–135

Anaraki S, Addiman S, Nixon G et al (2008) Investigations and control measures following a case of inhalation anthrax in East London in a drum maker and drummer. Euro Surveill 13(51): pii:19076

Antonis AFG, Kortekaas J, Kant J et al (2013) Vertical transmission of Rift Valley fever without detectable maternal viremia. Vector Borne Zoonotic Dis 13:601–606

Arent Z, Frizzell C, Gilmore C et al (2013) Isolation of leptospires from the genital tract of sheep. Vet Rec 173:582

Arricau Bouvery N, Souriau A, Lechopier P et al (2003) Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. Vet Res 34:423–433

Arricau-Bouvery N, Rodolakis A (2005) Is Q fever an emerging or re-emerging zoonosis? Vet Res 36:327–349

Azagi T, Hoornstra D, Kremer K et al (2020) Evaluation of disease causality of rare *Ixodes ricinus*-borne infections in Europe. Pathogens 9:150

Babudieri B (1959) Q fever: a zoonosis. Adv Vet Sci 5:181–182

Bakken JS, Dumler JS (2015) Human granulocytic anaplasmosis. Infect Dis Clin N Am 2:341–355

Bath GF (2007) Rift Valley fever. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 469–473

Bauer B, Prüfer L, Walter M et al (2020) Comparison of *Coxiella burnetii* excretion between sheep and goats naturally infected with one cattle-associated genotype. Pathogens 9:652

Beesley WN (1998) The myiases. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 881–891

Bente DA, Forrester NL, Watts DM et al (2014) Crimean-Congo hemorrhagic fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antivir Res 100:159–189

Berri M, Crochet D, Santiago S et al (2005) Spread of *Coxiella burnetii* infection in a flock of sheep after an episode of Q fever. Vet Rec 157:737–740

Bloodworth DL, Howard AJ, Davies A et al (1987) Infection in pregnancy caused by *Chlamydia psittaci* of ovine origin. Commun Dis Rep 10:3–4

Böhm B, Schade B, Bauer B et al (2017) Tick-borne encephalitis in a naturally infected sheep. BMC Vet Res 13:267

Borde JP, Zange S, Antwerpen MH et al (2017) Five cases of vector-borne *Francisella tularensis holarctica* infections in south-western Germany and genetic diversity. Ticks Tick Borne Dis 8 (5):808–812

Brunetti E, White AC Jr (2012) Cestode infestations. Hydatid disease and cysticercosis. Infect Dis Clin N Am 2:421–435

Butler T (1998) Yersiniosis and plague. In: Palmer SR, Lord S, DIH S (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 281–293

Buxton D (1986) Potential danger to pregnant women of *Chlamydia psittaci* from sheep. Vet Rec 118:510–511
Buxton D, Rodger SM (2007) Toxoplasmosis and neosporosis. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 112–119
Carrasco-Marín E, Rodriguez-Del Rio E, Frande-Cabanes E et al (2012) Phagosomes induced by cytokines function as anti-Listeria vaccines: novel role for functional compartmentalization of STAT-1 protein and cathepsin-D. J Biol Chem 287:14310–14324
Castrucci G (2007) *Brucella melitensis* infection. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 137–142
CDCP (Centers for Disease Control and Prevention) (1998) Rift Valley Fever – East Africa, 1997-1998. MMWR 47:261–264
Cerqueira GM, Picardeau M (2009) A century of *Leptospira* strain typing. Infect Genet Evol 9:760–768
Chalmers RM, Thomas DR, Salmon RL (2005) Borna disease virus and the evidence for human pathogenicity: a systematic review. Q J Med 98:255–274
Chen C, Li P, Li K-F et al (2019) Animals as amplification hosts in the spread of severe fever with thrombocytopenia syndrome virus: A systematic review and meta-analysis. Int J Infect Dis 79: 77–84
Cheng AC, Currie BJ (2005) Melioidosis: epidemiology, pathophysiology, and management. Clin Microbiol Rev 18:382–416
Chochlakis D, Ioannou I, Tselentis et al (2010) Human anaplasmosis and *Anaplasma ovis* variant. Emerg Infect Dis 16:1031–1032
Corbel MJ (1997) Brucellosis: an overview. Emerg Infect Dis 3:213–221
Cork SC (2011) Epidemiology of pathogens in the food supply. In: Krause DO, Hendrick S (eds) Zoonotic pathogens in the food chain. CAB International, Oxfordshire, pp 21–58
Cork SC, Checkley S (2011) Globalization of the food supply and the spread of disease. In: Krause DO, Hendrick S (eds) Zoonotic pathogens in the food chain. CAB International, Oxfordshire, pp 1–20
Desenclos JC, Bouvet P, Benz-Lemoine E et al (1996) Large outbreak of *Salmonella enterica* serotype paratyphi B infection caused by a goats' milk cheese, France, 1993: a case finding and epidemiological study. Brit Med J 312:91–94
Dorjee S, Heuer C, Jackson R et al (2008) Prevalence of pathogenic *Leptospira* spp. in sheep in a sheep-only abattoir in New Zealand. N Z Vet J 56:164–170
Dubey JP, Beattie CP (1988) Toxoplasmosis in animals and man. CRC Press, Boca Raton
Dürrwald R, Kolodziejek J, Herzog S et al (2007) Meta-analysis of putative human bornavirus sequences fails to provide evidence implicating Borne disease virus in mental illness. Rev Med Virol 17:181–203
Ebrahimi M, Nejad RB, Alamian S et al (2012) Safety and efficacy of reduced doses of *Brucella melitensis* strain Rev. 1 vaccine in pregnant Iranian fat-tailed ewes. Vet Ital 48:405–412
El-Koumi MA, Afify M, Al-Zahrani SH (2013) A prospective study of brucellosis in children: relative frequency of pancytopenia. Mediterr J Hematol Infect Dis 5(1):e2013011
Ellis WA (1998) Leptospirosis. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 115–126
Entrican G, Wheelhouse N, Wattegedera SR et al (2012) New challenges to prevent chlamydial abortion in sheep. Comp Immunol Microbiol Infect Dis 35:271–276
Espié E, Vaillant V (2005) International outbreak of *Salmonella* Stourbridge infection, April- July 2005: results of epidemiological, food and veterinary investigations in France. Euro Surveill 10(8):E050811.3
Félix SR, Hartwig DD, Argondizzo AP et al (2011) Subunit approach to evaluation of the immune protective potential of leptospiral antigens. Clin Vaccine Immunol 18:2026–2030
Fournier PE, Marrie TJ, Raoult D (1998) Diagnosis of Q fever. J Clin Microbiol 36:1823–1834
Franco MP, Mulder M, Gilman RH et al (2007) Human brucellosis. Lancet Infect Dis 7:775–786

Fratini F, Turchi B, Ferrone M et al (2016) Is *Leptospira* able to survive in raw milk? Study on the inactivation at different storage times and temperatures. Folia Microbiol 61:413–416
Fried B, Abruzzi A (2010) Food-borne trematode infections of humans in the United States of America. Parasitol Res 106:1263–1280
Gallagher J, Jenkins PA (1998) Mycobacterial diseases. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 155–164
Ganter M (2015) Zoonotic risks from small ruminants. Vet Microbiol 181:53–65
Genchi M, Prati P, Vicari N et al (2015) *Francisella tularensis*: No evidence for transovarial transmission in the tularemia tick vectors *Dermacentor reticulatus* and *Ixodes ricinus*. PLoS ONE 10(8):e0133593
Godfroid J, Cloeckaert A, Liautard JP et al (2005) From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet Res 36:313–326
Gomez G, Pei J, Mwangi W, Adams LG et al (2013) Immunogenic and invasive properties of *Brucella melitensis* 16M outer membrane protein vaccine candidates identified via a reverse vaccinology approach. PLoS ONE 8(3):e59751
Gray A, Capewell P, Loney C et al (2019) Sheep as host species for zoonotic *Babesia venatorum*, United Kingdom. Emerg Infect Dis 25:2257–2260
Guglielmone AA, Robbins RG, Apanaskevitch DA et al (2014) The hard ticks of the world (*Acar*i: *Ixodida*: *Ixodididae*). Springer
Guillet C, Join-Lambert O, Le Monnier A et al (2010) Human listeriosis caused by *Listeria ivanovii*. Emerg Infect Dis 16(1):136–138
Hilbink F, Penrose M, Kovacova E et al (1993) Q fever is absent from New Zealand. Int J Epidemiol 22:945–949
Humphrey TJ, Threlfall EJ, Cruickshank JG (1998) Salmonellosis. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 191–206
Hyde SR, Benirschke K (1997) Gestational psittacosis: case report and literature review. Mod Pathol 10:602–607
Hyslop NSG (1980) Dermatophilosis (streptothricosis) in animals and man. Comp Immunol Microbiol Infect Dis 2:389–404
Indran SV, Ikegami T (2012) Novel approaches to develop Rift Valley fever vaccines. Front Cell Infect Microbiol 2:131
Jahfari S, Coipan EC, Fonville M et al (2014) Circulation of for *Anaplasma phagocytophilum* ecotypes in Europe. Parasit Vectors 4:439
Jeffries CL, Mansfield KL, Phipps LP et al (2014) Louping ill virus: an endemic tick-borne disease of Great Britain. J Gen Virol 95:1005–1014
Kazar J (1999) Q fever – current concept. In: Raoult D, Brouqui P (eds) Rickettsiae and rickettsial diseases at the turn of the third millennium. Elsevier, Paris, pp 304–319
Kim S, Zuiani A, Carrero JA et al (2012) Single chain MHC I trimer-based DNA vaccines for protection against *Listeria monocytogenes* infection. Vaccine 30:2178–2186
King AA (1998) Rabies. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 437–458
Kirby FD (1985) Zoonoses in Britain. J Royal Soc Health 105:77–87
Kovacova E, Kazar J, Spanelova D (1998) Suitability of various *Coxiella burnetii* antigen preparations for detection of serum antibodies by various tests. Acta Virol 42:365–368
Kul O, Yildiz K, Ocal N et al (2013) In-vivo efficacy of toltrazuril on experimentally induced *Toxoplasma gondii* tissue cysts in lambs: a novel strategy for prevention of human exposure to meat-borne toxoplasmosis. Res Vet Sci 94:269–276
Lawson JR, Gemmell MA (1983) Hydatidosis and cysticercosis: the dynamics of transmission. Adv Parasitol 22:261–308

Lawson JR, Gemmell MA (1990) Transmission of taeniid tapeworm eggs via blowflies to intermediate hosts. Parasitology 100:143–146
Leake CJ (1998) Mosquito-borne arboviruses. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 401–413
Levett PS (2001) Leptospirosis. Clin Microbiol Rev 14:296–326
Li W, Ning Z, Hao W et al (2012) Isolation and phylogenetic analysis of orf virus from the sheep herd outbreak in northeast China. BMC Vet Res 8:229
Lightowlers MW, Jensen O, Fernandez E et al (1999) Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep. Int J Parasitol 29:531–534
Lilenbaum W, Varges R, Brandao FZ et al (2008) Detection of Leptospira spp. In semen and vaginal fluids of goats and sheep by polymerase chain reaction. Theriogenology 69:837–842
Lloyd S (1998) Other cestode infections hymenolepiosis, diphyllobothriosis, coenurosis, and other adult and larval cestodes. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 651–563
Lloyd S, Soulsby EJL (1998) Other trematode infections. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 731–746
Longbottom D (2008) Enzootic abortion of ewes (ovine chlamydiosis). In: Manual of diagnostic tests and vaccines for terrestrial animals, 6th edn. World Organisation for Animal Health (OIE), pp 1013–1020
Longbottom D, Coulter LJ (2003) Animal chlamydioses and zoonotic implications. J Comp Pathol 128:217–244
Longbottom D, Livingstone M, Maley S et al (2013) Intranasal infection with *Chlamydia abortus* induces dose-dependent latency and abortion in sheep. PLoS ONE 8(2):e57950
Low JC, Donachie W (1991) Listeriosis. In: Martin WB, Aitken ID (eds) Diseases of sheep, 2nd edn. Blackwell Scientific Publications, Oxford, pp 174–178
Lundén AL, Uggla A (1992) Infectivity of *Toxoplasma gondii* in mutton following curing, smoking, freezing or microwave cooking. Int J Food Microbiol 15:357–363
Luo X, Cai X (2012) A combined use of autolysin p60 and listeriolysin O antigens induces high protective immune responses against *Listeria monocytogenes* infection. Curr Microbiol 65:813–818
Mailles A, Vaillant V (2014) 10 years of surveillance of human tularaemia in France. Euro Surveill 13 19(45):20956
Martin WB (1991a) Human infection with orf virus. In: Martin WB, Aitken ID (eds) Diseases of sheep, 2nd edn. Blackwell Scientific Publications, Oxford, p 376
Martin WB (1991b) Hydatid disease. In: Martin WB, Aitken ID (eds) Diseases of sheep, 2nd edn. Blackwell Scientific Publications, Oxford, p 374
Mas-Coma S (2005) Epidemiology of fascioliasis in human endemic areas. J Helminthol 79:207–216
Maurin M, Raoult D (1999) Q fever. Clin Microbiol Rev 12:518–553
McLauchlin V, der Mee-Marquet N (1998) Listeriosis. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 127–140
Mearns R (2007) Other infectious causes of abortion. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 127–136
Milinovich GJ, Klieve AV (2011) Manure as a source of zoonotic pathogens. In: Krause DO, Hendrick S (eds) Zoonotic pathogens in the food chain. CAB International, Oxfordshire, pp 59–83
Mohamed W, Sethi S, Tchatalbachev S et al (2012) Protective immunity to *Listeria monocytogenes* infection mediated by recombinant *Listeria innocua* harboring the VGC locus. PLoS ONE 7(4): e35503

Moore JD, Barr BC, Daft BM et al (1991) Pathology and diagnosis of *Coxiella burnetii* infection in a goat herd. Vet Pathol 28:81–84
Moro P, Schantz PM (2009) Echinococcosis: a review. Int J Infect Dis 13:125–133
Morrill JC, Laughlin RC, Lokugamage N et al (2013) Safety and immunogenicity of recombinant Rift Valley fever MP-12 vaccine candidates in sheep. Vaccine 31:559–565
Nelson S, Clarke RC, Karmali MA (1998) Verocytotoxin-producing *Escherichia coli* (VTEC) infections. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 89–104
Palmer SR, Lord S, Simpson DIH (eds) (1998) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford
Papp JR, Shewen PE (1996) Pregnancy failure following vaginal infection of sheep with *Chlamydia psittaci* prior to breeding. Infect Immun 64:1116–1125
Papp JR, Shewen PE, Gartley CJ (1994) Abortion and subsequent excretion of chlamydiae from the reproductive tract of sheep during estrus. Infect Immun 62:3786–3792
Pearson A (1998) Tularaemia. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 267–279
Peng Y, Lu C, Yan Y et al (2021) The first detection of *Anaplasma capra*, an emerging zoonotic *Anaplasma* sp., in erythrocytes. Emerg Micro Inf 10:226–234
Plommet M, Diaz R, Verger J-M (1998) Brucellosis. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 23–35
Quevedo Diaz MA, Lukacova M (1998) Immunological consequences of *Coxiella burnetii* phase variant. Acta Virol 42:181–185
Rabinowitz PM, Conti LA (2010) Salmonellosis. In: Rabinowitz PM, Conti LA (eds) Human-animal medicine. Clinical approaches to zoonoses, toxicants and other shared health risks. Saunders Elsevier, Missouri, pp 248–254
Redkar R, Rose S, Bricker B et al (2001) Real-time detection of *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. Mol Cell Probes 15:43–52
Reid HW, Chianini F (2007) Louping-ill. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 250–255
Reid HW, Rodger SM (2007) Orf. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 297–302
Rodolakis A (2009) Q fever in dairy animals. Ann N Y Acad Sci 1166:90–93
Rodolakis A, Berri M, Héchard C et al (2007) Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine and ovine herds. J Dairy Sci 90:5352–5560
Roest HIJ, Tilburg JJHC, Ven der Hoeck W et al (2011) The Q-fever epidemic in the Netherlands: history, onset, response, and reflection. Epidemiol Infect 139:1–12
Rubbenstroth D, Schlottau K, Schwemmle M et al (2019) Human bornavirus research: Back on track! PLoS Pathog 1007873
Ruzek D, Avsic Zupanc T, Borde J et al (2019) Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. Antivir Res 164:23–51
Sachse K, Vretou E, Livingstone M et al (2009) Recent developments in the laboratory diagnosis of chlamydial infections. Vet Microbiol 135:2–21
Salat J, Ruzek D (2020) Tick-borne encephalitis in domestic animals. Acta Virol 64:226–232
Samish M, Ginsberg H, Glazer I (2004) Biological controls of ticks. Parasitology 129:S389–S403
Sampere M, Font B, Font J et al (2003) Q fever in adults: review of 66 clinical cases. Eur J Clin Microbiol Infect Dis 22:108–110
Sanford SE, Josephson GKA, MacDonald A (1994) *Coxiella burnetii* (Q fever) abortion storms in goat herds after attendance at an annual fair. Can Vet J 35:376–378
Sawyer LA, Fishbein DB, McDade JE (1987) Q fever: current concepts. Rev Infect Dis 9:935–946
Scharf W, Schauer S, Freyburger F et al (2011) Distinct host species correlate with *Anaplasma phagocytophilum* ankA gene clusters. J Clin Microbiol 49:790–796

Scott PR (2007) Listeriosis. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 255–259
Sellers RF (1980) Weather, hosts and vector – their interplay in the spread of insect-borne animal virus diseases. J Hyg 85:65–102
Seshadri R, Paulsen IT, Eisen JA et al (2003) Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. Proc Natl Acad Sci USA 100:5455–5460
Sharp JM (2007) Mycobacterial infections. In: Aitken ID (ed) Diseases of Sheep, 4th edn. Blackwell Publishing, Oxford, pp 168–173
Shope R (1991) Global climate change and infectious diseases. Environ Health Perspect 96:171–174
Sillis M, Longbottom D (2010) Chlamydiosis. In: Palmer SR, Lord S, Torgerson PR, Brown DWG (eds) Zoonoses: biology, clinical practice, and public health control, 2nd edn. Oxford University Press, Oxford, pp 146–157
Singh S, Gopinath K (2011) *Mycobacterium avium* subspecies *paratuberculosis* and Crohn´s regional ileitis: How strong is association? J Lab Phys 3:69–74
Skirrow MB (1998) Campylobacteriosis. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonosis, biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 37–46
Smith JL (1991) Foodborne toxoplasmosis. J Food Safety 2:17–57
Smith MC, Sherman DM (2009) Goat medicine, 2nd edn. Wiley-Blackwell, Iowa
Sören K, Lindblad M, Jernberg C et al (2015) Changes in the risk management of *Salmonella enterica* subspecies *diarizonae* serovar 61: (k):1,5, (7) in Swedish sheep herds and sheep meat due to the result of a prevalence study 2012. Acta Vet Scand 57:6
Sparkes AH (1998) Ringworm (Dermatophytosis). In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 907–919
Stanek G, Strle F, Gray J et al (2002) History and characteristics of Lyme borreliosis. In: Gray JS, Kahl O, Lane RS, Stanek G (eds) Lyme borreliosis. Biology, epidemiology and control. CABI Publishing, Oxon, pp 1–28
Stewart GH (1972a) Dermatophilosis: A skin disease of animals and man I. Vet Rec 91:537–544
Stewart GH (1972b) Dermatophilosis: A skin disease of animals and man II. Vet Rec 91:555–561
Stuen S (2020) Haemoparasites – a challenging and wasting infctions in small ruminants. A review. Animals 0(11):2179
Stuen S, Longbottom D (2011) Treatment and control of chlamydial and rickettsial infections in sheep and goats. Vet Clin Food Anim 27:213–233
Su C, Khan A, Zhou P et al (2012) Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proc Natl Acad Sci USA 109:5844–5849
Swaminathan B, Gerner-Smidt P (2007) The epidemiology of human listeriosis. Microbes Infect 9: 1236–1243
Swanepoel R (1998) Rift Valley fever. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonosis, biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 459–468
Taylor MG (1998) Schistosomosis. In: Palmer SR, Lord S, DIH S (eds) Zoonosis, biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 717–729
Taylor MA, Coop RL, Wall RL (2007) Veterinary parasitology, 3rd edn. Blackwell Publishing, Oxford
Thomas D (1998) Diseases caused by corynebacteria and related organisms. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 67–73
Thompson RCA (1998) *Giardia* infections. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 545–561
Tigertt WD, Benenson AS, Gochenour WS (1961) Airborne Q fever. Bacteriol Rev 25:285–293

Turnbull PCB (1998) Anthrax. In: Palmer SR, Lord S, Simpson DIH (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 3–16
van der Hoek W, Morroy G, Renders NH (2012) Epidemic Q fever in humans in the Netherlands. Adv Exp Med Biol 984:329–364
Wen H, Vuitton L, Tuxun T et al (2019) Echinococcosis: Advances in the 21st century. Clin Microbiol Rev 32:e00075–e00018
West DM, Bruère AN, Ridler AL (2009) The sheep. Health, disease & production. VetLearn, Wellington
Winter AC, Charnley JG (1999) Zoonoses. In: The Sheep Keeper's Veterinary Handbook. The Crowood Press Ltd, Ramsbury, pp 199–202
Woldehiwet Z (2010) The natural history of *Anaplasma phagocytophilum*. Vet Parasitol 167:108–122
Wright SE, Coop RL (2007) Cryptosporidiosis and coccidiosis. In: Aitken ID (ed) Diseases of sheep, 4th edn. Blackwell Publishing, Oxford, pp 179–185
Wright D, Kortekaas J, Bowden TA et al (2019) Rift Valley fever: biology and epidemiology. J Gen Virol 100:1187–1199
Yan W, Faisal SM, McDonough SP et al (2010) Identification and characterization of OmpA-like proteins as novel vaccine candidates for leptospirosis. Vaccine 28:2277–2283
Zhang W, McManus DP (2008) Vaccination of dogs against *Echinococcus granulosus*: a means to control hydatid disease? Trends Parasitol 24:419–424
Zhao K, He W, Gao W et al (2011) Orf virus DNA vaccines expressing ORFV 011 and ORFV 059 chimeric protein enhances immunogenicity. Virol J 8:562

Zoonoses Transmitted by Poultry

6

Risks Related to Poultry Rearing and Eating Poultry Products

Hafez M. Hafez and Rüdiger Hauck

Contents

H. M. Hafez (✉)
Institut für Geflügelkrankheiten, Freie Universität Berlin, Berlin, Germany
e-mail: hafez.mohamed@fu-berlin.de

R. Hauck
Department of Pathobiology and Department of Poultry Science, Auburn University, Auburn, AL, USA
e-mail: ruediger.hauck@auburn.edu

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_4

Abstract

Many factors and problems influence poultry production worldwide. These include strong global competition, continuous changes of consumer perceptions regarding food safety, animal welfare, and environmental protection. Poultry share a number of infectious diseases with humans, and most of the zoonotic diseases in poultry have additional reservoirs in other mammals than humans, which complicates their control. Roughly, there are three groups of zoonoses that humans can acquire from poultry:

The **first group** includes **food-borne diseases**, mainly caused by *Salmonella* serovars and *Campylobacter spp.,* which are the most common causes of human food-borne bacterial diseases linked to poultry. There are indications that *Escherichia coli* from poultry can cause disease in humans, in which case *E. coli* would have to be considered a potential food-borne pathogen. In addition, the development of antibiotic-resistant bacteria will also continue to be a hazard to public health.

The second group comprises diseases that are **transmitted by direct contact** between birds and humans. These include avian influenza, Newcastle disease, and chlamydiosis. Erysipelas has an exceptional position as it mainly infects people working in processing plants via skin injuries.

The third group comprises diseases **transmitted by insects**, especially ticks from mammals and birds, including poultry, to humans. These include West Nile Virus and Eastern and Western Equine Encephalitis.

Keywords

Chicken · Turkeys · Poultry · Birds · Salmonella · Campylobacter · *Escherichia coli* · Antimicrobial resistances · Avian influenza · Newcastle disease · Chlamydiosis · *Chlamydia psittaci* · Ornithosis · Erysipelas · Erysipeloid · *Erysipelothrix rhusiopathiae*

6.1 Introduction

In developed countries, most poultry is kept in large flocks on specialized farms. These husbandry conditions offer a high level of biosecurity, and only few humans or other animals have direct contact with the birds. Thus, the main transmission route from poultry to humans is via contaminated food, namely meat and eggs. *Salmonella* and Campylobacter are the two most important zoonotic agents in poultry causing food-borne infections. Despite significant improvements in technology and hygienic practice at all stages of the poultry production, these pathogens remain a persistent threat to human health. There have also been indications that Avian Pathogenic *Escherichia coli* (APEC) might have zoonotic properties, but more substantial evidence is needed. Furthermore, bacterial isolates from chicken can be resistant against antimicrobials and cause infections in humans that are difficult to treat or pass the resistance genes on to other bacteria.

In developing countries, where smaller backyard flocks are more common and where many of the birds raised for meat production are traded live to the consumer, direct contact between poultry and humans is more frequent. Thus, diseases are transmitted more often directly from poultry to humans. Avian influenza is the most important example and has received major attention in the media despite a relatively low number of infected humans. Other pathogens transmitted from poultry to humans by direct contact include Newcastle disease and chlamydiosis.

Erysipelas has an exceptional position since it mainly infects humans through skin injuries. Thus, besides persons handling infected birds, personnel at processing plants have an elevated risk of infection.

Further zoonotic diseases, which have lesser importance either, because they occur only infrequently or because they have a low virulence for humans, are listed in Table 1.

Table 1 Zoonotic pathogens detected in poultry

Pathogen	Host	Distribution	Transmission	Clinical symptoms (poultry)	Clinical symptoms (human)	Further information
Crimean Congo Hemorrhagic Fever Virus	Ostriches, migratory birds, several mammals	Asia, Africa	Ticks	Asymptomatic	Hemorrhagic fever	Estrada-Peña et al. (2012)
Eastern Equine Encephalitis Virus	Turkeys, wild birds and several mammals	North and South America	Ticks	Nervous	Nervous	Corrin et al. (2020)
West Nile Virus	Several birds and mammals	Africa, India, North America, rarely Europe	Ticks	Asymptomatic or nervous	Flue like, nervous	Habarugira et al. (2020)
Mycobacterium avium sp. avium	Several birds and mammals	Worldwide	Oral	Emaciation, tubercles in organs	Tuberculosis like pneumonia	Sanchez and Fulton (2020)
Dermanyssus gallinae	Birds	Worldwide	Contact	Skin irritations	Skin irritations	Cheikhrouhou et al. (2020)
Cryptococcus neoformans	Some birds, mammals	Worldwide	Oral	Asymptomatic	Nervous	Chang and Chen (2015)
Cryptosporidium meleagridis	Turkeys, other birds, humans	Worldwide	Oral	Diarrhea, respiratory	Diarrhea	Chalmers (2021)
Histoplasma capsulatum	Some birds, mammals	Worldwide, especially North America	Aerosol, oral	Asymptomatic	Respiratory	Mittal et al. (2019)

6.2 Salmonella

6.2.1 Epidemiology of Salmonella Infections in Poultry

Salmonellosis and *Salmonella* infections in poultry occur worldwide. The more than 2500 serovars of *Salmonella enterica* can roughly be classified into three categories or groups: **Group 1: highly host adapted and invasive serovars.** This group includes species restricted and invasive salmonella such as S. Pullorum and S. Gallinarum in poultry and S. Typhi in humans. **Group 2: nonhost-adapted and invasive serovars.** This group consists of approximately 10 to 20 serovars that can cause an invasive infection in poultry and may be capable of infecting humans. Currently, the most important serovars are S. Typhimurium, S. Enteritidis, S. Heidelberg, and S. Kentucky. Some serovars may be predominant for a few years in a region or country before they disappear and are replaced by another serovar. In the past, the dominant type worldwide of salmonella food poisoning was S. Typhimurium, but since the early 1980s, S. Enteritidis has become more important. S. Heidelberg and S. Kentucky are two serovars associated with poultry that are currently causing problems. **Group 3: nonhost-adapted and noninvasive serovars.** This group is the by far the largest but poses no or only a very low risk for human health. However, *Salmonella* of this group are detected when flocks are monitored. Laws and regulations may or may not apply to them.

The prevalence of *Salmonella*-infected flocks and the serovars involved varies widely between different countries. Current data obtained by monitoring programs in the European Union are published in regular intervals. Within an infected flock, the prevalence of infected birds can be well below 10%.

Transmission and spread of *Salmonella* in poultry occur by horizontal and/or vertical routes. Horizontal spread of infection takes place through contaminated feed, water, equipment, and the rearing environment. Significant reservoirs for these microorganisms are asymptomatically infected chickens, other avian species including pigeons and wild birds, and other farm animals and pets. Rodents are potential reservoirs transmitting infection between houses and contaminating stored feeding stuffs. In addition, insects are a potential source of *Salmonella* infection in chicken. Some invasive *Salmonella* can be transmitted vertically within the eggs, i.e., true vertical transmission. True vertical transmission occurs primarily by infection of the ovaries and the follicles, which become the yolks, by contact of the follicles with infected peritoneum or air sacs or alternatively in the oviduct where the egg white is produced. Pseudovertical transmission happens by contamination of the eggshell as a result of fecal contamination from cloaca and/or contaminated nests, floor, or incubators. Subsequently, Salmonella can penetrate the eggshell into the eggs.

6.2.2 Epidemiology of Salmonella Infections in Humans

Despite significant improvement in technology and hygienic practices at all stages of food production accompanied with advanced improvement in public sanitation,

Salmonellosis and *Salmonella* infections remain a persistent threat to human and animal health.

During the slaughter process, carcasses can become contaminated with *Salmonella*, if the equipment is contaminated from the same or a previous flock. Feather pickers and chilling of carcasses are among the processing steps with the highest risk of cross-contamination. Air-chilling or antimicrobial compounds like trichloroacetic acid in water chillers can decrease the risk. Eggs can be contaminated with *Salmonella* as described above. Egg-washing is a controversial preventative measure. While it removes fecal contaminations from the eggshell, it also removes the cuticle, a protective layer that reduces penetration of the eggshell by bacteria. Humans can contract the disease if they consume meat or eggs that are not thoroughly cooked. In addition to food-borne transmission, direct transmission from birds in pet- and backyard flocks to humans has been well documented and can be a major risk for children.

Humans will shed the bacteria in the feces and can infect other humans under unhygienic conditions.

6.2.3 Evidence of Zoonotic Transmission

Samples for the detection of *Salmonella* spp. in poultry flocks are crop, ceca, yolk sac, liver, or spleen from diseased birds. Samples for monitoring healthy flocks include composite samples of feces, boot swabs, or dust. In many countries, the sampling protocols for *Salmonella* monitoring are regulated by legislation. The laboratory procedure to detect *Salmonella enterica* serovars is described in ISO 6579.

There is ample evidence of zoonotic transmission from poultry to humans. Historically, the identity of strains was determined by restriction enzyme digestion of the genome and visualization after pulsed field gel electrophoresis (PFGE). Today, the whole genome is sequenced on a routine base to trace back outbreaks.

6.2.4 Disease Symptoms in Poultry

The course of the infection of salmonellosis in poultry depends on a number of factors such as the involved *Salmonella* serovar, age of birds, infectious dose, and route of infection. More often than not, infections of chickens with *Salmonella* are subclinical. In cases with disease, the incubation periods range between 2 and 5 days. Mortality in young birds varies from negligible to 10% to 20% and in severe outbreaks may reach 80% or higher.

Symptoms include an increased number of un-pipped and pipped hatching eggs with dead embryos, if infection was egg transmitted or occurred in the hatchers. Signs usually seen in young birds are somnolence, weakness, drooping wings, ruffled feathers, and huddling together near heat sources. Many birds that survive for several days will become emaciated, and the feathers around the vent will be soiled with fecal material (“pasty vent”). Furthermore, respiratory distress as well as

lameness as a result of arthritis may be present. Adult birds serve mostly as intestinal or internal organ carriers over longer periods with little or no clinical signs, but a drop in egg production can occur.

Birds that die in the acute phase of the disease show a persistent or inflamed yolk sac, catarrhal and hemorrhagic enteritis, necrotic foci in liver, spleen heart muscle, or granuloma in the lungs. Furthermore, congestion of the liver, kidney, gall bladder, and heart muscle is the most constant postmortem finding. Ceca may contain a caseous core and sometimes are filled with blood. In adult birds, lesions most frequently found in chronic carrier hens are misshapen, pedunculated, discolored cystic ova. The involved ova usually contain oily and caseous material enclosed in a thickened capsule. Ovarian and oviduct dysfunction may lead to abdominal ovulation or impassable oviduct, which in turn bring about extensive peritonitis and adhesions of the abdominal viscera. In male birds, the testes may be atrophied with thickening of tunica albuginea and multiple abscesses.

After recovery, salmonellae can persist in the intestines, especially in the ceca and the cecal tonsils, and birds continue to excrete *Salmonella* intermittently in their feces.

6.2.5 Disease Symptoms in Humans

Infections with S. Enterica serovars may cause intestinal inflammation with mucopurulent or bloody diarrhea accompanied by fever, vomiting, and abdominal cramps for several days. Incubation time is between less than 1 day and 3 days. If high amounts of water and electrolytes are lost, a hypovolemic shock can result. In severe cases, especially in infants and immunocompromised persons, sepsis and spread to other organs may occur, leading to a septic shock.

6.2.6 Unresolved Issues

Salmonellosis and *Salmonella* infections remain a persistent threat to human and animal health. According to the European Food Safety Agency, the proportion of human salmonellosis cases due to S. Enteritidis acquired in the EU remained at a similar level in recent years.

The EU prevalence of Salmonella target serovar-positive poultry flocks has been stable since 2015. The EU has set targets to reduce the prevalence of *Salmonella* in commercial poultry flocks; however, of the 26 member states reporting their data, only 18 met the reduction targets, whereas 8 failed to meet at least one.

In general, the major strategy to control salmonella should include cleaning the production chain from the top in aim to prevent the vertical transmission, hygienic measures throughout the production chain, vaccination, therapy, and eradication/reduction by legislations. In all cases, agent surveillance and monitoring programs must be adapted and followed strictly in aim to allow early intervention. In addition, since the success of any disease control program depends on farm and personal sanitation, it is essential to educate people involved in poultry production about

microorganisms, to educate people about modes of transmission, and to raise general awareness for the reasons behind such control programs. Finally, legislations, on its own, can never be sufficient to ensure the production of safe food. Rather, the industry itself, from producer to retailer, has a responsibility to ensure the safety of its products.

6.3 Campylobacteriosis

6.3.1 Epidemiology of Campylobacter Infections in Poultry

Campylobacter are curved, rod-shaped bacteria. Thermophilic *Campylobacter* has been found worldwide in poultry flocks. In most countries, prevalence in broiler, layer, and turkey flocks is higher than 50%. Of the 17 species and 6 subspecies, thermophilic species are the ones infecting avians and mammals. *C. jejuni* is predominant in poultry, while *C. coli* is less common, and *C. lari* is rare. Flocks younger than 3 weeks are rarely affected. Additionally, there is a seasonal variation with higher infection rates in spring and fall than in winter and summer.

In poultry, the highest prevalence of thermophilic *Campylobacter* has been detected in gallinaceous birds, but wild and commercial aquatic birds are also frequently affected. In addition, various mammalian species including cattle, sheep, pigs, pets, or rodents can be carriers.

It is not fully understood how thermophilic *Campylobacter* spp. are introduced into flocks. Due to their low tenacity, they probably depend strongly on living avian or mammalian vectors. Furthermore, various insects can serve as vectors for *Campylobacter* spp.

However, water supply sources, farm equipment such as trucks, forklifts, pallets, crates, and footwear have also been identified as potential sources of *Campylobacter* infection of poultry. Outside personnel like thinning crews pose a major risk for the introduction of *Campylobacter* into flocks. Furthermore, biofilms in water pipes offer *Campylobacter* an opportunity to survive for several weeks. It is very controversial if *Campylobacter* can be vertically transmitted.

6.3.2 Epidemiology of Campylobacter Infections in Humans

Globally, *Campylobacter* are one of the most frequent causes of diarrhea. In the EU, about 200,000 human cases of campylobacteriosis are diagnosed every year, making it the most frequently reported food-borne disease. Its incidence together with its duration and potential complications make it an important disease with a high socioeconomic impact. In the EU, the cumulative cost including direct cost to health systems as well as lost productivity is estimated to be around EUR 2.4 billion annually. In developing countries, *Campylobacter* infections in children under the age of 2 years are especially frequent.

Infection of humans generally occurs by the consumption of raw or undercooked meat, which originated from infected flocks and/or was contaminated during the

slaughter process. Contamination of carcasses or meat with *Campylobacter* during processing usually happens from feces.

Of the 17 *Campylobacter* spp. and 6 subspecies *C. jejuni* ssp. jejuni and *C. coli* are most frequent in humans. Other species such as *C. lari* and *C. upsaliensis* are found less frequently but have also been associated with diarrhea.

6.3.3 Evidence of Zoonotic Transmission

For the detection of thermophilic *Campylobacter,* various selective media are used. A standardized procedure for the isolation of *Campylobacter* from food and feed has been published in ISO 10272-1:2006. This method may be adapted for the investigation of clinical samples from birds.

As with *Salmonella*, there are many examples where the same clones were detected in live birds, poultry products, and infected humans. Isolates are typed by restriction enzyme digestion of the genome and visualization after pulsed field gel electrophoresis, multilocus sequence typing, or whole-genome sequencing, respectively.

An indirect indication is that in countries with specific strategies to reduce the prevalence of *Campylobacter* in live poultry, a similar reduction in human cases is observed.

6.3.4 Disease Symptoms in Poultry

Virtually all infections of poultry with thermophilic *Campylobacter* are without clinical signs or pathological lesions. Only chickens infected with virulent isolates at the time of hatch may develop enteritis with accumulation of mucus and fluid or focal hepatic focal necrosis and some mortality.

Avian vibrionic hepatitis was a disease that occurred in laying hens in the 1950s and 1960s. The hepatitis was characterized by small grayish-white focal lesions. *Campylobacter* spp. were regarded as the causative organisms, but for unknown reasons, the disease has not been observed in recent times.

6.3.5 Disease Symptoms in Humans

After an incubation period of 1 to 7 days, the most common clinical symptom of infections of humans with thermophilic *Campylobacter* is diarrhea. Other symptoms may be abdominal cramps, nausea, vomiting, fever, or headache.

Some postinfectious complications may be associated with *Campylobacter* infections. The most important of them is Guillain–Barré syndrome. The Guillain–Barré syndrome is a rapidly evolving paralysis without fever or other systemic symptoms and apparent causes. The symptoms are the consequence of an acute inflammatory demyelinating polyneuropathy. It is thought that antibodies against gangliosides are

involved in the pathogenesis, and that these autoantibodies are originally formed against *C. jejuni* strains possessing ganglioside-like epitopes or other pathogens.

6.3.6 Unresolved Issues

It is rarely known how *Campylobacter* are introduced into poultry flocks. Epidemiological investigations hardly ever provide a smoking gun, and estimation of the relative importance of the known sources is extremely difficult. In addition, epidemiological data also show circulation of multiple strains in the same farm or flock indicating repeated infections. This makes clear how difficult it is to prevent introduction into flocks with conventional biosecurity measures. Furthermore, *Campylobacter* are not easily isolated because they are fragile in the environment, forming viable-but-not-culturable stages, and are easily overgrown by other bacteria.

In addition, due to the high prevalence of *Campylobacter*, it is difficult to implement control strategies throughout the food chain. Processing plants receive birds from multiple farms. A single positive flock can contaminate carcasses from subsequent flocks, significantly increasing the risk of *Campylobacter* contamination of chicken meat. The different processing steps can all contribute to cross-contamination of *Campylobacter* until packaging.

6.4 *Escherichia coli*

6.4.1 Epidemiology of *Escherichia coli* in Poultry

E. coli are a ubiquitous, potentially beneficial part of the intestinal microbiota of chickens and turkeys. Chicks and poults can get infected right after hatch with environmental *E. coli* or *E. coli* from the eggshell surface. There is no true vertical transmission of *E. coli*.

Several diverse strains are present in each host, including strains of serotypes that are regarded as potentially pathogenic. Diseases in poultry are either caused by commensal *E. coli*, when predisposing factors weaken the birds, or by avian pathogenic *E. coli* (APEC) that can cause disease in uncompromised birds. There is no clear-cut criterion for APEC; APEC belong predominantly to serotypes O2, O18, and O78, but not all *E. coli* of these serotypes are APEC, and some APEC isolates belong to other serotypes. Detection of virulence genes and genes that enhance extraintestinal survival in the host allows a more etiological diagnosis.

6.4.2 Epidemiology of *Escherichia coli* in Humans

Epidemiology of *E. coli* in humans resembles epidemiology of *E. coli* in poultry. *E. coli* is a ubiquitous, mostly benign part of the intestinal microbiota, and only some strains, often belonging to serotypes O157, O4, or O18 are primary pathogens. These strains carry one or several of the known virulence genes. Based on the toxins and

course of the disease, a variety of pathotypes like Shiga toxin producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), or extraintestinal pathogenic *E. coli* (ExPEC) are differentiated.

6.4.3 Evidence of Zoonotic Transmission

Poultry and poultry products have been found to be a source of *E. coli* possessing or expressing the genes for various toxins or virulence factors associated with human disease like Shiga toxin or intimin, respectively.

In addition, ExPEC causing disease in humans and APEC, i.e., avian ExPEC, can share traits and are closely related. These traits include serogroups and phylogenetic groups. Some human ExPEC, especially of serogroups O1, O2, and O18, seem to be closer related to APEC strains than other human ExPEC strains. More importantly, the same virulence and fitness genes, e.g., adhesins, toxins, iron acquisition mechanisms, and invasins, can be found in APEC and human ExPEC isolates.

Considering the relatedness between APEC strains and some human ExPEC strains, it is not surprising that *E. coli* isolates similar to ExPEC and even isolates that were able to cause ExPEC-associated illnesses in animal models have been found on poultry products.

6.4.4 Disease Symptoms in Poultry

The most important diseases caused by *E. coli* in poultry are colisepticemia, inflammation of the upper and lower airways, omphalitis and yolk sac inflammation, as well as coliform cellulitis. Inflammation of the airways with involvement of *E. coli* is also known as swollen head syndrome, which is recognizable by edema of the skin of the head, or chronic respiratory disease causing respiratory distress. Coliform cellulitis is characterized by thickened and hard skin with a dark yellow to brown discoloration at the lower abdominal region and at the shanks. Mortality is usually under 5%, but can rise to more than 50%.

Pathological lesions of colisepticemia are polyserositis and swollen spleen, kidneys, and liver. Inflammation of the airways is visible by the accumulation of fibrinous exudate in the lungs and air sacs. Inflamed yolk sacs have fluid or past, greenish to brownish, and smelly contents. Salpingitis is characterized by oviducts that are thin-walled, dilated, and filled with fibrinous, scrambled egg-like material.

6.4.5 Disease Symptoms in Humans

E. coli strains can cause several diseases in humans. Infections with enteric *E. coli* can manifest themselves in various types of diarrheal disease, depending on the toxins. The most serious of these is hemolytic–uremic syndrome (HUS) caused by EHEC and characterized by bloody diarrhea, fever, and kidney failure. HUS can be a life-threatening disease.

Urinary tract infections (UTI), neonatal meningitis, and neonatal sepsis are the major diseases caused by ExPEC, but ExPEC can also be involved in infections of other organs. More than 50% of all UTI are caused by uropathogenic *E. coli* (UPEC). Neonatal meningitis occurs mostly in newborns between 6 and 9 days of age with 20% to 30% of infected babies dying. Signs of neonatal sepsis are lethargy, hypothermia, and poor feeding, and case fatality rates differ depending on age, but can be up to 25%.

6.4.6 Unresolved Issues

There is no smoking gun connecting *E. coli* in poultry with disease in humans yet. So far, all evidence is based on genetic and other similarities between APEC and ExPEC. Future research is needed to prove that poultry can be a reservoir for *E. coli* causing disease in humans, and how this risk can be minimized.

6.5 Antibacterial Resistances

6.5.1 Epidemiology of Antibacterial Resistances in Poultry

Many ad-hoc investigations and regularly conducted monitoring programs, e.g., the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) in Canada, Germ-Vet in Germany, or the National Antimicrobial Resistance Monitoring System (NARMS) in the United States, report data about the prevalence of antimicrobial-resistant bacteria in poultry flocks and on poultry products. On the European level, the Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals, and Food in 2018/2019 focused on poultry and poultry carcasses. The results vary widely between geographic regions, years, bacterial species, and antimicrobials. Antimicrobial resistances are more common in meat-type poultry, which is more likely to receive antimicrobials, than in laying hens because withdrawal periods on eggs result in major economic losses unless one of the few products with no withdrawal time is used.

There is the common tendency that a reduced use of antibiotics in poultry flocks decreases the incidence of bacteria-resistant against these antibiotics. However, it is also important to note that a reduction in the use of antimicrobials does not necessarily lead to a decrease of antimicrobial resistances. The most common reason is the localization of several resistance genes on the same plasmid causing coresistance. At best, there will be a lag of several years between the end to using an antimicrobial and the decrease of resistance rates.

6.5.2 Evidence of Zoonotic Transmission

While it is controversial to what extent the use of antimicrobials in food producing animals contributes to infections of humans with antimicrobial-resistant bacteria,

there is good evidence that resistant bacteria can be transmitted from animals to humans. Several studies found that bacteria, including *Salmonella* and *Campylobacter*, isolated from certified organic poultry meat, i.e. from flocks that had not received antimicrobials, had a lower prevalence of antimicrobial resistances than bacteria from conventional broiler meat.

In contrast, the detection of the same resistance genes in bacteria isolated from meat and in bacteria from human patients is no conclusive evidence because the direction of the transmission is unclear. This is especially true when genes coding for resistances against antimicrobials that are not used in poultry are detected in live birds or on food products. Further indications without being final evidence are correlations between prevalences of resistance against several antimicrobials in bacterial isolates from poultry and human patients.

6.5.3 Unresolved Issues

Even though the extent to which antimicrobial usage in food producing animals contributes to resistances in bacteria infecting humans remains controversial, there is a broad consensus that the use of antimicrobials that are important for human medicine needs to be minimized in animals.

Laying hen flocks have historically received fewer antimicrobial treatments, so efforts concentrate on meat-producing birds. Recently, improvements have been made in raising broilers without antibiotics; in the United States, 60% of broiler flocks are now raised without receiving prophylactic or therapeutic antibiotics, including ionophores, which are not used in human medicine. The challenge of the coming years will be to further reduce the need for antimicrobials through improved management and vaccines, while balancing this aim with animal welfare and economic constraints.

6.6 Avian Influenza

6.6.1 Epidemiology of Avian Influenza in Poultry

Influenza viruses can infect virtually all bird species. Wild aquatic birds like duck and geese have the highest prevalence of infection. When they are subclinically infected and migrate, they can transport the virus around the globe.

Avian influenza (AI) is caused by influenza A viruses of the subtypes H5 and H7 and occurs worldwide. Prevalence of infection in poultry varies widely between countries. Regions with high infection rates include Mexico, Egypt, and Southeast Asia. In Europe, North America except Mexico, and Australia, infections of domestic poultry with AI are comparatively rare, but their frequency has increased in recent years resulting in a series of smaller outbreaks in Europe and an epizootic causing about 3 billion US dollar economic damage in the United States in 2015.

The virus can be transmitted directly through contact with infected birds or indirectly through contaminated equipment. Contact with infected wild birds, especially waterfowl, is the major route how AI is introduced into flocks. A distinct relation between the proximity of poultry-rearing areas and migratory waterfowl routes as well as the method of rearing can be observed. Spread between commercial flocks seems to be less important but can happen by mechanical transfer of infective feces through movement of man and contaminated equipment. Rodents and insects may also mechanically carry the virus from infected to susceptible poultry.

There is little or no evidence of vertical transmission. However, eggshell surfaces can be contaminated with the virus.

6.6.2 Epidemiology of Avian Influenza in Humans

Transmission from birds to humans occurs only after close contact with infected live birds. This happens most frequently in rural Southeast Asia and in Egypt, where poultry is kept close to living quarters. Food-borne infections of humans have not been reported. However, this possibility should not be ruled out completely, since some felids like tigers and cats got infected this way.

AIV has been transmitted only in rare cases between humans. In the last 20 years, the World Health Organization registered less than 1000 cases of humans infected with H5 AIV, and since 2013, less than 200 cases of human infections with H7 subtypes. Human-to-human transmission seems to be exceedingly rare.

6.6.3 Evidence of Zoonotic Transmission

Influenza virus may be isolated in embryonated chicken eggs followed by typing by hemagglutination inhibition assay and classifying as highly or low pathogenic AI (HPAI or LPAI) in infection studies or by sequencing of the hemagglutinin gene. Alternatively, molecular biological detection and characterization can be done. Methods are described in detail by the OIE and are part of the legislation in many countries.

H5 and H7 influenza types do not usually infect humans, so detection of these subtypes is indicative of transmission from poultry. Sequencing of the hemagglutinin gene and the whole genome has shown that isolates from humans were identical to strains from poultry.

6.6.4 Disease Symptoms in Poultry

The severity of clinical signs, course of the disease, and mortality in poultry after infection with AI are extremely variable from highly acute to a very mild or even

inapparent form with few or no clinical signs. The most virulent form of AI in poultry is called Highly Pathogenic Avian Influenza (HPAI). Currently, only viruses of H5 and H7 subtype have been shown to cause HPAI in susceptible species, but not all H5 and H7 viruses cause HPAI. H5 and H7 influenza viruses not meeting the criteria of HPAI are designated Low Pathogenic Avian Influenza (LPAI). Infection of poultry with other influenza subtypes occurs, but is legally not defined as AI.

Clinical signs of HPAI may include high mortality, ruffled feathers, depression, diarrhea, sudden drop in egg production, cyanosis of comb and wattles, edema and swelling of head, blood-tinged discharge from nostrils, respiratory distress, incoordination, and pin-point hemorrhages mostly seen on the feet and shanks.

Lesions at postmortem may include swelling of the face. Removing skin from the carcass will show a clear straw-colored fluid in the subcutaneous tissues. Blood vessels are usually engorged. Hemorrhage may be seen in the trachea, proventriculus, and throughout the intestines. Young broilers may show signs of severe dehydration with other lesions less pronounced or entirely absent.

LPAI as well as some non-H5 or H7 influenza strains cause mild to severe respiratory disease.

6.6.5 Disease Symptoms in Humans

Infection of humans with AI causes disease of the lower respiratory tract, leading to cough, sore throat, breathing problems, and pneumonia. Other flu-like symptoms that may be caused by AI include fever and muscle aches. In atypical cases, respiratory symptoms may be absent, and diarrhea or neurologic signs have been reported in infected humans.

Lethality in confirmed cases is well above 50%; however, many nonfatal cases of the disease with milder symptoms may be undiagnosed.

6.6.6 Unresolved Issues

Control of AI is regulated at the national and international level, but most of the legislation is motivated by the disastrous consequences of the disease for infected poultry rather than by its public health significance. In countries with a low prevalence of AI, infected flocks usually are destroyed. This means that they are not slaughtered, and their meat cannot be used for human consumption. In the EU, table eggs laid during the presumed incubation period are also not to be used for human consumption unless they have been properly disinfected.

Countries with a high prevalence have adopted vaccination strategies which have led to frequent emergence of new variants. Before the emergence of COVID, it was feared that an AIV strain would mutate to be transmissible

between humans and cause a pandemic. The risk still exists with increasing case numbers of AI in Europe, partially due to increased free range husbandry of poultry and continuing close and frequent contact between poultry and humans in developing countries.

6.7 Newcastle Disease

6.7.1 Epidemiology of Newcastle Disease in Poultry

Newcastle disease (ND) is ranked as the major virus disease of poultry in many countries worldwide. In developed countries with established poultry industries, outbreaks with very virulent (velogenic) ND are comparatively rare or neglected. Due to widespread vaccination of commercial flocks against ND, it is difficult to assess its prevalence because clinical signs are rare. If NDV is tested for and detected, the isolate must be typed to determine if it is virulent or a circulating nonvirulent or vaccine strain. In developing countries, ND is one of the leading causes of mortality in small flocks.

Infections with NDV have been reported worldwide in at least 241 bird species from 27 different orders. It has been suggested that virtually all birds are susceptible to infection with NDV. Infections have also been reported in some nonavian species.

The infection can be transmitted primarily through direct contact between healthy and infected birds. The disease can also be spread by mechanical means and by vaccination and debeaking crews, manure haulers, rendering-truck drivers, or feed delivery personnel.

6.7.2 Epidemiology of Newcastle Disease in Humans

Transmission to humans requires close contact to infected birds that allows the virus to come into contact with the eye. Most cases in humans occur in persons working on poultry farms or in processing plants. Spray vaccination of poultry flocks poses a particular risk if no appropriate eye protection is worn, since lesions in humans can also be caused by vaccine strains. Additionally, laboratory accidents when working with isolated NDV have been reported.

6.7.3 Evidence of Zoonotic Transmission

The virus can be isolated in embryonated chicken eggs and identified by hemagglutination inhibition assay. Pathotyping can be done in infection studies in order to discriminate very virulent strains from less virulent strains, especially live vaccines. However, virulence, or lack thereof, for poultry is not connected to virulence for humans. The classical methods can be replaced by molecular biological detection and characterization. The methods are described in detail by the OIE.

Whenever NDV is detected in humans, zoonotic transmission can be assumed because human-to-human transmission has not been described.

6.7.4 Disease Symptoms in Poultry

The course of the disease is mainly determined by the virulence of the involved strain. Age and immune status of the birds as well as general health and environmental conditions play a comparatively minor role. Infection with velogenic and viscerotropic strains is accompanied with a high, peracute mortality rate without specific disease symptoms. Very virulent and neurotropic strains also cause high mortality, but birds show nervous signs such as tremors, twisting of the head and neck, abnormal movement like circling, rearing, somersaulting as well as paresis, and paralysis.

Clinical signs after infection with strains of lesser virulence include ruffled feathers, depression, diarrhea, and respiratory signs in form of nasal discharge, coughing, rales, and dyspnea.

Gross lesions may be absent or include hemorrhagic lesions on the heart, in the proventriculus, in the intestine, and in cecal tonsils. In addition, tracheitis and airsacculitis can be observed.

6.7.5 Disease Symptoms in Humans

The most common symptom of NDV infection of humans is conjunctivitis, which does not affect the cornea and is characterized by swollen and reddened eye lids and lacrimation. In rare cases, a generalized infection with sneezing, dyspnea headaches, and fever may occur after exposure to a high amount of virus.

6.7.6 Unresolved Issues

ND remains a problem in poultry. In commercial poultry, infections pose a significant economic risk masked by widely applied vaccines, while in small unvaccinated flocks, infections are frequent and cause severe losses. In contrast, because of the high infection doses and the usually mild course of the disease, infection of humans has a low priority for public health.

6.8 Chlamydiosis

6.8.1 Epidemiology of Chlamydiosis in Poultry

Infections of birds with *Chlamydia psittaci* occur worldwide, but incidence and distribution vary widely. Infections with *C. psittaci* have been described in more than 500 bird species.

Table 2 Association between *C. psittaci* genotypes and avian hosts

Genotypes							
	A	B	C	D	E	E/B	F
Psittacines	++[a]	+[b]			+		+
Pigeons, doves	+	++			++	+	
Waterfowl	+	+	++		+	++	
Turkeys	+	+	+	++	+	+	+
Chickens		++	+	++	+	+	
Passerines	+	++					
Ratites					++		
Wild birds		++			++		

[a]++ = Genotype most commonly associated with this bird species or group
[b]+ = Genotype less commonly associated with this bird species or group

C. psittaci has been classified based on genetic differences in the *omp1* gene into nine genotypes. Seven of them (A, B, C, D, E, E/B, and F) are found in avian species. Host species are mainly infected with one certain serotype, and each serotype seems to have a main host, but these connections are not absolute (Table 2). Infections have also been reported in more than 30 mammalian species, including mice and men.

In poultry, the infection is especially prevalent among turkey flocks, where outbreaks are mostly caused by isolates of serotype B or D. Outbreaks in turkeys usually involve several flocks, and free ranging flocks are at considerably greater risk. Clinically apparent and inapparent chlamydiosis in turkeys is more common than in chickens.

Occasional outbreaks in ducks as well as in geese have been caused by isolates of serotype C, and ducks and geese are considered the main host of serotype C. Outbreaks of Chlamydiosis in commercially reared ducks in North America are rare and were considered a problem in Europe. Serotypes A and F are mostly associated with psittacines and serotype E with pigeons.

C. psittaci is shed in large numbers in respiratory exudate, especially in the nasal secretions, and in the feces. The primary route of infection is inhalation of aerosols containing the bacterium, but infection by ingestion can also occur. Arthropods have been implicated as vectors, but indirect transmission does not seem to play an important role. The most important way *C. psittaci* is introduced into flocks seems to be by wild birds. Vertical transmission may happen at a low frequency in turkeys, chickens, and ducks. Birds may shed the bacterium intermittently.

6.8.2 Epidemiology of Chlamydiosis in Humans

Humans can get infected by direct contact with infected birds, mostly by inhalation of infectious aerosols, but infections through bite wounds have occurred. Humans contract infections most frequently from psittacines, but persons handling infected birds at the farm and personnel at processing plants have been infected from poultry. Consequently, mostly veterinarians, owners of pet birds, pet shop staff, and persons working in poultry processing plants are at risk.

Infections by consumption of contaminated meat or eggs are not known. Spread among humans is rare.

6.8.3 Evidence of Zoonotic Transmission

For a quick diagnosis, organ smears or smears of swabs from conjunctiva, oropharynx, or cloaca can be stained with Giemsa or related methods and investigated for red or purple chlamydial elementary bodies within infected cells. Staining of fixed tissues or immunohistochemistry is also possible.

For isolation in cell culture or embryonated chicken eggs, samples should be placed in special transport medium if they cannot be processed immediately.

At present, tests for the detection of chlamydial antigens lack sensitivity as well as specificity and are not recommended, while detection of chlamydial genes by PCR is the method of choice for routine diagnosis. Several protocols for different genes have been published.

Detection of antibodies against *C. psittaci* may identify inapparently infected birds and flocks. This can be done by complement fixation test, indirect immunofluorescence, or ELISA.

Because transmission between humans is rare, zoonotic infection is usually assumed whenever the disease is diagnosed in humans.

6.8.4 Disease Symptoms in Poultry

Generally, infections caused by isolates of serotype B have a longer incubation time of up to several weeks and are less severe than infections with isolates of serotype D, which have an incubation time of less than 10 days and cause more severe disease. Additionally, there are differences between different isolates of the same serovar.

Clinical symptoms of infected turkeys include severe respiratory disease with nasal and ocular discharges, conjunctivitis, and green droppings. Diseased birds are lethargic and anorectic and may become cachectic. Egg production in breeder hens is reduced. Morbidity may be up to 80% and mortality up to 30%. Gross lesions at postmortem are enlarged spleen, liver, and heart due to vascular congestions, congested, and inflamed lungs as well as fibrinous airsacculitis, pericarditis, and peritonitis.

Clinical signs and postmortem lesions in ducks are similar. Additionally, nervous signs can be observed. As in turkeys, morbidity may be up to 80% and mortality up to 30%.

6.8.5 Disease Symptoms in Humans

Historically, the disease in humans was called either psittacosis or ornithosis depending on the source of the infection, and it was assumed that ornithosis was a

less severe disease. Nowadays, this differentiation is no longer considered to be correct, and the disease is generally called chlamydiosis.

Incubation time usually is between 1 and 2 weeks. The outcome of infections depends on infection dose and route besides other factors. The disease may be inapparent or characterized by pneumonia accompanied by fever, headache, and myalgia. Symptoms may continue for several weeks. The disease is rarely fatal if treated correctly with antibiotics.

6.8.6 Unresolved Issues

Chlamydiosis in chickens causes only unspecific clinical signs and postmortem lesions. Diagnosis requires detection or isolation of the bacterium, and the tests are not part of routine procedures in diagnostic laboratories receiving poultry for necropsy. Detection of the intracellular inclusion bodies requires special stains. For this reason, the prevalence of chlamydiosis is likely to be underestimated, which puts poultry workers at risk of infections. At the same time, due to the underestimation and the unspecific signs, chlamydiosis might not be considered as differential diagnosis in poultry workers.

Infected flocks should be treated with chlortetracycline to reduce mortality. They will not clear the birds from the infection but reduce clinical symptoms and shedding. Therapy should continue until shortly before slaughter to prevent relapses and to minimize the risk for the workers at the processing plant. Personnel handling the birds and at processing plants must wear appropriate personal protective equipment. If an infected flock is allowed to be processed at all, it must be determined on a case-to-case base.

6.9 Erysipelas

6.9.1 Epidemiology of Erysipelas in Poultry

Erysipelas is an acute infection caused by *Erysipelothrix rhusiopathiae*, a ubiquitous gram-positive bacterial organism. The genus *Erysipelothrix* is classified into two species: *E. rhusiopathiae* and *E. tonsillarum*. Among them, 26 different serotypes are recognized. Some serotypes of *E. rhusiopathiae* are pathogenic for poultry, whereas *E. tonsillarum* strains are nonpathogenic. In poultry, serotypes 1, 2, and 5 are most prevalent; all of them belong to *E. rhusiopathiae*.

Infections of poultry with *E. rhusiopathiae* occur sporadically worldwide. In some regions, the disease is considered endemic. At risk are mostly free-range and older, i.e., laying hen and breeder flocks. The host spectrum of *E. rhusiopathiae* is extremely wide, comprising various species of mammals, birds, reptiles, and fish. All species of domestic poultry are susceptible to infection, even though the susceptibility differs between them.

The natural route(s) of infection are not entirely certain. It is assumed that the bacterium enters the host through injuries of the skin or mucous membranes. Experimental infections are done either subcutaneously or orally. *E. rhusiopathiae* can survive several weeks in soil, and this may be the main source of infection.

Turkey hens can become infected through lesions caused by artificial insemination. The infection can be introduced into flocks by the red fowl mite as potential mechanical vector of *E. rhusiopathiae*. In addition, infected rodents, pigs or sheep, as well as contaminated fish meal have been implicated as a source of infection.

6.9.2 Epidemiology of Erysipelas in Humans

Humans get infected through skin injuries. Particularly at risk are animal caretakers, veterinarians, and butchers. Infection by consumption of contaminated food or transmission between humans has not been reported.

6.9.3 Evidence of Zoonotic Transmission

Presumptive diagnosis can be done by detection of Gram-positive rod-shaped bacteria in smears of infected organs. *E. rhusiopathiae* can readily be isolated from heart, liver, spleen, and bone marrow of dead birds, but the small colonies are easily overlooked or overgrown by other bacteria. If dead birds have already started to decompose, culturing bone marrow samples will make it easier to obtain cultures.

PCR assays have been described that will detect almost all serotypes and differentiate *E. rhusiopathiae* from *E. tonsillarum*. Detection of antibodies against *E. rhusiopathiae* is not done in routine practice.

When *E. rhusiopathiae* infections are detected in humans, zoonotic transmission can be assumed because human-to-human transmission has not been described. If possible, serotypes of isolates from humans can be matched with isolates from birds.

6.9.4 Disease Symptoms in Poultry

The course of the disease is especially fulminant in turkeys, regardless of age and sex. However, outbreaks with high mortality have also occurred in chickens (especially layer flocks), ducks, and geese. Incubation time depends on the infection dose and on the infection route. In animal studies, it is shorter after subcutaneous infection than after oral infection. In turkeys, first signs may be observed 2 or 3 days after oral infection.

Older birds are more sensitive to the infection than the younger birds. Mortality in laying hens is age related and, just as the incubation time, dependent on the route of the infection with subcutaneous infection causing more severe disease.

Infected birds show unspecific signs like moderate general depression or decreased egg production in laying hens. Furthermore, diarrhea or cutaneous lesions, especially swollen, purple snoods in turkeys, can be observed. Unvaccinated birds showing clinical signs usually die, and some infected turkeys may suddenly die without previous clinical signs. Mortality in turkeys may be up to 50% over the course of several weeks.

The most prominent gross lesion at postmortem is generalized congestion of internal organs with hemorrhage in several organs. Liver, spleen, and kidneys are enlarged and may have areas of necrosis. Other lesions may be enteritis, endocarditis, or fibrinopurulent exudate in joints and pericardial sac. Histopathology shows vascular congestion in all organs with intravascular aggregations of bacteria and fibrin thrombi. Parenchymal cells may be damaged in liver, spleen, and kidney.

6.9.5 Disease Symptoms in Humans

Infections of humans with *E. rhusiopathiae* are called erysipeloid, while erysipelas in humans refers to infections with *Streptococci*. This is a potential source of confusion in conversations between veterinarians and medical doctors or when studying literature.

E. rhusiopathiae infections in humans most often are local infections of the skin at infection site characterized by swelling and purple discoloration. Usually, the hands are affected. Rarely infections become septicemic. In these cases, symptoms and lesions may be very diverse and include polyarthralgia, septic arthritis, renal failure, endocarditis, encephalitis, and peritonitis. Infections are usually successfully treated with penicillin.

6.9.6 Unresolved Issues

Infections of humans with *E. rhusiopathiae* have traditionally been regarded a problem in fish mongers and other persons handling raw fish. Due to the sporadic nature of the disease in poultry and the symptoms that resemble other infections, medical doctors might not consider infections with *E. rhusiopathiae* a differential diagnosis in poultry workers.

Treatment of infected flocks does not clear the infection so that infected flocks have to be processed. Extra precautions to prevent and cover skin injuries of workers processing flocks that are positive with *E. rhusiopathiae* should be taken.

6.10 Conclusions

Poultry share a number of infectious diseases with humans, and most of the zoonotic diseases in poultry have additional reservoirs in other mammals than humans, which complicates their control and makes eradication all but impossible.

Diseases that are transmitted from poultry to humans by the consumption of contaminated meat and eggs and diseases that are transmitted from poultry to humans by close contact have been described in some detail in this chapter. Food-borne pathogens, namely *Salmonella* and *Campylobacter*, receive much attention, and extensive control programs against *Salmonella* are in place that have contributed to a decrease of *Salmonella* infections of poultry and humans in the recent two decades. Prevention of campylobacteriosis will for the foreseeable future depend on hygiene during processing because control in live flocks seems not to be feasible. Because by far fewer humans have close contact with poultry, diseases that are directly transmitted from birds to humans are considered to be of minor importance.

A third group of diseases comprises pathogens transmitted by insects, especially ticks from mammals and birds, including poultry, to humans. Examples for these pathogens are West Nile Virus, which has rapidly spread in North America and is occasionally detected in migratory birds in Europe, as well as Eastern and Western Equine Encephalitis. Until now the importance of poultry in the epidemiology of these diseases is low, but the situation may change, especially in the light of climate change and a wider dissemination of insect vectors.

6.11 Cross-References

- ▶ *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control
- ▶ Crimean-Congo Hemorrhagic Fever Virus: An Emerging and Re-emerging Pathogen of Public Health Concern
- ▶ Influenza from a One Health Perspective: Infection by a Highly Versatile Virus
- ▶ The Zoonotic Agent Salmonella
- ▶ Vector-Borne Zoonoses
- ▶ West Nile Virus: From Africa to Europe, America, and Beyond
- ▶ Wild Birds and Zoonotic Pathogens
- ▶ Zoonotic Transmission of *Chlamydia* spp.: Known for 140 Years, but Still Underestimated

References

Chalmers R (2021) Chapter 3.10.2 – Cryptosporidiosis. In: OIE Terrestrial Manual. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/

Chang CC, Chen SCA (2015) Colliding epidemics and the rise of cryptococcosis. J Fungi (Basel) 23:1

Cheikhrouhou S, Trabelsi S, Aloui D, Bouchekoua M, Khaled S et al (2020) Avian mite bites acquired from pigeons: report of three cases and review of the literature. Tunis Med 98:241–245

Corrin T, Ackford R, Mascarenhas M, Greig J, Waddell LA et al (2020) Eastern equine encephalitis virus: a scoping review of the global evidence. Vector Borne Zoonotic Dis 21:305–320

Estrada-Peña A, Jameson L, Medlock J, Vatansever Z, Tishkova F et al (2012) Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the western Palearctic. Vector Borne Zoonotic Dis 12:743–752

Habarugira G, Suen WW, Hobson-Peters J, Hall RA, Bielefeldt-Ohmann H (2020) West Nile Virus: an update on pathobiology, epidemiology, diagnostics, control and "one health" implications. Pathogens 9:589

Mittal J, Ponce MG, Gendlina I, Nosanchuk JD et al (2019) Histoplasma Capsulatum: mechanisms for pathogenesis. Curr Top Microbiol Immunol 422:157–191

Sanchez S, Fulton RM (2020) Tuberculosis. In: Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, Suarez DL (eds) Diseases of poultry, 14th edn. Wiley Blackwell, Ames, pp 1033–1043

Bacterial Pathogens Associated with Aquaculture Products

7

Iddya Karunasagar

Contents

Abstract

According to FAO statistics, aquaculture is contributing to nearly half the global food fish production. Fish contributes to both nutritional and food security in many developing economies. Fish is also one of the extensively traded food commodities, and most of global aquaculture production takes place in developing countries and the major markets are in the developed world. European Union, Japan, and the USA together account for 70% of global fish imports. Generally, fish and fishery products have a very good safety record. But there are some bacterial hazards associated with aquaculture products. The chapter discusses the bacterial pathogens that may be associated with products of aquaculture, pathways of contamination, and risk management measures reported for these bacterial hazards. In terms of antibiotic usage, there is limited data from developing countries, and a number of studies have looked at antimicrobial resistance in bacterial pathogens associated with fish and fishery products. Aspects related to antimicrobial resistance in aquaculture products are also presented in this chapter.

I. Karunasagar (✉)
Nitte University, Mangalore, India
e-mail: iddya.karunasagar@nitte.edu.in

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_5

Keywords

Aquaculture · Bacterial pathogens · Vibrio spp · Salmonella · Risk analysis

7.1 Introduction

Fish constitute a highly nutritious food providing proteins, polyunsaturated fatty acids (PUFA), micronutrients, and vitamins. Though it is commonly believed that mostly marine fish like salmon are the major source of PUFA, particularly omega-3 fatty acids, it has been found that even fresh water farmed fish like carps that are widely produced and consumed in Asia provide much more omega-3 fatty acids than poultry or beef. Globally, fish contribute about 17% of animal protein intake of human population and this proportion is even higher in low-income food-deficit countries, reaching over 50% in some countries like Cambodia, Sierra Leone, Bangladesh, and Indonesia (FAO 2022). During 2020, the global fish production was 178 million tons of which 87.5 million tons came from aquaculture (FAO 2022). Global fish production by capture has been stagnating for last two decades and most of the fish stocks are either fully exploited or even over-exploited. Hence, to meet the increasing demand for fish as food, it is important to increase fish production by aquaculture. Diverse species of finfish and shellfish are produced by aquaculture. Fact sheets of 62 species of fish, crustaceans, and mollusks that are cultured in different parts of the world are available from FAO website (FAO 2023). Asia accounted for 89% of global aquaculture production by volume in 2020, and this was dominated by the contribution of China, which accounted for more than 64% of global aquaculture production by volume. Farmed finfishes dominate global aquaculture production (57.5 million tons), followed by mollusks (17.7 million tons), and crustaceans (11.2 million tons).

In general, fish is considered a safe food and there are very few epidemiological record illness associated with farmed fish. In the USA, during 1998–2015, 857 seafood-associated outbreaks occurred resulting in 4815 illnesses, 359 hospitalizations, and 4 deaths were recorded (Barrett et al. 2017). Of these, etiology could be confirmed in 637 outbreaks, and of these, scombrotoxin accounted for 349 outbreaks (55%) and ciguatoxin for 227 outbreaks (36%). Both these were associated with captured finfish such as tuna and mahi mahi. The bacterial pathogen, *Salmonella* was associated with 978 illness (26%) and 97% hospitalizations (31%). Norovirus was associated with 418 illnesses (11%). Illness due to Norovirus may be associated with cultured bivalves, but often, there could also be person-to-person spread and actual number of illness due to bivalve consumption is difficult to estimate. Data from the European Union (EU) show that in 2018, there were 113 outbreaks associated with fish and shellfish involving 1196 cases (EFSA and ECDC 2019) but data for aquaculture is not available separately. During 2011, 78.9% of the 71 outbreaks associated with finfish were due to scombrotoxin, and 4.2% due to ciguatoxin. *Salmonella* accounted for 4.2% of the illness. In case of shellfish (crustaceans, mollusks), 40.5% of the 42 outbreaks in 2011 were due to calciviruses (noroviruses) and 16.7% due to algal biotoxins

(EFSA and ECDC 2013). *Salmonella* and *Escherichia coli* each accounted for 4.8% illness. In some parts of Asia, where there is the practice of consuming raw fish, a large number of parasitic diseases caused by the fish-borne trematodes have been reported to be prevalent. For example, about 1.5 million people in Korea, six million people in China, and over five million in Thailand are reported to be infected with the liver flukes, either *Clonorchis sinensis*, *Opisthorchis viverrini*, or *O. felineus* (Chai et al. 2005). However, this chapter deals only with pathogenic bacteria that may be associated with farmed aquatic species.

7.2 Bacterial Hazards Associated with Aquaculture Products

Farmed fish live in an environment, where there are significant levels of aquatic bacteria and the microflora associated with fish are greatly influenced by the microflora in the surrounding environment. When fish are alive, aquatic bacteria may be associated with the skin surface, in the gill surface and in gut. Most of the bacteria that are associated naturally with aquatic environment are generally not pathogenic to humans with the exception of few *Vibrio* species that are discussed in later sections. Once the fish are harvested, they are handled, transported, and processed, and during this period, they will come in contact with human hands and various surfaces (containers, equipment), ice and water, which could influence the microflora associated with fishery products that reach the consumers. Bacteria of zoonotic potential that may be associated with fish and fishery products are discussed in the following sections.

7.2.1 *Vibrio* spp.

Vibrio spp. are autochthonous inhabitants of the aquatic environment, and of over 80 species included in the genus *Vibrio*, at least 12 are capable of causing human infections (Oliver and Kaper 2007). Most of the pathogenic species have environmental non-pathogenic strains. *Vibrio* spp. are commonly isolated from estuarine, coastal marine environments (some species like *Vibrio cholerae* are found in fresh waters) all over the world, and seafood-borne illnesses are primarily caused by *Vibrio parahaemolyticus*, *V. vulnificus,* and *V. cholerae* (FAO/WHO 2003). Of these, *V. parahaemolyticus* and *V. cholerae* cause gastrointestinal disease, while *V. vulnificus* causes septicemia. There are very few pathogens of fish that can also affect humans. For example, some strains of *V. vulnificus* cause infection in eels and can also infect humans. Most of the other *Vibrio* spp. pathogenic to aquaculture species, e.g., *V. harveyi* (causing disease in shrimp) and *V. anguillarum* (pathogenic to marine fish), are not human pathogens.

7.2.1.1 *V. cholerae*

V. cholerae is a heterogeneous species consisting of over 220 serotypes, of which only serotypes O1 and O139 are known to cause the disease cholera and these are

generally referred to as choleragenic *V. cholerae* (FAO/WHO 2005a). Strains belonging to non 01/non-0139 serotypes of *V. cholerae* are widely distributed in the aquatic environment including aquaculture systems (Gopal et al. 2005) and are mostly not pathogenic to humans, though occasionally, they may be associated with sporadic cases of gastroenteritis (Oliver and Kaper 2007). None of the *V. cholerae* serotypes are known to be pathogens of farmed aquatic animals. The O1 serovar is known to possess three antigenic forms: Inaba, Ogawa, and Hikojima. Based on their phenotypic characteristics, *V. cholerae* O1 strains are classified into two biotypes, Classical and El Tor (Kaper et al. 1995). Since the seventh pandemic of cholera, most outbreaks have been caused by El Tor strains and the Classical biotype strains are rarely isolated from any part of the world (Sack et al. 2003). The choleragenic El Tor biotype strains of *V. cholerae* are grouped in four major clonal groups: (i) the seventh pandemic, (ii) the US Gulf Coast, (iii) Australia, and (iv) Latin America, which seem to reflect broad demographic and epidemiological associations (Wachsmuth et al. 1994). When the O139 emerged in early 1990s and almost replaced O1 serotype in Southeast Asia, it was thought that it might represent a new pandemic, but this strain did not spread beyond Southeast Asia and even there, the cases due to O139 serotype have declined and O1 serotype has become dominant again (Oliver and Kaper 2007). The disease cholera is characterized by the passage of voluminous stools of rice water consistency leading to dehydration, hypovolemic shock, acidosis, and if appropriate treatment is not initiated, death. However, it has been estimated that only 2% of those infected with ElT or biotype and 11% of those infected with classical biotype develop severe disease. Five percent of El Tor infections and 15% of classical infections may result in moderate illness that can be managed in outpatient clinics (Kaper et al. 1995). Infected individuals shed the pathogen in their feces for 7–14 days. Symptoms due to O1 and O139 serotypes appear to be identical. About 80% of patients can be treated adequately through oral rehydration salts (ORS) or intravenous fluids depending on severity of symptoms. Antibiotic treatment can reduce the volume of diarrhea in patients with severe symptoms and reduce the period of fecal shedding. Doxycycline, tetracycline, trimethoprim-sulfamethaxazole and erythromycin are some of the antibiotics used. Antibiotic resistance in *V. cholerae* O1 El Tor has been reported from a number of countries (Kitaoka et al. 2011), but there are no such documented outbreaks associated with consumption of aquaculture products.

For differentiation of choleragenic *V. cholerae* from non-choleragenic types, serotyping has been commonly used, but some environmental stains could cross-react with O1 or O139 antisera (FAO/WHO 2005a). The most important virulence factor associated with *V. cholerae* O1 and O139 is the cholera toxin, which has two subunits, A and B. The *ctx* genes (*ctx*A and *ctx*B) encoding the production of the cholera toxin are present in a filamentous bacteriophage that is integrated into the genome of *V. cholerae* O1 and O139 (Faruque et al. 1998). Loss of bacteriophage may explain the presence of non-toxigenic O1 *V. cholerae* in the environment. Molecular identification methods based on probes or PCR primers binding to *ctx* gene have been widely used to detect toxigenic O1 or O139 *V. cholerae* in the environment and in foods (FAO/WHO 2005a). FDA Bacteriological Analytical

Manual recommends *ctx*-based PCR for determining toxigenicity of *V. cholerae* (Kaysner and DePaola 2004). Detection of choleragenic *V. cholerae* in fish homogenates containing less than 10 cells/ml was possible when PCR was performed after 6 h enrichment in alkaline peptone water (Karunasagar et al. 1995).

Toxigenic *V. cholerae* may persist in the environment for long periods of time in the absence of clinical cases and this explains the presence of *V. cholerae* O1 in waters in areas where cholera is not an endemic disease, e.g., US Gulf Coast and in Australia (Oliver and Kaper 2007). Adhesion to chitin has been shown to influence strongly the ecology of *V. cholerae* and strong association between levels of zooplankton like copepods, and incidence of *V. cholerae* has been observed in the aquatic environment. Choleragenic *V. cholerae* has also been reported to attach to the hindgut of crabs and it is noted that the hindgut of crustaceans is an extension of the exoskeleton and is lined with chitin (FAO/WHO 2005a). However, there are very few records of isolation of *V. cholera* O1 and O139 from aquaculture ponds and with shrimp. This could be because shrimp are benthic organisms. Studies from Southeast Asia indicate absence of *V. cholera* O1 from raw shrimp (Karunasagar et al. 1990, 1992; Fonseka 1990; Rattagool et al. 1990). Several studies on shrimp farms in India indicated an absence of choleragenic *V. cholerae* in shrimp culture ponds (Otta et al. 1999; Gopal et al. 2005). Dalsgaard et al. (1995a) found that *V. cholerae* O1 was present in 2% (2/107) of water, sediment, and shrimp samples collected from a major shrimp culture area in Southeast Asia. However, subsequent testing of the isolates indicated absence of the *ctx* genes in both the O1 strains (Dalsgaard et al. 1995b). Ravi Kiran (1992) and Dalsgaard et al. (1995a) analyzed shrimp gut content for the presence of potential human pathogens and noted the absence of *V. cholerae* O1.

Farmed fish could be contaminated with choleragenic *V. cholerae* due to improper hygiene during postharvest handling. Saravanan et al. (2007) noted the absence of choleragenic *V. cholerae* in shrimp processed under HACCP (Hazard Analysis Critical Control Point) conditions in India, but detected the organism in one shrimp sample in a domestic market. Chen et al. (2004) isolated *V. cholerae* O1 and O139 from shrimp in domestic market in Malaysia and possibly this could be due to postharvest contamination. During the Peruvian cholera epidemic, high levels of contamination (100%) were observed in a small number of raw seafood samples from street vendors, but only one out of 1011 seafood samples intended for export and processed under HACCP conditions was positive (DePaola et al. 1993). FAO/WHO risk assessment for choleragenic *V. cholerae* in warm water shrimp in international trade looked at the data from testing laboratories in shrimp importing countries during the period 1995–2000. Of a total of 21,857 samples tested, only two samples originating from an Asian country in 1995 (early days of HACCP) were positive for choleragenic *V. cholerae* (FAO/WHO 2005a).

Epidemiological data indicates that a variety of fish and fishery products have been involved in outbreaks of cholera in different parts of the world (FAO/WHO 2005a). Transmission of *V. cholerae* by seafood can be acute where fish and shellfish are consumed raw (DePaola 1981). Seventy-five of 336 passengers in an airline were affected in the Americas in 1992 in which cold seafood salad was implicated (Eberhart-Phillips et al. 1996). The shellfish most often associated with cholera

cases are molluscan shellfish (oysters) and crabs. Oysters are consumed raw in many countries, but crabs are generally cooked. However, studies (Blake et al. 1980) have shown that even after boiling crabs for up to 10 min or steaming for up to 30 min, *V. cholerae* O1 may still retain viability due to issues related to heat penetration. There are very few outbreaks linked to crustacean shellfish. In an outbreak linked to the consumption of raw shrimp in the USA in 1986, the source was found to be domestic. Contaminated crab salad served in an airplane flying from Peru to California caused 75 cases. An outbreak in Japan in 1978 was associated with lobsters imported from Indonesia and another outbreak was linked to the consumption of raw shrimp in the Philippines in 1962. However, in most cases it is not possible to assess whether *V. cholerae* O1 was naturally present or cross-contaminated after harvest (FAO/WHO 2005a). Kim et al. (2018) reported three cases of cholera in Korea associated with raw seafood and the source of contamination was traced to be seawater in the area.

There is very little data on the level of *V. cholerae* associated with aquatic animals and most studies reporting isolations were done following enrichment. Hence, it is expected that levels are generally very low. The dose–response model developed in FAO/WHO risk assessment (FAO/WHO 2005a) indicates that 10^6 choleragenic *V. cholerae* is required to produce disease. This suggests that if products of aquaculture are contaminated with *V. cholerae*, multiplication of the organism has to occur before infective dose is reached. In raw shrimp, *V. cholerae* has to compete with other organisms for growth. The optimum temperature for growth of *V. cholerae* is 37 °C with a range of 10–43 °C (ICMSF 1996). Kolvin and Roberts (1982) measured growth of *V. cholerae* O1 in raw and cooked seafood. No growth was observed in raw prawns, mussels and oysters, but growth occurred in cooked shellfish. Levels of 10^{10} cells/g were reported in cooked prawns and mussels stored at 37 °C. At 22 °C, there was a lag phase of 8 h for classical biotype and 4 h for the El Tor biotype. The organism is sensitive to desiccation and to heat with a D value of 2.65 min at 60 °C (ICMSF 1996). *V. cholerae* survives refrigeration, though some decline in numbers is seen. Viable cells could be recovered from raw shrimp spiked with 10^5 cells/g *V. cholerae* O1 after 4–9 days at 5–10 °C (ICMSF 1996). Similarly, though freezing causes initial decline in numbers, the organism may survive over 180 days in fish (ICMSF 1996).

FAO/WHO risk assessment of choleragenic *V. cholerae* in warm water shrimp in international trade indicated that the risk of transmission cholera through this commodity is very low (FAO/WHO 2005a). Use of a spread sheet-based risk assessment tool (Ross and Sumner 2002) for quantitative risk assessment predicted the likelihood of illness to be 1–2 cases in a decade in Japan and the USA, considering the volumes of warm water shrimp imported and consumed, and one case in 25 years in other shrimp importing countries (FAO/WHO 2005a). Quantitative approach using model based on import to consumption pathway (prevalence estimated based on data from testing laboratories in importing countries – 2 samples in 1995 positive out of 21,857 samples tested between 1995 and 2000) predicted the illness to be 1–5 cases every 5 years, based on the assumption that 10% shrimp are consumed raw and 90% after cooking (FAO/WHO 2005a). Thus,

quantitative risk assessment indicates a very low risk, and there are no epidemiological records of illness linked to imported warm water shrimp supporting the very low risk predicted.

7.2.1.2 *V. parahaemolyticus*

Vibrio parahaemolyticus is found in the estuarine and coastal environments in the tropical to temperate zones (Joseph et al. 1982) where it is considered to be part of the autochthonous microflora. There is no correlation between the presence of this organism and fecal contamination of the environments (Joseph et al. 1982; Oliver and Kaper 2007). *V. parahaemolyticus* has been isolated from seawater, sediment, marine animals, plankton, various fish and shellfish species (Joseph et al. 1982). Thus, *V. parahaemolyticus* is naturally present in shellfish (shrimp and molluscan shellfish) growing and harvesting areas. The level of this organism in various fish and shellfish may vary. Certain areas may have more favorable environmental conditions that support establishment, survival, and growth of the organism such as temperature, salinity, zooplankton, tidal flushing, and dissolved oxygen (Garay et al. 1985; Kaneko and Colwell 1977; Venkateswaran et al. 1990). In temperate waters, the ecology is strongly influenced by temperature and salinity. In these environments, *V. parahaemolyticus* is often detected in warmer months and the organism has been reported to survive in the sediment during winter (Kaneko and Colwell 1977; DePaola et al. 2003); however, in tropical waters, *V. parahaemolyticus* can be detected throughout the year (Natarajan et al. 1980; Deepanjali et al. 2005). Salinity may influence the levels in tropical waters, low counts being recording during post-monsoon period (Deepanjali et al. 2005). *V. parahaemolyticus* can grow in sodium chloride concentrations ranging from 0.5% to 10% with optimum levels between 1% and 3% (Colwell et al. 1984). Adsorption of *V. parahaemolyticus* on to plankton or chitin containing materials occurs with higher efficiency under conditions of estuarine salinity (Kaneko and Colwell 1977). In tropical shrimp culture environments, *V. parahaemolyticus* is often present. This organism accounted for 0–27% of the flora in water and sediment of shrimp ponds in India (Otta et al. 1999; Gopal et al. 2005). The level of *V. parahaemolyticus* in seafood may vary depending on the type of seafood and geographical location. In US Gulf Coast oysters, during warm months, levels such as 1.1×10^4/100 g have been reported, but in Pacific oysters that are at lower temperatures, the levels were 2.1×10^3/100 g (Drake et al. 2007). In Indian oysters, the levels range from 10^2 to 10^4/g (Deepanjali et al. 2005). In shrimp, the levels range from undetectable to 10^4/g, high counts being rare (Cann et al. 1981; Karunasagar et al. 1984) and in finfish levels of ~88/g have been reported (Chan et al. 1989).

Most of the environmental strains may not be pathogenic to man. Early studies in Japan showed that 96% of clinical strains produce a thermostable direct hemolysin (TDH), while only 1% of the environmental strains produce this hemolysin (Joseph et al. 1982). Low prevalence of TDH positive strains in the environment has been confirmed from different geographical regions. In the Gulf Coast in the USA, the percentage has been generally less than 1%, but in Pacific North west, up to 3.2% strains could be TDH positive (FAO/WHO 2007). Six to 10% of oysters from India were positive for *V. parahaemolyticus* carrying *tdh* gene (Karunasagar et al.

1996; Deepanjali et al. 2005; Raghunath et al. 2008). Some TDH negative strains from clinical cases were found to produce a TDH-related hemolysin, TRH (Honda et al. 1988). Presently, strains producing TDH and TRH are considered pathogenic to man. But there may be strain variations. There are five sequence variants of the *tdh* gene (*tdh*1 – *tdh*5) and two sequence variants of the *trh* gene (*trh*1 – *trh*2) (Nishibuchi and Kaper 1990, 1995). Some strains carry both *tdh* and *trh* genes. Most clinical strains carry *tdh*-2 gene. Diverse serotypes may be associated with human infections, but recently, strains belonging to O3:K6 serotype and its variants have been found to be the causative agent of several outbreaks in different countries (Nair et al. 2007). Though several publications refer to these strains as "pandemic" strains, Nair et al. (2007) pointed out that this is misleading in the epidemiological sense, because outbreaks have not affected exceptionally high proportion of the population. Nevertheless, strains belonging to this group show clonality in molecular typing methods like arbitrarily primed (AP) PCR, ribotyping, or pulse field gel electrophoresis (PFGE) and are characterized by the presence of only *tdh* gene (and not *trh* gene), some mismatches in nucleotides in the *tox*RS gene, an open reading frame ORF8 derived from a filamentous bacteriophage f237 (Nair et al. 2007).

Outbreaks of shrimp mortality at early stage (<30 days) were reported from China in 2009, which spread to Vietnam, Thailand, and Mexico during following years. The disease was called "Early Mortality Syndrome (EMS)" and a case definition was adopted in 2012 (NACA 2012). The causative agent was identified as specific types of *V. parahaemolyticus* (Tran et al. 2013) possessing a unique virulence plasmid bearing genes encoding a *Photorhabdus* insect-related (PIR) toxin (Yang et al. 2014). The disease is now called Acute Hepatopancreatic Necrosis Disease (AHPND) and all *V. parahaemolyticus* strains causing AHPND tested so far contain the virulence plasmid and are negative for *tdh* and *trh* genes (Kumar et al. 2021). This shows that *V. parahaemolyticus*$_{AHPND}$ is not of food safety concern.

Based on data from human volunteer studies and using beta-Poisson model, a dose–response relationship has been established in FAO/WHO risk assessment of *V. parahaemolyticus* in seafood (FAO/WHO 2011). This suggests that there is a low risk (0.001%) of illness following consumption of 10^4 *tdh* + *V. parahaemolyticus* and high risk (50%) when 10^8 cells are consumed. Since the levels of this pathogen found in freshly harvested or frozen seafood are generally low, growth of the organism due to mishandling at temperatures permitting growth would be necessary before the organism reaches infective dose. *V. parahaemolyticus* can grow at a temperature range of 5–43 °C and optimum temperature for growth is 37 °C (ICMSF 1996). At optimum temperature, the doubling time in shrimp was 9–10 min and at 18.3 °C, it was 144 min (Katoh 1965). At 20 °C, the doubling time was 34 min in raw shrimp and 28 min in cooked shrimp (Liston 1974). Growth rates in a range of seafoods and tryptic soy broth with 2.5% salt (NaCl) have been recorded and these data indicate that moderate populations of 10^2–10^3 organisms/g on seafood can increase to $>10^5$ organisms/g in 2–3 h at ambient temperatures between 20 °C and 35 °C (ICMSF 1996). A number of studies indicate that *V. parahaemolyticus* dies when exposed to temperatures <5–7 °C, with highest mortality rate being in the range 0–5 °C (ICMSF 1996). A 1–2 $\log_{10}$ drop in numbers

occur during freezing, but the organism can persist in frozen seafood for long periods of time (ICMSF 1996). Both pathogenic and non-pathogenic strains have been observed to respond similarly to freezing (FDA 2005). *V. parahaemolyticus* is very sensitive to heat with a D value of <1.0 min at 65 °C in crab homogenate with an initial inoculum of 10^6 cells (ICMSF 1996), hence cooking would greatly reduce the hazard due to this pathogen.

Symptoms of *V. parahaemolyticus* infection include watery diarrhea, nausea, vomiting, abdominal cramps, and less frequently headache, fever, and chills (FAO/WHO 2011). Generally, the gastroenteritis is self-limiting and severe cases requiring hospitalization are rare. Depending on seafood consumption habits, the source of infection could vary, but mostly involving consumption of raw products and cooked products subjected to postprocess contamination. Oysters are the most common source in outbreaks in the USA and South America, but there have been reports of involvement of other types of seafood including clams, shrimp, lobster, crayfish, scallops, crabs, and finfish (Daniels et al. 2000; Oliver and Kaper 2007). In Japan (Anonymous 2000), implicated foods include *sashimi*, pieces of raw fish fillet (responsible for 26% of outbreaks), followed by *sushi,* vinegary rice ball with pieces of raw fish fillet (23%), shellfish (16%), and cooked seafood (12%). A large outbreak linked to shrimp occurred in Louisiana in 1978 in which 1133 of the 1700 persons attending a dinner were affected and this appears to have been caused by cross contamination between raw and boiled shrimp. Shrimp boiled in the morning were kept in the same wooden seafood box used to transport raw shrimp and transported 40 miles in an unrefrigerated truck and held additional 7–8 h before serving for dinner (Oliver and Kaper 2007). In Japan, *V. parahaemolyticus* is one of the most common causes of gastroenteritis and annually 500–800 outbreaks affecting 10,000 people are reported annually (FAO/WHO 2011). This organism is the leading cause of food-borne illness in Taiwan causing 197 outbreaks during 1986–1995 (Pan et al. 1997) and accounted for 69% of the food-borne cases between 1981 and 2003 (Su and Liu 2007). *V. parahaemolyticus* accounted for 31.1% of 5770 food-borne outbreaks that occurred in China from 1991 to 2001 (Liu et al. 2004).

FDA (2005) carried out quantitative risk assessment of *Vibrio parahaemolyticus* in raw oysters in which a model for predicting *V. parahaemolyticus* levels in oyster based on water temperature was developed. The postharvest oyster handling practices in the USA and the effect of these practices on levels of *V. parahaemolyticus* were modelled. Data from two regions in the USA (Pacific Northwest and Gulf Coast) were used to estimate the proportion of strains that are pathogenic. It was estimated that about 50% of oysters are consumed raw and each serving would be about 200 g. The risk assessment suggested that in the absence of subsequent post-harvest mitigations, "at-harvest" guidance levels of 10^5, 10^3, 10^2 total *V. parahaemolyticus* per g could potentially reduce the illness rate by 1.6, 68, and 98% with corresponding impact of 0.25, 21, and 66% of the harvest, respectively. If the control is applied on the basis of *V. parahaemolyticus* levels at retail, a standard to 10^4/g would reduce illness by 99% and 43% of the harvest would have to be diverted from the raw market. A 5000/g standard could almost eliminate almost 100% of illness, with 70% of the harvest having to be diverted from the raw market (FDA 2005).

The FAO/WHO risk assessment of *V. parahaemolyticus* in raw oysters used a similar approach to estimate risk of illness in Australia, New Zealand, Canada, and Japan (FAO/WHO 2011). Local data on water and air temperature, local harvest practices and prevalence of *V. parahaemolyticus* in oysters in these countries was used. The US data on proportion of pathogenic *V. parahaemolyticus*, multiplication of *V. parahaemolyticus* in oysters, consumption patterns, and underreporting of illness was used. The risk assessment also looked at the impact of applying microbiological criteria, e.g., 100/g, 1000/g, and 10,000/g. The data showed that a criterion of 100/g would lead to 99% reduction in illness in Australia, but this would lead to rejection of 67% of product currently going to the market. Considering that epidemiological records of illness are very rare in Australia, risk management based on microbiological criterion would not be a reasonable approach there. Noting wide variations in the occurrence of *V. parahaemolyticus* in different geographical regions, adopting a global microbiological criterion could not be recommended. This led the Codex Alimentarius Commission to develop a Code of Practice (CAC/GL 73-2010) for minimizing the risk rather than adopt a microbiological criterion.

Since the illness caused by *V. parahaemolyticus* is generally mild, it does not require antibiotic treatment. In severe or prolonged cases, tetracycline, ampicillin, or ciprofloxacin may be used. Though antimicrobial resistance has been detected in environmental strains (Baker-Austin et al. 2008), there is very little evidence that this is an issue with clinical strains. A study of *V. parahaemolyticus* strains isolated from outbreaks of illness in Chile during 2005 and 2007 showed that they were sensitive to tetracycline, sulfamethaxazole-trimethoprim, and ciprofloxacin, but were resistant to ampicillin (Dauros et al. 2011).

7.2.1.3 *Vibrio vulnificus*

Vibrio vulnificus is a common inhabitant of warm water estuarine environments all over the world. The organism has been isolated from coastal marine and estuarine waters, sediment, plankton, and various shellfish (both molluscan and crustacean) and finfish species in areas where the temperatures range from 9 °C to 31 °C. *V. vulnificus* proliferates in waters where temperature exceeds 18 °C (Kaspar and Tamplin 1993; Strom and Paranjpaye 2000; FAO/WHO 2005b; Drake et al. 2007). The abundance varies considerably and is greatly influenced by temperature and salinity. In North America, higher densities are observed in mid-Atlantic, Chesapeake Bay, and Gulf Coast waters, where temperatures are warmer throughout the year, while densities are lower in Pacific, Canadian, and North Atlantic waters (FAO/WHO 2005b). The lowest temperature at which *V. vulnificus* has been isolated varies geographically, being 8 °C at Chesapeake Bay (Wright et al. 1996) and < 12.5 °C in Gulf Coast (Simonson and Siebeling 1986) and the organism survives in sediment during winter. In tropical waters, where temperature does not go below 18 °C, abundance of *V. vulnificus* is influenced by salinity (Parvathi et al. 2004). In south India, highest *V. vulnificus* levels were found during monsoon season when the salinities were less than 5 ppt, and at salinities exceeding 25 ppt, these organisms were not detectable (Parvathi et al. 2004). Salinity has a significant effect on the abundance of the organism even in temperate waters. In the waters of the USA,

numbers of *V. vulnificus* were high at salinity between 5 and 25 ppt, but dropped by 58–88% at salinities over 30 ppt (FAO/WHO 2005b). *V. vulnificus* produces chitinase, which might help the organism to colonize zooplankton (Strom and Paranjpaye 2000) and can colonize plankton and fish gut (FAO/WHO 2005b). Through fish, the organism even reaches the gut of birds since Miyasaka et al. (2006) found 14.1% aquatic birds in Japan to be positive for *V. vulnificus*.

Presently three biotypes are recognized based on a combination of phenotypic, serologic, and host range characters (Drake et al. 2007). Biotype 1 strains are indole positive, serologically diverse, and are associated with human infections. Biotype 2 strains are indole negative and considered mainly as eel pathogens, but may also be opportunistic human pathogens, being associated with infections in eel handlers. This biotype has three serotypes and strains associated with eel and human infections belong to serotype E (Sanjuan and Amaro 2004). Biotype 3 has five atypical biochemical characters, genetically clonal, and has been isolated from 62 Israeli patients with wound infection or septicemia. This biotype has not been associated with food-borne infections (Drake et al. 2007). The virulence of this organism seems to be related to multiple factors such as presence of a polysaccharide capsule, ability to obtain iron from transferrin, and ability to produce extracellular enzymes and exotoxin (Drake et al. 2007). Most of the virulence-associated factors are present in over 95% of environmental strains. Rosche et al. (2005) using nucleotide sequence analysis showed that Biotype 1 strains can be distinguished into two types that strongly correlate with clinical (C) or environmental (E) origin. C-genotypes showed greater resistance to human serum than E-genotypes and had lower LD_{50} suggesting that C-genotype strains may be more virulent (Rosche et al. 2010). While similar levels of C- and E-genotypes were found in estuarine waters, oysters had 85% E-genotypes (Warner and Oliver 2008).

V. vulnificus can cause primary septicemia and wound infections. The disease rarely (<5%) occurs in healthy individuals and risk factors for *V. vulnificus* infection include liver disease, cirrhosis due to alcohol consumption, diabetes, gastrointestinal disorders (ulcer, surgery), hematological conditions, and immunocompromised condition associated with cancer and therapy with immunosuppressive drugs. Epidemiological data suggests that men are more susceptible than women to *V. vulnificus* infection. The fatality rate (about 50%) is the highest among food-borne pathogens (FAO/WHO 2005b) while the attack rate is low with one illness occurring per 10,000 meals of raw US Gulf Coast oysters (containing *V. vulnificus*) served to the highest risk population, i.e., people with liver diseases (FAO/WHO 2005b). The incubation period ranges from 7 h to 10 days, with symptoms appearing in 36 h in most cases (Oliver and Kaper 2007). The symptoms include sudden onset of fever and chills, generally accompanied with nausea, vomiting, abdominal pain, hypotension (systolic pressure < 85 mm) and in over 60% cases, secondary lesions appear, mostly on the legs that often develop necrotizing fasciitis or vasculitis that may require surgical debridement or amputation (Strom and Paranjapaye 2000; Oliver and Kaper 2007). *V. vulnificus* can be isolated from blood and cutaneous lesions. Rare cases of atypical infections have been reported and these include septic arthritis, meningoencephalitis, and ocular infection following consumption of raw oysters or raw fish. Sixty-nine

percent of wound infections were associated with occupational exposures among oyster shuckers and commercial fishermen (Strom and Paranjapye 2000). Wound infections may progress to echymoses, cellulitis, bullae, and necrotizing fasciitis, but mortality rate (25%) is much lower than in case of primary septicemia, though 50% of cases may require surgical debridement or amputation (Jones and Oliver 2009).

Antibiotic therapy is important for both wound infections and septicemia. While tetracycline has been the most effective drug, in some cases, this has been used in combination with third-generation cephalosporin or gentamycin or chloramphenicol (Strom and Paranjapye 2000; Liu et al. 2006). Roig et al. (2009) noted that the *V. vulnificus* biotype 2 serovar E eel pathogenic strains can develop resistance to quinolones by spontaneous mutation of *gyr*A gene and suggested avoiding quinolones for treatment of vibriosis in eel farms. Baker-Austin et al. (2009) screened 151 environmental isolates and 10 primary septicemia isolates for antimicrobial susceptibility. Several isolates showed resistance to antibiotics routinely prescribed for *V. vulnificus* infections such as tetracycline, doxycycline, aminoglycosides, and cephalosporins. The resistance was seen at similar frequencies in C-type and E-type strains. Among environmental isolates, there was no consistent difference in the frequency of resistance between strains from pristine and anthropologically impacted areas suggesting natural rather than human-derived source of resistance traits.

Epidemiological data suggests that about 100 cases of primary septicemia due to *V. vulnificus* occur per year in the USA (Drake et al. 2007). The Korean Centres for Disease Control estimates 40–70 confirmed cases per year and this high rate is suspected to be due to consumption of raw seafood or higher prevalence of predisposing factors (Drake et al. 2007). However, in Japan, Inoue et al. (2008) estimated 12–24 cases per year and in Taiwan, there was a peak occurrence in 2000 with 26 cases per million population (Hsueh et al. 2004). While in the USA, oysters are the main source, this is not the case in Japan since raw oysters are eaten only in winter and most infections occur during June–November with a peak in July. A mud shrimp *Upogebia major* was the common agent associated with *V. vulnificus* infections (Inoue et al. 2008). 72.3 percent of infections had septicemia and mortality rate was 75%. Most patients (86.5%) had liver function impairment with 56.9% having liver cirrhosis and 10.1% liver cancer (Inoue et al. 2008). In Europe, *V. vulnificus* infections are rare and mostly wound infections (Baker-Austin et al. 2010). Rare cases of septicemia have been reported from Thailand (Thamlikitkul 1990) and India (Saraswathi et al. 1989).

V. vulnificus is a natural inhabitant of the estuarine environment and hence fecal coliforms/*Escherichia coli* cannot be used as indicator organism for this pathogen. Since molluscan shellfish are filter feeding organisms, when environmental conditions are favorable, they may harbor high levels of *V. vulnificus* with levels in oysters being 100 times higher than in water surrounding them. On the US Gulf Coast, the levels in oysters may reach 10^4cfu/g during summer months (Drake et al. 2007), and in tropical waters of India, similar levels were reached in oysters when salinities were less than 10 ppt (Parvathi et al. 2004). *V. vulnificus* counts exceeding 10^6/g have been reported from the intestines of benthic fish inhabiting oyster reefs (DePaola et al.

1994). If the temperature of oysters is not controlled immediately after harvest, growth of *V. vulnificus* could occur. Cook (1997) demonstrated that *V. vulnificus* levels in oyster shell stock held without refrigeration for 3.5, 7, 10.5, and 14 h increased 0.75, 1.3, 1.74, and 1.94 log units. It has also been reported that *V. vulnificus* levels in retail oysters originating from Gulf of Mexico were 1–2 log units greater than at harvest (Cook et al. 2002). The organism does not grow in oysters at temperatures below 13 °C and prolonged refrigeration could lead to reduction in numbers and the levels could become non-detectable (<3/g) in 14–21 days (Cook 1994; Cook and Ruple 1992). However, Kaysner et al. (1989) observed survival in artificially contaminated oysters for 14 days at 2 °C, suggesting that refrigeration cannot be relied upon for elimination of this pathogen in oysters. The rate of decline in refrigerated oyster shell stock has been estimated to be 0.041log unit per day (Cook et al. 2002). It has been estimated that commercial cooling of oyster stocks could take an average of 5.5 h (FDA 2005) and therefore the time shell stock is unrefrigerated on boat deck is an issue in control plans.

Freezing could reduce levels of *V. vulnificus* in oysters, but this cannot eliminate the organisms completely. Four to five $\log_{10}$ reductions in numbers of natural *V. vulnificus* population in oysters occur when frozen to −40 °C and stored for 3 weeks (Cook and Ruple 1992). A combination of vacuum packaging and freezing can bring down *V. vulnificus* counts by 3–4 $\log_{10}$ units in 7 days but complete elimination cannot be achieved (Parker et al. 1994). *V. vulnificus* is sensitive to heat with 6 $\log_{10}$ reduction in numbers occurring when subjected to 50 °C for 5 min in shucked oyster meat (Cook and Ruple 1992). Natural populations of *V. vulnificus* (4.3×10^3cfu/g) could be reduced to non-detectable levels by exposing them to 50 °C for 10 min (Cook and Ruple 1992). In North and South Carolina, commercial shell stock is subjected to heat shock by submerging about 70 chilled oysters in wire baskets into a heat-shock tank containing about 850 L of potable water at a temperature of 67 °C for about 5 min depending on oyster size and condition. This process has been shown to reduce *V. vulnificus* levels by 2–4 $\log_{10}$ units (Drake et al. 2007). *V. vulnificus* cells are acid-sensitive and can be inactivated at pH 2.0 (Koo et al. 2000). *V. vulnificus* is sensitive to ionizing radiation and irradiation doses of 1.0 kGy applied on whole shell oysters can reduce the cell numbers from 10^7cfu/g to undetectable levels (Andrews et al. 2003). Hydrostatic pressure of 250 Mpa for 120 s reduced *V. vulnificus* >5 $\log_{10}$ units in oyster (Cook 2003).

Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) carried out a quantitative risk assessment for *V. vulnificus* in raw oysters (FAO/WHO 2005b) and this study modified the FDA *V. parahaemolyticus* risk assessment model to assess the risk of *V. vulnificus* primary septicemia in the USA. The geographical coverage was limited because quantitative data for *V. vulnificus* levels in oysters at the point of consumption and the data for the susceptible population was available only for the USA (FAO/WHO 2005b). The risk assessment model used the data on *V. vulnificus* levels in oysters from four Gulf States and assumed that all strains were equally virulent. Harvest and postharvest module used for exposure assessment was based on postharvest practices (duration oysters in harvest vessel in water, time to first refrigeration, cool down time) derived based on surveys conducted in Gulf

Coast. *V. vulnificus* growth in oysters, survival during refrigeration, and levels at consumption were estimated based on data from studies along the US Gulf Coast (FAO/WHO 2005b). The model predicted that the mean *V. vulnificus* levels in oysters would be 5.7×10^4/g in summer and 8.0×10^1/g in winter. At a serving size of 196 g, the ingested dose would be 1.1×10^7 *V. vulnificus* in summer and 1.6×10^4 in winter. FDA data on the prevalence of risk factors in the US population and oyster consumption data from surveys was used in the model (FAO/WHO 2005b). The dose–response relationship was modeled by estimating the exposure per eating occasion and number of eating occasions for oyster-associated *V. vulnificus* cases reported to the US Centers for Disease Control and Prevention (CDC) during 1995–2001. The risk assessment also predicted the reductions in illness that could be achieved by postharvest treatments to reduce *V. vulnificus* levels to target values such as 3/g or 30/g or 300/g. In the USA, there are three validated methods to achieve end-point criterion of <3 MPN/g *V. vulnificus* and these include mild heat treatment (50 °C), freezing with extended frozen storage and high hydrostatic pressure. If all oysters are treated to achieve target level of 3/g, the model predicted that the number of cases could be reduced from current 32 reported cases per year to one case every 6 years. If the target is shifted to 30 or 300/g, then the predicted cases would increase to 1.2 and 7.7 cases per year, respectively (FAO/WHO 2005b).

The FAO/WHO risk assessment model suggested that immediate cooling of oysters alone is not adequate to achieve substantial reduction in the number of *V. vulnificus* illnesses. The predicted illness ranged from 17.7 to 59.3 at a time to refrigeration range of 0–20 h. Since *V. vulnificus* levels in oysters harvested from waters with a salinity of >30 ppt is greatly reduced, it is predicted that if all oysters are harvested from waters at salinity of >30 ppt, irrespective of the water temperature, *V. vulnificus* illness would be <1 case per year (FAO/WHO 2005b). Relaying oysters to high salinity waters (>32 ppt) has been shown to reduce *V. vulnificus* levels by 3–4 log units (<10/g) within 2 weeks. Based on FAO/WHO risk assessment, Codex Committee on Food Hygiene developed Code of Hygienic practice for control of *Vibrio* spp. in seafood with an annex on control measures for *V. parahaemolyticus* and *V. vulnificus* in bivalve mollusks. This Code recommends assessment of the need for control measures based on (a) number of sporadic illnesses associated with bivalve mollusks in the area, (b) water temperature at harvest, air temperature, and harvest and post-harvest practices, and (c) water salinity at harvest. Since there is wide geographical variation in prevalence and levels of *V. vulnificus* in bivalves, control measures that have been validated and appropriate for the region may be adopted by the competent authority having jurisdiction and implemented under HACCP system. Validation of control measure should be carried out in accordance with the Codex Guidelines for the validation of food safety control measures (CAC/GL 69-2008).

V. vulnificus resides inside various tissues of oysters, hence depuration is ineffective in elimination of this pathogen, but relaying oysters in high salinity (>30 ppt) waters for 17–49 days caused a decrease in population from 10^3cfu/g to <10 MPN/g (Motes and De Paola 1996). The US National Shellfish Sanitation Programme (NSSP) guide

(2011) includes the following strategies for minimizing the risk due to *V. vulnificus* in molluscan shellfish in states reporting two or more cases of *V. vulnificus* illness per year: (a) increased educational efforts targeted toward the population at risk to improve their awareness of the risks of eating raw molluscan shellfish and to change their eating behavior to reduce or stop eating raw or untreated molluscan shellfish, (b) limited harvest restrictions on areas incriminated in outbreaks, (c) requirement for the temperature of shell stock to be brought down to 10 °C or less by using ice, mechanical refrigeration, or other means within specified period (12 h when water temperature is >28 °C; 18 h when water temperature is 15 and 27 °C; 14 h when water temperature is 18–23 °C; and 36 h when water temperature is <18 °C), and (d) phased-in postharvest treatment requirements or other controls.

7.2.2 *Salmonella*

Currently, two species are recognized in the genus *Salmonella,* a member of the family Enterobacteriaceae (Tindall et al. 2005): *Salmonella enterica* and *Salmonella bongori*. Six subspecies are recognized in *S. enterica*, viz. subsp. *enterica*, subsp. *salamae*, subsp. *arizone*, subsp. *diarizonae,* subsp. *Houtenae,* and subsp. *indica*. More than 2500 serotypes have been recorded, of which majority (59%) belong to *S. enterica* subsp. *enterica*, which are also responsible for 99% of *Salmonella* infections in humans and warm-blooded animals (Brenner et al. 2000). Other subspecies may also be associated with cold-blooded animals and environment, but isolates from both species and all subspecies have occurred in humans (Brenner et al. 2000).

The clinical outcomes of *Salmonella* can be considered as two separate groups: (a) Typhoid fever (enteric fever) caused by *Salmonella* Typhi/Paratyphi strains is a serious systemic illness. Incubation period ranges from 7 to 28 days. Symptoms include malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, and bloody stools. Typhoid fever is generally transmitted through water. (b) Non-typhoid *Salmonella* caused by other strains and characterized by gastroenteritis in humans. Incubation period ranges from 8 to 72 h. The symptoms include abdominal pain, diarrhea, chills, fever, nausea, vomiting and malaise. Systemic infection such as septicemia may occur especially in susceptible patients such as the very young, very old, and immunocompromised. The available data measuring illness as the endpoint suggests that no response is observed until a dose of 10^6 is reached (Coleman and Marks 1998). However, outbreak investigations show that lower number of cells can cause infection depending upon the food matrix. There is no data with seafood matrix alone but in an outbreak of *S. enteritidis* associated with scallop and egg yolk, a 56% attack rate was observed at a dose of 6.3 log CFU (FAO/WHO 2002). Severe dehydration due to diarrhea can on occasion require medical intervention through the administration of intravenous fluids and antibiotic treatment. However, occasionally some serovars of this pathogen may cause sepsis after entering the blood stream from the intestine and require intense medical intervention. Mortality is rare if patient is promptly hydrated and provided antibiotic treatment.

Though the normal habitat of *S. enterica* subspecies *enterica* is the gut of warm-blooded animals, very few serovars are host adapted and others may be found in the environment for long periods of time. The habitat for other subspecies is cold-blooded animals and environment. *Salmonella* has been isolated from several aquatic environments in different parts of the world (FAO 2010). Water bodies contaminated with fecal matter from humans, animals including birds and aquatic mammals may contain this pathogen. *Salmonella* can survive in human waste for 10–15 days in septic system and through seepage from septic tanks, sewage and storm runoff, reach surface waters. It can survive and even multiply in aquatic environment, e.g. it can adhere to soil particles and survive and multiply in this ecosystem for at least 1 year (Winfield and Groisman 2003). In Tech River (France), 574 isolates of *Salmonella* belonging to 41 serotypes were obtained during 1996–1997, some serotypes being specific to flood events (Baudart et al. 2000). In a 4-year study of coastal waters of Galecia, North western Spain, a prevalence of 2.4% in mollusks and seawater was found with *S.* Senftenberg being the most predominant (42%) among 20 different serotypes (Martinez-Urtaza et al. 2004a). The presence of S. Senftenberg could not be correlated with environmental parameters, while presence of other serotypes was associated with wind and rainfall events. *S.* Senftenberg has been very rarely reported in human infections and is halotolerant since it has been isolated from brines with a salt concentration of 30% (Martinez-Urtaza et al. 2004b). *Salmonella* Senftenberg has been one of the predominant serovars detected in the coastal waters of Portugal (Catalao Dionisio et al. 2000). This serovar has been isolated from crustaceans from India (Hatha and Lakshmanperumalsay 1995), seafood imported into the USA especially from tropical countries (Heinitz et al. 2000), and from environmental samples in France and Brazil (Baudart et al. 2000; Tavechio et al. 2002). Detection of *Salmonella* in 16% shrimp and 22.1% in mud/water in Southeast Asia led Reilly and Twiddy (1992) to suggest that *Salmonella* are part of the normal aquatic flora in tropical environments. But recent reports of detection of *Salmonella* in fish gut in natural river system in Texas (Gaertner et al. 2008) suggest that *Salmonella* are more widely present in aquatic systems than earlier thought to be. Seventeen to thirty-three percent of fish sampled in San Marcos River, Texas were positive for *Salmonella* and presence in fish gut has been attributed to ingestion of *Salmonella* present in detritus. Byappanahally et al. (2009) reported that the filamentous alga, *Cladospora* in Lake Michigan is a reservoir for *Salmonella*, a 3-year study during 2005–2007 indicating presence in 23–72% samples at densities ranging from 0.16 to 89.46 the most probable number (MPN) per gram. This alga can be found in fresh and marine waters. Therefore, present evidence suggests that *Salmonella* are widely distributed in the aquatic environment and could be part of normal flora in aquaculture systems.

Specific seasonal patterns or climate characteristics have been reported to affect the dynamics of contamination of *Salmonella* in natural environments. The presence of *Salmonella* in the environment in both temperate and tropical regions has been linked to the periods of rains, and more specifically, after the days of the first heavy rains signaling the washing effect of torrential rains as one of the principal environmental drivers of *Salmonella* contamination in coastal areas (FAO 2010). There may

also be other sources in the marine environment. For example, *Salmonella* may colonize marine mammals like killer whale, bottlenose dolphins, seals, sea lions, elephant seals and porpoises (Higgins 2000; Old et al. 2001; Fenwick et al. 2004; Stoddard et al. 2005) and the organisms shed by these mammals may contaminate other marine fish. 21.7% of harbor porpoises in England and Wales were positive for *Salmonella* during 1990–2002. In San Miguel Island, California, 33% of fur seal pups and 40% of sea lion pups were positive for *Salmonella* (Higgins 2000).

Most of the studies looking at the presence of *Salmonella* in aquatic environments have evidenced two main observations: only a small but constant number of serovars have been found in these environments and, in most cases, these do not coincide with the main zoonotic serovars identified in the surrounding areas (FAO 2010). In spite of the variability in sampling size ($n = 37$ to 251), in most of these studies the maximum number of serotypes identified has been around 20 (FAO 2010). Among the clinically important serovars, typhimurium has been shown to be the most common but mostly this accounted for only a small percentage of serotyped strains (FAO 2010); nevertheless, this attests to their capacity of adaptation survival in external environments (Baudart et al. 2000). *Salmonella* Weltevreden has been identified in recent years as one of the prevailing serovars in seafood products from Asian countries. Serovar Weltevreden has been detected as the dominant *Salmonella* serotype in fish and shrimps samples collected in India (Shabarinath et al. 2007), and in other Asian countries (Reilly and Twiddy 1992; Koonse et al. 2005) and this serovar has been involved in several clinical cases in Asia (Bangtrakulnonth et al. 2004; Phan et al. 2005).

Salmonella has been isolated from aquaculture systems in both developing countries and developed countries and the prevalence rates reported vary depending on the methodology used for detection. In aquaculture systems of Southeast Asia, 16.1% of shrimp and 22.2% of water/mud samples were positive for *Salmonella* (Reilly and Twiddy 1992). In US fresh water catfish ponds, Wyatt et al. (1979) reported a prevalence of 5% while a relatively high percentage of 33% in US catfish and 50% in Vietnamese catfish were reported by Pal and Marshall (2009) and this may be due to the methodology used for isolation. From eel culture ponds in Japan, a prevalence of 21% (Saheki et al. 1989) has been documented. *Salmonella* has also been isolated from pond water in a trout farm in Spain (Cesar-Javier et al. 1999). Long-term persistence of *Salmonella* in fish feed plants in Norway has been reported (Nesse et al. 2003). During 2000–2004, 3.78% of environmental samples from Norwegian fish feed production facilities were positive for *Salmonella*. But the serovars recovered were mostly *S*. Senftenberg and *S*. Montevedeo that account for 2% of human cases in Norway (Lunestad et al. 2007). Thus, fish feed could be a source of *Salmonella* in aquaculture systems. These studies provide evidence for the rather common prevalence of *Salmonella* in aquaculture systems across the globe. Contamination of aquaculture systems with *Salmonella* could involve multiple pathways such as runoff of organic matter into ponds during rainfall events, animal wastes introduced directly through bird droppings, frogs living in ponds or indirectly through runoff, fertilization using no-composted manures, integrated aquaculture systems, where animals such as poultry are housed directly over aquaculture pond,

toilets discharging into ponds, contaminated source water through wildlife runoff, untreated domestic sewage, discharge from animal farms, contaminated feed or unhygienic handling practices in farm (FAO 2010). Noor Uddin et al. (2015) reported that *Salmonella* could be detected in both extensive and intensive shrimp culture systems in Vietnam and isolates of *S*. Weltevreden from these systems were clonal in nature. They suggested that *S*. Weltevreden could survive and possibly even multiply in shrimp culture systems. Studies of Hounmanou et al. (2020) indicated occurrence of *S*. Weltevreden as the most common *Salmonella* associated with shrimp culture systems in China and the isolates are genetically related. Though detection of *Salmonella* in aquaculture products has been reported, this has not led to any major public health problem.

Salmonella are often detected in seafood in markets and the prevalence rates reported vary widely. In Malaysia, 25% raw prawns in market were positive, the serovars found being *S*. Blockley, *S*. Weltevreden, *S*. Agona (Armugaswamy et al. 1995), and in India, 1% of the 500 market prawns tested were positive the serovars being *S*. Newport and *S*. *infantis* (Prasad and Pandurangarao 1995). *Salmonella* present in seafood at market level could be a result of postharvest contamination. *Salmonella* including serovar Weltevreden can form biofilms on food contact surfaces and resist sanitizer treatment in biofilms (Joseph et al. 2001). In a study of 353 imported seafood in Japan, 2/47 black tiger shrimp were positive, both with *S*. Weltevreden, and contamination level in seafood were <30–40 MPN/100 g (Asai et al. 2008). Analysis of 11,312 imported and 768 domestic seafood in the USA during 1990–1998 revealed that 10% of imported and 2.8% domestic raw seafood was positive for *Salmonella* and the overall incidence was 7.2% for imported and 1.3% for domestic seafood (Heinitz et al. 2000). The most frequent serotypes in imported seafood were *S*. Weltevreden, *S*. Senftenberg, *S*. Lexington, and *S*. Paratyphi B. These most common serotypes were rarely (<0.5%) observed in human illness in the USA (Helfrick et al. 1997). *S. enteritidis* ranked fifth and *S. typhimurium* ranked 12th (Heinitz et al. 2000). S. Weltevreden was also the most common serotype isolated from imported food including seafood in USA in 2000 (24/187) followed by *S*. Thompson (13/187), *S*. Lexington (12/187), and number of other serotypes (Zhao et al. 2003). Though *S*. Weltevreden is rarely associated with human cases in the USA, it is frequently isolated from human cases in Thailand (Bangtrakulnonth et al. 2004).

Most studies on *Salmonella* in foods including seafood have been carried out using enrichment procedure and quantitative estimation of the concentration of *Salmonella* in foods has rarely been reported. One study of imported seafood (353 samples of 29 types of seafood) in Japan found two samples of black tiger shrimp and the levels estimated by Most Probable Number (MPN) were < 30 to 40/100 g (Asai et al. 2008). Considering that $>10^5$ cells are required to cause infection (FAO/WHO 2002), it can be suggested that multiplication in fish would be necessary before the food is consumed. *Salmonella* being a mesophilic organism, the growth rate of this organism is markedly reduced at temperatures <15 °C while the growth of most strains is prevented at <7 °C (ICMSF 1996). In raw seafoods containing a variety of bacteria, *Salmonella*, if present has to compete with other

flora for growth. *S*. Heidelberg had a generation time of 28 h and 31 h in the fish English sole and sterile crab, respectively, at 8 °C (ICMSF 1996). Ingham et al. (1990) reported proliferation of *Salmonella* in cooked crab inoculated with *Salmonella* and stored at 8–11 °C under modified atmospheres containing low levels of CO_2 (20–50%). The optimum pH for the proliferation of *Salmonella* is 7.0–7.5 though the organism can grow at pH values ranging from 3.8 to 9.5 with (ICMSF 1996). The minimum water activity for growth is 0.94 (ICMSF 1996) and the growth is generally inhibited at 3–4% NaCl, but salt tolerance increases with increasing temperature in the range 10–30 °C (D'Aoust and Maurer 2007). Though the resistance of *Salmonella* to drying varies, this organism may survive for months or even years in dried products and has been frequently isolated from fish meal, meat and bone meal, maize and soy products (Lunestad et al. 2007). *Salmonella* is sensitive to lower temperatures causing decrease in numbers during freezing and frozen storage, but this process does not guarantee elimination of salmonellae in foods (ICMSF 1996). *Salmonella* are heat-sensitive and typical D-values reported are 0.176 min in chicken at 70 °C, 0.36 min in ground beef at 63 °C (FAO/WHO 2002). Some strains of *Salmonella* like *S*. Senftenberg 775 W may show higher heat resistance (ICMSF 1996). Interestingly, *S*. Senftenberg is the serovar often isolated from fish feed (Lunestad et al. 2007). D-values are influenced by the water activity, nature of the solutes, and pH of the suspending medium (ICMSF 1996).

Though *Salmonella* has been isolated from seafood at both farm level and retail level, seafood account for only a small proportion of salmonellosis outbreaks. Greig and Ravel (2009) analyzed food-borne outbreaks reported in International literature between 1988 and 2007, for which a source could be identified ($n = 4093$). This study indicated that 46.9% outbreaks were due to *Salmonella* of which, seafood accounted for 1.7% compared to 14% associated with eggs. In the USA over a three-decade period (1973–2006), *Salmonella* accounted for 18 of a total of 188 outbreaks involving seafood. Three hundred seventy four illness were associated with *Salmonella* in seafood out of 4020 seafood associated illnesses (Iwamoto et al. 2010). In EU, during 2011, there were 1501 food-borne outbreaks of salmonellosis, and these were grouped as 283 outbreaks with strong evidence and 1218 outbreaks with weak evidence (EFSA and ECDC 2013). Of the outbreaks with strong evidence, 17 (6%) were due to seafood.

Considering that *Salmonella* in aquatic systems are derived from human or animal source, antibiotic resistance in seafood would be reflective of the situation in other sectors. Antimicrobial resistance was detected in 9% of the total of strains isolated from environmental sources and shellfish over different studies in Spain (Martinez-Urtaza et al. 2004b; Martinez-Urtaza and Liebana 2005). On the other hand, the presence of antimicrobial resistant strains among strains isolated from the marine environment in Morocco reached 49.1% of the strains (Setti et al. 2009), whereas in Mexico, 50.4% of the strains recovered from water samples showed resistance to antimicrobials (FAO 2010). In a study carried in Cochin, India, 82% of the strains isolated from seafood product presented antimicrobial resistance (Kumar et al. 2009), whereas in Vietnam, antimicrobial resistance was observed in 11.1% of strains (Van et al. 2007). It should however be noted that the methodology and

spectrum of antibiotics used by different investigators vary and this may contribute to the high degree of variation observed. Khan et al. (2006, 2009) recorded varying antibiotic resistance in *Salmonella* strains belonging to several serovars isolated from imported seafood in the USA, but the frequency of resistance in the serovar Weltevreden was low (Ponce et al. 2008).

Biosecurity and control measures to minimize the risk of Salmonella contamination of aquaculture products have been elaborated by FAO (2010). A number of aspects covering farm design, layout, source of water, hygiene of equipment, personnel, feed as well as good hygienic practices during harvesting, transport, and processing would be important.

7.2.3 *Listeria monocytogenes*

The genus *Listeria* has seven species, of which only *L. monocytogenes* is considered as a pathogen to human, and *L. ivanovii* is considered an animal pathogen (Swaminathan et al. 2007). *L. monocytogenes* is a common inhabitant of moist environments like decaying vegetation, soil and can be isolated from various aquatic environments and therefore, the organism is commonly associated with aquaculture environments and in freshly harvest fish (Reilly and Kaferstein 1997) and fish in retail markets (Dhanashree et al. 2003; Parihar et al. 2008). In the US catfish industry, *L. monocytogenes* has been found at a frequency of 76.7% in chilled fresh catfish fillets and 43.3% in unchilled fillets. The organism was also detected in fish contact surfaces such as deheading machine, trimming board, chiller water, and conveyor belts at different stages (Chen et al. 2010). In some studies, raw fish have been found to be a source of contamination of fish processing plants (Gudmundsdóttir et al. 2005). Prevalence of *L. monocytogenes* in fish processing environments has been a particular problem, particularly for the fish smoking industry. To understand the source of contamination of the final product, various molecular techniques such as Pulse Field Gel Electrophoresis (PFGE) and Multi Locus Sequence Typing (MLST) have been used. Chen et al. (2010) noted that in the US catfish industry, chiller water and processing table are important sources of contamination for the final fillets rather than the raw material. Ciccio et al. (2012) noted that though raw fish could be source of contamination of the processing environment, certain strains can persist longer than others and the strains found in the final product are generally these persistent strains. *L. monocytogenes* has been reported to form biofilms and survive in fish processing environment for years (Wilks et al. 2006). PEGE studies on isolates provide evidence for the persistence of strains for 11 years (Vongkamjan et al. 2013).

Unlike most other food-borne pathogens, *L. monocytogenes* is psychrotrophic and capable of growing at refrigerator temperatures. The temperature range at which growth can occur is between 0 °C and 45 °C with an optimum temperature of 37 °C. The organism is resistant to environmental conditions such as high salinity or acidity, which enables its survival for long periods in the environment. Possibly, due to its

psychrotrophic nature, the organism seems to be more prevalent in seafood from temperate environments compared to that from tropical regions. For example, in India, the reported prevalence ranges from absence to 8.6% (Karunasagar and Karunasagar 2000; Parihar et al. 2008), while in cold water shrimp, the prevalence could be 20.9% (Gudmundsdottir et al. 2006).

Despite the wide prevalence of *Listeria monocytogenes* in foods, natural and food processing environments, and its asymptomatic carriage in 5–10% of humans and domestic animals, listeriosis is a rare disease. The incidence is typically in the range of 0.1–11 cases per million people per year (FAO/WHO 2004). A severe form of listeriosis is characterized by an invasive infection often leading to septicemia with or without infections of the central nervous system such as meningitis, meningoencephalitis, rhomboencephalitis or brain abscess. In the case of pregnant women, while the mother will often experience mild flu-like symptoms, her fetus may be stillborn, aborted or be born with generalized infections. Less common symptoms include localized infections including endocarditis, peritonitis, and arthritis. Skin infections may also occur in some patients. The incubation period is very variable ranging from 3 to 70 days, and since most people do not remember their food consumption from months earlier, it is often difficult to trace the source of infection. The median incubation period is approximately 3 weeks. If diagnosed, the disease can usually be treated effectively with a range of common antibiotics. Severe form of listeriosis occurs mostly in susceptible population that includes elderly, pregnant women, people with underlying illness (chronic conditions such as cardiovascular disease, congestive heart failure, diabetes, cirrhosis, and alcoholism) and immunocompromised individuals. Fatality rate of 20–30% is common in severe form of listeriosis (FAO/WHO 2004).

L. monocytogenes may also cause a non-invasive febrile gastroenteritis in healthy individuals. An outbreak of gastrointestinal illness from a tuna and corn salad, affecting >1500 schoolchildren and adults in Italy, provided conclusive evidence for the existence of a febrile gastroenteritis form of listeriosis (Drevets and Bronze 2008; Allerberger and Wagner 2009). The incubation period for this form of the disease ranges from 6 to 50 h, and symptoms usually resolve without treatment after 1 to 2 days. Symptoms are described as "mild flu-like," including diarrhea, abdominal pain, fever, muscle pain, and headaches.

Most outbreaks of listeriosis are associated with ready-to-eat foods that can support the growth of *L. monocytogenes,* have a long refrigerated shelf life and are consumed without further listericidal treatment. Even in products that receive listericidal treatment, post-process contamination, cross contamination at distribution level and home level is an important issue (FAO/WHO 2004). This applies to a range of seafood products, including marinated fish and mussels, prawns, pasteurized crustacea, and smoked fish products. Cold-smoked products have received particular attention in this regard due to a high prevalence of *L. monocytogenes* in such products and persistent contamination of fish processing plants. Contamination rates range from 0.4% to 78.7% but more typically in the range of 4–30%. Studies by Jørgensen and Huss (1998) suggest that at the point of production, 34% of samples are positive with 28% having <10 CFU/g, 5% having 10–100 CFU/g, and 1%

having >100 CFU/g. After 14–20 days of storage at 5C, 40% were positive and the level exceeded 100 CFU/g in 10.5% samples and the levels were 10–100 CFU/g in 20% cases. Epidemiological evidence suggests that listeriosis has been associated with consumption of shrimps, smoked mussels, "gravid" trout and smoked trout (FAO/WHO 2004). Many of these outbreaks, however, involved the gastrointestinal form of the disease and, despite the interest in RTE smoked fish as a source of listeriosis, there are very few documented cases of *systemic* listeriosis due to seafood.

Based on somatic (O) and flagellar (H) antigens *L. monocytogenes* has been differentiated into 13 serovars (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 6a, 6b). Most isolates involved in human disease belong to four serotypes, 1/2a, 1/2b, 1/2c, and 4b. Dose–response models for *L. monocytogenes* inferred from epidemiological data and estimates of total food-borne exposure (FAO/WHO 2004) have generated ID_{50} estimates for immunocompromised people of $>10^{10}$ cells. Since the levels of *L. monocytogenes* found in seafood are generally low, multiplication of the organism would have occurred in products involved in outbreaks. Therefore, prevention of growth in the products would be an important risk management strategy. A number of hurdles are employed to increase the shelf life of lightly preserved seafood, often in combination, including refrigeration, salt, phenolic (smoke) compounds, acidification with organic acids including lactate, acetate, sorbate, benzoate, citrate, or addition of salts of organic acids, addition of nitrite and modified atmosphere packaging including CO_2. Models to predict the effect of these hurdles on the growth of *L. monocytogenes* have been developed and evaluated (Mejlholm et al. 2010).

When *L. monocytogenes* was identified as a food-borne pathogen, the first response of regulatory agencies was to establish a "zero tolerance" policy, i.e., the organism should be absent in 25 g sample of the product. This caused trade disruptions and the issue came up before the Codex Alimentarius Commission, which asked FAO/WHO to perform risk assessment of *L. monocytogenes* in foods and specifically to "estimate the risk of serious illness from *L. monocytogenes* in food when the number of organisms ranges from absence in 25 grams to 1000 colony forming units (CFU) per gram or millilitre, or does not exceed specified levels at the point of consumption." The risk assessment report (FAO/WHO 2004) noted that in areas where a regulatory level of "absence in 25g" or 0.04 CFU/g is applied and all products in market comply with the requirement, the listeriosis cases would be less than 1 case per year, but in the USA, where such regulatory limit is applied, cases were still being seen and this indicated that a portion of ready-to-eat food contained a substantially greater number of the pathogen than the regulatory limit. Thus, the public health impact of *L. monocytogenes* is almost exclusively a function of the foods that greatly exceed the "absence in 25g" limit. The report also examined "what if scenario" using two often discussed regulatory limits – 0.04 CFU/g and 100 CFU/g. The risk assessment model indicated that at 100% compliance, the number of predicted cases is low for both limits, with an approximate tenfold difference between them, that is, 0.5 cases versus 5.7 cases. But as the number of "defectives" (proportion of products not meeting the criteria) increased, the difference narrowed. For example, at a presumed defective level of 0.0001%, the predicted number of cases would be 12.3 and 17.4. The model predicted that if a microbiological limit of 0.04 CFU/g with

a 0.018% defect rate (2133 cases) was replaced with a 100 CFU/g limit and a 0.001% defect rate (124 cases), the predicted result based on the scenario is an approximate 95% reduction in food-borne listeriosis. In view of the widespread occurrence of *L. monocytogenes* in fish processing environments, there would be significant percentage of "defectives," if "absence in 25 g" is used as criterion. Considering these findings, the Codex Alimentaruis Commission agreed to have a criterion of 100 CFU/g for ready-to-eat products that do not support the growth of *L. monocytogenes* and absence in 25 g for products that support the growth of the organism.

Ampicillin is the antibiotic of choice for treatment of listeriosis. Many investigators have screened clinical as well as food isolates of *L. monocytogenes* for antimicrobial susceptibility and generally the reported prevalence of resistance is very low (Walsh et al. 2001; Hansen et al. 2005). Lungu et al. (2011) compiled data regarding reported antibiotic resistance in *L. monocytogenes* isolated from food. Resistance to a variety of antimicrobials including tetracyclines, cephalosporins, β Lactams, aminoglycosides, and quinolones has been noted in many food-borne strains. Interestingly, there were no such strains reported from fish or seafood.

7.2.4 *Streptococcus agalactiae*

Group B Streptococci (GBS) have been known to cause neonatal septicemia and have been involved in sepsis in adults with co-morbidities. In 2015, *S. agalactiae* were implicated in an outbreak involving over 140 apparently healthy adults in Singapore. The affected individuals had serious systemic disease like septic arthritis and meningitis. Epidemiological investigations indicated strong link to consumption of raw freshwater fish. Multilocus sequence typing (MLST) of the isolates involved in the outbreak showed that they belong to Sequence Type 283 (FAO 2021). Based on capsular polysaccharide, GBS have been assigned to ten serotypes Ia, Ib, II to IX. All ST283 isolates belong to serotype III-4.

GBS have also been reported to cause fish infections, particularly in fresh water fish like tilapia in aquaculture. The affected fish show anorexia, abdominal distension, exophthalmia (protruding eyes), hemorrhages, and meningoencephalitis. Three major serotypes of *S. agalactiae* have been detected in fish, serotype Ia, Ib, and III. Isolates of serotype Ia belong to ST7, isolates of serotype Ib belong to ST 260, and isolates of serotype III belong to ST283 or closely related STs.

Betalactams have been the drug of choice for treatment of GBS in humans. GBS ST283 have been reported to be generally susceptible to erythromycin, tetracycline, and penicillins (FAO 2021).

7.3 Antimicrobial Resistance Microorganisms Associated with Products of Aquaculture

The importance of antimicrobial agents in protection of animal health has been widely acknowledged, but the negative impacts of the use of these agents in animals raised for food have been a cause of concern. The use of antimicrobials in

agriculture, animal husbandry, and aquaculture in many developing countries is often unregulated and there is very little data on their usage. Schar et al. (2020) estimated that aquaculture accounts for 5.7% of global consumption of antimicrobials. In 2017, the consumption was 10,259 t and the consumption intensity varied in different species, e.g., 157 mg/kg for catfish, 103 mg/kg for tilapia, 59 mg/kg for tilapia, 46 mg/kg for shrimp, and 27 mg/kg for salmon.

The current risk management strategy for antimicrobial residues in aquaculture products is based on the precautionary principle, and there are no epidemiological records of illnesses in fish consumers due to residues. The FAO/OIE/WHO consultation on scientific issues related to non-human usage of antimicrobials held in Geneva, in December 2003, concluded that residues of antimicrobials in foods, under present regulatory regimes, represent a significantly less important human health risk than the risk related to antimicrobial resistant bacteria in food. Resistance of bacteria to antimicrobial agents is a complex issue. Some bacteria have intrinsic resistance to certain antibiotics, e.g., most gram-negative bacteria have intrinsic resistance against penicillin G, due to the nature of cell wall in this group of bacteria. Resistance to antimicrobial compounds is a very ancient trait in environmental bacteria. Genes conferring resistance to antibiotics that are critical for human medicine today have been found in bacteria billions of years before antimicrobial usage (D'Costa et al. 2011). Bacteria resistant to β-lactams, aminoglycosides, and macrolides, as well as newer drugs such as daptomycin, linezolid, telithromycin, and tigecycline, have been isolated from the Lechuguilla caves in New Mexico that were totally isolated for >4 million years (Bhullar et al. 2012). Recent molecular biological studies on antibiotic resistance genes provide very interesting insights into the evolution and ecology of antibiotic resistance genes. It is estimated that Class A β-lactamases evolved approximately 2.4 billion years ago and were horizontally transferred into the gram-positive bacteria about 800 million years ago. The family of genes, including the progenitors of CTX-Ms (cefotaxime resistance genes), diverged 200–300 million years ago. In addition to being involved in hydrolysis of the β lactam ring, metallo-β lactamases are involved in various basic cellular processes such as hydrolysis, DNA repair, and RNA processing and these enzymes can be found in all the three domains of life, i.e., Bacteria, Archaea, and Eucarya (Garau et al. 2005). Ribosomal protection proteins (RPP) that mediate resistance to tetracyclines were derived through duplication and divergence of GTPase, before the divergence of the three superkingdoms: Bacteria, Archaea, and Eucarya (Kobayashi et al. 2007).

There is evidence to show that clinical bacteria have acquired resistance genes from environmental bacteria. The *qnr* gene responsible for resistance to quinolones are widely distributed in aquatic bacteria such as members of Vibrionaceae, Aeromonas, and Shewanella species (Poirel et al. 2012). $bla_{CTX\text{-}M}$ gene coding for resistance to extended spectrum β-lactamases (ESBL) have originated from the environmental bacteria belonging to *Kluyvera* species (Canton et al. 2012).

Though antibiotic resistance genes may emerge as a process of natural genetic changes occurring in bacteria, presence of antibiotics would exert selective pressure favoring resistant bacteria and their spread. Multiple antibiotic resistance in bacteria causing human infections is a great public health concern. The widespread use of

antibiotics in different sectors such as animal husbandry, agriculture, and human medicine has contributed to selection and spread of antibiotic-resistant bacteria in the environment. Antibiotic resistance genes can spread among unrelated bacteria without any phylogenetic, ecological, or geographical barriers. The Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance held in 2006 identified two types of hazards with respect of antimicrobial resistance:

(a) Development of acquired resistance in bacteria in aquatic environments that can infect humans – This can be regarded as a direct spread of resistance from aquatic environments to humans; and
(b) Development of acquired resistance in bacteria in aquatic environments whereby such resistant bacteria can act as a reservoir of resistance genes from which the genes can be further disseminated and ultimately end up in human pathogens – This can be viewed as an indirect spread of resistance from aquatic environments to humans caused by horizontal gene transfer.

The consequences of antimicrobial resistance in bacteria causing human infections could include increased severity of infection and increased frequency of treatment failures (FAO/OIE/WHO 2006). However, there are no recorded cases of human infections caused by antibiotic-resistant bacteria from aquaculture products.

There are few human pathogenic bacteria that are commonly found in the aquatic environment (e.g., *Vibrio parahaemolyticus*, *V. vulnificus*, *V. cholerae*, motile *Aeromonas* spp., *Edwardsiella tarda*). Antibiotic resistance that cannot be linked to the use of antimicrobials in aquaculture may be found in these aquatic bacteria. Baker-Austin et al. (2008) found antibiotic resistance in *V. parahaemolyticus* isolated from water and sediment along the coasts of Georgia and South Carolina, and resistance frequency was slightly reduced among virulent strains compared to non-virulent strains. Study of antibiotic resistance in *V. vulnificus* from different sites found no difference in antibiotic resistance frequency in isolates from pristine and anthropologically impacted areas and suggested that the resistance traits are naturally derived, rather than from human-derived sources (Baker-Austin et al. 2009). A recent FAO/WHO risk assessment has shown that the risk of transmission of cholera through warm water shrimp in international trade is very low (FAO/WHO 2005a). Motile *Aeromonas* spp. and non-O1 *V. cholerae* are rarely involved in gastrointestinal infections that are mostly self-limiting, and such infections do not require antibiotic therapy.

Indirect spread of antibiotic resistance from aquatic bacteria and human pathogens has been considered a possible hazard. A number of investigators have reported increased prevalence of bacteria carrying antibiotic resistance genes in fish/shrimp ponds and in water and sediments surrounding aquaculture sites in Japan (Kim et al. 2004), Europe (Schmidt et al. 2000), the USA (Chuah et al. 2016), South America (Miranda and Zemelman 2002), China (Dang et al. 2009), and Southeast Asia (Karunasagar et al. 1984; Lee et al. 2005). Though experimental transfer of antibiotic resistance from fish pathogenic bacteria to human gut-associated *E. coli* has been

demonstrated (Kruse and Sorum 1994), the link between antibiotic resistance in aquatic bacteria and human pathogens in nature is yet to be clearly established. Often, similarity in genetic elements is taken as evidence of transfer, but one cannot be sure in which direction the gene flow has occurred, considering that hospital effluents also discharge antibiotic resistant bacteria to the aquatic environment. Although some authors (e.g., Cabello 2006) have tried to link the antibiotic resistance seen in *V. cholerae* involved in the cholera outbreak in Latin America in 1991 with bacteria present in shrimp farms in Ecuador, Smith (2007) presented evidence that resistance plasmids found in these bacteria were earlier reported from pandemic *V. cholerae* strains in other countries and concluded that no link to the pool of resistance genes in the aquaculture environment could be established. Conclusions based on similarity of genetic determinants found in aquatic bacteria and human pathogens need to be evaluated carefully due to the fact that the aquatic environment receives effluents from various sectors of antimicrobial use, e.g., human medicine (hospital effluents), agricultural use, animal husbandry, and aquaculture (fish farm effluents). Thus, the water source used in aquaculture may be contaminated with antibiotic residues or antibiotic-resistant bacteria derived from different sectors (Karunasagar 2012, Fig. 1). Complexities involved in source attribution of antimicrobial resistance in aquaculture have been discussed recently by Karunasagar et al. (2020).

FAO (2008) noted that a risk analysis of the release of human and animal effluents into aquatic environments serving as water sources for aquaculture needs to be performed, particularly with respect to the antimicrobials identified as critically

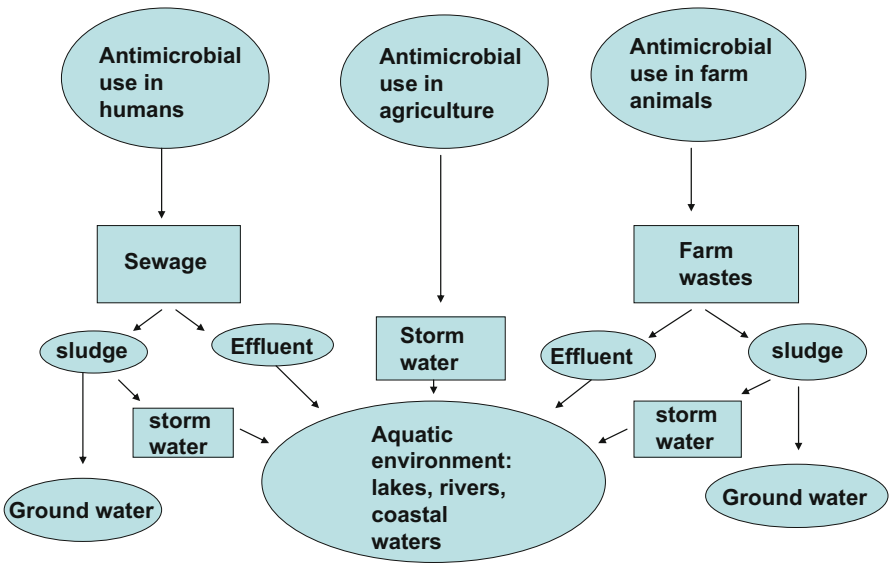

Fig. 1 Pathways for spread of antimicrobial residues and resistant bacteria in the aquatic environment

important by WHO and OIE. Such a risk analysis would determine the appropriate management options through which improved effluent management measures should be implemented (e.g., measures dealing with hospital effluents). Thus, the issue of antimicrobial resistance cannot be addressed for one sector (e.g., aquaculture) alone, but requires a comprehensive approach involving all sectors of antimicrobial usage.

References

Allerberger F, Wagner M (2009) Listeriosis: a resurgent food-borne infection. Clin Microbiol Infect 16:16–23

Andrews LS, Jahncke M, Millikarjunan K (2003) Low-dose gamma irradiation to reduce pathogenic *Vibrios* in live oysters (*Crassostrea virginica*). J Aquat Food Prod T12:71–82

Anonymous (2000) Ten year summary of outbreaks due to *V. parahaemolyticus in* Japan. (1989–1999), The Ministry of Health and Welfare, Japan

Armugaswamy RK, Rusul G, Abdul Hamid SN, Cheh CT (1995) Prevalence of *Salmonella* in raw and cooked food in Malaysia. Food Microbiol 12:3–8

Asai Y, Kaneko M, Ohtsuka K, Morita Y, Kaneko S, Noda H, Furukawa I, Takatori K, Hara-Kudo Y (2008) *Salmonella* prevalence in seafood imported into Japan. J Food Prot 71:1460–1464

Baker-Austin C, McArthur JV, Tuckfield RC, Najarro M, Lindell AH, Gooch J, Stepanauskas R (2008) Antibiotic resistance in the shellfish pathogen *Vibrio parahaemolyticus* isolated from the coastal water and sediment of Georgia and South Carolina, USA. J Food Protect 71:2552–2558

Baker-Austin C, McArther JV, Lindell AH, Wright MS, Tuckfield RC, Gooch J, Warner L, Oliver J, Stepanauskas R (2009) Multi-site analysis reveals widespread antibiotic resistance in the marine pathogen *Vibrio vulnificus*. Microb Ecol 57:151–159

Baker-Austin C, Stockley L, Rangdale R, Martinez-Urtaza J (2010) Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. Environ Microbiol Rep 2:7–18

Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, Sawanpanyalert P, Hendriksen RS, Lo Fo Wong DMA, Aarestrup FM (2004) *Salmonella* serovars from humans and other sources in Thailand, 1993–2002. Emerg Infect Dis 10(1):131–136

Barrett KA, Nakao JH, Taylor EV, Eggers C, Gould LH (2017) Fish-associated foodborne disease outbreaks: United States, 1998–2015. Foodborne Pathog Dis 14:537–543

Baudart J, Lemarchand K, Brisabois A, Lebaron P (2000) Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. Appl Environ Microbiol 66:1544–1552

Benbrook CM (2002) Antibiotic drug use in US aquaculture. In*:* Institute of Agriculture and Trade Policy Report. 18 pp. http://www.healthobservatory.org/library.cfm?RefID=37397

Bhullar K, Waglechner L, Pawlowski L, Koteva K, Banks ED, Johnston MD, Barton HA, Wright GD (2012) Antibiotic resistance is present in an isolated cave microbiome. PLoS One 7:e34953

Blake PA, Allegra DT, Snyder JD, Barrett TJ, McFarland L, Caraway CT, Feeley JC, Craig JP, Lee JV, Puhr ND, Feldman RA (1980) Cholera – a possible endemic focus in the United States. N Engl J Med 302:305–309

Bondad-Reantaso MG, Arthur JR, Subasinghe RP (2008). Understanding and applying risk analysis in aquaculture. FAO Fisheries andAquaculture Technical Paper. No. 519. Rome, FAO. 2008. 304p.

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B (2000) *Salmonella* nomenclature. J Clin Microbiol 38:2465–2467

Burridge L, Weis J, Cabello F, Pizzaro J, Bostick K (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306:7–23

Byappanahally MN, Sawdey R, Ishii S, Shively DA, Ferguson JA, Whitman RL, Sadowsky MJ (2009) Seasonal stability of *Cladophora*-associated *Salmonella* in Lake Michigan watersheds. Water Res 43:806–814

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: growing problem for human and animal health and for the environment. Environ Microbiol 8:1134–1147

Cann DC, Taylor LY, Merican Z (1981) A study of the incidence of *Vibrio parahaemolyticus* in Malaysian shrimp undergoing processing for export. J Hyg (London) 87:485–491

Canton R, Gonzalez-Alba JM, Galan JC (2012) CTX-M enzymes: origin and diffusion. Front Microbiol 3:110

Catalao Dionisio PL, Joao M, Ferreiro VS, Hidalgo ML, García Rosado ME, Borrego JJ (2000) Occurrence of *Salmonella* spp in estuarine and coastal waters of Portugal. Anton Leeuw Int J G 78:99–106

Cesar-Javier G, Teresa-Maria L, Garcia-Lopez M, Miguel P, Andrei O (1999) Bacterial flora of wild brown trout (*Salmo trutta*), Wild Pike (*Esox Lucius*), and aquaculture rainbow trout (*Oncorhynchus mykiss*). J Food Prot 62:1270–1277

Chai JY, Murrell KD, Lymbery AJ (2005) Fish-borne parasitic zoonoses: status and issues. Int J Parasitol 35:1233–1254

Chan KY, Woo ML, Lam LY, French GL (1989) *Vibrio parahaemolyticus* and other halophilic vibrios associated with seafood in Hong Kong. J Appl Bacteriol 66:57–64

Chen CH, Shimada T, Elhadi N, Radu S, Nichibuchi M (2004) Phenotypic and genotypic characteristics and epidemiological significance of ctx+ strains of *Vibrio cholerae* isolated from seafood in Malaysia. Appl Environ Microbiol 70:1964–1972

Chen B, Pyla R, Kim T, Sylva JL, Jung Y (2010) Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environments. Food Microbiol 27:645–652

Chuah L, Effarizah ME, Goni M, Rusul G (2016) Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. Curr Environ Health Rep 3:118–127

Ciccio PD, Meloni D, Festino AR, Conter M, Zanardi E, Ghidini S, Vergara A, Mazzette R, Ianieri A (2012) Longitudinal study on the sources of contamination in cold-smoked salmon and its processing environment in Italy. Int J Food Microbiol 158:79–84

Coleman M, Marks H (1998) Topics in dose-response modelling. J Food Prot 61:1550–1559

Colwell RR, West PA, Maneval D, Remmers EF, Elliot EL, Carlson NE (1984) Ecology of pathogenic vibrios in Chesapeake Bay. In: Colwell RR (ed) Vibrios in the environment. Wiley, New York, pp 367–387

Cook DW (1994) Effect of time and temperature on multiplication of *Vibrio vulnificus* in post-harvest gulf coast shellstock oysters. Appl Environ Microbiol 60:3483–3484

Cook DW (1997) Refrigeration of oyster shellstock: conditions which minimize the outgrowth of *Vibrio vulnificus*. J Food Prot 60:349–352

Cook DW (2003) Sensitivity of *Vibrio* species in phosphate-buffered saline and in oysters to high pressure treatment. J Food Prot 66:2276–2292

Cook DW, Ruple AD (1992) Cold storage and mild heat treatment as processing aids to reduce the numbers of *Vibrio vulnificus* in raw oysters. J Food Prot 55:985–989

Cook DW, Bowers JC, DePaola A (2002) Density of total and pathogenic (tdh+) *Vibrio parahaemolyticus* in Atlantic and Gulf coast molluscan shellfish at harvest. J Food Prot 65:1873–1880

D'Aoust J-Y, Maurer J (2007) *Salmonella* species. In: Doyle MP, Beuchat LR (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 187–236

D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD (2011) Antibiotic resistance is ancient. Nature 477:457–461

Dalsgaard A, Huss HH, H-Kithikun A, Larsen JL (1995a) Prevalence of *Vibrio cholera* and *Salmonella* in a major shrimp production area in Thailand. Int J Food Microbiol 28:101–113

Dalsgaard A, Serichantalergs O, Shimada T, Sethabuthr O, Echeverria P (1995b) Prevalence of *Vibrio cholera* with heat-stable enterotoxin (NAG-ST) and cholera toxin genes: restriction fragment length polymorphisms (RFLP) of NAG-ST genes among *V. cholerae* Oserogroups from a major shrimp production area in Thailand. J Med Microbiol 43:216–220

Dang H, Zhao J, Song L, Chen M, Chang Y (2009) Molecular characterisation of chloramphenicol- and oxytetracycline resistant bacteria and resistance genes in mariculture waters of China. Mar Pollut Bull 58:987–994

Daniels NA, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammod RM, Thompson S, Wilson S, Bean NH, Griffin PM, Slutsker L (2000) *Vibrio parahaemolyticus* infections in United States, 1973–1998. J Infect Dis 181:1661–1666

Dauros P, Bello H, Dominguez M, Hormazabal JC, Ganzales G (2011) Characterisation of *Vibrio parahaemolyticus* strains isolated in Chile in 2005 and in 2007. J Infect Dev Ctries 5:502–510

Deepanjali A, Sanath Kumar H, Karunasagar I, Karunasagar I (2005) Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. Appl Environ Microbiol 71:3575–3580

DePaola A (1981) *Vibrio cholera* in marine foods and environmental waters: a literature review. J Food Sci 46:66–70

DePaola A, Rivadeneyra C, Gelli DS, Zuazua H, Grahn M (1993) Peruvian cholera epidemic: role of seafood. In: Proceedings of the 16th annual tropical and subtropical fisheries technology conference of the Americas, pp 28–33

DePaola A, Capers GM, Alexander D (1994) Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf Coast. Appl Environ Microbiol 60:984–988

DePaola A, Nordstrom JL, Bowers JC, WellsJG, Cook DW (2003) Seasonal abundance of Vibrio parahaemolyticus in Alabama oysters. Appl Environ Microbiol 69:1521–1526

Dhanashree B, Otta SK, Karunasagar I, Goebel W, Karunasagar I (2003) Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India. Food Microbiol 20:447–453

Drake SL, DePaola A, Jaykus L (2007) An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Compr Rev Food Sci Food Saf 6:120–144

Drevets DA, Bronze MS (2008) *Listeria monocytogenes*: epidemiology, human disease and mechanisms of brain invasion. FEMS Immunol Med Microbiol 53:151–165

Eberhart-Phillips J, Besser RE, Tormey MP, Feikin D, Araneta MR, Wells J, Kilman L, Rutherford GW, Griffin PM, Baron R, Mascola L (1996) An outbreak of cholera from food served on an international aircraft. Epidemiol Infect 116:9–13

EFSA, ECDC (2013) The European Union Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA Journal 11:3129

EFSA, ECDC (2019) The European Union one health zoonoses report. EFSA J 17(12):5926, 276 pp

FAO (2010) Report of FAO expert workshop on the application of biosecurity measures to control *Salmonella* contamination in sustainable aquaculture. FAO fisheries and aquaculture report no. 937

FAO (2022) The State of World Fisheries and Aquaculture. https://doi.org/10.4060/cc0461en

FAO (2023) Cultured aquatic species – fact sheets. http://www.fao.org/fishery/culturedspecies/search/en

FAO (2021) Risk profile – Group B Streptococcus – *Streptococcus agalactiae* Sequence Type (ST) 283 in fresh water fish. https://doi.org/10.4060/cb5067en

FAO/OIE/WHO (2006) Report of Joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance: Seoul, Republic of Korea, 13-16 June 2006, FAO, Rome.

FAO/WHO (2002) Risk assessments of *Salmonella* in eggs and broiler chicken. Microbiological risk assessment series no 2. FAO, Rome, 302 pp

FAO/WHO (2003) Risk assessment of *Campylobacter* spp in broiler chickens and *Vibrio* spp in seafood. FAO Food Nutr Pap 75:1–57

FAO/WHO (2004) Risk assessment of *Listeria monocytogenes* in ready to eat foods. Microbiological risk assessment series 5. FAO, Rome, pp 1–269

FAO/WHO (2005a) Risk assessment of choleragenic *Vibrio cholerae* O1 and O139 in warm water shrimp in international trade. Microbiological risk assessment series 9. FAO, Rome, pp 1–90

FAO/WHO (2005b) Risk assessment of choleragenic *Vibrio vulnificus* in raw oysters. Microbiological risk assessment series 8. FAO, Rome, pp 1–114

FAO/WHO (2011) Risk assessment of *Vibrio parahaemolyticus* in seafood. Microbiological risk assessment series 16. FAO, Rome, pp 1–183

Faruque SM, Albert MJ, Mekalanos JJ (1998) Epidemiology, genetics, and ecology of choleragenic *Vibrio cholerae*. Microbiol Mol Biol Rev 62:1301–1314
Fenwick SG, Duignan PJ, Nocol CM, Leyland MJ, Hunter JEB (2004) A comparison of *Salmonella* serotypes isolated from New Zealand Sea lions and feral pigs on the Auckland Islands by pulse field gel electrophoresis. J Wildl Dis 40:566–570
Fonseka TSG (1990) Microbial flora of pond cultured prawn (*Penaeus monodon*). FAO Fisheries Report 40124-31
Food and Drug Administration (FDA) (2005) Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. http://www.cfsan.fda.gov/~dms/vpra-toc.html
Gaertner J, Wheeler PE, Obafemi S, Valdez J, Forstner RJ, Bonner TH, Hahn D (2008) Detection of *Salmonella* in fish in a natural river system. J Aquat Anim Health 20:150–157
Garau G, Di Guilmi AM, Hall BG (2005) Structure based phylogeny of metallo- β lactamases. Antimicrob Agents Chemother 49:2778–2784
Garay E, Arnau A, Amaro C (1985) Incidence of *Vibrio cholerae* and related vibrios in a coastal lagoon and seawater influenced by lake discharges along an annual cycle. Appl Environ Microbiol 50:426–430
Gopal S, Otta SK, Kumar S, Karunasagar I, Karunasagar I (2005) The occurrence of *Vibrio* spp in tropical aquaculture environments: implications for food safety. Int J Food Microbiol 102:151–159
Gudmundsdóttir S, Gudbjörnsdóttir B, Lauzon HL, Einarsson H, Kristinsson KG, Kristjánsson M (2005) Tracing *Listeria monocytogenes* isolates from cold-smoked salmon and its processing environment in Iceland using pulsed-field gel electrophoresis. Int J Food Microbiol 101:41–51
Gudmundsdottir S, Gudmundsdottir B, Einarsson H, Kristinsson KG, Kristjansson M (2006) Contamination of cooked peeled shrimp (*Pandalus borealis*) by *Listeria monocytogenes* during processing at two processing plants. J Food Prot 69:1304–1311
Hansen JM, Gerner-Smidt P, Bruun B (2005) Antibiotic susceptibility of *Listeria monocytogenes* in Denmark 1958–2001. APMIS 113:31–36
Hatha AA, Lakshmanperumalsay P (1995) Antibiotic resistance of Salmonella strains isolated from fish and crustaceans. Lett App Microbiol 21:47–49
Heinitz ML, Ruble RD, Wagner DE, Tatini SR (2000) Incidence of *Salmonella* in fish and seafood. J Food Prot 63:579–592
Helfrick D, Bean NH, Slutsker L, Tauxe RV (1997) Annual tabulation summary 1997. *Salmonella surveillance*. Centers for Disease Control and Prevention, Atlanta
Higgins R (2000) Bacteria and fungi of marine mammals: a review. Can Vet J 45:101–106
Honda T, Ni YX, Miwatani T (1988) Purification and characterisation of hemolysin produced by a clinical isolate of Kanagawa phenomenon negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin. Infect Immun 56:961–965
Hounmanou YMG, Dalsgaard A, Sopacua TH, Uddin GMN, Leekitcharoenphon P, Hendriksen RF, Olsen JE, Larsen MH (2020) Molecular characteristics and zoonotic potential of *Salmonella* Weltevreden from cultured shrimp and tilapia in Vietnam and China. Front Microbiol 11:1985
Hsueh PR, Lin CY, Tang HJ, Lee H, Liu J, Liu Y, Chuang Y (2004) *Vibrio vulnificus* in Taiwan. Emerg Infect Dis 10:1363–1369
ICMSF (1996) Microorganisms in foods 5. Characteristics of microbial pathogens. Blackie Academic & Professional, London, pp 1–513
Ingham SC, Alford RA, McCown AP (1990) Comparative growth rates of *Salmonella typhimurium* and *Pseudomonas fragi* on cooked crab meat stored under air and modified atmosphere. J Food Prot 53:566–567, 625
Inoue Y, Ono T, Matsui T, Miyasaka J, Kinoshita Y, Ihn H (2008) Epidemiological survey of *Vibrio vulnificus* infection in Japan, between 1999–2003. J Dermatol 25:129–139
Iwamoto M, Ayers T, Mahon BE, Swerdlow DL (2010) Epidemiology of seafood associated infections in the United States. Clin Microbiol Rev 23:399–411
Jones MK, Oliver JD (2009) *Vibrio vulnificus*: disease and pathogenesis. Infect Immun 77:1723–1733
Jørgensen LV, Huss HH (1998) Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. Int J Food Microbiol 42:127–131

Joseph SW, Colwell RR, Kaper JB (1982) *Vibrio parahaemolyticus* and related halophilic vibrios. CRC Crit Rev Microbiol 10:77–124

Joseph B, Otta SK, Karunasagar I, Karunasagar I (2001) Biofilm formation by *Salmonella* spp on food contact surfaces and their sensitivity to sanitisers. Int J Food Microbiol 64:367–372

Kaneko T, Colwell RR (1977) The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. Microb Ecol 4:135–155

Kaper JB, Morris JG, Levine MM (1995) Cholera. Clin Microbiol Rev 8:48–86

Karunasagar I (2012) Public health and trade impact of antimicrobial use in aquaculture. In: Improving biosecurity through prudent and responsible use of veterinary medicines in aquaculture. FAO fisheries and aquaculture technical paper 547. https://www.fao.org/3/ba0056e/ba0056e.pdf

Karunasagar I, Karunasagar I (2000) *Listeria* in tropical fish and fishery products. Int J Food Microbiol 62:177–181

Karunasagar I, Venugopal MN, Karunasagar I (1984) Levels of *Vibrio parahaemolyticus* in Indian shrimp undergoing processing for export. Can J Microbiol 30:713–715

Karunasagar I, Susheela M, Malathi GR, Karunasagar I (1990) Incidence of human pathogenic vibrios in seafoods harvested along coast of Karnataka (India). FAO Fish Rep 401(Suppl):53–56

Karunasagar I, Ismail SM, Amarnath HV, Karunasagar I (1992) Bacteriology of tropical shrimp and marine sediments. FAO Fish Rep 470(Suppl):1–8

Karunasagar I, Sugumar G, Karunasagar I, Reilly A (1995) Rapid detection of Vibrio cholerae contamination of seafood by polymerase chain reaction. Mol Mar Biol Biotechnol 4:365–368

Karunasagar I, Sagumar G, Karunasagar I, Reilly A (1996) Rapid polymerase chain reaction method for detection of Kanagawa positive *Vibrio parahaemolyticus* in seafoods. Int J Food Microbiol 31:317–323

Karunasagar I, Karunasagar I, Bodand-Reantaso MG (2020) Complexities involved in source attribution of antimicrobial resistance genes in aquaculture products. Fish Sci 33S1:16–21

Kaspar CW, Tamplin ML (1993) Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. Appl Environ Microbiol 59:2425–2429

Katoh H (1965) Studies on growth rate of various food bacteria. I. On the generation time of *Vibrio parahaemolyticus* Fujino. Jpn J Bacteriol 20:94–100

Kaysner CA, DePaola A Jr (2004) *Vibrio*. Chapter 9. Bacteriological analytical manual. Online. http://www.cfsan.fda.gov/~ebam/bam-24.html

Kaysner CA, Tamplin ML, Wekell MM, Stott RF, Colburn KG (1989) Survival of *Vibrio vulnificus* in shellstock and shucked oysters (*Crassostrea gigas* and *Crassostrea virginica*) and effects of isolation medium on recovery. Appl Environ Microbiol 55:3072–3079

Khan AA, Cheng CM, Van KT, West CS, Nawaz MS, Khan SA (2006) Characterization of class 1 integron resistance gene cassettes in *Salmonella enterica* serovars Oslo and Bareily from imported seafood. J Antimicrob Chemother 58:1308–1310

Khan AA, Ponce E, Nawaz MS, Cheng C, Khan JA, West CS (2009) Identification and characterisation of class I integron resistance gene cassettes among *Salmonella* strains isolated from imported seafood. Appl Environ Microbiol 75:1192–1196

Kim JH, Lee J, Hong S, Lee S, Na HY, Jeong YI, Choi EJ, Kim J, Kawk HS, Cho E (2018) Cholera outbreak due to raw seafood consumption in South Korea. Am J Trop Med Hyg 99:168–170

Kim SR, Nonaka L, Suzuki S (2004) Occurrence of tetracycline resistance genes tet (M) and tet (S) in bacteria from marine aquaculture sites. FEMS Microbiol Lett 237:147–156

Kitaoka M, Miyata ST, Unteweger D, Pukatzki S (2011) Antibiotic resistance mechanisms of *Vibrio cholerae*. J Med Microbiol 60:397–407

Kobayashi T, Nonaka L, Maruyama F, Suzuki S (2007) Molecular evidence for the ancient origin of the ribosomal protection protein that mediates tetracycline resistance in bacteria. J Mol Evol 65: 228–235

Kolvin JL, Roberts D (1982) Studies on growth of *Vibrio cholerae* biotype El Tor and biotype classical in foods. J Hyg Camb 89:243–252

Koo J, DePaola A, Marshall DL (2000) Impact of acid on survival of *Vibrio vulnificus* and *Vibrio vulnificus* phage. J Food Prot 63:1049–1052

Koonse B, Burkhardt W, Chirtel S, Hoskin GP (2005) *Salmonella* and the sanitary quality of aquacultured shrimp. J Food Prot 68:2527–2532
Kruse H, Sorum S (1994) Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Appl Environ Microbiol 60:4015.4021
Kumar R, Surendran PK, Thampuran N (2009) Analysis of antimicrobial resistance and plasmid profiles in *Salmonella* serovars associated with tropical seafood of India. Foodborne Pathog Dis 6:621–625
Kumar V, Roy S, Behera BK, Bossier P, Das BK (2021) Acute hepatopancreatic necrosis disease (AHPND): virulence, pathogenesis and mitigation strategies in shrimp aquaculture. Toxins 13:524
Liston J (1974) Influence of U.S. seafood handling procedures on *Vibrio parahaemolyticus*. In: Fujino T, Sakaguchi G, Sakazaki R, Takeda Y (eds) International symposium on *Vibrio parahaemolyticus*. Saikon Publishing, Tokyo, pp 123–128
Liu X, Chen Y, Wang X, Ji R (2004) Food-borne disease outbreaks in China from 1992–2001-national food-borne disease surveillance system. J Hygiene Res 33:725–727
Liu JW, Lee IK, Tang HJ, Ko WC, Lee HC, Liu YC, Hseuh PR, Chang YC (2006) Prognostic factors and antibiotics in *V. vulnificus* septicaemia. Arch Intern Med 166:2117–2123
Lunestad BT, Nesse L, Lassen J, Svihus B, Nesbakken T, Fossum K, Rosnes JT, Krusse H, Yazdankhah S (2007) *Salmonella* in fish feed; occurrence and implications for fish and human health in Norway. Aquaculture 265:1–8
Lungu B, O'Bryan CA, Muthaiyan A, Milillo SR, Johnson MG, Crandall PG, Ricke SC (2011) *Listeria monocytogenes*: antibiotic resistance in food production. Foodborne Pathog Dis 8:569–578
Martinez-Urtaza J, Liebana E (2005) Investigation of clonal distribution and persistence of *Salmonella* Senftenberg in the marine environment and identification of potential sources of contamination. FEMS Microbiol Ecol 52:255–263
Martinez-Urtaza J, Saco M, Novova J, Perez-Peniero P, Peiteado J, Lozano-Lean A, Garcia-Martin O (2004a) Influence of environmental factors and human activity on the presence of *Salmonella* serovars in the marine environment. Appl Environ Microbiol 70:2089–2097
Martinez-Urtaza J, Peiteado J, Lozano-Lean A, Garcia-Martin O (2004b) Detection of *Salmonella* Senftenberg associated with high saline environments in mussel processing facilities. J Food Prot 67:256–263
Mejlholm O, Gunvig A, Borggaard C, Blom-Hanssen J, Mellefont L, Ross T, Leroi F, Else T, Visser D, Dalgaard P (2010) Predicting growth rates and growth boundary of *Listeria monocytogenes* – an international validation study with focus on processed and ready-to-eat meat and seafood. Int J Food Microbiol 141:137–150
Miranda CD, Zemelman R (2002) Bacterial resistance to oxytetrcycline in Chilean salmon farming. Aquaculture 212:31–47
Miyasaka J, Yahiro J, Arahira Y, Tokunaga H, Katsuki K, Hara-Kudo Y (2006) Isolation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in wild birds in Japan. Epidemiol Infect 134:780–785
Motes ML, DePaola A (1996) Offshore suspension relaying to reduce levels of Vibrio vulnificus (Crassostrea virginica). Appl Environ Microbiol 62:3875–3877
NACA (2012) Report of the Asia Pacific emergency regional consultation on the emerging shrimp disease: Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS), 9–10 August. https://enaca.org/enclosure/?id=719
Nair GB, Ramamurthy T, Bhattacharya SK, Dutta B, Takeda Y, Sack DA (2007) Global dissemination of *Vibrio parahaemolyticus* O3:K6 and its serovariants. Clin Microbiol Rev 20:39–48
Natarajan R, Abraham M, Nair GB (1980) Distribution of *Vibrio parahaemolyticus* in Porto Novo environment. Indian J Med Res 71:679–687
Nesse LL, Nordby K, Heir E, Bergsjoe B, Wardund T, Nyagaard H, Holstad G (2003) Molecular analysis of *Salmonella enterica* isolates from fish feed factories and fish feed ingredients. Appl Environ Microbiol 69:1075–1081
Nishibuchi M, Kaper JB (1990) Duplication and variation of the thermostable direct haemolysin (*tdh*) gene in *Vibrio parahaemolyticus*. Mol Microbiol 4:87–99
Nishibuchi M, Kaper JB (1995) Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. Infect Immun 63:2093–2099

Noor Uddin GM, Larsen MH, Barco L, Minh Phu T, Dalsgaard A (2015) Clonal occurrence of *Salmonella* Weltevreden in cultured shrimp in Mekong Delta Vietnam. PLoS One 10:e0134252

Old DC, Crichton PB, Taylor A, Mather H (2001) An attempt to identify the evolutionary origin of a novel serotype of *Salmonella enterica* isolated from harbour porpoises. J Med Microbiol 50: 415–420

Oliver JD, Kaper JB (2007) *Vibrio* spp. In: Doyle MP, Beuchat LR (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 343–379

Otta SK, Karunasagar I, Karunasagar I (1999) Bacterial flora associated with shrimp culture ponds growing *Penaeus monodon* in India. J Aquac Tropics 14:309–318

Pal A, Marshall DL (2009) Comparison of culture media for enrichment and isolation of *Salmonella* spp. from frozen channel catfish and Vietnamese basa fillets. Food Microbiol 26:317–319

Pan T-M, Wang T-K, Lee C-L, Chien S-W, Horng C-B (1997) Food-borne disease outbreaks due to bacteria in Taiwan, 1986 to 1995. J Clin Microbiol 35:1260–1262

Parihar VS, Barbuddhe SB, Danielsson-Tham ML, Tham W (2008) Isolation and characterisation of *Listeria* species from tropical seafoods. Food Control 19:566–569

Parker RW, Maurer EM, Childers AB, Lewis DH (1994) Effect of frozen storage and vacuum packaging on survival of *Vibrio vulnificus* in Gulf Coast oysters (*Crassostrea virginica*). J Food Prot 57:604–606

Parvathi A, Kumar HS, Karunasagar I, Karunasagar I (2004) Detection and enumeration of *Vibrio vulnificus* in oysters from two estuaries along the southwest coast of India, using molecular methods. Appl Environ Microbiol 70:6909–6913

Phan TT, Khai LT, Ogasawara N, Tam NT, Okatani AT, Akiba M, Hayashidani H (2005) Contamination of *Salmonella* in retail meats and shrimp in Mekong delta, Vietnam. J Food Prot 68: 1077–1080

Poirel L, Cattoir V, Nordmann P (2012) Plasmid-mediated quinolone resistance: interactions between human, animal and environmental ecologies. Front Microbiol 3:24

Ponce E, Khan AA, Cheng CM, West SC, Cerniglia CE (2008) Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiol 25:29–35

Prasad MM, Pandurangarao CC (1995) Occurrence of *Salmonella infantis* and *S. newport* in market prawns. J Food Sci Technol 32:135–137

Raghunath P, Acharya S, Bhanumathi A, Karunasagar I, Karunasagar I (2008) Detection and molecular characterization of *Vibrio parahaemolyticus* isolated from seafood harvested along southwest coast of India. Food Microbiol 25:824–830

Rattagool P, Wongchinda N, Methatip P, Sanghtong N (1990) Hygienic processing of shrimp in Thailand. FAO Fish Rep 401(Suppl):32–46

Ravi Kiran K (1992) Potential pathogens and indicator bacteria associated with prawn gut. M.F. Scthesis University of Agricultural Sciences, College of Fisheries, Mangalore

Reilly A, Kaferstein F (1997) Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aquac Res 28:735–752

Reilly PJA, Twiddy DR (1992) *Salmonella* and *Vibrio cholerae* in brackish water tropical prawns. Int J Food Microbiol 16:293–301

Roig JF, Fouz LB, Amaro C (2009) Spontaneous quinolone resistance in the zoonotic serovar of *Vibrio vulnificus*. Appl Environ Microbiol 75:2577–2580

Rosche TM, Yano Y, Oliver JD (2005) A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. Microbiol Immunol 49:381–389

Rosche TM, Binder EA, Oliver JD (2010) *Vibrio vulnificus* genome suggests two distinct ecotypes. Environ Microbiol Rep 2:128–132

Ross T, Sumner J (2002) A simple, spreadsheet-based, risk assessment tool. Int J Food Microbiol 77:39–53

Sack RB, Siddique AK, Longini IM Jr, Nizam A, Yunus M, Islam MS, Morris JG Jr, Ali A, Huq A, Nair GB, Qadri F, Faruque SM, Sack DA, Colwell RR (2003) A 4-year study of the epidemiology of *Vibrio cholerae* in four rural areas of Bangladesh. J Infect Dis 187:96–101

Saheki K, Kobayashi S, Kawanishi T (1989) *Salmonella* contamination of eel culture ponds. Nippon Suisan Gakkashi 55:675–679

Sanjuan E, Amaro C (2004) Protocol for specific isolation of virulent strains of *Vibrio vulnificus* serovar E (Biotype 2) from environmental samples. Appl Environ Microbiol 70:7024–7032

Saraswathi K, Barve SM, Deodhar LP (1989) Septicaemia due to *Vibrio vulnificus*. Trans R Soc Trop Med Hyg 83:714

Saravanan V, Kumar SH, Karunasagar I, Karunasagar I (2007) Putative virulence genes of *Vibrio cholerae* from seafoods and coastal environments of Southwest India. Int J Food Microbiol 119: 329–333

Schar D, Klein EY, Laxminarayan R, Gilbert M, Van Boeckel TP (2020) Global trends in antimicrobial use in aquaculture. Sci Rep 10:21878

Schmidt AS, Bruun MS, Dalsgaard I, Pedersen K, Larsen JL (2000) Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Appl Environ Microbiol 66:4908–4915

Setti I, Rodriguez-Castro A, Pata MP, Cadarso-Suarez C, Yacoubi B, Bensmael L, Moukrim A, Martinez-Urtaza J (2009) Characteristics and dynamics of *Salmonella* contamination along the coast of Agadir, Morocco. Appl Environ Microbiol 75:7700–7709

Shabarinath S, Kumar SH, Khushiramani R, Karunasagar I, Karunasagar I (2007) Detection and characterisation of *Salmonella* associated with tropical seafood. Int J Food Microbiol 114:227–233

Simonson J, Siebeling RJ (1986) Rapid serological identification of *Vibrio vulnificus* by anti-H coagglutination. Appl Environ Microbiol 52:1299–1304

Smith P (2007) Antimicrobial use in shrimp farming in Ecuador and emerging multi-resistance during the cholera epidemic of 1991 – a re-examination of data. Aquaculture 271:1–7

Stoddard AR, Gulland FMD, Atwill RE, Lawrance J, Jang S, Conrad PA (2005) *Salmonella* and *Campylobacter* spp. in northern elephant seals. Emerg Infect Dis 11:1967–1969

Strom MS, Paranjpye RN (2000) Epidemiology and pathogenesis of Vibrio vulnificus. Microbes Infect 2:177–188

Su Y, Liu C (2007) *Vibrio parahaemolyticus*, a concern for seafood safety. Food Microbiol 24:549–558

Swaminathan B, Cabanes D, Zhang W, Cossart P (2007) Listeria monocytogenes. In: Doyle MP, Beuchat LR (eds) Food microbiology: fundamentals and Frontiers, 3rd edn. ASM Press, Washington, DC, pp 457–491

Tavechio AT, Ghilardi Ac, Peresi JT, Fuzihara TO, Yonamine EK, Jacabi M, Fernandes SA (2002) *Salmonella* serotypes isolated from non-human sources in Sao Paulo, Brazil from 1996 through 2000. J Food Prot 65:1041–1044.

Thamlikitkul V (1990) *Vibrio* bacteraemia in Siriraj hospital. J Med Assoc Thailand 73:136–138

Tindall BJ, Grimont PAD, Garrity GM, Euzeby JP (2005) Nomenclature and taxonomy of the genus *Salmonella*. Int J Syst Bacteriol 55:521–524

Tran L, Nunan L, Redman RM, Mohney LL, Pantoja CR, Fitzsimmons K, Lightner DV (2013) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis Aquat Org 105:45–55

Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ (2007) Detection of *Salmonella* spp in retail raw food samples from Vietnam and characterisation of their antibiotic resistance. Appl Environ Microbiol 73:6885–6890

Venkateswaran K, Kiiyukia C, Nakanishi K, Nakano H, Matsuda O, Hashimoto H (1990) The role of sinking particles in the overwintering process of *Vibrio parahaemolyticus* in a marine environment. FEMS Microbiol Ecol 73:159–166

Vongkamjan K, Roof S, Staciewicz MJ, Wiedmann M (2013) Persistent *Listeria monocytogenes* subtypes isolated from a smoked fish processing facility included both phage susceptible and resistant isolates. Food Microbiol 35:38–48

Wachsmuth K, Olsvik O, Evins GM, Popovic T (1994) Molecular epidemiology of cholera. In: Wachsmuth IK, Blake PA, Olsvik B (eds) *Vibrio cholerae* and cholera*:* molecular to global perspectives. ASM Press, Washington, pp 357–370

Walsh D, Duffy G, Sheridan JJ, Blair IS, McDowell DA (2001) Antibiotic resistance among *Listeria*, including *Listeria monocytogenes* in retail foods. J Appl Microbiol 90:517–522

Warner E, Oliver JD (2008) Population structures of two genotypes of *Vibrio vulnificus* in oysters (*Crassostrea virginica*) and seawater. Appl Environ Microbiol 74:80–85

Wilks SA, Michels HT, Keevil CW (2006) Survival of *Listeria monocytogenes* Scott A on metal surfaces: implications for cross-contamination. Int J Food Microbiol 111:93–98

Winfield MD, Groisman EA (2003) Role of non-host environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl Environ Microbiol 69:3687–3694

Wright AC, Hill RT, Johnson JA, Roghman MC, Colwell RR, Morris JG Jr (1996) Distribution of *Vibrio vulnificus* in the Chesapeake Bay. Diag Microbiol Infect Dis 24:165–167

Wyatt LE, Nickelson R, Vanderzant C (1979) Occurrence and control of *Salmonella* in freshwater catfish. J Food Sci 44:1067–1073

Yang YT, Chen IT, Lee CT, Chen CY, Lin SS, Hor LI, Tseng TC, Huang YT, Sritunyalucksana K, Thitamadee S, Wang HC (2014) Draft genome sequences of four strains of *Vibrio parahaemolyticus*, three of which cause early mortality syndrome/acute hepatopancreatic necrosis disease in shrimp in China and Thailand. Genome Announc 2(5):e00816-14

Zhao S, Dutta AR, Ayers S, Friedman S, Walker RD, White DG (2003) Antibiotic-resistant *Salmonella* serovars isolated from imported foods. Int J Food Microbiol 84:87–92

Campylobacter: Animal Reservoirs, Human Infections, and Options for Control

8

Jaap A. Wagenaar, Diane G. Newell, Ruwani S. Kalupahana, and Lapo Mughini-Gras

Contents

J. A. Wagenaar (✉)
Division of Infectious Diseases and Immunology, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Wageningen Bioveterinary Research, Lelystad, The Netherlands

WHO Collaborating Center for Reference and Research on Campylobacter and Antimicrobial Resistance from a One Health Perspective/WOAH Reference Laboratory for Campylobacteriosis, Utrecht, The Netherlands
e-mail: j.wagenaar@uu.nl

D. G. Newell
School of Veterinary Medicine, University of Surrey, Guildford, UK
e-mail: diane.newell@btinternet.com

R. S. Kalupahana
Department of Veterinary Public Health and Pharmacology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka
e-mail: rskalupahana@vet.pdn.ac.lk

L. Mughini-Gras
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
e-mail: lapo.mughini.gras@rivm.nl

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_6

Abstract

Campylobacteriosis is a frequently diagnosed disease in humans. Most infections are considered foodborne and are caused by *Campylobacter jejuni* and *C. coli*. The animal reservoirs of these *Campylobacter* species, and the sources and routes of transmission, are described and discussed in this chapter. Most warm-blooded animals can be colonized by *Campylobacter,* but avian species, and in particular poultry, are preferred hosts. Much of the world's poultry production is colonized by *Campylobacter.* Source attribution studies estimate that 20–40% of cases are attributed to the handling and consumption of chicken meat, while up to 80% of cases are due to *Campylobacter* found in the chicken reservoir. The difference suggests that routes other than through the food chain, i.e., environmental contamination, are important. The epidemiology of infections in humans differs between industrialized and low- and middle-income countries. Thus, the most effective interventions would be targeted to primary production. To date, only improved biosecurity is available. If effectively implemented, strict biosecurity can reduce the number of *Campylobacter*-positive flocks, but implementation to this level has proved difficult for the poultry industry. Available interventions in chicken processing plants can substantially reduce *Campylobacter* numbers on carcasses and consequently reduce the risk to humans. Public health strategies therefore utilize control programs, which aim at reducing the level of *Campylobacter* by measures along the food chain. It is now recognized that commercially acceptable complementary interventions for primary production, such as vaccines and feed additives, are urgently needed. Once *Campylobacter* in poultry is controlled then other minor sources of *Campylobacter* including contaminated drinking water, direct contact with (pet) animals, and other food items (e.g., red meat and milk) can be addressed.

Keywords

Campylobacter · Food borne disease · Poultry · Livestock · Source attribution · Environment · Low- and middle-income countries

8.1 Campylobacteriosis: The Disease and Its Burden in Humans

Human campylobacteriosis is primarily caused by *Campylobacter jejuni* (*C. jejuni*) and to a much lesser extent by its close relative *Campylobacter coli* (*C. coli*). Human infection with either pathogen largely presents as gastrointestinal illness (Gillespie et al. 2002). *C. jejuni* and *C. coli* together account for more than 90% of all cases of

human campylobacteriosis. Infections with other *Campylobacter* species may also occur, but they occur in either specific risk groups, for example, people with impaired immunity (e.g., *C. fetus*) (Wagenaar et al. 2014), or are very rare (e.g., *C. lari*), or cluster in specific geographical areas (e.g., *C. upsaliensis*) (Man 2011). This chapter will focus on *C. jejuni* and *C. coli*, and hereafter *Campylobacter* refers to these two species only.

Campylobacter is the most commonly reported cause of bacterial infectious intestinal disease (IID). However, disease surveillance programs, which include campylobacteriosis, are largely limited to industrialized countries, such as the United States (USA) and Member States of the European Union (EU) (EFSA and ECDC 2021; CDC 2022a). In industrialized countries, *Campylobacter* is isolated 3–4 times more frequently from patients with IID than *Salmonella* or *Escherichia coli*. However, it is well recognized that underreporting of such diseases is frequent. Adjusting for this, the true prevalence of campylobacteriosis was estimated to be 9.2 million in the EU in 2009 (Havelaar et al. 2013) and 1.3 million in the USA in 2011 (Scallan et al. 2011). Nevertheless, serological evidence suggests that exposure to this pathogen is substantially more frequent (Teunis et al. 2013), such that based on serological data virtually all individuals have been exposed to the organism by 20 years of age (Ang et al. 2011) and that the average infection pressure is estimated at around 1.6 *Campylobacter* infections per person/year (Monge et al. 2018). Such exposure can lead to protective immunity, which might affect the outcome and impact on disease incidence and could explain the low reported prevalence of disease in developing countries despite obvious regular exposure (Havelaar et al. 2009).

There are some additional interesting epidemiological features of campylobacteriosis, many of which have yet to be fully explained. These include a seasonal peak, which varies between countries and seems to be inconsistent with seasonal peaks observed in potential sources (Djennad et al. 2019).

In the past campylobacteriosis was largely considered a mild illness, but the severity of this disease is clearly reflected in the relatively high rate of *Campylobacter*-infected individuals seeking medical attention. Surveys show that one in four cases in the Netherlands and one in seven cases in the United Kingdom (UK) visit a general practitioner and approximately 1% of these individuals are hospitalized (Tam et al. 2012; Havelaar et al. 2012). In the acute phase, campylobacteriosis is primarily characterized by gastrointestinal symptoms, such as watery (sometimes bloody) diarrhea, abdominal cramps, nausea, vomiting, and fever. The disease is usually self-limiting, lasting a week or less. Antimicrobial treatment is only indicated in severe cases (e.g., bloody diarrhea or systemic infection). However, *Campylobacter* infections can also have serious sequelae, including Guillain-Barré and Miller-Fisher syndromes, reactive arthritis, and functional gastrointestinal disorders, including irritable bowel syndrome (Helms et al. 2006; Doorduyn et al. 2008; Haagsma et al. 2010; Berumen et al. 2021).

The burden of campylobacteriosis has been quantified in terms of disability-adjusted life-years (DALYs), which is a metric of health loss caused by the disease comprising years of life lost by the population due to disability and premature death. The different manifestations of campylobacteriosis were estimated to cause an

average disease burden of 3300 DALYs in the Netherlands in 2019, with sequelae accounting for approximately 80% of this burden (Lagerweij et al. 2020). Among foodborne pathogens investigated in the Netherlands, this DALY estimate was the highest. Similar studies in the USA in 2011 showed *Campylobacter* to cause a burden second only to *Salmonella*, with a cost of illness of $1.7 billion annually (Hoffmann et al. 2012).

Despite the relative importance of campylobacteriosis, unlike for salmonellosis, there have been no effective intervention programs implemented, with the exception of Iceland and New Zealand where very specific conditions prevailed (Stern et al. 2003; Sears et al. 2011). This is all the more surprising given that the incidence of human campylobacteriosis increased significantly during the 1980s–1990s, stabilized around the start of this century, and has tended to increase again in the second decade of this century in the USA, while remaining stable in Europe (EFSA and ECDC 2021; CDC 2022b). There has been a remarkable sudden decrease in human campylobacteriosis associated with the COVID-19 pandemic in the USA and Europe, as observed also, for example *Salmonella* (Mughini Gras et al. 2021a). The reasons for the lack of specific intervention for *Campylobacter* are debatable, but include the complexity of foodborne and environmental sources and transmission routes, the financial imbalance accruing from interventions where the cost is to the poultry industry while the benefit is to the public health sector, and lack of consumer/political acceptance of effective measures like irradiation or chemical decontamination. In addition, there is a general lack of public interest, which is in part due to the scarcity of major outbreaks.

8.2 Characteristics of *Campylobacter*

Campylobacter comprises a genus of Gram-negative, motile, non-spore forming, mostly microaerophilic, spiral bacteria (diameter 0.2–0.5 μm, length 0.5–8 μm). To date (January 2023), the genus includes 43 species (https://lpsn.dsmz.de/genus/campylobacter) and with the use of molecular approaches, this number is rapidly expanding. Both *C. jejuni* and *C. coli* are thermophilic, showing optimal growth at 42 °C. For the purposes of isolation this thermotolerance, especially in combination with resistance to cephalosporin, is often used to reduce contaminating flora and improve recovery, particularly from fecal material.

Campylobacter readily generates resistance against an increasing number of classes of antimicrobials. Although antimicrobials are infrequently prescribed for campylobacteriosis, such resistance can have clinical consequences. There are clear differences in antimicrobial resistance in different geographical areas. Generally, resistance is higher in Asia and Africa compared to Europe, the USA, and Australia and New Zealand (Nhung et al. 2016; Gahamanyi et al. 2020; EFSA 2021). This parallels the amount of antimicrobials used in animals and humans in these regions. Resistance to fluoroquinolones and tetracyclines is increasing in most regions of the world. An association between the licensed use of fluoroquinolones in poultry and increased fluoroquinolone resistance in strains isolated from humans was noticed in the 1980s

(Endtz et al. 1990). This association was strengthened by a low fluoroquinolone resistance in *C. jejuni* isolates from humans in Australia, a country where fluoroquinolones were never licensed for use in production animals (Cheng et al. 2012).

Campylobacter is sensitive to many environmental stresses, including desiccation, heat, ultraviolet radiation, atmospheric oxygen, and high salinity. As a consequence, *Campylobacter* is unable to grow naturally outside a host and is considered generally fragile compared with, for example, *Salmonella*. Nevertheless, *Campylobacter* can survive in the environment for prolonged periods, especially in moist conditions. Survival has been recorded for up to 3 months in slurries and water contaminated with organic materials (Nicholson et al. 2005) and up to 10 months in manure compost (Douglas Inglis et al. 2010).

The fastidious nature of the organism is reflected in its demanding requirements at culture. Diagnosis of infection is usually based on isolation from fecal samples using selective media, containing appropriate antimicrobials, and incubated under reduced oxygen tension, at 42 °C for 48–72 h. However, the isolation technique and media constituents may vary depending on the matrix under investigation and may affect both the efficacy of recovery and the species and/or strain types recovered (Newell et al. 2001). Numerous rapid detection tests, using a variety of technologies, are now commercially available. For application in food chain settings, e.g., slaughterhouses or chicken farms, such tests need to be cheap and user-friendly as well as sensitive and specific (Llarena et al. 2022).

The typing of *Campylobacter* has proved challenging. The organisms demonstrate considerable variation at both the phenotypic and genotypic levels and many attempts have been used to exploit this diversity to characterize *Campylobacter* for epidemiological studies. Initial typing methods included serotyping and phage typing. However, these methods were largely superseded by molecular techniques, such as *fla*-typing and Pulsed Field Gel Electrophoresis (PFGE) (Wassenaar and Newell 2000). Subsequently, as DNA sequencing became cheaper and quicker, Multi Locus Sequence Typing (MLST), based on variations in the sequences of seven housekeeping genes, was used to establish the population structures of *C. jejuni* and *C. coli* (Dingle et al. 2001). The significant advantage of this technique was its portability due to the use of globally available internet-based databases, which allowed easy strain comparison. Not surprisingly, this technique was quickly exploited for epidemiological purposes and, with the application of highly sophisticated statistical methods, its use was expanded to determine potential infection sources and to provide a global public health tool. Many *C. jejuni* MLST sequence types (STs) have been cataloged to date. Most STs are generalists and can colonize several hosts but some are specialized to defined hosts, such as cattle and chicken (Mourkas et al. 2020). However, the use of just the sequences of seven housekeeping genes has raised issues regarding resolution for the purpose of source identification. With continued improvements in DNA sequencing, rapid whole-genome sequencing (WGS) of campylobacters has become routine (Didelot et al. 2012). However, due to the high genome diversity of *Campylobacter*, SNP-based comparisons are problematic. In 2017, a core-genome MLST (cgMLST) approach was proposed expanding the number of gene sequences analyzed to 1343 (Cody et al. 2017). The cgMLST

typing approach has now been validated and types present in a wide range of animals identified (Hsu et al. 2020) and compared with those found causing human disease using increasingly sophisticated analytical techniques, including machine learning techniques (Arning et al. 2021). Nevertheless, the large number of "generalist" sequence types continue to elude source attribution. As a consequence, efforts to further improve the resolution by incorporating additional sequences, for example, from potential host-associated genes, continue.

8.3 The Disease and Carriage in Animals

The primary habitat of *Campylobacter* and its main amplification site is the intestinal tract of warm-blooded animals. Both *C. jejuni* and *C. coli* are normal inhabitants of the guts of healthy livestock, pets, and wild animals. There appears to be some host preference with *C. jejuni* more commonly isolated from most animals, like cattle, dogs. and cats, while pigs predominantly carry *C. coli*. The reason for this is unclear. Certainly, a significant proportion of livestock animals is colonized and the prevalence varies with factors like age, husbandry, country, etc. (Plishka et al. 2021; Mota-Gutierrez et al. 2022; Knipper et al. 2022). Similarly, up to 45% of dogs are colonized (Marks et al. 2011).

The role of *C. jejuni* and *C. coli* as pathogens in these animals is considered of relatively minor importance. They can cause abortion in cattle and sheep, but are usually less frequently isolated from aborted fetuses than *C. fetus*. An exception is the spread of a single tetracycline-resistant *C. jejuni* clone causing abortion in sheep throughout the USA (Wu et al. 2014). This hypervirulent clone is also reported in other countries such as the UK, Japan, and China (Stone et al. 2014; Wu et al. 2016, 2020; Sahin et al. 2017; Tang et al. 2017; Hsu et al. 2020; Yaeger et al. 2021a, b). Interestingly, this clone has also been recovered from diarrheic humans in the USA, but the route of transmission has not yet been identified. The role of *Campylobacter* as a pathogen in dogs remains debatable (Burch 2005; Marks et al. 2011). The high level of asymptomatic carriage (Marks et al. 2011) suggests that any association with disease is coincidental rather than causative. Nevertheless, there is certainly evidence of such companion animals as a source for human infections (Mughini Gras et al. 2013, 2021b).

Poultry, in particular and (wild) avian species in general, are the preferred hosts for these organisms. This is a reflection of the bacterium's thermophilic character, as 41–42 °C is the normal body temperature of a bird. Colonization occurs throughout the gut, but primarily in the cecum of a broiler, where levels of up to 10^9 colony forming units per gram have been reported. All the evidence indicates that *Campylobacter* act as a commensal in the avian gut, although this is occasionally disputed. The prevalence of *Campylobacter*-positive broiler flocks varies considerably, for example, with age, season of the year, latitude, extensive or intensive rearing, etc. In an EU-wide survey of broiler flocks undertaken in 2008, the prevalence of *C. jejuni*/*C. coli* colonization varied between 5% and 100% among Member States (EFSA 2010). The prevalence is particularly high if the flocks are free-ranging

(Vandeplas et al. 2010). The organism is highly infectious and in each colonized flock up to 100% of birds can be *Campylobacter*-positive. Thus, overall, it is reasonable to assume that a significant proportion of broilers produced worldwide are colonized with these organisms.

8.4 *Campylobacter* Epidemiology in Low- and Middle-Income Countries

Country-specific epidemiological data on infectious enteric diseases, especially those transmitted through the food chain, has been sparse in Low- and Middle-Income Countries (LMIC) but the effects of these diseases, as leading causes of morbidity and mortality, has long been recognized.

Campylobacteriosis is generally considered to be a major contributor to those diseases, especially in young children, but evidence from large global case-controlled studies has been poorly available. There have been multiple barriers to such investigations, including costs, organizational structures, perceptions of importance, etc. One barrier has been access to modern rapid diagnostic/surveillance technologies. For example, qPCR can have twice the sensitivity of *Campylobacter* detection than the more conventional culture methods generally available in laboratories in LMIC (Liu et al. 2016). Recently, the microbiological causes of diarrheal diseases in LMIC have been investigated in two such global studies using improved diagnostic and statistical tools. In the Global Enteric Multicentre Study (GEMS), the etiology and population-based burden of pediatric diarrheal disease in Sub-Saharan Africa and South Asia were investigated (Kotloff et al. 2013) in 9439 children with moderate-to-severe diarrhea and 13,129 children without diarrhea. Interestingly *C. jejuni* was only identified as a statistically significant cause of pediatric diarrhea in children of 0–11 months and 24–59 months in sites in India. Five other enteropathogens, including rotavirus and *Cryptosporidia*, were considered substantially more important targets for intervention. However, when qPCR was applied rather than more conventional methods, *Campylobacter* was identified as the sixth most common cause of illness. Similarly, the Malnutrition and Consequences for Child Health and Development (MAL-ED) consortium study (Platts-Mills et al. 2015), comparing 7318 diarrheal and 24,310 non-diarrheal stools from 2145 children (aged 0–24 months) from eight sites in South America, Sub-Saharan Africa, and Asia indicated that *Campylobacter* was among the most important causes of pediatric diarrhea, especially in the second year of life. These recent epidemiological surveys support reports from the WHO's Foodborne Disease Burden Epidemiology Reference Group (FERG), which considers *Campylobacter* one of the most common organisms causing diarrhea, especially in children (Havelaar et al. 2015), with the geographical regions most highly affected by campylobacteriosis in LMIC.

These recent large epidemiological studies have also confirmed some differences in the presentation of campylobacteriosis between high- and low- and middle-income countries. For example, although it had been previously well recognized that in LMIC adults excreting *Campylobacter* are usually asymptomatic, many

infected children also show no symptoms. In addition, the seasonal distribution in *Campylobacter* infections generally seen in the higher income world is not observed elsewhere (Havelaar et al. 2015; Platts-Mills et al. 2015).

The extent of the public health burden due to campylobacteriosis in LMIC is only just begun to be understood. Not only are symptomatic *Campylobacter* infections associated with poor linear growth in children over the first 2 years of life (Amour et al. 2016; Rogawski et al. 2018), but repeated exposure to such enteropathogens, even if subclinical, can cause substantial enteric dysfunction and malnutrition (Walson and Pavlinac 2018). Such life changing effects reinforce calls for interventions against foodborne enteropathogens, including *Campylobacter*, in LMIC (WHO 2017). Another potentially significant health issue is Guillain–Barré syndrome (GBS), which is most commonly caused by a preceding *Campylobacter* infection. Unfortunately, data on post-infectious GBS in LMIC is sparce and largely confined to South Asia (Bangladesh and India) (Papri et al. 2021).

Worldwide, the control and prevention of the public health burden of campylobacteriosis requires surveillance and monitoring especially of *Campylobacter* throughout the food chain. Unfortunately, LMIC rarely include foodborne enteropathogens, such as *Campylobacter,* in disease surveillance (Deolalikar et al. 2021). As a consequence, the national prevalence of such diseases in the population is generally unknown. Among South-East Asian countries in 2017, apparently only Singapore included campylobacteriosis in its national disease surveillance program (Premarathne et al. 2017).

The sources and routes of *Campylobacter* transmission in LMIC are poorly understood. Although epidemiological data from Africa, Asia, and the Middle East are incomplete, it is widely accepted that infection with *Campylobacter* is endemic in these regions, and traveling to Asia, Africa, Latin America and the Caribbean, and Southern Europe poses an increased risk of campylobacteriosis compared to traveling within Western Europe (Mughini Gras et al. 2014). It is generally believed that in such countries, campylobacteriosis is limited to children, because exposure in early life leads to protective immunity (Havelaar et al. 2009), which would also be consistent with endemicity.

The prevalence of human campylobacteriosis in LMIC may be attributed to many factors, including poor food hygiene, environmental contamination, animal rearing and handling practices, wet markets, etc. In high-income countries, human-to-human transmission is not considered an important route of *Campylobacter* infection, except in some institutional situations. Nevertheless, high levels of asymptomatic infections in those locations where sanitary facilities are inadequate could contribute to environmental contamination and result in higher exposure.

Campylobacter is generally considered a foodborne enteropathogen. To date, there is very little information available on potential sources of infection in LMIC and the little available data comes primarily from poultry, presumably because this is considered the primary source in high-income countries. Poultry production is thriving in South-East Asia, with livestock production in these regions being largely extensive (Gilbert et al. 2015), but frequently also as backyard or small local units for economic reasons (Alders et al. 2018). In such systems, biosecurity is either unfeasible or very difficult to apply (Kalupahana et al. 2013; Wang et al. 2015). Even commercial poultry production will use deep litter open-house systems where

biosecurity is minimal and the birds are constantly in contact with the outdoor environment, wild animals, and insects. Moreover, new flocks, including day-old chicks, are generally exposed to already *Campylobacter*-colonized chickens in the same farms (Kottawatta et al. 2017). Therefore, a high prevalence of *Campylobacter* colonization of broilers at slaughter in LMIC should be expected. Consistent with this, surveys conducted in Sri Lanka have reported >65% *Campylobacter* prevalence in broilers at slaughter (Kottawatta et al. 2017; Kalupahana et al. 2018).

Published surveys of *Campylobacter* contamination in retail poultry meats and their by-products (such as ground or frozen poultry meats) indicate that in most countries, regardless of social-economic status, the majority of samples are contaminated with *Campylobacter* (Suzuki and Yamamoto 2009) and there is no obvious difference between countries in the prevalence of sample contamination. However, few such retail surveys have been undertaken in LMIC compared to high-income countries.

Because *Campylobacter* is a common gut colonizer of many domestic animal species, not just poultry, multiple attributable sources and routes of transmission can occur especially in those countries where animal-to-human contact levels might be high. For example, in India *Campylobacter* colonization is frequent in dogs and calves, as well as poultry (Begum et al. 2015), though whether these strains can cause human disease is not known (Begum et al. 2015). To understand the attributable role of potential sources, time-related strain collections from humans and animals/environment need to be compared using typing techniques of appropriate discriminatory power, such as WGS. Unfortunately, such techniques may not be widely available in LMIC and, because of their low discriminatory power, little if any, useful conclusions can be drawn on sources from the use of low-technology techniques, such as serotyping (Bodhidatta et al. 2013).

Effective cheap and easy-to-apply interventions for the control and prevention of campylobacteriosis remain a major challenge for LMIC, where food chain regulations would be difficult to implement. Nevertheless, the eating and handling of raw or improperly cooked poultry meat has been shown to be the most common source of human campylobacteriosis throughout the world. One (apparently) simple approach, therefore, is education to encourage the effective cooking of poultry meat. In Sri Lanka, the absence of *Campylobacter* contamination in chicken curries (Kulasooriya et al. 2019) indicated that such approaches were effective. However, *Campylobacter* contamination of chicken dishes identified in, both local and branded, Pakistani restaurants (Arshad and Zahoor 2019) indicate that kitchen hygiene is also important.

Overall, the paucity of information available on the epidemiology of campylobacteriosis in LMIC highlights the need for active food safety surveillance in these countries using state-of-art technologies and approaches.

8.5 Sources and Transmission Pathways of Human Campylobacteriosis

Although *Campylobacter* is considered mainly a foodborne pathogen, there is evidence for other transmission pathways, including contact with colonized animals and environments contaminated by their waste products, as well as, rarely, infected

people in conditions of poor hygiene (Mughini Gras et al. 2012, 2013, 2014, 2021b). It is well recognized that *Campylobacter*-containing gut contents can enter the food chain by contaminating various food products of animal origin, including meats and dairy products. Cross-contamination during food preparation at home is also an important transmission route (Bai et al. 2021). Alternative routes with animals as sources include exposure to environments contaminated by primary production (e.g., run-off from livestock in farms and at pasture, water used for cleaning animal-containment areas, stockpiled sewage, etc.). *Campylobacter* survives for long periods in surface waters, so such contamination might pose a risk to humans through the drinking of untreated water, recreational activities, or the consumption of fresh produce irrigated or washed with manure-contaminated water.

8.5.1 *Campylobacter* Source Attribution

A general framework for source attribution of campylobacteriosis has been designed (Wagenaar et al. 2013). Based on this framework, animals (e.g., cattle, sheep, poultry, etc.) are defined as *reservoirs* or *amplifying hosts*; the environment, the food chain, and direct contact with animals are given as examples of *pathways*; drinking water, meat, milk, and occupation are given as examples of *exposure*; and examples of *risk factors* include swimming in rivers, eating chicken meat, beef, etc. In a typical example, cattle (reservoir) may contaminate the food chain (pathway) resulting in a hazard in the milk supply (exposure), which manifests itself as an increased risk associated with the consumption of unpasteurized milk (risk factor) (Wagenaar et al. 2013).

Source attribution models provide an estimate of the relative contribution of the different known reservoirs to the burden of human illness. They can be used to inform decision makers in order to target the most effective intervention strategies and are, therefore, an important tool for risk management (Pires et al. 2009). Several approaches can be used for source attribution, including microbiological (e.g., microbial subtyping) and epidemiological (e.g., outbreak investigations and case-control studies) approaches and intervention studies (Pires et al. 2009). Structured expert opinions and comparative exposure assessment can also be used for source attribution, but will not be considered here.

8.5.1.1 Source Attribution Based on Outbreak Data

Most *Campylobacter* infections are sporadic. As an example, in Europe in 2019, the total number of reported campylobacteriosis cases was 220,682, of which only 1254 were related to outbreaks (EFSA and ECDC 2021). Outbreak data is, therefore, generally considered of limited value for campylobacteriosis because of the rarity of reported outbreaks (Pires et al. 2010). *Campylobacter* outbreaks, however, may occur more frequently, but are often unreported due to the generally intermittent typing of clinical isolates. Indeed, the added value of high-throughput sequencing methods for campylobacteriosis outbreak investigation has been shown in several occasions, such as during the large waterborne campylobacteriosis outbreaks that

occurred in New Zealand, in 2016 (Gilpin et al. 2020). An estimated 6260–8320 campylobacteriosis cases were linked to the contamination of an untreated, groundwater-derived drinking water supply. Of the 12 different *Campylobacter* genotypes observed in the clinical cases, four were also retrieved from water, three from sheep, and one from both water and sheep. The outbreak was traced back to contamination of the water supply after a heavy rainfall event that caused drainage of sheep feces into a shallow aquifer. The existence of a routine clinical surveillance for campylobacteriosis, coupled with early testing of water for pathogens and genotyping of *Campylobacter* isolates from human cases and potential sources, facilitated outbreak detection and helped define its source, as well as confirm outbreak periods and cases. Similar experiences are increasingly being documented for foodborne campylobacteriosis outbreaks as well (Sorgentone et al. 2021). Moreover, using data of the New Zealand outbreak, it has been shown that alternative data sources (i.e., general practitioner consultations, consumer helpline, Google Trends, Twitter microblogs, and school absenteeism) can provide earlier indications of the outbreak as compared to conventional case notifications (Adnan et al. 2020). Routine application of WGS to *Campylobacter* isolates is already a reality in several governmental agencies, industry, and academia. The ever-growing availability of sequencing data as well as the creative exploitation of alternative data sources are expected to improve our ability to detect and characterize *Campylobacter* outbreaks, including source tracing and root cause determination of contamination events (Franz et al. 2016).

Although scarce, campylobacteriosis outbreak data is collected annually in Europe and has been used to estimate the causative vehicles for the years 2005–2006 (Pires et al. 2010). Putative sources rank differently depending on whether the data was analyzed in terms of either the proportion of outbreaks or the proportion of infected individuals reported. The majority (~64%) of outbreaks had no identified source, while ~12% were attributed to meat products as a whole and ~10% specifically to chicken. In contrast, in terms of ill individuals, the majority (~44%) was attributed to travel, ~17% to putatively contaminated drinking water, 10% each to meat and chicken, and 36% were of unknown source. Although the ranking of source importance seems different, chicken remains an important source regardless of the approach taken. Indeed, the authors report that "among illnesses that could be attributed to a source, 29% of campylobacteriosis cases were attributed to chicken" (Pires et al. 2010).

8.5.1.2 Source Attribution Based on Case-Control Studies

Case-control studies have been used in several countries to identify those risk factors associated with sporadic *Campylobacter* infections. Overall, these studies indicate that the handling and consumption of chicken meat is a very important risk factor (Doorduyn et al. 2010; Domingues et al. 2012; MacDonald et al. 2015; Mossong et al. 2016; Rosner et al. 2017; Kuhn et al. 2018). Other frequently identified risk factors include the consumption of unpasteurized milk (Friedman et al. 2004; Mughini Gras et al. 2021b), eating in restaurants (Friedman et al. 2004; Danis et al. 2009), contact with pet dogs (especially puppies) (Friedman et al. 2004;

Doorduyn et al. 2010; Mughini Gras et al. 2013; MacDonald et al. 2015; Mossong et al. 2016; Kuhn et al. 2018), contact with livestock (Friedman et al. 2004; Danis et al. 2009; Mughini Gras et al. 2012; Rosner et al. 2017), and foreign travel (Friedman et al. 2004; Doorduyn et al. 2010). The calculations of the attributable fractions for each risk factor also indicate that, like the outbreak data, chicken consumption accounts for 28–31% of sporadic cases (Doorduyn et al. 2010; MacDonald et al. 2015; Rosner et al. 2017; Kuhn et al. 2018). In contrast, the contribution of dog ownership is 4–8% (Doorduyn et al. 2010; MacDonald et al. 2015), but it can go up to 21% in children under 5 years (Kuhn et al. 2018). Of course, many factors can influence source attribution studies using case-control data. For instance, individuals taking proton-pump inhibitors or having a chronic gastrointestinal disease have increased risk of campylobacteriosis (Doorduyn et al. 2010; Mughini Gras et al. 2012; Rosner et al. 2017; Kuhn et al. 2018; Fravalo et al. 2021), probably as a consequence of reduced gastric acidity allowing the survival of *Campylobacter* during passage through the stomach and/or disturbed gut function facilitating intestinal infection.

A recent systematic review and meta-analysis (Fravalo et al. 2021), which synthesized the evidence provided by 71 eligible case-control studies on risk factors for sporadic *Campylobacter* infection, highlighted the importance of other, less common risk factors beyond chicken consumption. These include consumption of food products like beef, eggs, and dairy, especially when consumed raw/undercooked, but also non-foodborne transmission routes like contact with animals and environmental sources. For example, occupational exposure to animals or products thereof, such as working in a slaughterhouse, farm, pet shop, or zoo, as well as working in food handling/preparation, emerged as significant risk factors. The same applied to (non-occupational) contact with farm animals, wild animals and pets, and environmental exposure to playground sandpits, rural environments, or recreational waters, with these non-foodborne risk factors, as well as person-to-person transmission, being particularly important among children (Fravalo et al. 2021).

Specific immunity against *Campylobacter,* acquired as a result of prior exposure, is another very important confounder of case-control studies (Havelaar and Swart 2016). Certainly, repeated exposure to pathogens, such as *Campylobacter*, may lead to sufficient immunity to provide protection against severe clinical illness (Swift and Hunter 2004). Such immunity can lead to individuals being protected from disease, even when colonized (Havelaar et al. 2009; Havelaar and Swart 2016), and this has been proposed as an explanation of why, in some instances, the regular consumption of poultry meat (at home) is identified as a protective, rather than a risk factor (Friedman et al. 2004). Acquired immunity also provides an explanation of why either the very frequent consumption of chicken meat or never consuming it, are risk factors for campylobacteriosis (Mughini Gras et al. 2021b). Indeed, people who frequently consume chicken are highly exposed to chicken-associated *Campylobacter* strains and therefore are at increased risk of falling ill with these strains because the levels of exposure to these strains are too high to allow acquired immunity to exert any protective effect. Conversely, people who do not eat chicken meat would not be exposed to these strains at all, and therefore would be unable to develop any

immunity against them, thereby falling ill more easily upon incidental exposure to them via, e.g., cross-contamination of other food items or non-foodborne transmission. It has also been shown that consumption of chicken meat is a risk factor for campylobacteriosis only or predominantly when this is consumed outside the household (Swift and Hunter 2004; Friedman et al. 2004; Mossong et al. 2016; Lake et al. 2021), which indicates that exposure to chicken-associated *Campylobacter* strains outside the household (e.g., at restaurants, catering events, etc.) would increase the chance of being exposed to (possibly higher doses of) specific *Campylobacter* strains different from those to which people are (usually) exposed at home (Mughini Gras et al. 2021b).

8.5.1.3 Source Attribution Based on Microbial Subtyping

As previously indicated, *Campylobacter* are highly phenotypically and genotypically variable. This variability has been exploited to develop subtyping strategies with the aim of determining sources of human infection. However, for various reasons including the high plasticity of the *Campylobacter* genome, the lateral transfer of genetic material among strains, the time delay to diagnosis, and the poor recovery from putative sources, the direct tracking of strains from source to human has not been feasible. However, the widespread application of MLST, as well as other genotyping methods with higher discriminatory power like cgMLST, allowed for the study of *Campylobacter* population structures and the conduction of source attribution analyses. Studies of the evolutionary relationships within populations reported that some *Campylobacter* strain features are preferentially associated with certain animal hosts. Thus, using complex statistical methods, the probable sources can be inferred by comparison of the *Campylobacter* strains recovered from diseased humans with those recovered from a range of animal, food, and environmental sources. Several MLST-based studies, reviewed by Cody et al. (2019), have provided in the past the first source attribution results for campylobacteriosis, showing that most (50–80%) strains infecting humans come from the chicken reservoir, 20–30% from cattle, and the remainder from other reservoirs (e.g., sheep, pigs, wild animals, etc.) (EFSA BIOHAZ 2010). However, in more recent years, the growing availability of WGS data allowed for genomic data with a much higher discriminatory power than MLST, such as cgMLST and wgMLST, to be used in source attribution studies (Pérez-Reche et al. 2020; Lake et al. 2021; Mughini Gras et al. 2021b; Harrison et al. 2021; Arning et al. 2021). While most human cases are still attributed to poultry, followed by cattle, the ability to better differentiate isolates based upon more than just seven MLST genes, coupled with the use of more powerful models, allow for more accurate attribution estimates. This includes better differentiation of host generalist, commonly occurring or clonally related strains.

While there is an apparent conflict between the importance of poultry as a source from case-control studies (20–40%) and from the genotyping studies (50–80%), this is explained by case-control studies being able to trace human cases back only to the level of exposure (e.g., food items consumed, contact with animals, etc.), while genotyping data indicates the original host reservoir. It has been hypothesized that

the difference reflects that *Campylobacter* strains may reach humans through pathways other than food, for example, through environmental exposure (EFSA BIOHAZ 2010) (section "Role of the Environment").

8.5.1.4 Intervention Studies

On the presumption that poultry is the major source of sporadic campylobacteriosis, there have been several incidents that have acted as "natural experiments," which have been investigated to determine the effect of reduced population exposure to *Campylobacter* in the food chain. For example, in 1999, contamination of animal feed with dioxin in Belgium resulted in a nationwide withdrawal of broiler meat from the market, which was concomitant with a 40% decrease in campylobacteriosis, countrywide (Vellinga and Van Loock 2002). Similarly, in 2003 in the Netherlands, an avian influenza outbreak led to a massive poultry cull, which was associated with a subsequent 30% decrease overall in campylobacteriosis (Friesema et al. 2012). This disease reduction varied between regions from 10% to 70%, with the largest fall reported in those laboratories' serving areas where the flocks were actually culled. This observation supports the hypothesis that there were important transmission routes other than the handling and consuming poultry meat (EFSA BIOHAZ 2010; Friesema et al. 2012). As yet, the transmission routes of such alternative pathways are unclear.

Other interventions targeted at the poultry production sector and/or to the poultry meat consumer, resulted in reduced exposure to national populations in Iceland and New Zealand. Following these interventions, the number of reported campylobacteriosis cases fell by 72% in Iceland (Stern et al. 2003) and by 54% in New Zealand (Sears et al. 2011). Furthermore, in New Zealand there was a concurrent 74% reduction in the proportion of poultry-associated campylobacteriosis cases as determined by source attribution using MLST (Sears et al. 2011) and 13% decline in hospitalizations for Guillain-Barré syndrome (Baker et al. 2012).

8.5.2 Role of the Environment

Campylobacter is often found in the environment, including surface water, where it usually indicates recent fecal contamination from animals, sewage, or agricultural run-off. *Campylobacter*'s fate in the environment is typically the one of die-off rather than growth. Although *Campylobacter* survives poorly outside the host, some specialist strains can survive better in certain sylvatic (Hepworth et al. 2011), farmland (French et al. 2005), and environmental (French et al. 2005; Sopwith et al. 2008; Colles et al. 2011) niches. These strains are generally more resistant to physical stress (Sopwith et al. 2008). *Campylobacter* can also assume a viable, but non-culturable state in response to advert conditions outside the host (Murphy et al. 2006).

Human *Campylobacter* infections of environmental origin exhibit strong seasonality (Mughini Gras et al. 2012). Indeed, *Campylobacter* survival in the environment is compromised by factors like high temperatures and sunlight, among others, and shedding from animals varies seasonally depending on stress, changes in diet,

housing conditions, rearing period, etc. Moreover, the pattern of human exposure to environmental sources (e.g., outdoor activities) is largely weather-dependent. Although the primary transmission route for human *Campylobacter* infection is contaminated food, source attribution studies have estimated that on top of the contributions of livestock and wild animals, the environment may account for a further 5–10% of human campylobacteriosis morbidity, with open water swimming, consuming game meat, and exposure to storm water overflows being a source of environment-borne campylobacteriosis (Mughini Gras et al. 2012, 2021b; Sales-Ortells et al. 2015; Mossong et al. 2016). Studies have also shown that heavy rainfall may lead to *Campylobacter* entering the drinking water supply system (Gilpin et al. 2020). Perhaps more importantly, water may act as a source for *Campylobacter* (re)colonization in livestock (Bull et al. 2006). Yet, the environment at large serves more as a vehicle of transmission for *Campylobacter* among animals, from animals to humans and vice versa, rather than as an amplifying reservoir per se.

Surface water represents a "sink" that collects *Campylobacter* strains from different (animal) hosts, whose individual contributions have been quantified in source attributions studies based on MLST (Mughini Gras et al. 2016) and cgMLST (Mulder et al. 2020). This latter study, conducted in the Netherlands, provides the most comprehensive data on the prevalence, genotypes, and animal sources of *Campylobacter* in surface water. Prevalence is the highest in agricultural waters (77%) and in autumn and winter (74%), and lowest in recreational (swimming) waters (46%) and in summer (54%), which concurs with *Campylobacter* being highly sensitive to sunlight and high temperatures. Overall, water isolates are mainly attributed to wild birds (84%) and poultry (10%). However, the probability for water isolates to originate from poultry is significantly higher in high poultry density areas, i.e., a geographical association exists between the magnitude of the local poultry industry and its role as source of microbial contamination of the environment. Similarly in the USA, it has been shown that communities with high-density poultry operations have higher incidences of campylobacteriosis and infectious diarrhea (Poulsen et al. 2018).

8.6 *Campylobacter* in Poultry and Intervention in Primary Production

Given that the majority of the infecting strains in humans come from chicken, targeting *Campylobacter* in poultry production has become the preferred public health measure (Koutsoumanis et al. 2020). The poultry meat chain can be viewed as two distinct stages: chicken rearing and production (largely on-farm to entry to the slaughter house) and poultry meat processing (largely lairage to retail). Theoretically, control measures focused on the primary production stage will prevent up to 80% of human cases, by preventing or reducing *Campylobacter* entering the food chain and the environment, while those measures targeted at the processing stage, can prevent only an estimated 42% of cases (Mughini Gras et al. 2012). Control of

Campylobacter in primary poultry production, however, has proved to be very difficult (Wagenaar et al. 2013).

Campylobacter colonization occurs in all types of commercially produced poultry (e.g., broilers, turkeys, ducks) (Wagenaar et al. 2006), but clearly the focus for intervention is broiler, as it forms the largest source of human infections. The prevention of *Campylobacter* in poultry is solely targeted at meat-producing birds. This is because vertical transmission is extremely rare, if at all (Callicott et al. 2006; Cox et al. 2012). Thus, each new broiler production cycle starts with *Campylobacter*-free chicken. In all-in/all-out production systems, poultry houses are cleaned, disinfected, and dried before the arrival of a new flock. Such preparation seems to be largely effective at preventing the carry-over of *Campylobacter* from previous flocks (Newell et al. 2011; Georgiev et al. 2017). Nevertheless, birds subsequently become colonized with the bacteria. Experimental studies indicate that the ingestion of as few as 40 organisms can cause colonization (Cawthraw et al. 1996). Once the first bird has been colonized, it sheds large numbers of bacteria in its feces (up to 10^7 cfu per gram), and most, if not all, the other birds in the flock become colonized within a few days. Thus, preventing the first bird becoming colonized seems to be a prerequisite for a *Campylobacter*-negative flock.

Broiler flocks are frequently exposed to the *Campylobacter* from their external environment throughout their limited lifespan (Newell et al. 2011). However, colonization does not usually become detectable until 2–3 weeks of age of the flock. This so-called "lag-phase" appears to be due to an inherent resistance in young chickens (Kalupahana et al. 2013) which is, at least in part, a result of maternal immunity (Cawthraw and Newell 2010).

By comparing *Campylobacter*-negative with -positive flocks, many risk factors and farm practices have been identified, which increase the chance of flock positivity (Newell et al. 2011; Sibanda et al. 2018). One major risk factor is the age of broilers at slaughter, which is most likely associated with exposure to external contamination over time and is a measure of the effectiveness of biosecurity. Other biosecurity-associated risk factors, such as multiple broiler houses on the farm, the presence of other livestock, partial depopulation (thinning), pets on the farm, etc., are also important. Nevertheless, no one biosecurity-related factor seems to predominate. Moreover, although improved biosecurity can decrease the risk of a flock becoming *Campylobacter*-positive, it seems that even strict biosecurity cannot guarantee a *Campylobacter*-free flock at the time of slaughter (Newell et al. 2011). In many countries, the biosecurity challenge seems even more difficult in the summer months, when the prevalence of *Campylobacter*-positive flocks increases significantly in response to some temperature-related factors (Jore et al. 2010). Some of this seasonal increase may be associated with transmission by flies. In Denmark, this risk has been significantly reduced by the application of fly-screens around broiler house ventilation systems (Bahrndorff et al. 2013). The efficacy may be country-dependent, i.e., related to weather conditions, as well as dependent on the biosecurity level already applied.

In Europe, improved biosecurity has been strongly recommended as the only currently available intervention measure to reduce flock positivity (Koutsoumanis

et al. 2020). However, the appropriate targeting of biosecurity measures has proved very frustrating for the poultry industry. Anecdotal evidence suggests the compliance of farmers with general biosecurity measures is essential and such compliance would be even more important in summer months (Koutsoumanis et al. 2020). The challenge is likely to become even greater in the future given consumer-driven concerns for animal welfare leading to an increasing trend toward the production of slower-growing animals with a longer lifespan and with outdoor access. Under such conditions good biosecurity is impractical (Kalupahana et al. 2013).

It is widely recognized that biosecurity alone cannot produce *Campylobacter*-negative flocks and that complementary measures will be required to increase the resistance to, or reduce the colonization of, birds with the bacterium (Koutsoumanis et al. 2020; Lu et al. 2020). Research into vaccination against *Campylobacter* is progressing, but not yet ready for practice (de Zoete et al. 2007; Nothaft et al. 2021). Neither is it yet possible to influence the intestinal flora to generate a *Campylobacter*-resistant avian gut (Schneitz 2005). The use of bacteriophages and bacteriocins looks promising (Wagenaar et al. 2005), but research to solve key issues in safety, efficacy, and sustainability is still needed (Olson et al. 2021). The use of medium chain fatty acids has been reported to have at least some effect on *Campylobacter* colonization (van Gerwe et al. 2010; Hermans et al. 2012; Jansen et al. 2014; Guyard-Nicodème et al. 2016), but the results require validation in the field.

Thus, it currently seems that improved biosecurity is the only credible measure available to decrease the prevalence of *Campylobacter*-positive flocks. However, as indicated above, the identification of specific and effective biosecurity approaches has proved very difficult. Thus, a wide range of high-level biosecurity measures need to be consistently maintained throughout the life of intensively reared flocks. This is often impractical, especially when *Campylobacter* colonization is asymptomatic, and therefore with no consequent economic loss to providing an incentive for the poultry farmer.

8.7 Post-Harvest Control Measures in Poultry

When *Campylobacter* colonization cannot be prevented at the farm level, post-harvest treatment becomes very important. Such treatments include the prevention of cross-contamination and the application of chemical or physical methods of decontamination in the slaughterhouse. The availability and effectiveness of such methods, with particular relevance to Europe, have been reviewed previously (Koutsoumanis et al. 2020).

Cross-contamination can be a significant problem associated with the huge throughput of carcasses (circa 13,000 per hour in many processing plants), slaughter line automation, and the high concentrations of *Campylobacter* in cecal contents. Any leakage of fecal material, or rupture of the gut during evisceration, can lead to surface contamination of the meat. Interestingly, there are statistically significant differences, in the level of carcass contamination between slaughterhouses (EFSA 2010), suggesting that some processing plants are better than others at controlling

this problem. However, the basis of these differences has yet to be determined (Koutsoumanis et al. 2020).

The decontamination of carcasses with chemicals is allowed in the USA and currently practiced using several chemicals, such as organic acids, quaternary ammonium compounds, acidified sodium chlorite, and trisodium phosphate. Although the decontamination of carcasses with chemicals is allowed in the EU, specific approval is required and currently no chemic decontaminants have been approved for use on chicken carcasses.

Some physical treatments (e.g. ,ultraviolet, ultrasound, etc.) have been specifically applied to reduce *Campylobacter* on chicken carcasses, but their effectiveness is usually limited to a reduction of only 1–2 $\log_{10}$. Highly effective irradiation procedures are poorly accepted by consumers and difficult to implement under high throughput conditions. The freezing of carcasses from positive flocks can reduce *Campylobacter* concentrations by 2–3 $\log_{10}$ and this strategy has been effectively used in Iceland as part of a program to reduce human campylobacteriosis (Stern et al. 2003). However, from both the logistic and the economic (i.e., the preference of consumers for fresh meat) viewpoints, such a strategy would be difficult to implement, especially in those countries with high prevalence of *Campylobacter*-positive flocks (Havelaar et al. 2007).

8.8 Interventions and Public Health Impact

The potential public health impact of intervention measures in the poultry production chain are clearly demonstrated in two successful examples from Iceland and New Zealand (see section "Intervention Studies").

In Iceland, multiple-level measures were implemented (including producer and consumer education, enhanced biosecurity, changes in poultry processing, and the identification and freezing of products from *Campylobacter*-positive flocks) in response to a sharp increase in campylobacteriosis in 1999 (Tustin et al. 2011). As mentioned before, this spectrum of measures resulted in a 72% reduction in the incidence of campylobacteriosis (Stern et al. 2003). Of all these measures, the freezing of contaminated products is considered the most important (Tustin et al. 2011). In New Zealand, a 54% reduction in the incidence of campylobacteriosis was similarly achieved as a consequence of the introduction of a range of voluntary and regulatory measures (Müllner et al. 2010; Sears et al. 2011; Baker et al. 2012).

Given these successes, it is tempting to extrapolate those approaches implemented in New Zealand and Iceland to other countries. However, in both cases, specific conditions prevailed and, therefore, success in disease reduction in other countries may not be predictable. While highly effective interventions against *Campylobacter* in broiler farms remain elusive, slaughterhouses in the EU have been set up to keep *Campylobacter* contamination in broiler carcasses under control. Indeed, since 2018, a process hygiene criterion (Commission Regulation EU 2017/1495), with a limit of 1000 CFU/g of neck skin, has been implemented among EU Member States. This limit was based on a Scientific Opinion of the European Food

Safety Agency (EFSA) on control options for *Campylobacter* along the poultry meat production chain and their estimated impact on the reduction of the number of human campylobacteriosis cases (EFSA BIOHAZ 2011). The EFSA estimated a public health risk reduction of more than 50% if carcases complied with the aforementioned process hygiene criterion. Moreover, a cost-benefit analysis indicated that a process hygiene criterion for *Campylobacter* in broiler carcases would provide one of the best balances between reduction of human campylobacteriosis cases attributed to broiler meat and the economic consequences of the application of such criterion (EC Europe 2012). A step-by-step approach would also be recommendable, making the process hygiene criteria gradually stricter over time.

8.9 *Campylobacter* in Poultry – The Future

Given that *Campylobacter* is a part of the normal gut flora of birds (and is a highly successful colonizer of that site), the increasing consumer demand worldwide for low cost chicken meat (while expecting higher animal welfare during production) and the steady reduction in human populations with acquired immunity (either due to lack of natural exposure or to increased susceptibility through age, disease or medication), campylobacteriosis will remain a major foodborne pathogen in most countries (Newell et al. 2010). At the moment, the reliable production of *Campylobacter*-negative flocks, through best-practice biosecurity alone, seems unlikely. In the future, effective vaccines and/or other complementary measures should be achievable outcomes of current research. Although, such measures may not totally eliminate colonization, significant reductions in colonization levels may be feasible. In this case, risk assessment studies show that a significant reduction in public health risk can still be achieved (Nauta and Havelaar 2008). Once chicken is no longer a major source of *Campylobacter*, the importance of other animal reservoirs and transmission routes can be identified and tackled.

Acknowledgments This study was supported by the Netherlands' Organization for Health Research and Development (ZonMw) with grant number 50-52200-98-316 (project name: "DEPiCT – Discerning Environmental Pathways of Campylobacter Transmission").

References

Adnan M, Gao X, Bai X et al (2020) Potential early identification of a large *Campylobacter* outbreak using alternative surveillance data sources: autoregressive modelling and spatiotemporal clustering. JMIR Public Health Surveill 6. https://doi.org/10.2196/18281

Alders RG, Dumas SE, Rukambile E et al (2018) Family poultry: multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. Matern Child Nutr 14(Suppl 3). https://doi.org/10.1111/MCN.12668

Amour C, Gratz J, Mduma ER et al (2016) Epidemiology and impact of *Campylobacter* infection in children in 8 low-resource settings: results from the MAL-ED study. Clin Infect Dis 63:1171–1179. https://doi.org/10.1093/CID/CIW542

Ang CW, Teunis PFM, Herbrink P et al (2011) Seroepidemiological studies indicate frequent and repeated exposure to *Campylobacter* spp. during childhood. Epidemiol Infect 139:1361–1368. https://doi.org/10.1017/S0950268810002359

Arning N, Sheppard SK, Bayliss S et al (2021) Machine learning to predict the source of campylobacteriosis using whole genome data. PLoS Genet 17. https://doi.org/10.1371/JOURNAL.PGEN.1009436

Arshad F, Zahoor T (2019) Assessing the microbiological safety status of most commonly consumed food items sold at local and branded restaurants of Faisalabad, Pakistan. J Food Saf 39: e12587. https://doi.org/10.1111/JFS.12587

Bahrndorff S, Rangstrup-Christensen L, Nordentoft S, Hald B (2013) Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. Emerg Infect Dis 19:425. https://doi.org/10.3201/EID1903.111593

Bai Y, Lin XH, Zhu JH et al (2021) Quantification of cross-contamination of *Campylobacter jejuni* during food preparation in a model kitchen in China. J Food Prot 84:850–856. https://doi.org/10.4315/JFP-20-280

Baker MG, Kvalsvig A, Zhang J et al (2012) Declining Guillain-Barré syndrome after campylobacteriosis control, New Zealand, 1988–2010. Emerg Infect Dis 18:226–233. https://doi.org/10.3201/EID1802.111126

Begum S, Sekar M, Gunaseelan L et al (2015) Molecular identification of *Campylobacter jejuni* and *coli* from chicken, calves and dogs to determine its potential threat on human being. Vet World 8:1420–1423. https://doi.org/10.14202/VETWORLD.2015.1420-1423

Berumen A, Lennon R, Breen-Lyles M et al (2021) Characteristics and risk factors of post-infection irritable bowel syndrome after *Campylobacter* enteritis. Clin Gastroenterol Hepatol 19:1855. https://doi.org/10.1016/J.CGH.2020.07.033

Bodhidatta L, Srijan A, Serichantalergs O et al (2013) Bacterial pathogens isolated from raw meat and poultry compared with pathogens isolated from children in the same area of rural Thailand. Southeast Asian J Trop Med Public Health 44(2):259–272. PMID: 23691636

Bull SA, Allen VM, Domingue G et al (2006) Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. Appl Environ Microbiol 72:645–652. https://doi.org/10.1128/AEM.72.1.645-652.2006

Burch D (2005) Avian vibrionic hepatitis in laying hens. Vet Rec 157:528. https://doi.org/10.1136/VR.157.17.528-A

Callicott KA, Frioriksdóttir V, Reiersen J et al (2006) Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. Appl Environ Microbiol 72:5794. https://doi.org/10.1128/AEM.02991-05

Cawthraw SA, Newell DG (2010) Investigation of the presence and protective effects of maternal antibodies against *Campylobacter jejuni* in chickens. Avian Dis 54:86–93. https://doi.org/10.1637/9004-072709-REG.1

Cawthraw SA, Wassenaar TM, Ayling R, Newell DG (1996) Increased colonization potential of *Campylobacter jejuni* strain 81116 after passage through chickens and its implication on the rate of transmission within flocks. Epidemiol Infect 117:213–215. https://doi.org/10.1017/S0950268800001333

CDC (2022a) FoodNet Homepage|CDC. https://www.cdc.gov/foodnet/index.html. Accessed 14 Jan 2023

CDC (2022b) FoodNet Fast|CDC. https://wwwn.cdc.gov/foodnetfast/. Accessed 14 Jan 2023

Cheng AC, Turnidge J, Collignon P et al (2012) Control of fluoroquinolone resistance through successful regulation, Australia. Emerg Infect Dis 18:1453–1460. https://doi.org/10.3201/EID1809.111515

Cody AJ, Bray JE, Jolley KA et al (2017) Core genome multilocus sequence typing scheme for stable, comparative analyses of *Campylobacter jejuni* and *C. coli* human disease isolates. J Clin Microbiol 55:2086–2097. https://doi.org/10.1128/JCM.00080-17

Cody AJ, Maiden MCJ, Strachan NJC, McCarthy ND (2019) A systematic review of source attribution of human campylobacteriosis using multilocus sequence typing. Euro Surveill 24. https://doi.org/10.2807/1560-7917.ES.2019.24.43.1800696

Colles FM, Ali JS, Sheppard SK et al (2011) *Campylobacter* populations in wild and domesticated Mallard ducks (*Anas platyrhynchos*). Environ Microbiol Rep 3:574–580. https://doi.org/10.1111/J.1758-2229.2011.00265.X

Cox NA, Richardson LJ, Maurer JJ et al (2012) Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. J Food Prot 75:1896–1902. https://doi.org/10.4315/0362-028.JFP-11-322

Danis K, Di Renzi M, O'Neill W et al (2009) Risk factors for sporadic *Campylobacter* infection: an all-Ireland case-control study. Euro Surveill 14:19123. https://doi.org/10.2807/ESE.14.07.19123-EN/CITE/PLAINTEXT

de Zoete MR, van Putten JPM, Wagenaar JA (2007) Vaccination of chickens against *Campylobacter*. Vaccine 25:5548–5557. https://doi.org/10.1016/J.VACCINE.2006.12.002

Deolalikar A, Desiraju K, Laxminarayan R et al (2021) Infectious diseases in the South-East Asia region. Cent Dis Dyn Econ Policy

Didelot X, Bowden R, Wilson DJ et al (2012) Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet 13:601–612. https://doi.org/10.1038/NRG3226

Dingle KE, Colles FM, Wareing DRA et al (2001) Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol 39:14–23. https://doi.org/10.1128/JCM.39.1.14-23.2001

Djennad A, Lo Iacono G, Sarran C et al (2019) Seasonality and the effects of weather on *Campylobacter* infections. BMC Infect Dis 19. https://doi.org/10.1186/S12879-019-3840-7

Domingues AR, Pires SM, Halasa T, Hald T (2012) Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. Epidemiol Infect 140:970–981. https://doi.org/10.1017/S0950268811002676

Doorduyn Y, Van Pelt W, Siezen CLE et al (2008) Novel insight in the association between salmonellosis or campylobacteriosis and chronic illness, and the role of host genetics in susceptibility to these diseases. Epidemiol Infect 136:1225–1234. https://doi.org/10.1017/S095026880700996X

Doorduyn Y, Van Den Brandhof WE, Van Duynhoven YTHP et al (2010) Risk factors for indigenous *Campylobacter jejuni* and *Campylobacter coli* infections in The Netherlands: a case-control study. Epidemiol Infect 138:1391–1404. https://doi.org/10.1017/S095026881000052X

Douglas Inglis G, McAllister TA, Larney FJ, Topp E (2010) Prolonged survival of *Campylobacter* species in bovine manure compost. Appl Environ Microbiol 76:1110–1119. https://doi.org/10.1128/AEM.01902-09

EC Europe (2012) Analysis of the costs and benefits of setting certain control measures for reduction of *Campylobacter* in broiler meat at different stages of the food chain Final Report. https://food.ec.europa.eu/system/files/2016-10/biosafety_food-borne-disease_campy_cost-bene-analy.pdf. Accessed 23 Jan 2023

EFSA (2010) Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008 – part A: *Campylobacter* and *Salmonella* prevalence estimates. EFSA J 8. https://doi.org/10.2903/J.EFSA.2010.1503

EFSA (2021) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA J 19. https://doi.org/10.2903/J.EFSA.2021.6490

EFSA BIOHAZ (2010) Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA J 8. https://doi.org/10.2903/J.EFSA.2010.1437

EFSA BIOHAZ (2011) Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA J 9. https://doi.org/10.2903/J.EFSA.2011.2105

EFSA, ECDC (2021) The European Union one health 2020 Zoonoses report. EFSA J 19. https://doi.org/10.2903/J.EFSA.2021.6971

Endtz HP, Mouton RP, Van Der Reyden T et al (1990) Fluoroquinolone resistance in *Campylobacter* spp isolated from human stools and poultry products. Lancet (London, England) 335:787. https://doi.org/10.1016/0140-6736(90)90897-E

Franz E, Mughini Gras L, Dallman T (2016) Significance of whole genome sequencing for surveillance, source attribution and microbial risk assessment of foodborne pathogens. Curr Opin Food Sci 8:74–79. https://doi.org/10.1016/J.COFS.2016.04.004

Fravalo P, Kooh P, Mughini-Gras L et al (2021) Risk factors for sporadic campylobacteriosis: a systematic review and meta-analysis. Microb Risk Anal 17:100118. https://doi.org/10.1016/J.MRAN.2020.100118

French N, Barrigas M, Brown P et al (2005) Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ Microbiol 7:1116–1126. https://doi.org/10.1111/J.1462-2920.2005.00782.X

Friedman CR, Hoekstra RM, Samuel M et al (2004) Risk factors for sporadic *Campylobacter* infection in the United States: a case-control study in FoodNet sites. Clin Infect Dis 38(Suppl 3). https://doi.org/10.1086/381598

Friesema IHM, Havelaar AH, Westra PP et al (2012) Poultry culling and campylobacteriosis reduction among humans, The Netherlands. Emerg Infect Dis 18:466–468. https://doi.org/10.3201/EID1803.111024

Gahamanyi N, Mboera LEG, Matee MI et al (2020) Prevalence, risk factors, and antimicrobial resistance profiles of thermophilic *Campylobacter* species in humans and animals in sub-Saharan Africa: a systematic review. Int J Microbiol. https://doi.org/10.1155/2020/2092478

Georgiev M, Beauvais W, Guitian J (2017) Effect of enhanced biosecurity and selected on-farm factors on *Campylobacter* colonization of chicken broilers. Epidemiol Infect 145:553–567. https://doi.org/10.1017/S095026881600251X

Gilbert M, Conchedda G, Van Boeckel TP et al (2015) Income disparities and the global distribution of intensively farmed chicken and pigs. PLoS One 10. https://doi.org/10.1371/JOURNAL.PONE.0133381

Gillespie IA, O'Brien SJ, Frost JA et al (2002) A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. Emerg Infect Dis 8:937–942. https://doi.org/10.3201/eid0809.010817

Gilpin BJ, Walker T, Paine S et al (2020) A large scale waterborne Campylobacteriosis outbreak, Havelock North, New Zealand. J Infect 81:390–395. https://doi.org/10.1016/J.JINF.2020.06.065

Guyard-Nicodème M, Keita A, Quesne S et al (2016) Efficacy of feed additives against *Campylobacter* in live broilers during the entire rearing period. Poult Sci 95:298–305. https://doi.org/10.3382/PS/PEV303

Haagsma JA, Siersema PD, De Wit NJ, Havelaar AH (2010) Disease burden of post-infectious irritable bowel syndrome in The Netherlands. Epidemiol Infect 138:1650–1656. https://doi.org/10.1017/S0950268810000531

Harrison L, Mukherjee S, Hsu CH et al (2021) Core genome MLST for source attribution of *Campylobacter coli*. Front Microbiol 12. https://doi.org/10.3389/FMICB.2021.703890

Havelaar AH, Swart A (2016) Impact of waning acquired immunity and asymptomatic infections on case-control studies for enteric pathogens. Epidemics 17:56–63. https://doi.org/10.1016/J.EPIDEM.2016.11.004

Havelaar AH, Mangen MJJ, De Koeijer AA et al (2007) Effectiveness and efficiency of controlling *Campylobacter* on broiler chicken meat. Risk Anal 27:831–844. https://doi.org/10.1111/J.1539-6924.2007.00926.X

Havelaar AH, Van Pelt W, Ang CW et al (2009) Immunity to *Campylobacter*: its role in risk assessment and epidemiology. Crit Rev Microbiol 35:1–22. https://doi.org/10.1080/10408410802636017

Havelaar AH, Haagsma JA, Mangen MJJ et al (2012) Disease burden of foodborne pathogens in The Netherlands, 2009. Int J Food Microbiol 156:231–238. https://doi.org/10.1016/J.IJFOODMICRO.2012.03.029

Havelaar AH, Ivarsson S, Löfdahl M, Nauta MJ (2013) Estimating the true incidence of campylobacteriosis and salmonellosis in the European Union, 2009. Epidemiol Infect 141:293–302. https://doi.org/10.1017/S0950268812000568

Havelaar AH, Kirk MD, Torgerson PR et al (2015) World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med 12. https://doi.org/10.1371/JOURNAL.PMED.1001923

Helms M, Simonsen J, Mølbak K (2006) Foodborne bacterial infection and hospitalization: a registry-based study. Clin Infect Dis 42:498–506. https://doi.org/10.1086/499813

Hepworth PJ, Ashelford KE, Hinds J et al (2011) Genomic variations define divergence of water/wildlife-associated *Campylobacter jejuni* niche specialists from common clonal complexes. Environ Microbiol 13:1549–1560. https://doi.org/10.1111/J.1462-2920.2011.02461.X

Hermans D, Martel A, Garmyn A et al (2012) Application of medium-chain fatty acids in drinking water increases *Campylobacter jejuni* colonization threshold in broiler chicks. Poult Sci 91: 1733–1738. https://doi.org/10.3382/PS.2011-02106

Hoffmann S, Batz MB, Morris JG (2012) Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J Food Prot 75:1292–1302. https://doi.org/10.4315/0362-028X.JFP-11-417

Hsu CH, Harrison L, Mukherjee S et al (2020) Core genome multilocus sequence typing for food animal source attribution of human *Campylobacter jejuni* infections. Pathogens (Basel, Switzerland) 9:1–12. https://doi.org/10.3390/PATHOGENS9070532

Jansen W, Reich F, Klein G (2014) Large-scale feasibility of organic acids as a permanent preharvest intervention in drinking water of broilers and their effect on foodborne *Campylobacter* spp. before processing. J Appl Microbiol 116:1676–1687. https://doi.org/10.1111/JAM.12490

Jore S, Viljugrein H, Brun E et al (2010) Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007. Prev Vet Med 93:33–41. https://doi.org/10.1016/J.PREVETMED.2009.09.015

Kalupahana RS, Kottawatta KSA, Kanankege KST et al (2013) Colonization of *Campylobacter* spp. in broiler chickens and laying hens reared in tropical climates with low-biosecurity housing. Appl Environ Microbiol 79:393–395. https://doi.org/10.1128/AEM.02269-12

Kalupahana RS, Mughini-Gras L, Kottawatta SA et al (2018) Weather correlates of *Campylobacter* prevalence in broilers at slaughter under tropical conditions in Sri Lanka. Epidemiol Infect 146: 972–979. https://doi.org/10.1017/S0950268818000894

Knipper AD, Ghoreishi N, Crease T (2022) Prevalence and concentration of *Campylobacter* in faeces of dairy cows: a systematic review and meta-analysis. PLoS One 17. https://doi.org/10.1371/JOURNAL.PONE.0276018

Kotloff KL, Nataro JP, Blackwelder WC et al (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet (London, England) 382:209–222. https://doi.org/10.1016/S0140-6736(13)60844-2

Kottawatta KSA, Van Bergen MAP, Abeynayake P et al (2017) *Campylobacter* in broiler chicken and broiler meat in Sri Lanka: influence of semi-automated vs. wet market processing on *Campylobacter* contamination of broiler neck skin samples. Foods 6. https://doi.org/10.3390/FOODS6120105

Koutsoumanis K, Allende A, Alvarez-Ordóñez A et al (2020) Update and review of control options for *Campylobacter* in broilers at primary production. EFSA J Eur Food Saf Auth 18:1–89. https://doi.org/10.2903/J.EFSA.2020.6090

Kuhn KG, Nielsen EM, Mølbak K, Ethelberg S (2018) Determinants of sporadic *Campylobacter* infections in Denmark: a nationwide case-control study among children and young adults. Clin Epidemiol 10:1695–1707. https://doi.org/10.2147/CLEP.S177141

Kulasooriya GDBN, Amarasiri MKUT, Abeykoon AMH, Kalupahana RS (2019) *Salmonella*, *Campylobacter* and *Escherichia coli* in raw chicken meat, chicken products and cooked chicken in retail markets in Kandy, Sri Lanka. Sri Lanka Vet J 66:19. https://doi.org/10.4038/SLVJ.V66I1.33

Lagerweij G, Pijnacker R, Frieseman I et al (2020) Disease burden of food-related pathogensin the Netherlands, 2019. RIVM Briefrapport 0117

Lake RJ, Campbell DM, Hathaway SC et al (2021) Source attributed case-control study of campylobacteriosis in New Zealand. Int J Infect Dis 103:268–277. https://doi.org/10.1016/J.IJID.2020.11.167

Liu J, Platts-Mills JA, Juma J et al (2016) Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet (London, England) 388:1291–1301. https://doi.org/10.1016/S0140-6736(16)31529-X

Llarena AK, Skjerve E, Bjørkøy S et al (2022) Rapid detection of *Campylobacter* spp. in chickens before slaughter. Food Microbiol 103. https://doi.org/10.1016/J.FM.2021.103949

Lu T, Marmion M, Ferone M et al (2020) On farm interventions to minimise *Campylobacter* spp. contamination in chicken. Br Poult Sci 62:53–67. https://doi.org/10.1080/00071668.2020.1813253. https://doi-org.proxy.library.uu.nl/101080/0007166820201813253

MacDonald E, White R, Mexia R et al (2015) Risk factors for sporadic domestically acquired *Campylobacter* infections in Norway 2010–2011: a national prospective case-control study. PLoS One 10. https://doi.org/10.1371/JOURNAL.PONE.0139636

Man SM (2011) The clinical importance of emerging *Campylobacter* species. Nat Rev Gastroenterol Hepatol 8:669–685. https://doi.org/10.1038/NRGASTRO.2011.191

Marks SL, Rankin SC, Byrne BA, Weese JS (2011) Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. J Vet Intern Med 25:1195–1208. https://doi.org/10.1111/J.1939-1676.2011.00821.X

Monge S, Teunis P, Friesema I et al (2018) Immune response-eliciting exposure to *Campylobacter* vastly exceeds the incidence of clinically overt campylobacteriosis but is associated with similar risk factors: a nationwide serosurvey in The Netherlands. J Infect 77:171–177. https://doi.org/10.1016/J.JINF.2018.04.016

Mossong J, Mughini-Gras L, Penny C et al (2016) Human campylobacteriosis in Luxembourg, 2010–2013: a case-control study combined with multilocus sequence typing for source attribution and risk factor analysis. Sci Rep 6(1):1–12. https://doi.org/10.1038/SREP20939

Mota-Gutierrez J, Lis L, Lasagabaster A et al (2022) *Campylobacter* spp. prevalence and mitigation strategies in the broiler production chain. Food Microbiol 104. https://doi.org/10.1016/J.FM.2022.103998

Mourkas E, Taylor AJ, Méric G et al (2020) Agricultural intensification and the evolution of host specialism in the enteric pathogen *Campylobacter jejuni*. Proc Natl Acad Sci 117:11018–11028. https://doi.org/10.6084/m9.figshare.9929054

Mughini Gras L, Smid JH, Wagenaar JA et al (2012) Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. PLoS One 7. https://doi.org/10.1371/JOURNAL.PONE.0042599

Mughini Gras L, Smid JH, Wagenaar JA et al (2013) Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. Epidemiol Infect 141:2526–2535. https://doi.org/10.1017/S0950268813000356

Mughini Gras L, Smid JH, Wagenaar JA et al (2014) Campylobacteriosis in returning travellers and potential secondary transmission of exotic strains. Epidemiol Infect 142:1277–1288. https://doi.org/10.1017/S0950268813002069

Mughini Gras L, Penny C, Ragimbeau C et al (2016) Quantifying potential sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*. Water Res 101:36–45. https://doi.org/10.1016/J.WATRES.2016.05.069

Mughini Gras L, Chanamé Pinedo L, Pijnacker R et al (2021a) Impact of the COVID-19 pandemic on human salmonellosis in The Netherlands. Epidemiol Infect 149:e254. https://doi.org/10.1017/S0950268821002557

Mughini Gras L, Pijnacker R, Coipan C et al (2021b) Sources and transmission routes of campylobacteriosis: a combined analysis of genome and exposure data. J Infect 82:216–226. https://doi.org/10.1016/J.JINF.2020.09.039

Mulder A, Franz E, de Rijk S et al (2020) Tracing the animal sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*. Water Res 187:116421. https://doi.org/10.1016/J.WATRES.2020.116421

Müllner P, Collins-Emerson JM, Midwinter AC et al (2010) Molecular epidemiology of *Campylobacter jejuni* in a geographically isolated country with a uniquely structured poultry industry. Appl Environ Microbiol 76:2145–2154. https://doi.org/10.1128/AEM.00862-09

Murphy C, Carroll C, Jordan KN (2006) Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. J Appl Microbiol 100:623–632. https://doi.org/10.1111/J.1365-2672.2006.02903.X

Nauta MJ, Havelaar AH (2008) Risk-based standards for *Campylobacter* in the broiler meat chain. Food Control 19:372–381. https://doi.org/10.1016/J.FOODCONT.2007.04.016

Newell DG, Shreeve JE, Toszeghy M et al (2001) Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. Appl Environ Microbiol 67:2636–2640. https://doi.org/10.1128/AEM.67.6.2636-2640.2001

Newell DG, Koopmans M, Verhoef L et al (2010) Food-borne diseases – the challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol 139(Suppl 1). https://doi.org/10.1016/J.IJFOODMICRO.2010.01.021

Newell DG, Elvers KT, Dopfer D et al (2011) Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. Appl Environ Microbiol 77:8605–8614. https://doi.org/10.1128/AEM.01090-10

Nhung NT, Cuong NV, Thwaites G, Carrique-Mas J (2016) Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. Antibiotics (Basel, Switzerland) 5. https://doi.org/10.3390/ANTIBIOTICS5040037

Nicholson FA, Groves SJ, Chambers BJ (2005) Pathogen survival during livestock manure storage and following land application. Bioresour Technol 96:135–143. https://doi.org/10.1016/j.biortech.2004.02.030

Nothaft H, Perez-Muñoz ME, Yang T et al (2021) Improving chicken responses to glycoconjugate vaccination against *Campylobacter jejuni*. Front Microbiol 12. https://doi.org/10.3389/FMICB.2021.734526

Olson EG, Micciche AC, Rothrock MJ et al (2021) Application of bacteriophages to limit *Campylobacter* in poultry production. Front Microbiol 12. https://doi.org/10.3389/FMICB.2021.458721

Papri N, Islam Z, Leonhard SE et al (2021) Guillain-Barré syndrome in low-income and middle-income countries: challenges and prospects. Nat Rev Neurol 17:285–296. https://doi.org/10.1038/S41582-021-00467-Y

Pérez-Reche FJ, Rotariu O, Lopes BS et al (2020) Mining whole genome sequence data to efficiently attribute individuals to source populations. Sci Rep 10. https://doi.org/10.1038/S41598-020-68740-6

Pires SM, Evers EG, Van Pelt W et al (2009) Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathog Dis 6:417–424. https://doi.org/10.1089/FPD.2008.0208

Pires SM, Vigre H, Makela P, Hald T (2010) Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. Foodborne Pathog Dis 7:1351–1361. https://doi.org/10.1089/FPD.2010.0564

Platts-Mills JA, Babji S, Bodhidatta L et al (2015) Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health 3:e564–e575. https://doi.org/10.1016/S2214-109X(15)00151-5

Plishka M, Sargeant JM, Greer AL et al (2021) The prevalence of *Campylobacter* in live cattle, Turkey, chicken, and swine in the United States and Canada: a systematic review and meta-analysis. Foodborne Pathog Dis 18:230–242. https://doi.org/10.1089/FPD.2020.2834

Poulsen MN, Pollak J, Sills DL et al (2018) Residential proximity to high-density poultry operations associated with campylobacteriosis and infectious diarrhea. Int J Hyg Environ Health 221:323–333. https://doi.org/10.1016/J.IJHEH.2017.12.005

Premarathne JMKJK, Satharasinghe DA, Huat JTY et al (2017) Impact of human *Campylobacter* infections in Southeast Asia: the contribution of the poultry sector. Crit Rev Food Sci Nutr 57:3971–3986. https://doi.org/10.1080/10408398.2016.1266297

Rogawski ET, Liu J, Platts-Mills JA et al (2018) Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. Lancet Glob Health 6:e1319–e1328. https://doi.org/10.1016/S2214-109X(18)30351-6

Rosner BM, Schielke A, Didelot X et al (2017) A combined case-control and molecular source attribution study of human *Campylobacter* infections in Germany, 2011–2014. Sci Rep 7:5139. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5505968/

Sahin O, Terhorst SA, Burrough ER et al (2017) Key role of capsular polysaccharide in the induction of systemic infection and abortion by hypervirulent *Campylobacter jejuni*. Infect Immun 85. https://doi.org/10.1128/IAI.00001-17/ASSET/AD5A3732-BDCA-4BA1-9578-8DCCCDA622E1/ASSETS/GRAPHIC/ZII00617-2051-T01.JPEG

Sales-Ortells H, Agostini G, Medema G (2015) Quantification of waterborne pathogens and associated health risks in urban water. Environ Sci Technol 49:6943–6952. https://doi.org/10.1021/ACS.EST.5B00625

Scallan E, Hoekstra RM, Angulo FJ et al (2011) Foodborne illness acquired in the United States – major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/EID1701.P11101

Schneitz C (2005) Competitive exclusion in poultry – 30 years of research. Food Control 16:657–667. https://doi.org/10.1016/J.FOODCONT.2004.06.002

Sears A, Baker MG, Wilson N et al (2011) Marked campylobacteriosis decline after interventions aimed at poultry, New Zealand. Emerg Infect Dis 17:1007–1015. https://doi.org/10.3201/EID/1706.101272

Sibanda N, McKenna A, Richmond A et al (2018) A review of the effect of management practices on *Campylobacter* prevalence in poultry farms. Front Microbiol 9. https://doi.org/10.3389/FMICB.2018.02002

Sopwith W, Birtles A, Matthews M et al (2008) Identification of potential environmentally adapted *Campylobacter jejuni* strain, United Kingdom. Emerg Infect Dis 14:1769–1773. https://doi.org/10.3201/EID1411.071678

Sorgentone S, Busani L, Calistri P et al (2021) A large food-borne outbreak of campylobacteriosis in kindergartens and primary schools in Pescara, Italy, May–June 2018. J Med Microbiol 70. https://doi.org/10.1099/JMM.0.001262

Stern NJ, Hiett KL, Alfredsson GA et al (2003) *Campylobacter* spp. in Icelandic poultry operations and human disease. Epidemiol Infect 130:23–32. https://doi.org/10.1017/S0950268802007914

Stone DM, Chander Y, Bekele AZ et al (2014) Genotypes, antibiotic resistance, and ST-8 genetic clone in *Campylobacter* isolates from sheep and goats in Grenada. Vet Med Int. https://doi.org/10.1155/2014/212864

Suzuki H, Yamamoto S (2009) *Campylobacter* contamination in retail poultry meats and by-products in the world: a literature survey. J Vet Med Sci 71:255–261. https://doi.org/10.1292/JVMS.71.255

Swift L, Hunter PR (2004) What do negative associations between potential risk factors and illness in analytical epidemiological studies of infectious disease really mean? Eur J Epidemiol 19:219–223. https://doi.org/10.1023/B:EJEP.0000020453.84296.F6

Tam CC, Rodrigues LC, Viviani L et al (2012) Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 61:69–77. https://doi.org/10.1136/GUT.2011.238386

Tang Y, Meinersmann RJ, Sahin O et al (2017) Wide but variable distribution of a hypervirulent *Campylobacter jejuni* clone in beef and dairy cattle in the United States. Appl Environ Microbiol 83. https://doi.org/10.1128/AEM.01425-17

Teunis PFM, Falkenhorst G, Ang CW et al (2013) Campylobacter seroconversion rates in selected countries in the European Union. Epidemiol Infect 141:2051–2057. https://doi.org/10.1017/S0950268812002774

Tustin J, Laberge K, Michel P et al (2011) A national epidemic of campylobacteriosis in Iceland, lessons learned. Zoonoses Public Health 58:440–447. https://doi.org/10.1111/J.1863-2378.2010.01387.X

van Gerwe T, Bouma A, Klinkenberg D et al (2010) Medium chain fatty acid feed supplementation reduces the probability of *Campylobacter jejuni* colonization in broilers. Vet Microbiol 143: 314–318. https://doi.org/10.1016/J.VETMIC.2009.11.029

Vandeplas S, Dubois-Dauphin R, Palm R et al (2010) Prevalence and sources of *Campylobacter* spp. contamination in free-range broiler production in the southern part of Belgium. Biotechnol Agron Soc Environ 14:279–288

Vellinga A, Van Loock F (2002) The dioxin crisis as experiment to determine poultry-related *Campylobacter* enteritis. Emerg Infect Dis 8:19–22

Wagenaar JA, Bergen MAPV, Mueller MA et al (2005) Phage therapy reduces *Campylobacter jejuni* colonization in broilers. Vet Microbiol 109:275–283. https://doi.org/10.1016/J.VETMIC.2005.06.002

Wagenaar JA, Mevius DJ, Havelaar AH (2006) *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. Rev Sci Tech Off Int Epiz 25:581–594

Wagenaar JA, French NP, Havelaar AH (2013) Preventing campylobacter at the source: why is it so difficult? Clin Infect Dis 57:1600–1606. https://doi.org/10.1093/cid/cit555

Wagenaar JA, Van Bergen MAP, Blaser MJ et al (2014) *Campylobacter* fetus infections in humans: exposure and disease. Clin Infect Dis 58:1579–1586. https://doi.org/10.1093/cid/ciu085

Walson JL, Pavlinac PB (2018) Targeting enteric pathogens to improve childhood survival and growth. Lancet Glob Health 6:e1258–e1259. https://doi.org/10.1016/S2214-109X(18)30453-4

Wang L, Basuno E, Nguyen T et al (2015) An ecohealth assessment of poultry production clusters (PPCs) for the livelihood and biosecurity improvement of small poultry producers in Asia. Infect Dis Poverty 4. https://doi.org/10.1186/2049-9957-4-6

Wassenaar TM, Newell DG (2000) Genotyping of *Campylobacter* spp. Appl Environ Microbiol 66: 1–9. https://doi.org/10.1128/AEM.66.1.1-9.2000

WHO (2017) Strategic action plan to reduce the double burden of in the. WHO

Wu Z, Sippy R, Sahin O et al (2014) Genetic diversity and antimicrobial susceptibility of *Campylobacter jejuni* isolates associated with sheep abortion in the United States and Great Britain. J Clin Microbiol 52:1853–1861. https://doi.org/10.1128/JCM.00355-14

Wu Z, Periaswamy B, Sahin O et al (2016) Point mutations in the major outer membrane protein drive hypervirulence of a rapidly expanding clone of *Campylobacter jejuni*. Proc Natl Acad Sci U S A 113:10690–10695. https://doi.org/10.1073/PNAS.1605869113/-/DCSUPPLEMENTAL

Wu Z, Yaeger MJ, Sahin O et al (2020) A homologous bacterin protects sheep against abortion induced by a hypervirulent *Campylobacter jejuni* clone. Vaccines 8:662. https://doi.org/10.3390/VACCINES8040662

Yaeger M, Mochel JP, Wu Z et al (2021a) Pharmacokinetics of tulathromycin in pregnant ewes (*Ovis aries*) challenged with *Campylobacter jejuni*. PLoS One 16:e0256862. https://doi.org/10.1371/JOURNAL.PONE.0256862

Yaeger MJ, Sahin O, Plummer PJ et al (2021b) The pathology of natural and experimentally induced *Campylobacter jejuni* abortion in sheep. J Vet Diagn Investig 33:1096. https://doi.org/10.1177/10406387211033293

The Zoonotic Agent Salmonella

9

Sandra Simon, Marina C. Lamparter, Michael Pietsch, Maria Borowiak, Angelika Fruth, Wolfgang Rabsch, and Jennie Fischer

Contents

S. Simon (✉) · M. Pietsch · A. Fruth · W. Rabsch
Robert Koch Institute, Department of Infectious Diseases, Unit for Enteropathogenic Bacteria and Legionella, National Reference Centre for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany
e-mail: SimonS@rki.de; PietschM@rki.de; FruthA@rki.de; rabschw@rki.de

M. C. Lamparter · J. Fischer
Federal Institute for Risk Assessment, Department Biological Safety, Unit Food Microbiology, Pathogen-Host Interactions, National Reference Laboratory for Salmonella, Berlin, Germany
e-mail: Marina.Lamparter@bfr.bund.de; Jennie.Fischer@bfr.bund.de

M. Borowiak
Federal Institute for Risk Assessment, Department Biological Safety, National Study Centre for Sequencing in Risk Assessment, Berlin, Germany
e-mail: Maria.Borowiak@bfr.bund.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_7

Abstract

Salmonella are ubiquitous and robust pathogens, which are often transmitted via food. Especially poultry and poultry products, but also pork and plant-based foods, play an important role as vehicles. They are widely distributed and often spread unnoticed along the farm to fork continuum. Their entry can take place at various points along the food chain ending up in animal and nonanimal derived, *Salmonella*-contaminated food. *Salmonella* can be host-restricted, host-adapted, or of broad host range, but only a relatively small proportion of the about 2600 described serovars have significant clinical relevance. Among these, serovars *S.* Enteritidis and *S.* Typhimurium represent the most prevalent serovars worldwide, together accounting for about 75% of all reported cases with a specified serovar in Germany. Both have a broad host range and likewise infect humans and various animal species (including invertebrates). Depending on serovar, ingested dose, and immunocompetence of the host, *Salmonella* infections differ substantially in their clinical manifestations, ranging from an asymptomatic state to severe illness. Foodborne outbreaks provoked by *Salmonella* are frequently described and range from temporally restricted regional occurrences to protracted, multinational events with hundreds of cases. Whole-genome sequencing (WGS) data is an important tool for bacterial typing, outbreak investigation, source tracking, and surveillance, and its application is highly encouraged by international authorities. By now, numerous examples of the utile application of WGS have been given in different countries and contexts for *Salmonella,* emphasizing its capacity to identify potential outbreak vehicles, support epidemiological studies, and food safety activities, finally aiming for the prevention of further cases.

Keywords

Salmonella enterica · Salmonellosis

9.1 The Zoonotic Agent *Salmonella*

Salmonella is a facultatively anaerobic, rod-shaped, and flagellated gram-negative bacterium. Based on the currently applied nomenclature for the genus *Salmonella,* two species have been described: *Salmonella (S.) bongori* and *Salmonella (S.) enterica*, with *S. enterica* further divided into six subspecies by biochemical characteristics (Tindall et al. 2005). Recent genome-based studies even propose 11 subspecies (Alikhan et al. 2018; Pearce et al. 2021). In total, both species comprise more than 2600 serovars (Issenhuth-Jeanjean et al. 2014). While *S. bongori* and the *S. enterica subspecies II (salamae), IIIa (arizonae), IIIb (diarizonae), IV (houtenae)*, and *VI (indica)* are predominantly related to cold-blooded animals and considered to be of minor clinical relevance (Brenner et al. 2000), *S. enterica subspecies I (enterica)* is responsible for about 99% of human salmonellosis cases (Lamas et al. 2018). The >1500 *subspecies I* serovars form a group of pathogens that differ widely

in their host range within mammals, birds, and reptiles. They can also differ substantially in clinical manifestations, ranging from an asymptomatic state to severe illness (Jones et al. 2008). Serovars can be host-restricted (e.g., *S.* Typhi in humans and higher primates), host-adapted (e.g., *S.* Choleraesuis in pigs and infrequently in humans), or of broad host range (e.g., *S.* Typhimurium, *S.* Enteritidis), infecting various avian and mammalian hosts with a wide spectrum of diseases. Up to now, the traditional *Salmonella* serotyping scheme according to White-Kauffmann-Le Minor (Grimont and Weill 2007) is accepted worldwide as the "gold standard" for the classification of *Salmonella* below the subspecies level. However, this phenotypic approach has been challenged by the emergence of molecular typing methods (Achtman et al. 2012b) and will be presumably replaced by whole genome-based methods in the near future (Ashton et al. 2015). The majority of human cases are caused by only a few non-typhoidal serovars and the overall number of reported human *Salmonella* infections in Germany dropped dramatically during the last three decades from 195,000 in 1992 (Bundesamt 1993) and still 77,000 in 2001 (Robert Koch-Institut 2002) to 13,500 cases in 2019 (Robert Koch-Institut 2020). Among these, *S.* Enteritidis and *S.* Typhimurium represent the most prevalent serovars, together counting for about 75% of all reported cases with specified serovars (Robert Koch-Institut 2020). In accordance to this, meat/meat products and eggs/egg products are still regarded as the most common vehicles of transmission (EFSA and ECDC 2013). However, in recent years, transmission through foods of nonanimal origin has been increasingly reported (Da Silva Felício et al. 2015; Dyda et al. 2020; Krtinić et al. 2010) and typically rare serovars account for a considerable number of large foodborne outbreaks (see Table 1). In this chapter, we focus on the zoonotic potential of *Salmonella enterica,* emphasizing its prevalence in different animal hosts, various foods, and humans, and the shift of surveillance tools to next-generation sequencing as new standard.

9.2 *Salmonella* in Animal and Food

Salmonella is widely spread and often unnoticed along the farm to fork continuum. Their entry can take place at various points along the food chain ending up in animal- and nonanimal derived, *Salmonella*-contaminated food (Hazards 2013).

Salmonella are ubiquitous and are able to withstand harsh conditions in the environment. Growth requirements of *Salmonella* are low, compared to other bacteria. They are able to grow in temperature ranges from 10 °C to 48 °C or even 6–8 °C, at pH values of 4.5–9, and a minimal a_w value (depending on substrate and temperature) of >0.93 (Alvarez-Ordóñez et al. 2010; Alvseike et al. 2000; Dewanti and Doyle 1992; Himathongkham et al. 1999; Matches and Liston 1972; Oscar 2003). However, survival in dried food was even possible at far less water activity levels ($a_w < 0.6$) (Santillana Farakos et al. 2013). Thus, *Salmonella* is able to survive extended time in farm surroundings, dust, soil, feed, or food processing plants. This allows horizontal transmission to the livestock sector and productions systems of

Table 1 Exemplary *Salmonella* outbreaks from the past two decades

Year	Serovar	Country	Suspected vehicle	Reference
2001/2002	*S.* Oranienburg	Germany, Sweden, Finland, Denmark, Netherlands, Belgium, Austria	Chocolate	Werber et al. (2005)
2002/2003	*S.* Agona	Germany	Aniseed-fennel-caraway infusion	Koch et al. (2005), Rabsch et al. (2005)
2003	*S.* Newport	USA	Mangoes	Sivapalasingam et al. (2003)
2004	*S.* Thompson	Norway	Rocket	Nygård et al. (2008)
2004	*S.* Newport	UK, Ireland	Lettuce	Irvine et al. (2009)
2004	*S.* Senftenberg	Serbia	Fennel seed tea	Ilic et al. (2010)
2004	*S.* Braenderup	USA	Tomatoes	Gupta et al. (2007)
2005	*S.* Newport	USA	Tomatoes	Greene et al. (2008)
2005	*S.* Enteritidis	Canada	Mung bean sprouts	Rohekar et al. (2008)
2005	*S.* Typhimurium, *S.* Saintpaul	USA	Orange juice	Jain et al. (2009)
2006	*S.* Enteritidis	Sweden	Almonds	Ledet Muller et al. (2007)
2006	*S.* Saintpaul	Australia	Cantaloupe	Munnoch et al. (2009)
2007	*S.* Senftenberg	UK, Denmark, Netherlands, USA	Basil	Pezzoli et al. (2008)
2007	*S.* Weltvreden, S. Stanley	Norway, Denmark, Finland, Sweden	Alfalfa sprouts	Emberland et al. (2007)
				Werner et al. (2007)
2007	*S.* Paratyphi B var. Java	Sweden, UK, Denmark	Spinach	Denny et al. (2007)
2008	*S.* Newport, *S.* Reading	Finland	Iceberg lettuce	Lienemann et al. (2011)
2008	*S.* Panama	Netherlands	Fresh fruit juice	Noel et al. (2010)
2009	*S.* Bovismorbificans	Finland	Alfalfa sprouts	Rimhanen-Finne et al. (2011)

(continued)

Table 1 (continued)

Year	Serovar	Country	Suspected vehicle	Reference
2009/2010	*S.* Montevideo	USA	Black and red pepper	Gieraltowski et al. (2012)
2010	*S.* Bareilly	UK	Bean sprouts	Cleary et al. (2010)
2010	*S.* Paratyphi B var. Java	UK	Salad vegetables	Gobin et al. (2011)
2010	*S.* Montevideo	Germany	Herbal Food supplement	Stocker et al. (2011)
2011	S. Strathcona	Denmark, Germany, Austria, Italy	Tomatoes	Müller et al. (2016)
2011	*S.* Newport	Germany, UK, Ireland	Watermelon	Byrne et al. (2013)
2011	*S.* Newport	Germany, Netherlands	Mung bean sprouts	Bayer et al. (2013)
2012	*S.* Bareilly	USA	Scraped tuna	
2014	*S.* Muenchen	Germany	Pork products	Schielke et al. (2017)
2015	*S.* Dublin	France	Raw-milk cheese	Ung et al. (2019)
2015/2016	*S. Poona*	USA	Cucumbers	Laughlin et al. (2019)
2015–2019	*S.* Agona, *S.* Anatum, *S.* Gaminara, *S.* Infantis, *S.* Kiambu, *S.* Newport, *S.* Senftenberg, *S.* Uganda, *S.* Urbana	USA	Papaya (five outbreaks)	Whitney et al. (2021)
2016	*S.* Give	Malta	Ready-to-eat antipasti	Donachie et al. (2018)
2016	*S.* Vari	Greece, Germany, Czech Republic, Luxembourg, UK	Sesame products	Meinen et al. (2019)
2017	*S.* Saintpaul	UK	Person-to-person spread	Thomson et al. (2019)
2017	*S.* Agona	France	Infant formula	Jourdan-Da Silva et al. (2018)
2018	*S.* Poona	France, Belgium, Luxembourg	Infant formula (based on rice proteins)	ECDC and EFSA (2019)
2018	*S.* Agbeni	Norway	Dried exotic fruit mix	Johansen et al. (2021)
2020	*S.* Muenchen	Germany	Dried coconut pieces	(manuscript in preparation)

(continued)

Table 1 (continued)

Year	Serovar	Country	Suspected vehicle	Reference
2019–2021	*S.* Senftenberg *S.* Kintambo *S.* Orion *S.* Havana *S.* Mbandaka *S.* Amsterdam	Germany, Sweden, Denmark, Norway, Netherlands, Canada, USA	Sesame products (tahini, halva)	EFSA (2021)
2021	*S.* Thompson	USA	Seafood	CDC (2021b)
2021	*S.* Braenderup	12 EU countries and UK	Small melons (Galia melons)	ECDC and EFSA (2021)
2021	*S.* Weltevreden	USA	Frozen cooked shrimps	CDC (2021a)
2022	*S.* Typhimurium	EU countries and UK	Chocolate	Larkin et al. (2022)

food of nonanimal origin (FoNAO) (Finn et al. 2013; Habimana et al. 2014; Jechalke et al. 2019; Liljebjelke et al. 2005; Marin et al. 2009; Skov et al. 2008). *Salmonella* are also found in cold-blooded animals such as reptiles or insects, which may act as vectors to reach or spread from their main reservoir, the gastrointestinal tract of warm-blooded animals (Corrente et al. 2017; Pulford et al. 2019; Vandeweyer et al. 2021). Wildlife animals are also frequently reported to carry *Salmonella* (Skov et al. 2008; Uelze et al. 2021b). However, the main focus is set on livestock animals, due to the risk of subsequent transmission to derived foodstuff. In this context, vertical transmission from parents to progeny (e.g., from chicken to eggs) plays an important role (Liljebjelke et al. 2005). Infected farm animals can be asymptomatic, healthy-looking carriers, but acute and chronical disease symptoms or dying animals due to a *Salmonella* infection are possible as well (Gutema et al. 2019; Suzuki 1994). Animals can shed relatively large numbers of *Salmonella* in the feces over a long period of time. Infection with host-adapted serovars, such as *S.* Gallinarum (in poultry) or *S.* Dublin (in cattle), can severely affect the livestock production with high mortality rates (Mcdonough et al. 1999; Schat et al. 2021). Meanwhile, non-adapted serovars – among them the most relevant ones in human infections: Enteritidis and Typhimurium – often spread asymptomatically within herds replacing the eradicated host-specific serovars as matter of concern. Although causing no symptoms in animals, those non-adapted serovars cause severe health issues in humans. The incidence of *Salmonella* in livestock depends on several factors such as the conditions for intensive animal husbandry, hygiene measures, and climate conditions. The incidence of *Salmonella* in Northern countries is often lower than in those located in warmer climate zones. Furthermore, *Salmonella* cases are subject to a seasonal variation with higher rates in the summer and autumn, and a decline in the winter months (EFSA and ECDC 2021c).

Consequently, the control of *Salmonella* in food-producing animals preharvest is imperative to avoid the transmission along the food chain to humans. Usually, in

developed countries, livestock is regularly monitored in order to estimate the prevalence of the pathogen and serovars and to perform control measures. Such control measures include the application of vaccine strains of relevant serovars to herds of livestock to improve subsequent foodstuff safety, rather than the prevention of salmonellosis in animals.

Food processing, such as the slaughter process of the animals, raw fruits and vegetables, or infant formula processing lines are further possible points of contamination (Bolton et al. 2003; Ehuwa et al. 2021; Kent et al. 2015; Artes et al. 2007). Therefore, at the food processing and retail levels, *Salmonella* surveillance is continued, and, e.g., food safety criteria are laid down in the context of the EU Commission Regulation (EC) No 2073/2005. Here, i.e., the absence of *Salmonella* in 25 g of most listed food types is regulated. This includes a variety of foods such as meat products, eggs, infant formula, milk products, ready-to-eat-food, seafood and snails, sprouts, as well as cut fruits and vegetables. In the last few years, *Salmonella*-associated outbreaks have additionally shed a light on uncommon *Salmonella* serovars. These are often associated with "new" vehicles that attracted attention and should be considered in food safety management, notably for foodstuffs with low water activity (tahini, chocolate, dried fruits, food supplements, and tea) (EFSA 2021; EFSA and ECDC 2018).

9.2.1 Salmonella in Poultry and Poultry Products

Poultry represents the major putative source for non-typhoidal salmonellosis infections in humans (CDC and FDA 2021; Thomas et al. 2020). Especially, *S.* Enteritidis, which had been found since the 1980s to contaminate poultry flocks worldwide (often asymptomatically), is today a dominant serovar in, e.g., laying hens and broilers in the EU and still remains a global problem for public health (Baumler et al. 2000; Ferrari et al. 2019; Li et al. 2021). Contamination in poultry production might take place via horizontal transmission through feed, the farm environment, and live vectors (e.g., rodents or insects), as well as via vertical transfer in hen reproduction (Liljebjelke et al. 2005; Okamura et al. 2001).

In Europe, baseline surveys (2004–2005) on the prevalence of *Salmonella* in, e.g., commercial large-scale laying hen holdings showed that 30.8% of the flocks of *Gallus gallus* were *Salmonella* positive (EFSA 2007). Subsequently, control programs were established in Europe (Directive 2003/99/EC and Regulation (EC) No 2160/2003) aiming at reducing the prevalence of predominant target serovars such as *S.* Enteritidis and *S.* Typhimurium in poultry (i.e., in laying hens (Commission regulation (EU) No 517/2011) and broiler flocks (Commission regulation (EU) No 200/2012) of *Gallus gallus* and in breeding and fattening flocks of turkeys (Commission regulation (EU) No 1190/2012). In breeding hen, further target serovars were included, namely *S.* Infantis, *S.* Hadar, and *S.* Virchow (Commission Regulation (EU) No 200/2010).

Since the beginning of the control programs in 2008, target serovar prevalence has shown an overall decreasing trend since 2009. Target prevalence for breeding (target prevalence of 1%) and laying hen (target prevalence of 2%) has been met,

with a few states missing the criteria (e.g., in 2019, up to 5 out of 27 states reported data for breeding flocks of *Gallus gallus*). Otherwise, the trends in prevalence of *Salmonella* target serovar-positive flocks were rather stable from 2015 for all poultry categories. However, increasing *Salmonella* prevalence trends point at the issue of certain successful clonal lineages with high fitness spreading in different animal populations, such as *S.* Infantis in broiler flocks (EFSA and ECDC 2021b). Predominant serovars in poultry all over the world are reported to vary among different regions. For example, in the USA, *S.* Kentucky is often isolated, as well as *S.* Heidelberg, which is also common in, e.g., Brazil (Foley et al. 2013; Golden and Mishra 2020; Shah et al. 2017). Poultry-adapted serovars *S.* Gallinarum and *S.* Pullorum, which in the last century have caused great damage to the poultry industry, are, in contrast to, e.g., Asia, no longer an issue in European and North American poultry flocks nowadays due to past eradication programs (Kumar et al. 2019; Wang et al. 2020).

In the USA, data from 2017 and 2018 show a prevalence of *Salmonella* in chicken (cecal) of 38% and 46% and 14% and 15% in turkey (cecal content) (FDA 2022). Thomas et al. (2020) reported an adjusted *Salmonella* prevalence of 13.9% in poultry samples in Africa.

While eggs and egg products account for most of the strong-evidence salmonellosis foodborne outbreaks in the EU (EFSA 2018–2020), findings of *Salmonella* in eggs in the EU are rare. From 2016 to 2019, 78 (0.28%) out of 28,190 eggs and egg product samples (non-ready-to-eat-food) were *Salmonella*-positive. Nevertheless, several *Salmonella* outbreaks linked to eggs have caught attention in recent years (EFSA 2014a; EFSA and ECDC 2020, 2022). Despite control programs on poultry primary production levels in the EU, percentage of *Salmonella* positive samples is still the highest in poultry meat, e.g., fresh broiler meat at 6.79% or turkey at 5.99% between 2016 and 2019. TOP serovars found in both matrices are *S.* Infantis and *S.* Enteritidis (EFSA and ECDC 2021c).

9.2.2 Salmonella in Pork and Pork Products

Pork is regarded as another important source for human salmonellosis (EFSA and ECDC 2021c; Pires et al. 2011). Pig meat and products thereof range second in the EU TOP food vehicle (following egg and egg products) causing strong-evidence *Salmonella* outbreaks, while in the USA, pork is the third food category (first chicken, second fruits) attributed to *Salmonella* illnesses (CDC and FDA 2021; EFSA and ECDC 2021c). In animal production, European data from 2019 and 2020 revealed 36% (17 states, 66,624 samples) and 27.9% (10 states, 56,008 samples) positive pig samples (EFSA and ECDC 2021b, c). In the USA, data from the NARMS Update listed up to 50% of market swine cecal samples in 2017 (1008 samples) and up to 61% of sows cecal samples in 2018 *Salmonella*-positive (FDA 2022). *S.* Typhimurium together with its monophasic variant 4,[5],12:i:- is one of the most common serovars isolated from pigs in both, Europe and the USA (EFSA and ECDC 2021c; Morningstar-Shaw et al. 2016). The monophasic variant started to

emerge in Europe in the mid-1990s beginning from Spain (Echeita et al. 1999). In the EU, *S.* Typhimurium 4,[5],12:i:- meanwhile became one of the most frequently isolated serovars from pigs and pork. Noteworthy, the dominant host-adapted serovar Choleraesuis in Europe (Sojka et al. 1977) decreased dramatically since the 1950s and 1960s and is today very rarely isolated in European pig production. However, it remains predominant in wild boars. The second predominant serovar in pigs is *S.* Derby, which causes mainly asymptomatic infections in pigs (EFSA and ECDC 2021c). In pigs and pig meat, *Salmonella* Derby ranks in the top two in Europe (EFSA and ECDC 2021c). Similarly, *S.* Derby is the third most frequently isolated in clinical and nonclinical cases of pigs in the USA, while *S.* Typhimurium monophasic and biphasic variants ranked separately first and second in 2016 (Morningstar-Shaw et al. 2016). In fresh pig meat, the rate of *Salmonella*-positive tested samples in Europe from 2016 to 2019 was 1.94% (EFSA and ECDC 2021c). The serovar distribution in pig meat is similar compared to pigs at the primary production level, reflecting the transmission of the serovars along the food chain.

9.2.3 Salmonella in Cattle and Bovine Products

Although foodborne illness due to contaminated beef products is less often reported compared to poultry or pork, bovine-associated *Salmonella* outbreaks have caught attention in the past. In cattle, the prevalence of *Salmonella* is generally significantly lower than in poultry or pigs. A rate of 3.3% and 3.4% in 2019 and 2020, respectively, on animals and 7.6% (2019) at the slaughterhouse level (animals) was detected in the EU (EFSA and ECDC 2021c). In North America, *Salmonella* pooled prevalence in healthy cattle was determined in a meta-analysis at 16% (2000–2017), while in the USA, 9% of cecal beef samples were *Salmonella*-positive in 2018 (Gutema et al. 2019; FDA 2022). Bovine salmonellosis is often associated with cattle-host-adapted serovar *S.* Dublin, but *S.* Typhimurium (including the monophasic variant) infection may also cause bovine *Salmonella* symptoms (Wallis and Barrow 2005). In the EU, the overall prevalence of bovine foodstuffs was low. Between 2016 and 2019, it was 0.34% in non-ready-to-eat meat and meat products from bovine animals, and in fresh meat from bovine animals 0.28% (EFSA and ECDC 2021c). In the USA, ground beef was analyzed at 1% positive for *Salmonella* in 2018 (FDA 2022).

9.2.4 Salmonella in Plant-Based Food

Salmonella infections are increasingly reported to be linked to the consumption of food of nonanimal origin (FoNAO) (Olaimat and Holley 2012; Lynch et al. 2009; CDC and FDA 2021). In the EU, *Salmonella* ranked as the most common causative agent causing outbreaks linked to FoNAO. Leafy greens eaten raw as salads, bulb and stem vegetables, tomatoes, and melons were the most common FoNAO products involved in outbreaks (2013). In the USA, fruits are estimated to be the main cause

of plant-based foodborne salmonellosis, responsible for 13.5% of human *Salmonella* infections in 2019 (CDC and FDA 2021). *Salmonella* is detected in further FoNAO matrices, such as spices and herbs, bakery products, sprouts, cereals, and nuts, but at very low levels (0.1–0.8%) in the EU (EFSA and ECDC 2021c). Various serovars could be detected in diverse spice matrices in the USA, from domestic as well as imported products. Worldwide, different studies on the prevalence of *Salmonella* in spices and herbs ranged from 0% to 5.6% (Zweifel and Stephan 2012).

Worldwide trade, large-scale productions, and changing consumption behaviors aiming at a convenient and healthy diet (ready-to-eat products, increasing demand for fresh produce) contribute to the rising infection risk. Epidemic outbreaks of foodborne infections are not only a threat to public health but also erode consumer confidence in the causal food product and thus affect the economic viability of the industry. Contamination of plant-based food with *Salmonella* might occur at various production stages. Applications of manure or compost, fertilizers, pesticides, or the use of contaminated irrigation water are described as the main risk factors (2013). Furthermore, environmental factors, such as proximity to animal rearing operations, seasonality, and associated climatic conditions might increase the transfer of *Salmonella* from those reservoirs. Further contact with animal reservoirs such as wild life or insects poses another contamination source. Post-harvest cross-contamination on the farm or by food handlers and equipment can occur through washing, packaging, and transport processes (Zweifel and Stephan 2012; Park et al. 2012; Beuchat 2002).

Although the low water activity of spices or dried herbs does not support *Salmonella* growth and many spices have inhibitory compounds that show antibacterial activity against *Salmonella*, contamination levels of up to 10% could be detected. The survival of *Salmonella* in those dried products for an extended period of time is promoted by its resistance against desiccation (Keller et al. 2013). In general, concentrations of *Salmonella* in spices are low, and due to the low amount of spices used for food preparation, the dose of *Salmonella* intake might be smaller than in other foodstuffs with similar *Salmonella* contamination levels (Chitrakar et al. 2019).

Colonization of crop plants with *Salmonella* are mainly due to surface (cross-) contaminations, but internalization of *Salmonella* into plant tissues was also proven and poses a concern for public health (Zarkani and Schikora 2021; Park et al. 2012). Consequently, cleaning and disinfection can be ineffective to remove the pathogen before consumption. The identification of routes of plant contamination by *Salmonella* is crucial to the design of intervention strategies to prevent contamination from taking place (Brandl and Sundin 2014). Zarkani and Schikora (2021) summarized mechanisms implicated in *Salmonella* interactions with crop plants that enable *Salmonella* colonization or persistence on or in plant tissues. Different strategies based on *Salmonella* adaptation mechanisms, avoidance, or suppression of the plant's immune system are described in the literature, considering the genetic variation of both the plant and the microorganism in determining the efficiency of colonization and persistence (Zarkani and Schikora 2021). Further external influences like the co-colonization of other established epiphytic bacteria such as *Pseudomonas fluorescens* and *Erwinia herbicola* (*Pantoea agglomerans*) influence the ability of *Salmonella* to persist on plants (Poza-Carrion et al. 2013).

9.2.5 Salmonella in Seafood

Salmonella in seafood is rarely detected in the EU and is only reported from single Member States, underlining that seafood is not a common reservoir for *Salmonella*. However, occurrence of *Salmonella* from seafood is described worldwide with different serovars dominating in different continents, such as the serovar *S.* Hadar in Latin America and Africa, *S.* Typhimurium in Europe, *S.* Weltevreden in Asia, and *S.* Newport in North America (Ferrari et al. 2019). In some countries, especially India and African countries, *Salmonella* may reach contamination levels of up to 24% at retail markets (Olgunoğlu 2012; Prabhakar et al. 2020). *Salmonella*-related foodborne outbreaks related to seafood consumption occur occasionally and were reported from the USA, Japan, and the EU (Ferrari et al. 2019; Barrett et al. 2017; Li et al. 2013). In the USA, *Salmonella* is the most common bacterial causative agent in fish and fishery products, leading to large human outbreaks. The percentage of foodborne *Salmonella* illnesses caused by fish or other seafood in the USA was estimated as 2.6% or 1.4%, respectively, with tuna being one of the main sources of infection (Barrett et al. 2017). Shrimp can be another major source of *Salmonella*. The prevalence of *Salmonella* in fresh shrimp at processing plants can reach 10–14%, and in the USA, seafood was described as the product with the most violations for *Salmonella* (Barrett et al. 2017; Wan Norhana et al. 2010). The occurrence of *Salmonella* in fish and shellfish is often a sign of low control measurement at the primary production level and poor standards of hygiene and sanitation during processing, handling, and transport and might be intensified by anthropogenic contamination of coastal waters (Olgunoğlu 2012; Prabhakar et al. 2020).

9.2.6 Salmonella in Insects

Nowadays, insect-based food is discussed intensively and might capture the European market due to insects as alternative protein sources to combat issues coming up with growing world population and increasing demands on an optimized nutrient composition in food, enabling simultaneously sustainable food and feed production systems and the reduction of greenhouse gases (Bessa et al. 2020). Although *Salmonella* in edible insects are considered being a lower risk, occurrence of different *Salmonella* serovars with public health relevance, such as *S.* Agona, but also *S.* Stanley and *S.* Wandsworth being endemic in Asian countries (Frentzel et al., not yet published) have been detected in edible insects or insects rearing residues (Wynants et al. 2019; Vandeweyer et al. 2021). Transfer from substrate to insects and survivability or transmission of main *Salmonella* serovars in or via insects are known to occur (EFSA 2015; Wynants et al. 2019). On the other hand, several studies have shown that, for example, black soldier flies possess antimicrobial potential to reduce *Salmonella* and other pathogenic bacteria from their substrate (Vandeweyer et al. 2021). This open gap in the knowledge of *Salmonella* contamination during mass produced insects needs to be filled in the future (Vandeweyer et al. 2021).

9.2.7 Salmonella in Wild Animals

Occurrence of *Salmonella* in wildlife animals is generally not of human health relevance. However, several broad host range *Salmonella* serovars or sequence types including those most often associated with disease in humans are detected among diverse wildlife species and also whole-genome sequencing analysis reveal isolates from wildlife being genetically highly similar to isolates from infected humans, foodstuff, or livestock animals (Hilbert et al. 2012; Uelze et al. 2021b). Apart from human infections caused by consumption of wildlife-derived meat, transmission of *Salmonella* isolates from wildlife to humans may occur by direct contact with wild animals and their natural environment, with children being predominantly affected (Hauser et al. 2009; Williams et al. 2015; Lawson et al. 2018). Indirect transmission of *Salmonella* from wildlife to food-producing animal housings or consumption of crop plants contaminated by wildlife animals is further possible transmission pathways (EFSA 2014b; Hilbert et al. 2012). However, transmission from contaminated animal farms to surrounding wildlife was proven (Skov et al. 2008). Besides birds and rodents, the continuous expansion of omnivorous wildlife species like foxes, raccoons, and wild boars into urban settings, where they to a considerable extent feed on waste and ort and verifiably contaminate public zones like sunbathing areas or playgrounds, may pose a risk for pathogen transmission (including *Salmonella*) to humans, especially young children. Some *Salmonella* sequence types are described to be associated with certain animal species, such as *S.* Typhimurium ST128 with pigeons, *S.* Enteritidis ST183 with hedgehogs, or *S.* Choleraesuis ST145 with wild boars (Uelze et al. 2021b; Longo et al. 2019; Leekitcharoenphon et al. 2019).

9.3 Human Salmonellosis

9.3.1 Transmission and Symptoms

Salmonella enterica can provoke gastrointestinal as well as systemic disease. Bloodstream infections are regularly caused by the typhoid serovars *S.* Typhi and *S.* Paratyphi A, B, and C. These infections are mostly related to people coming or returning from areas with poor sanitary conditions where typhoid and paratyphoid are still endemic. Typhoid serovars are host-restricted to humans and higher primates; they have no reservoir in other wildlife or livestock animals. Transmission occurs via fecal contamination of food/water or through (chronic) carriers. Due to their lack of zoonotic potential, typhoid serovars are not further discussed here.

Non-typhoid *Salmonella* (NTS) typically remain restricted to the gastrointestinal tract, where they cause self-limiting gastroenteritis characterized by diarrhea, abdominal pain, and fever (Velge et al. 2012). Symptoms like nausea, vomiting, and headache may also occur. Illness usually lasts 4–7 days and does not require antibiotic treatment. Nevertheless, dysfunction of the mucosal barrier can result in life threating infections, especially in vulnerable groups like infants, elderly, and

immunocompromised people (Santos et al. 2009). Severe infections, like bacteremia, meningitis, osteomyelitis, and bronchopulmonary salmonellosis have been described (Gilchrist and Maclennan 2019; Gordon 2008; Fabrega and Vila 2013).

The main reservoir for NTS is warm-blooded animals, including livestock. Food of animal origin is still supposed to be the most common source of human infections, but infections due to plant-derived foods have been increasingly reported. Direct or indirect animal contact also bears a potential risk for infection, and although fecal-oral human-to-human transmission rarely occurs in industrialized countries, it poses a problem in areas with poor hygiene conditions.

Surveillance programs that detect *Salmonella* contaminations in a timely manner in the entire food chain (crop plants, animal feed, breeding, rearing and fattening plants, hatcheries, layer flocks, slaughterhouses, food processing plants, whole sale, and retail sector) together with sanitary measures are essential for preventing human *Salmonella* infections (Newell et al. 2010; Wattiau et al. 2011).

9.3.2 Pathogenesis

The incubation time for NTS mostly varies from 6 to 72 h, but in cases with low-dose exposure, symptom onset may be delayed for up to 16 days (Abe et al. 2004; Siira et al. 2019). Infections with NTS lead to an acute intestinal inflammation in human and animal hosts. Following ingestion via contaminated food, the bacteria need to overcome the highly acidic pH of the stomach as well as the adverse conditions in the intestine (high bile concentration, competition with commensal bacteria) to reach their infection site. It is proposed that foods with a high fat or protein content may protect *Salmonella* from digestion in the stomach or that the uptake with liquid foods facilitates survival due to a shorter passaging time. But more importantly, *Salmonella* has developed complex strategies to survive in these hostile environments (Álvarez-Ordóñez et al. 2011). Crucial to *Salmonella* virulence is its ability to invade and break through the intestinal mucosal barrier. Adhesins and fimbriae are necessary to mediate attachment to epithelial cells in the gut. Once in the lower intestine, the bacteria adhere to enterocytes or M cells. From there, they translocate to the lamina propria, where they are taken up by phagocytes, disseminate from the gastrointestinal tract to mesenteric lymph nodes, and colonize systemic sites, like the liver and spleen (Agbor and Mccormick 2011; Dong et al. 2022).

Therefore, *Salmonella enterica* has developed ingenious virulence mechanisms to manipulate host cell functions to its own benefit (Agbor and Mccormick 2011). Two type III secretion systems (T3SS) encoded within the ***S****almonella* **p**athogenicity **i**slands SPI-1 (Mills et al. 1995) and SPI-2 (Shea et al. 1996) are responsible for the delivery of a series of bacterial effectors into host cells with the intention to reprogram eukaryotic cell functions. While the T3SS apparatus is highly conserved across bacterial genera, the translocated effectors are unique proteins with very specialized functions critical to virulence. Moreover, considerable evidence indicates that individual effectors secreted by the T3SS are modular proteins composed

of functionally distinct domains that may act in different stages of the infection process (Agbor and Mccormick 2011).

SPI-1 effectors play a fundamental role in the early stages of mammalian infection mediating the remodeling of the actin cytoskeleton of the host cell, thus leading to internalization of the bacteria and subsequent penetration of the ileal mucosa. SPI-1 effectors also have pro-inflammatory potential via activation of the host cell inflammasome, and they cause a very strong proapoptotic effect in monocytic cells (Hautefort et al. 2008; Kaiser and Hardt 2011; Bierschenk et al. 2017).

The SPI-2-encoded T3SS and its effectors allow the intracellular survival of *Salmonella enterica* within phagocytic cells, creating a niche where the bacteria are inaccessible for the host cell defense mechanisms. Within this so-called *Salmonella* containing vacuole (SCV), the bacteria replicate and translocate various effectors into and across the SCV membrane, thereby interfering with host cell functions like antimicrobial defense mechanisms, intracellular transport processes, integrity of the cytoskeleton, and host cell death (Kuhle and Hensel 2004).

Besides these two major pathogenicity islands altogether 24 SPIs have been identified in *Salmonella enterica* so far. Some of them are widespread, others are restricted to certain subspecies or even serovars and contribute to virulence processes in different hosts (Cheng et al. 2019).

Like other enterobacteria, *Salmonella* can exchange genetic material via horizontal gene transfer. The acquisition or loss of plasmids, prophages, and other mobile genetic elements may result in changes to the antibiotic resistance profile or affect the virulence or fitness properties of these strains.

9.3.3 Salmonellosis Worldwide

9.3.3.1 Global Burden of Disease

Assessing the burden of foodborne disease is a complex task because many different pathogens can be transmitted by food, leading to different health outcomes. But doubtlessly, human salmonellosis has a very high economical and public health impact worldwide (Majowicz et al. 2010), and burden-of-disease estimates combining indicators of mortality, morbidity, and health care costs are increasingly used to instruct public health officials and politicians. Based on WHO data, in 2010, the estimated number of foodborne illnesses caused worldwide by NTS was 78.7 Mio. They accounted for 59,000 fatal casualties and about 4 Mio. years of life lost (YLL) (Havelaar et al. 2015). The public health burden of non-typhoidal *Salmonella* infections is exceptionally high in low-income countries and urban settings with high population density and poor sanitary conditions, especially in areas where other severe infectious diseases (e.g., Malaria and AIDS) are endemic. Data collected between 2009 and 2012 from the World Health Organization (WHO) Foodborne Disease Burden Epidemiology Reference Group (FERG) showed that of all foodborne diseases, diarrheal and invasive infections due to non-typhoidal *S. enterica* infections resulted in the highest burden. They cause 4.07 million (95% UI 2.49–6.27 million) disability adjusted life years (DALYs), with a most

considerable burden of foodborne disease in children less than 5 years of age. In addition, as major cause of global morbidity and mortality an increasing number of invasive non-typhoidal salmonellosis (iNTS) was observed in recent years worldwide, but mostly in the sub-Sahara region. Especially patients infected with HIV are at risk. Improved access to antiretroviral therapy (ART) has probably helped to reduce the burden of iNTS disease among those persons (Havelaar et al. 2015).

9.3.3.2 Salmonellosis in Germany and the European Union

In Germany and the European Union (EU), salmonellosis is the second most commonly reported bacterial foodborne infection in humans after campylobacteriosis with about 13,500 and 88,000 reported cases in 2019, respectively (Robert Koch-Institut 2020; EFSA and ECDC 2021a). After a long period of remarkable decline, the number of salmonellosis cases in humans has been stable for several years now, and *Salmonella enterica* still represents an important cause of foodborne outbreaks (Robert Koch-Institut 2020; EFSA and ECDC 2021a).

In Germany, salmonellosis ranked fourth among six major enteric pathogens considering YPLL (years of potential life lost) with the highest mortality rate (Werber et al. 2013).

In Europe, salmonellosis is a largely seasonal disease with most cases reported during the summer months (EFSA and ECDC 2021b).

S. Enteritidis and *S*. Typhimurium represent the far most prevalent serovars, together counting for >70% of all reported cases with specified serovars in Germany and also in the EU. Although eggs and egg products are still considered as the main source for *S*. Enteritidis infections while pork and pork products are regarded as the most common food vehicles for *S*. Typhimurium, other food categories (also of nonanimal origin) may as well be contaminated with these serovars.

Although the main focus of this chapter is on foodborne *Salmonella* infections, it should be mentioned that infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm environments, or other public, professional, or private settings. Clinically affected animals may exhibit a higher prevalence of shedding than apparently healthy animals, but both can shed *Salmonella* over long periods of time. The public health risk varies by mammals, birds, and reptile species, age group, husbandry practice, and health status (Hoelzer et al. 2011). Numerous reports exist on the prevalence of *Salmonella* sp. in reptiles, and many different serovars have been described, usually rarely isolated from humans or livestock. Publications demonstrate a higher prevalence in lizards in comparison to tortoises and turtles: in a study in captive lizards, *Salmonella* spp. were isolated from 76% of all cloacal swabs, including 44 serovars (Pasmans et al. 2005).

While most reports of reptile-associated salmonellosis are from infants and children, adults, especially immunocompromised individuals and patients with impaired gastric acid production (Stam et al. 2003), may also be affected. Clinical symptoms include mainly gastroenteritis, but severe outcomes such as septicemia, meningitis, and subdural empyema (Chiodini and Sundberg 1981) have been described, especially in children younger than 5 years (Tabarani et al. 2010; Van

Meervenne et al. 2009). Fatalities due to reptile-associated salmonellosis in infants have been reported, for instance, from Austria.

A study exploring the evidence for transmission of *Salmonella* from pet reptiles to children in Germany showed that almost 50% of the interviewed households kept at least one reptile. Sixty-eight percent of the examined reptiles were bearded dragons (*Pogona vitticeps*). Altogether, 319 *Salmonella* isolates were recovered and 44 different serovars were identified (Pees et al. 2013).

9.4 *Salmonella* Outbreaks

Salmonella enterica is an important and frequent cause for foodborne outbreaks on regional, national, or international level and a leading cause for outbreak-related hospitalization. In the EU, *S. enterica* accounts for the largest proportion of bacterial foodborne outbreaks: In 2019, 926 foodborne salmonellosis outbreaks were reported to the ECDC, representing 17.9% of all foodborne outbreaks but counting for 50.5% of all outbreak-related hospitalizations (EFSA and ECDC 2021b). In Germany, 277 *Salmonella* outbreaks were reported in 2019. On EU level, almost three-fourths of *Salmonella* outbreaks were caused by the predominant serovar *S.* Enteritidis, followed by *S.* Typhimurium (bi- or monophasic). Commonly associated food vehicles for *Salmonella* Enteritidis outbreaks were eggs, egg products, egg dishes, and bakery products. Implicated food vehicle for *S.* Typhimurium outbreaks were commonly pork products, such as ground pork or different types of raw sausage. Further serovars observed in more than one outbreak in the EU member states in 2019 were: *S.* Infantis, *S.* Newport, *S.* Coeln, *S.* Mikawasima, *S.* Agona, *S.* Muenchen, and *S.* Poona. However, many different serovars were observed in foodborne outbreaks over the last years. In addition to classical food vehicles for *Salmonella* outbreaks, food of plant origin, like fresh vegetables and fruits, but also herbal infusions, spices, and sweets have been identified as sources for foodborne illness and associated with (multinational) outbreaks (Table 1).

9.4.1 Molecular Tools for Outbreak Investigations

The ability to distinguish strains or clonal lineages of a bacterial pathogen is essential for addressing many questions in food microbiology, epidemiology, infection prevention, and control. Traditional typing systems were based on phenotypic characteristics, such as serotype, phage type, or phenotypic antibiotic resistance profiles. However, phage typing schemes had been developed only for a few *S. enterica* serotypes, and the discriminatory power of these phenotypic methods was quite limited. Genotyping methods had been successfully established within the past decades to characterize subsets of defined strains. They provided better discriminatory power to differentiate closely related *Salmonella* strains and give more information with respect to the genetic relatedness within the population (Wattiau et al. 2011).

The detection of sequence variation within housekeeping genes is highly suitable for such studies because they are considered to be neutral in evolution and generally their function is well understood. This concept is utilized by multilocus sequence typing (MLST). Following PCR amplification and sequencing of seven housekeeping genes, MLST generates allelic profiles from these genes and can thus identify phylogenetic lineages. Further, MLST has shown that certain serovars originate from more than one common ancestor (termed as polyphyletic serovar), meaning that different lineages of the same serovar are only distantly related (Achtman et al. 2012b). However, due to its limited resolution 7-locus MLST is not an appropriate tool for outbreak investigations.

Methods for outbreak investigations and for tracing contamination within the food chain should be highly discriminative. This had been achieved by digesting the DNA from the strain(s) of interest with specific restriction endonucleases and separating the fragments, e.g., using pulsed-field gel electrophoresis (PFGE), fluorescent amplified fragment length polymorphism (fAFLP), or ribotyping, each providing distinct band patterns. PFGE fingerprinting was the gold standard for molecular subtyping of *Salmonella* serovars (especially for outbreak investigations) for about two decades because it had been internationally standardized and provided the highest level of strain discrimination in the pre-NGS era (Gerner-Smidt and Scheutz 2006; Swaminathan et al. 2006).

Another molecular approach was the multilocus variable number of tandem repeat analysis (MLVA), which is based on the determination of short repetitive tandem DNA units within defined loci by capillary electrophoresis. The more differences in the number of tandem repeat units within the set of analyzed loci are detected, the more distantly related the strains are interpreted to be. Although, MLVA schemes had been developed for a couple of serovars such as *S.* Typhimurium (Lindstedt et al. 2004), *S.* Enteritidis (Boxrud et al. 2007; Malorny et al. 2008), *S.* Infantis (Ross and Heuzenroeder 2008), *S.* Typhi (Ramisse et al. 2004), *S.* Newport (Davis et al. 2009), and *S.* Heidelberg (Young et al. 2012), only those for the epidemiologically most important serovars *S.* Typhimurium and (to a smaller degree) *S.* Enteritidis achieved international standardization and acceptance. In several countries, MLVA was applied instead (or in addition to) phage typing for strain characterization below serotype level and as a tracing tool (with limited discriminatory power) in outbreak studies (Heck 2009; Hopkins et al. 2011; Sintchenko et al. 2012).

Also another family of DNA repeats named CRISPR (clustered regularly interspaced short palindromic repeats) is highly polymorphic in *Salmonella spp.* (Fabre et al. 2012). CRISPR are supposed to confer resistance to foreign DNA, such as plasmids and phages. The spacer content of a strain reflects previous DNA insertions and therefore can provide evolutionary information. It strongly correlates with both, serovar and multilocus sequence types. The discriminatory power based on variations in the spacer content (loss, acquisition, duplication of spacers, or point mutations within spacers) is regarded similar to that of former gold standard methods, such as PFGE. CRISPR strain characterization was therefore regarded as a potential alternative to both serotyping and PFGE (Fabre et al. 2012).

9.4.2 Whole-Genome Sequencing: A Game Changer for Molecular Typing and Outbreak Investigation of Foodborne Disease

In recent decades, a variety of immunological, biochemical, and molecular techniques have been developed for the detection, characterization, and typing of foodborne bacteria (Li et al. 2009). However, especially the introduction of whole-genome sequencing (WGS) in routine diagnostics in public health, veterinary, and food safety laboratories has revolutionized the surveillance of foodborne pathogens and microbial food safety (Ronholm 2018; Taboada et al. 2017).

Bacterial whole-genome sequencing data can be used for bacterial typing, outbreak investigation, source tracking, and surveillance, and its application is highly encouraged by international authorities (EFSA et al. 2019; ECDC 2016).

By now, there are several examples for the valued application of WGS for outbreak investigation and routine surveillance typing in different countries and contexts (Leekitcharoenphon et al. 2019; Hoffmann et al. 2020; Simon et al. 2018; Meinen et al. 2019). For WGS of bacteria such as *Salmonella*, genomic DNA extracted from a bacterial isolate culture is yet the basis for the routine utilization of sequencing data. However, with new technologies rising, metagenomic approaches will also soon find their way into routine applications (EFSA et al. 2019). The isolated DNA is the starting point for the device-dependent sequencing preparation procedure (also called library preparation process). After preparation, the DNA is sequenced using a suitable sequencing platform (EFSA et al. 2019) (Fig. 1). While short-read sequencing is

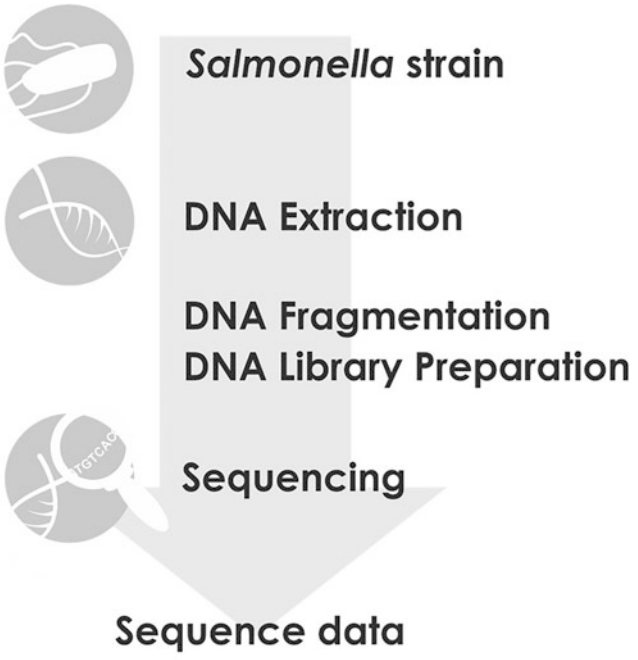

Fig. 1 Whole genome sequencing workflow

standard procedure for routine application today, long-read procedures are developed to generate complete bacterial genomes. These long-read sequencing approaches have specific application areas including the reconstruction of gene order and orientation and localization of virulence factors and antimicrobial resistance genes on mobile elements for the evaluation of potential transmission risks (Ben Khedher et al. 2022).

International standardization efforts to define universally applicable quality criteria are currently developed (EFSA et al. 2019, DIN EN ISO 23418:2020). These are needed not only for quality-assured data generation and metadata sharing but also for data evaluation software and pipelines. This is necessary in order to share and compare the generated WGS data nationally and globally across the food safety, environmental and public health sectors (EFSA et al. 2019).

9.4.2.1 WGS-Based *Salmonella* Typing

With the entire genetic information of the organism at hand, the analysis of WGS data is able to replace or extend classical methods used in *Salmonella* typing. *Salmonella* serovars can be predicted using bioinformatics software such as SeqSero (Zhang et al. 2015, 2019) or the *Salmonella* in silico *typing resource* SISTR (Yoshida et al. 2016). Classical 7-gene multilocus sequencing typing (MLST) used to further discriminate *Salmonella* isolates (Achtman et al. 2012a) was previously assessed by means of PCR and can be now determined in silico by comparing WGS data against the Enterobase MLST database also available at PubMLST (Jolley et al. 2018; Jolley and Maiden 2010).

Additionally, conventional phenotypic screening methods can be complemented. Antibiotic resistance markers and corresponding phenotypic traits of *Salmonella* can be predicted by directly searching *Salmonella* WGS data for the presence of antimicrobial resistance genes (Anjum 2015). For this purpose, bioinformatics tools searching comprehensive databases containing sequence data of resistance genes and chromosomal point mutations associated with resistance phenotypes are provided to the scientific community. Examples are the ResFinder tool, searching the ResFinder and PointFinder databases (Bortolaia et al. 2020; Zankari et al. 2017), and AMRFinderPlus, searching the NCBI Pathogen Detection Reference Gene Catalog (Feldgarden et al. 2021). The latter can also be used to identify acid, biocide, metal, and stress resistance and virulence genes (Feldgarden et al. 2021). Moreover, mobile genetic elements (plasmids, transposons, phages, pathogenicity islands), which often carry resistance or virulence genes and are transmissible between bacterial hosts, can be classified. This allows to investigate the epidemiology of plasmid-mediated antibiotic resistance and virulence (Orlek et al. 2017). To further analyze genomes, genome annotation programs are available to predict genes and get a deeper insight into the microbial gene content, genomic organization, and metabolic traits (Ruiz-Perez et al. 2021).

Users can choose to install bioinformatics tools locally, if they have a suitable computer infrastructure available. However, there are also possibilities to use web-based typing and characterization tools as provided by the Center of Genomic Epidemiology (CGE) (https://www.genomicepidemiology.org/) or the Pathosystems Resource Integration Center (PATRIC) (https://www.patricbrc.org/).

9.4.2.2 WGS-Based *Salmonella* Outbreak Detection

Thanks to the high resolution and rapid data provision of WGS methods, outbreaks can be analyzed in a much higher discriminatory manner and quicker as with previously applied subtyping methods, like phage typing or pulsed-field gel electrophoresis (PFGE). This helps to carry out intervention in a more targeted and rapid way (U.N., Food and Agriculture Organization 2016). To determine the phylogenetic relationship of isolates based on WGS data, two main approaches exist: (a) reference-based mapping approach with subsequent variant identification and (b) whole or core genome multilocus sequence typing (wg/cgMLST) (Fig. 2).

In the reference-based mapping approach, sequencing reads are aligned against a reference genome to identify single nucleotide polymorphisms (SNPs). By alignment of sequence reads of different isolates against the same reference genome and comparison of the detected SNP positions, distance matrices can be calculated and/or phylogenetic trees can be constructed to investigate the relatedness of different *Salmonella* isolates (WHO 2018) (Fig. 3).

In cgMLST, de novo assembled WGS data is used to determine the allelic variation of a predefined set of genes from the *Salmonella* core genome. Based on the allelic differences of the analyzed isolates, a distance matrix can be calculated and a phylogenetic tree can be inferred (Schürch et al. 2018; Alikhan et al. 2018).

Both, SNP analysis and cgMLST can be applied for *Salmonella* outbreak analysis by individual laboratories using available commercial or open source software. Due to their different approaches, each method comes with its own advantages: Fitted with the best possible reference genome for the data set, the mapping-based

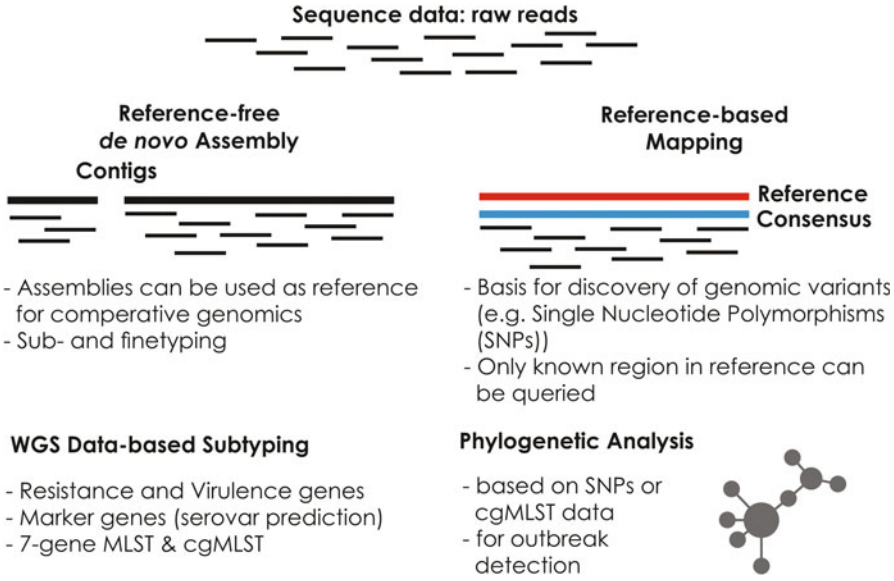

Fig. 2 Bioinformatic analysis of sequence data: *De novo* assembly and reference-based mapping are exemplary bioinformatics approaches for the determination of phylogenetic relationships

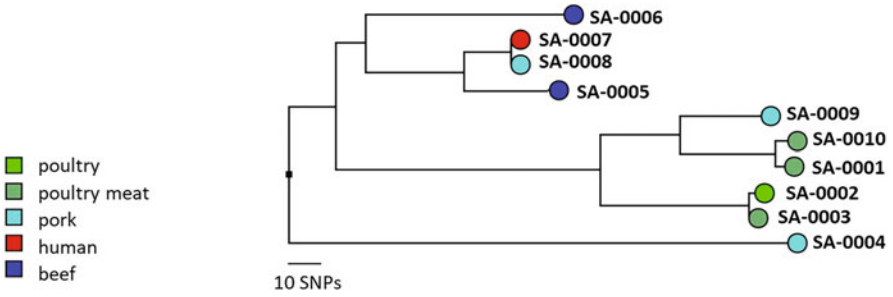

Fig. 3 Schematic SNP-based phylogenetic tree of *Salmonella* Infantis from human, animal and food samples. Isolates SA-0007 from human and SA-0008 from pork are indistinguishable (0 SNPs)

approach enables the highest possible resolution by including intergenic regions into the analysis. However, an interlaboratory comparison with SNP-based analysis is more difficult as well as time and computing intensive to achieve. The cgMLST approach on the other hand offers a sufficiently high (but not so in depth as the SNP approach) resolution for outbreak analyses and comes (usually) with the advantage of a common nomenclature (likewise to STs) and easier data sharing possibilities due to the stable core genome set.

WGS is already used for *Salmonella* outbreak analysis by government agencies in several countries on routine basis (Uelze et al. 2021a; Brown et al. 2019; Gymoese et al. 2017; Simon et al. 2018).

In order to take the full advantage offered by WGS, the data must be placed in an international context to perform real-time outbreak investigation in a global manner. Therefore, different public online platforms and surveillance networks have been established. The GenomeTrakr network and the NCBI Pathogen Detection Browser use SNP-based approaches, which allow participating laboratories to compare their *Salmonella* isolate data to the international NCBI pathogen detection database, which already includes data for more than 400,000 *Salmonella* isolates (Timme et al. 2019) (https://www.ncbi.nlm.nih.gov/pathogens/isolates/#taxgroup_name:"Salmonella enterica" 18.03.2022). Similarly, Enterobase allows international outbreak investigation based on cgMLST by providing a public and stable *Salmonella* cgMLST scheme, a public allele database and a *Salmonella* isolate database including already more than 300,000 *Salmonella* isolates (Alikhan et al. 2018) (https://enterobase.warwick.ac.uk/, 18.03.2022).

9.4.2.3 WGS-Based Salmonella Surveillance and Source Tracking

Moreover, WGS-based Salmonella surveillance allows to globally monitor the emergence and spread of Salmonella lineages (Li et al. 2021; Rantsiou et al. 2018) and thus the emergence and spread of novel serovars (Meinen et al. 2019) or of multidrug-resistant or highly virulent Salmonella lineages (EFSA et al. 2019).

As WGS data offers numerous of characteristic markers of an organism and enables phylogenetic differentiation in depth, the data can be used to trace the

transmission routes of foodborne bacterial pathogens and develop source attribution models extending classical source attribution approaches. So far, only few pilot and benchmarking studies in this field were conducted (Merlotti et al. 2020; Munck et al. 2020; Sévellec et al. 2020). However, these source attribution models for Salmonella are promising for future development in the context of identifying contamination source in the food chain (Brown et al. 2021; EFSA 2019; Rantsiou et al. 2018).

References

Abe K, Saito N, Kasuga F, Yamamoto S (2004) Prolonged incubation period of salmonellosis associated with low bacterial doses. J Food Prot 67:2735–2740

Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Uesbeck A, Dougan G, Harrison LH, Brisse S, Group, S. E. M. S (2012a) Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. PLoS Pathog 8:e1002776

Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Uesbeck A, Dougan G, Harrison LH, Brisse S, The SEMSG (2012b) Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. PLoS Pathog 8:e1002776

Agbor TA, Mccormick BA (2011) *Salmonella* effectors: important players modulating host cell function during infection. Cell Microbiol 13:1858–1869

Alikhan NF, Zhou Z, Sergeant MJ, Achtman M (2018) A genomic overview of the population structure of *Salmonella*. PLoS Genet 14:e1007261

Alvarez-Ordóñez A, Fernández A, Bernardo A, López M (2010) Acid tolerance in *Salmonella* Typhimurium induced by culturing in the presence of organic acids at different growth temperatures. Food Microbiol 27:44–49

Álvarez-Ordóñez A, Begley M, Prieto M, Messens W, López M, Bernardo A, Hill C (2011) *Salmonella* spp. survival strategies within the host gastrointestinal tract. Microbiology (Reading) 157:3268–3281

Alvseike O, Nerbrink E, Skjerve E, Nesbakken T (2000) Growth of *Salmonella choleraesuis* subspecies diarizonae serovar 61:k:1,5,(7) in broth and fresh mutton. Int J Food Microbiol 57: 159–167

Anjum MF (2015) Screening methods for the detection of antimicrobial resistance genes present in bacterial isolates and the microbiota. Future Microbiol 10:317–320

Artes F, Gomez PA, Artes-Hernandez F (2007) Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. Food Sci Technol Int 13:177–188

Ashton P, Nair S, Peters T, Tewolde R, Day M, Doumith M, Green J, Jenkins C, Underwood A, Arnold C, De Pinna E, Dallman T, Grant K (2015) Revolutionising public health reference microbiology using whole genome sequencing: *Salmonella* as an exemplar. bioRxiv 2015. https://doi.org/10.1101/033225

Barrett KA, Nakao JH, Taylor EV, Eggers C, Gould LH (2017) Fish-associated foodborne disease outbreaks: United States, 1998-2015. Foodborne Pathog Dis 14:537–543

Baumler AJ, Hargis BM, Tsolis RM (2000) Tracing the origins of *Salmonella* outbreaks. Science 287:50–52

Bayer CBH, Prager R, Rabsch W, Hiller P, Malorny B, Pfefferkorn B, Frank C, De Jong A, Friesema I, Stark K, Rosner BM (2013) An outbreak of *Salmonella* Newport associated with mung bean sprouts in Germany and the Netherlands, October-November 2011. Eurosurveillance (submitted)

Ben Khedher M, Ghedira K, Rolain J-M, Ruimy R, Croce O (2022) Application and challenge of 3rd generation sequencing for clinical bacterial studies. Int J Mol Sci 23:1395

Bessa LW, Pieterse E, Sigge G, Hoffman LC (2020) Insects as human food; from farm to fork. J Sci Food Agric 100:5017–5022

Beuchat LR (2002) Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect 4:413–423

Bierschenk D, Boucher D, Schroder K (2017) *Salmonella*-induced inflammasome activation in humans. Mol Immunol 86:38–43

Bolton DJ, Pearce R, Sheridan JJ, Mcdowell DA, Blair IS (2003) Decontamination of pork carcasses during scalding and the prevention of *Salmonella* cross-contamination. J Appl Microbiol 94:1036–1042

Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykasenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM (2020) ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500

Boxrud D, Pederson-Gulrud K, Wotton J, Medus C, Lyszkowicz E, Besser J, Bartkus JM (2007) Comparison of multiple-locus variable-number tandem repeat analysis, pulsed-field gel electrophoresis, and phage typing for subtype analysis of *Salmonella enterica* serotype Enteritidis. J Clin Microbiol 45:536–543

Brandl MT, Sundin GW (2014) Focus on food safety: human pathogens on plants. Phytopathology. PHYTO10340304test

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B (2000) *Salmonella* nomenclature. J Clin Microbiol 38:2465–2467

Brown E, Dessai U, Mcgarry S, Gerner-Smidt P (2019) Use of whole-genome sequencing for food safety and public health in the United States. Foodborne Pathog Dis 16:441–450

Brown B, Allard M, Bazaco MC, Blankenship J, Minor T (2021) An economic evaluation of the whole genome sequencing source tracking program in the U.S. PLoS One 16:e0258262

Byrne L, Fisher I, Peters T, Mather A, Thomson N, Rosner B, Bernard H, Aiyedun V, Lane C (2013) A multi-country outbreak of *Salmonella* Newport gastroenteritis in Europe associated with watermelon from Brazil, confirmed by whole genome sequencing: October 2011–January 2012. Eurosurveillance (submitted)

Centre for Disease Control and Prevention (CDC) (2021a) *Salmonella* outbreak linked to Frozen cooked shrimp [Online]. Available: https://www.cdc.gov/salmonella/weltevreden-06-21/index.html. Accessed 15 Aug 2022

Centre for Disease Control and Prevention (CDC) (2021b) *Salmonella* outbreak linked to seafood [Online]. Available: https://www.cdc.gov/salmonella/thompson-10-21/index.html. Accessed 15 Aug 2022

Centre for Disease Control and Prevention (CDC) and U.S. Food and Drug Administration (FDA) (2021) Interagency Food Safety Analytics Collaboration. Foodborne illness source attribution estimates for 2019 for *Salmonella*, *Escherichia coli* O157, *Listeria monocytogenes* and *Campylobacter* using multi-year outbreak surveillance data, United States. GA and D.C

Cheng RA, Eade CR, Wiedmann M (2019) Embracing diversity: differences in virulence mechanisms, disease severity, and host adaptations contribute to the success of nontyphoidal salmonella as a foodborne pathogen. Front Microbiol 10:1368

Chiodini RJ, Sundberg JP (1981) Salmonellosis in reptiles: a review. Am J Epidemiol 113:494–499

Chitrakar B, Zhang M, Adhikari B (2019) Dehydrated foods: are they microbiologically safe? Crit Rev Food Sci Nutr 59:2734–2745

Cleary P, Browning L, Coia J, Cowden J, Fox A, Kearney J, Lane C, Mather H, Quigley C, Syed Q, Tubin-Delic D, Outbreak Control, T (2010) A foodborne outbreak of *Salmonella* Bareilly in the United Kingdom, 2010. Euro Surveill 15:19732

Corrente M, Sangiorgio G, Grandolfo E, Bodnar L, Catella C, Trotta A, Martella V, Buonavoglia D (2017) Risk for zoonotic *Salmonella* transmission from pet reptiles: a survey on knowledge, attitudes and practices of reptile-owners related to reptile husbandry. Prev Vet Med 146:73–78

Da Silva Felício MT, Hald T, Liebana E, Allende A, Hugas M, Nguyen-The C, Johannessen GS, Niskanen T, Uyttendaele M, Mclauchlin J (2015) Risk ranking of pathogens in ready-to-eat unprocessed foods of non-animal origin (FoNAO) in the EU: initial evaluation using outbreak data (2007-2011). Int J Food Microbiol 195:9–19

Davis MA, Baker KN, Call DR, Warnick LD, Soyer Y, Wiedmann M, Grohn Y, Mcdonough PL, Hancock DD, Besser TE (2009) Multilocus variable-number tandem-repeat method for typing *Salmonella enterica* serovar Newport. J Clin Microbiol 47:1934–1938

Denny J, Threlfall J, Takkinen J, Lofdahl S, Westrell T, Varela C, Adak B, Boxall N, Ethelberg S, Torpdahl M, Straetemans M, Van Pelt W (2007) Multinational *Salmonella* Paratyphi B variant Java (Salmonella Java) outbreak, August - December 2007. Euro Surveill 12:E071220.2

Dewanti R, Doyle MP (1992) Influence of cultural conditions on cytotoxin production by *Salmonella* Enteritidis. J Food Prot 55:28–33

Donachie A, Melillo T, Bubba L, Hartman H, Borg ML (2018) National outbreak of *Salmonella* give linked to a local food manufacturer in Malta, October 2016. Epidemiol Infect 146:1425–1432

Dong K, Zhu Y, Deng Q, Sun L, Yang S, Huang K, Cao Y, Li Y, Wu S, Huang R (2022) *Salmonella* pSLT-encoded effector SpvB promotes RIPK3-dependent necroptosis in intestinal epithelial cells. Cell Death Dis 8:44

Dyda A, Nguyen P-Y, Chughtai AA, Macintyre CR (2020) Changing epidemiology of *Salmonella* outbreaks associated with cucumbers and other fruits and vegetables. Global Biosecurity 2(1)

Echeita MA, Aladueña A, Cruchaga S, Usera MA (1999) Emergence and spread of an atypical *Salmonella enterica* subsp. *enterica* serotype 4,5,12:i:- strain in Spain. J Clin Microbiol 37:3425

Ehuwa O, Jaiswal AK, Jaiswal S (2021) *Salmonella*, food safety and food handling practices. Foods 10:907

Emberland KE, Ethelberg S, Kuusi M, Vold L, Jensvoll L, Lindstedt BA, Nygard K, Kjelso C, Torpdahl M, Sorensen G, Jensen T, Lukinmaa S, Niskanen T, Kapperud G (2007) Outbreak of *Salmonella* Weltevreden infections in Norway, Denmark and Finland associated with alfalfa sprouts, July-October 2007. Euro Surveill 12(E071129):4

European Centre for Disease Prevention and Control (ECDC) (2016) Expert opinion on whole genome sequencing for public health surveillance: strategy to harness whole genome sequencing to strengthen EU outbreak investigations and public health surveillance. Centre for Disease Prevention and Control, European

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) (2019) Multi-country outbreak S. Poona infections linked to consumption of infant formula. EFSA Supporting Publications

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) (2021) Multi-country outbreak of *Salmonella* Braenderup ST22, presumed to be linked to imported melons. EFSA Support Publ 18:6807E

European Food Safety Authority (EFSA) (2007) Report of the task force on zoonoses data collection on the analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*. EFSA J 5:97r

European Food Safety Authority (EFSA) (2014a) Multi-country outbreak of *Salmonella* Enteritidis infections associated with consumption of eggs from Germany. EFSA Support Publ 11:646E

European Food Safety Authority (EFSA) (2014b) Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in leafy greens eaten raw as salads). EFSA J 12:3600

European Food Safety Authority (EFSA) (2015) Risk profile related to production and consumption of insects as food and feed. EFSA J 2015;13(10):4257

European Food Safety Authority (EFSA) (2021) Multi-country outbreak of multiple *Salmonella enterica* serotypes linked to imported sesame-based products. EFSA Support Publ 18:6922E

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2013) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J 3129 [250 pp.]

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2018) Multi-country outbreak of *Salmonella* Agona infections linked to infant formula. EFSA Support Publ 15:1365E

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2020) Multi-country outbreak of *Salmonella* Enteritidis infections linked to eggs, third update – 6 February 2020. EFSA Support Publ 17:1799E

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2021a) The European Union one health 2019 zoonoses report. EFSA J 19:6971
European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2021b) The European Union one health 2019 zoonoses report. EFSA J 19:e06406
European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2021c) The European Union one health 2020 zoonoses report. EFSA J 19:e06971
European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2022) Multi-country outbreak of *Salmonella* Enteritidis sequence type (ST)11 infections linked to eggs and egg products – 8 February 2022. EFSA Support Publ 19:7180E
European Food Safety Authority (EFSA), P. O. B. H, Koutsoumanis K, Allende A, Alvarez-Ordonez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Jenkins C, Malorny B, Ribeiro Duarte AS, Torpdahl M, Da Silva Felicio MT, Guerra B, Rossi M, Herman L (2019) Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms. EFSA J 17:e05898
Fabre L, Zhang J, Guigon G, Le Hello S, Guibert V, Accou-Demartin M, De Romans S, Lim C, Roux C, Passet V, Diancourt L, Guibourdenche M, Issenhuth-Jeanjean S, Achtman M, Brisse S, Sola C, Weill FX (2012) CRISPR typing and subtyping for improved laboratory surveillance of *Salmonella* infections. PLoS One 7:e36995
Fabrega A, Vila J (2013) *Salmonella enterica* serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev 26:308–341
Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W (2021) AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep 11:12728
Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA (2019) Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. Appl Environ Microbiol 85:e00591-19
Finn S, Condell O, McClure P, Amezquita A, Fanning S (2013) Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. Front Microbiol 4:331
Foley SL, Johnson TJ, Ricke SC, Nayak R, Danzeisen J (2013) *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev 77:582–607
Food and Agriculture Organization of the United Nations (2016) FAO_Applications of Whole Genome Sequencing in food safety management.pdf
Gerner-Smidt P, Scheutz F (2006) Standardized pulsed-field gel electrophoresis of Shiga toxin-producing *Escherichia coli*: the PulseNet Europe Feasibility Study. Foodborne Pathog Dis 3: 74–80
Gieraltowski L, Julian E, Pringle J, Macdonald K, Quilliam D, Marsden-Haug N, Saathoff-Huber L, Von Stein D, Kissler B, Parish M, Elder D, Howard-King V, Besser J, Sodha S, Loharikar A, Dalton S, Williams I, Barton Behravesh C (2012) Nationwide outbreak of *Salmonella* Montevideo infections associated with contaminated imported black and red pepper: warehouse membership cards provide critical clues to identify the source. Epidemiol Infect: 2013 Jun;141(6):1244–52.
Gilchrist JJ, Maclennan CA (2019) Invasive nontyphoidal *Salmonella* disease in Africa. EcoSal Plus 2019 Jan;8(2)
Gobin M, Launders N, Lane C, Kafatos G, Adak B (2011) National outbreak of *Salmonella* Java phage type 3b variant 9 infection using parallel case-control and case-case study designs, United Kingdom, July to October 2010. Euro Surveill 16:20023
Golden CE, Mishra A (2020) Prevalence of *Salmonella* and *Campylobacter* spp. in alternative and conventionally produced Chicken in the United States: a systematic review and meta-analysis. J Food Prot 83:1181–1197
Gordon MA (2008) *Salmonella* infections in immunocompromised adults. J Infect 56:413–422
Greene SK, Daly ER, Talbot EA, Demma LJ, Holzbauer S, Patel NJ, Hill TA, Walderhaug MO, Hoekstra RM, Lynch MF, Painter JA (2008) Recurrent multistate outbreak of Salmonella Newport associated with tomatoes from contaminated fields, 2005. Epidemiol Infect 136:157–165

Grimont PAD, Weill FX (2007) Antigenic formulae of the Salmonella serovars. WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France

Gupta SK, Nalluswami K, Snider C, Perch M, Balasegaram M, Burmeister D, Lockett J, Sandt C, Hoekstra RM, Montgomery S (2007) Outbreak of *Salmonella* Braenderup infections associated with Roma tomatoes, northeastern United States, 2004: a useful method for subtyping exposures in field investigations. Epidemiol Infect 135:1165–1173

Gutema FD, Agga GE, Abdi RD, De Zutter L, Duchateau L, Gabriël S (2019) Prevalence and serotype diversity of *Salmonella* in apparently healthy cattle: systematic review and meta-analysis of published studies, 2000–2017. Front Vet Sci:6

Gymoese P, Sørensen G, Litrup E, Olsen JE, Nielsen EM, Torpdahl M (2017) Investigation of outbreaks of *Salmonella enterica* Serovar typhimurium and its monophasic variants using whole-genome sequencing, Denmark. Emerg Infect Dis J 23:1631

Habimana O, Nesse LL, Moretro T, Berg K, Heir E, Vestby LK, Langsrud S (2014) The persistence of *Salmonella* following desiccation under feed processing environmental conditions: a subject of relevance. Lett Appl Microbiol 59:464–470

Hauser E, Hühn S, Junker E, Jaber M, Schroeter A, Helmuth R, Rabsch W, Winterhoff N, Malorny B (2009) Characterisation of a phenotypic monophasic variant belonging to *Salmonella enterica* subsp. enterica serovar typhimurium from wild birds and its possible transmission to cats and humans. Berl Munch Tierarztl Wochenschr 122:169–177

Hautefort I, Thompson A, Eriksson-Ygberg S, Parker ML, Lucchini S, Danino V, Bongaerts RJ, Ahmad N, Rhen M, Hinton JC (2008) During infection of epithelial cells *Salmonella enterica* serovar Typhimurium undergoes a time-dependent transcriptional adaptation that results in simultaneous expression of three type 3 secretion systems. Cell Microbiol 10:958–984

Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, Praet N, Bellinger DC, De Silva NR, Gargouri N, Speybroeck N, Cawthorne A, Mathers C, Stein C, Angulo FJ, Devleesschauwer B (2015) World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med 12:e1001923

Hazards EPOB (2013) Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). EFSA J 11:3025

Heck M (2009) Multilocus variable number of tandem repeats analysis (MLVA)--a reliable tool for rapid investigation of *Salmonella* Typhimurium outbreaks. Euro Surveill 14:19177

Hilbert F, Smulders FJM, Chopra-Dewasthaly R, Paulsen P (2012) *Salmonella* in the wildlife-human interface. Food Res Int 45:603–608

Himathongkham S, Nuanualsuwan S, Riemann H (1999) Survival of *Salmonella* enteritidis and Salmonella typhimurium in chicken manure at different levels of water activity. FEMS Microbiol Lett 172:159–163

Hoelzer K, Moreno Switt AI, Wiedmann M (2011) Animal contact as a source of human non-typhoidal salmonellosis. Vet Res 42:34

Hoffmann M, Miller J, Melka D, Allard MW, Brown EW, Pettengill JB (2020) Temporal dynamics of Salmonella enterica subsp. enterica serovar agona isolates from a recurrent multistate outbreak. Front Microbiol 11:478

Hopkins KL, Peters TM, De Pinna E, Wain J (2011) Standardisation of multilocus variable-number tandem-repeat analysis (MLVA) for subtyping of *Salmonella enterica* serovar Enteritidis. Euro Surveill 16:19942

Ilic S, Duric P, Grego E (2010) *Salmonella* Senftenberg infections and fennel seed tea, Serbia. Emerg Infect Dis 16:893–895

International Organization for Standardization (2020) ISO/DIS 23418:2020, Microbiology of the Food Chain - Whole Genome Sequencing for Typing and Genomic Characterization of Foodborne Bacteria - General Requirements and Guidance version EN ISO 23418:2020. International Organization for Standardization, Geneva

Irvine WN, Gillespie IA, Smyth FB, Rooney PJ, Mcclenaghan A, Devine MJ, Tohani VK, Outbreak Control T (2009) Investigation of an outbreak of *Salmonella enterica* serovar Newport infection. Epidemiol Infect 137:1449–1456

Issenhuth-Jeanjean S, Roggentin P, Mikoleit M, Guibourdenche M, De Pinna E, Nair S, Fields PI, Weill FX (2014) Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. Res Microbiol 165:526–530

Jain S, Bidol SA, Austin JL, Berl E, Elson F, Lemaile-Williams M, Deasy M 3rd, Moll ME, Rea V, Vojdani JD, Yu PA, Hoekstra RM, Braden CR, Lynch MF (2009) Multistate outbreak of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice--United States, 2005. Clin Infect Dis 48:1065–1071

Jechalke S, Schierstaedt J, Becker M, Flemer B, Grosch R, Smalla K, Schikora A (2019) *Salmonella* establishment in agricultural soil and colonization of crop plants depend on soil type and plant species. Front Microbiol 10:967

Johansen TB, Brandal LT, Macdonald E, Naseer U, Stefanoff P, Røed MH, Berglund TM, Johannessen GS, Bergsjø B, Vold L, Lange H (2021) Exotic dried fruits caused *Salmonella* Agbeni outbreak with severe clinical presentation, Norway, December 2018 to March 2019. Eur Secur 26:2000221

Jolley KA, Maiden MC (2010) BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595

Jolley KA, Bray JE, Maiden MCJ (2018) Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124

Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'angelo M, Hurd S, Medus C, Cronquist A, Angulo FJ (2008) Salmonellosis outcomes differ substantially by serotype. J Infect Dis 198:109–114

Jourdan-Da Silva N, Fabre L, Robinson E, Fournet N, Nisavanh A, Bruyand M, Mailles A, Serre E, Ravel M, Guibert V, Issenhuth-Jeanjean S, Renaudat C, Tourdjman M, Septfons A, De Valk H, Le Hello S (2018) Ongoing nationwide outbreak of *Salmonella* Agona associated with internationally distributed infant milk products, France, December 2017. Euro Surveill:23, 17-00852

Kaiser P, Hardt WD (2011) Salmonella typhimurium diarrhea: switching the mucosal epithelium from homeostasis to defense. Curr Opin Immunol 23:456–463

Keller SE, Vandoren JM, Grasso EM, Halik LA (2013) Growth and survival of Salmonella in ground black pepper (Piper nigrum). Food Microbiol 34:182–188

Kent RM, Fitzgerald GF, Hill C, Stanton C, Ross RP (2015) Novel approaches to improve the intrinsic microbiological safety of powdered infant milk formula. Nutrients 7:1217–1244

Koch J, Schrauder A, Alpers K, Werber D, Frank C, Prager R, Rabsch W, Broll S, Feil F, Roggentin P, Bockemuhl J, Tschape H, Ammon A, Stark K (2005) *Salmonella* agona outbreak from contaminated aniseed, Germany. Emerg Infect Dis 11:1124–1127

Krtinić G, Durić P, Ilić S (2010) Salmonellae in food stuffs of plant origin and their implications on human health. Eur J Clin Microbiol Infect Dis 29:1321–1325

Kuhle V, Hensel M (2004) Cellular microbiology of intracellular *Salmonella enterica*: functions of the type III secretion system encoded by Salmonella pathogenicity island 2. Cell Mol Life Sci 61:2812–2826

Kumar Y, Singh V, Kumar G, Gupta NK, Tahlan AK (2019) Serovar diversity of *Salmonella* among poultry. Indian J Med Res 150:92–95

Lamas A, Miranda JM, Regal P, Vázquez B, Franco CM, Cepeda A (2018) A comprehensive review of non-enterica subspecies of Salmonella enterica. Microbiol Res 206:60–73

Larkin L, Pardos De La Gandara M, Hoban A, Pulford C, Jourdan-Da Silva N, De Valk H, Browning L, Falkenhorst G, Simon S, Lachmann R, Dryselius R, Karamehmedovic N, Borjesson S, Van Cauteren D, Laisnez V, Mattheus W, Pijnacker R, Van Den Beld M, Mossong J, Ragimbeau C, Vergison A, Thorstensen Brandal L, Lange H, Garvey P, Nielsen CS, Herrera Leon S, Varela C, Chattaway M, Weill FX, Brown D, Mckeown P (2022) Investigation of an international outbreak of multidrug-resistant monophasic *Salmonella* Typhimurium associated with chocolate products, EU/EEA and United Kingdom, February to April 2022. Euro Surveill. 2022 Apr;27(15):2200314.

Laughlin M, Bottichio L, Weiss J, Higa J, Mcdonald E, Sowadsky R, Fejes D, Saupe A, Provo G, Seelman S, Concepcion-Acevedo J, Gieraltowski L, Outbreak Investigation T (2019) Multistate outbreak of *Salmonella* Poona infections associated with imported cucumbers, 2015-2016. Epidemiol Infect 147:e270

Lawson B, Franklinos LHV, Rodriguez-Ramos Fernandez J, Wend-Hansen C, Nair S, Macgregor SK, John SK, Pizzi R, Núñez A, Ashton PM, Cunningham AA, De Pinna EM (2018) *Salmonella* Enteritidis ST183: emerging and endemic biotypes affecting western European hedgehogs (*Erinaceus europaeus*) and people in Great Britain. Sci Rep 8:2449

Ledet Muller L, Hjertqvist M, Payne L, Pettersson H, Olsson A, Plym Forshell L, Andersson Y (2007) Cluster of *Salmonella* Enteritidis in Sweden 2005–2006 – suspected source: almonds. Euro Surveill 12:E9–E10

Leekitcharoenphon P, Sorensen G, Lofstrom C, Battisti A, Szabo I, Wasyl D, Slowey R, Zhao S, Brisabois A, Kornschober C, Karssin A, Szilard J, Cerny T, Svendsen CA, Pedersen K, Aarestrup FM, Hendriksen RS (2019) Cross-border transmission of *Salmonella choleraesuis* var. Kunzendorf in European pigs and wild boar: infection, genetics, and evolution. Front Microbiol 10:179

Li W, Raoult D, Fournier PE (2009) Bacterial strain typing in the genomic era. FEMS Microbiol Rev 33:892–916

Li H, Wang H, D'aoust J-Y, Maurer J (2013) *Salmonella* species. In: Doyle MP, Buchanan RL (eds) Food microbiology. ASM Press, Washington, DC.

Li S, He Y, Mann DA, Deng X (2021) Global spread of *Salmonella* Enteritidis via centralized sourcing and international trade of poultry breeding -stocks. Nat Commun 12:5109

Lienemann T, Niskanen T, Guedes S, Siitonen A, Kuusi M, Rimhanen-Finne R (2011) Iceberg lettuce as suggested source of a nationwide outbreak caused by two Salmonella serotypes, newport and reading, in Finland in 2008. J Food Prot 74:1035–1040

Liljebjelke KA, Hofacre CL, Liu T, White DG, Ayers S, Young S, Maurer JJ (2005) Vertical and horizontal transmission of salmonella within integrated broiler production system. Foodborne Pathog Dis 2:90–102

Lindstedt BA, Vardund T, Aas L, Kapperud G (2004) Multiple-locus variable-number tandem-repeats analysis of *Salmonella* enterica subsp. enterica serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. J Microbiol Methods 59:163–172

Longo A, Losasso C, Vitulano F, Mastrorilli E, Turchetto S, Petrin S, Mantovani C, Dalla Pozza MC, Ramon E, Conedera G, Citterio CV, Ricci A, Barco L, Lettini AA (2019) Insight into an outbreak of *Salmonella choleraesuis* var. Kunzendorf in wild boars. Vet Microbiol 238: 108423

Lynch MF, Tauxe RV, Hedberg CW (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137:307–315

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'brien SJ, Jones TF, Fazil A, Hoekstra RM, International Collaboration on Enteric Disease 'Burden of Illness'Studies (2010) The Global Burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis 50:882–889

Malorny B, Junker E, Helmuth R (2008) Multi-locus variable-number tandem repeat analysis for outbreak studies of *Salmonella* enterica serotype enteritidis. BMC Microbiol 8:84

Marin C, Hernandiz A, Lainez M (2009) Biofilm development capacity of Salmonella strains isolated in poultry risk factors and their resistance against disinfectants. Poult Sci 88:424–431

Matches JR, Liston J (1972) Effect of pH on low temperature growth of salmonella. J Milk Food Technol 35:49–52

Mcdonough PL, Fogelman D, Shin SJ, Brunner MA, Lein DH (1999) *Salmonella enterica* serotype Dublin infection: an emerging infectious disease for the northeastern United States. J Clin Microbiol 37:2418–2427

Meinen A, Simon S, Banerji S, Szabo I, Malorny B, Borowiak M, Hadziabdic S, Becker N, Luber P, Lohr D, Harms C, Plenge-Bonig A, Mellou K, Mandilara G, Mossong J, Ragimbeau C, Weicherding P, Hau P, Dedicova D, Safarikova L, Nair S, Dallman TJ, Larkin L, Mccormick J, De Pinna E, Severi E, Kotila S, Niskanen T, Rizzi V, Deserio D, Flieger A, Stark K (2019) Salmonellosis outbreak with novel *Salmonella enterica* subspecies *enterica* serotype (11:z41:e,n,z15) attributable to sesame products in five European countries, 2016 to 2017. Euro Surveill. 2019 Sep;24(36):1800543

Merlotti A, Manfreda G, Munck N, Hald T, Litrup E, Nielsen EM, Remondini D, Pasquali F (2020) Network approach to source attribution of *Salmonella enterica* serovar *Typhimurium* and its monophasic variant. Front Microbiol. 2020 Jun 16;11:1205.

Mills DM, Bajaj V, Lee CA (1995) A 40 kb chromosomal fragment encoding *Salmonella* Typhimurium invasion genes is absent from the corresponding region of the Escherichia coli K-12 chromosome. Mol Microbiol 15:749–759

Morningstar-Shaw B, Mackie T, Barker D, Palmer E (2016) *Salmonella* serotypes isolated from animals and related sources. United States Department of Agriculture (USDA), National Veterinary Services Laboratories, Ames

Müller L, Kjelsø C, Frank C, Jensen T, Torpdahl M, Søborg B, Dorleans F, Rabsch W, Prager R, Gossner CM, Ethelberg S (2016) Outbreak of Salmonella Strathcona caused by datterino tomatoes, Denmark, 2011. Epidemiol Infect 144:2802–2811

Munck N, Leekitcharoenphon P, Litrup E, Kaas R, Meinen A, Guillier L, Tang Y, Malorny B, Palma F, Borowiak M, Gourmelon M, Simon S, Banerji S, Petrovska L, Dallman TJ, Hald T (2020) Four European Salmonella typhimurium datasets collected to develop WGS-based source attribution methods. Scientific Data 7:75

Munnoch SA, Ward K, Sheridan S, Fitzsimmons GJ, Shadbolt CT, Piispanen JP, Wang Q, Ward TJ, Worgan TL, Oxenford C, Musto JA, Mcanulty J, Durrheim DN (2009) A multi-state outbreak of *Salmonella* Saintpaul in Australia associated with cantaloupe consumption. Epidemiol Infect 137:367–374

Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, Opsteegh M, Langelaar M, Threfall J, Scheutz F, Van Der Giessen J, Kruse H (2010) Food-borne diseases – the challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol 139(Suppl 1):S3–S15

Noel H, Hofhuis A, De Jonge R, Heuvelink AE, De Jong A, Heck ME, De Jager C, Van Pelt W (2010) Consumption of fresh fruit juice: how a healthy food practice caused a national outbreak of *Salmonella* Panama gastroenteritis. Foodborne Pathog Dis 7:375–381

Nygård K, Lassen J, Vold L, Andersson Y, Fisher I, Löfdahl S, Threlfall J, Luzzi I, Peters T, Hampton M, Torpdahl M, Kapperud G, Aavitsland P (2008) Outbreak of Salmonella Thompson infections linked to imported rucola lettuce. Foodborne Pathog Dis 5:165–173

Okamura M, Kamijima Y, Miyamoto T, Tani H, Sasai K, Baba E (2001) Differences among six Salmonella serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. Avian Dis 45:61–69

Olaimat AN, Holley RA (2012) Factors influencing the microbial safety of fresh produce: a review. Food Microbiol 32:1–19

Olgunoğlu İA (2012) Salmonella in fish and fishery products. *Salmonella* – A dangerous foodborne pathogen. IntechOpen, London

Orlek A, Stoesser N, Anjum MF, Doumith M, Ellington MJ, Peto T, Crook D, Woodford N, Walker AS, Phan H, Sheppard AE (2017) Plasmid classification in an era of whole-genome sequencing: application in studies of antibiotic resistance epidemiology. Front Microbiol 8:182

Oscar TP (2003) Comparison of predictive models for growth of parent and green fluorescent protein–producing strains of *Salmonella*. J Food Prot 66:200–207

Park S, Szonyi B, Gautam R, Nightingale K, Anciso J, Ivanek R (2012) Risk factors for microbial contamination in fruits and vegetables at the preharvest level: a systematic review. J Food Prot 75:2055–2081

Pasmans F, Martel A, Boyen F, Vandekerchove D, Wybo I, Immerseel FV, Heyndrickx M, Collard JM, Ducatelle R, Haesebrouck F (2005) Characterization of *Salmonella* isolates from captive lizards. Vet Microbiol 110:285–291

Pearce ME, Langridge GC, Lauer AC, Grant K, Maiden MCJ, Chattaway MA (2021) An evaluation of the species and subspecies of the genus *Salmonella* with whole genome sequence data: proposal of type strains and epithets for novel S. enterica subspecies VII, VIII, IX, X and XI. Genomics 113:3152–3162

Pees M, Rabsch W, Plenz B, Fruth A, Prager R, Simon S, Schmidt V, Muench S, Braun P (2013) Evidence for the transmission of *Salmonella* from reptiles to children in Germany. Eur Secur 18 (46):20634

Pezzoli L, Elson R, Little CL, Yip H, Fisher I, Yishai R, Anis E, Valinsky L, Biggerstaff M, Patel N, Mather H, Brown DJ, Coia JE, Van Pelt W, Nielsen EM, Ethelberg S, De Pinna E, Hampton MD, Peters T, Threlfall J (2008) Packed with *Salmonella* – investigation of an international

outbreak of *Salmonella* Senftenberg infection linked to contamination of prepacked basil in 2007. Foodborne Pathog Dis 5:661–668

Pires SM, De Knegt L, Hald T (2011) Estimation of the relative contribution of different food and animal sources to human *Salmonella* infections in the European Union. EFSA Support Publ 8:184E

Poza-Carrion C, Suslow T, Lindow S (2013) Resident bacteria on leaves enhance survival of immigrant cells of *Salmonella* enterica. Phytopathology 103:341–351

Prabhakar P, Lekshmi M, Ammini P, Nayak BB, Kumar S (2020) *Salmonella* contamination of seafood in landing centers and retail markets of Mumbai, India. J AOAC Int 103:1361–1365

Pulford CV, Wenner N, Redway ML, Rodwell EV, Webster HJ, Escudero R, Kröger C, Canals R, Rowe W, Lopez J, Hall N, Rowley PD, Timofte D, Harrison RA, Baker KS, Hinton JCD (2019) The diversity, evolution and ecology of *Salmonella* in venomous snakes. PLoS Negl Trop Dis 13:e0007169

Rabsch W, Prager R, Koch J, Stark K, Roggentin P, Bockemuhl J, Beckmann G, Stark R, Siegl W, Ammon A, Tschape H (2005) Molecular epidemiology of *Salmonella enterica* serovar Agona: characterization of a diffuse outbreak caused by aniseed-fennel-caraway infusion. Epidemiol Infect 133:837–844

Ramisse V, Houssu P, Hernandez E, Denoeud F, Hilaire V, Lisanti O, Ramisse F, Cavallo JD, Vergnaud G (2004) Variable number of tandem repeats in *Salmonella* enterica subsp. enterica for typing purposes. J Clin Microbiol 42:5722–5730

Rantsiou K, Kathariou S, Winkler A, Skandamis P, Saint-Cyr MJ, Rouzeau-Szynalski K, Amézquita A (2018) Next generation microbiological risk assessment: opportunities of whole genome sequencing (WGS) for foodborne pathogen surveillance, source tracking and risk assessment. Int J Food Microbiol 287:3–9

Rimhanen-Finne R, Niskanen T, Lienemann T, Johansson T, Sjoman M, Korhonen T, Guedes S, Kuronen H, Virtanen MJ, Makinen J, Jokinen J, Siitonen A, Kuusi M (2011) A nationwide outbreak of *Salmonella bovismorbificans* associated with sprouted alfalfa seeds in Finland, 2009. Zoonoses Public Health 58:589–596

Robert Koch-Institut (2002) Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2001, Berlin

Robert Koch-Institut (2020) Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2019, Berlin

Rohekar S, Tsui FW, Tsui HW, Xi N, Riarh R, Bilotta R, Inman RD (2008) Symptomatic acute reactive arthritis after an outbreak of salmonella. J Rheumatol 35:1599–1602

Ronholm J (2018) Editorial: game changer – next generation sequencing and its impact on food microbiology. Front Microbiol 9:363

Ross IL, Heuzenroeder MW (2008) A comparison of three molecular typing methods for the discrimination of Salmonella enterica serovar Infantis. FEMS Immunol Med Microbiol 53: 375–384

Ruiz-Perez CA, Conrad RE, Konstantinidis KT (2021) MicrobeAnnotator: a user-friendly, comprehensive functional annotation pipeline for microbial genomes. BMC Bioinformatics 22:11

Santillana Farakos SM, Frank JF, Schaffner DW (2013) Modeling the influence of temperature, water activity and water mobility on the persistence of *Salmonella* in low-moisture foods. Int J Food Microbiol 166:280–293

Santos RL, Raffatellu M, Bevins CL, Adams LG, Tukel C, Tsolis RM, Baumler AJ (2009) Life in the inflamed intestine, *Salmonella* style. Trends Microbiol 17:498–506

Schat KA, Nagaraja KV, Saif YM (2021) Pullorum disease: evolution of the eradication strategy. Avian Dis 65:227–236

Schielke A, Rabsch W, Prager R, Simon S, Fruth A, Helling R, Schnabel M, Siffczyk C, Wieczorek S, Schroeder S, Ahrens B, Oppermann H, Pfeiffer S, Merbecks SS, Rosner B, Frank C, Weiser AA, Luber P, Gilsdorf A, Stark K, Werber D (2017) Two consecutive large outbreaks of *Salmonella* Muenchen linked to pig farming in Germany, 2013 to 2014: is something missing in our regulatory framework? Eur Secur 22:30528

Schürch AC, Arredondo-Alonso S, Willems RJL, Goering RV (2018) Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene–based approaches. Clin Microbiol Infect 24:350–354

Sévellec Y, Granier SA, Le Hello S, Weill FX, Guillier L, Mistou MY, Cadel-Six S (2020) Source attribution study of sporadic *Salmonella* Derby cases in France. Front Microbiol 11:889

Shah DH, Paul NC, Sischo WC, Crespo R, Guard J (2017) Population dynamics and antimicrobial resistance of the most prevalent poultry-associated *Salmonella* serotypes. Poult Sci 96:687–702

Shea JE, Hensel M, Gleeson C, Holden DW (1996) Identification of a virulence locus encoding a second type III secretion system in *Salmonella* Typhimurium. Proc Natl Acad Sci U S A 93:2593–2597

Siira L, Macdonald E, Holmbakken GM, Sundar T, Meyer-Myklestad L, Lange H, Brandal LT, Naseer U, Johannessen GS, Bergsjø B, Espenhain L, Vold L, Nygård K (2019) Increasing incubation periods during a prolonged monophasic *Salmonella* Typhimurium outbreak with environmental contamination of a commercial kitchen at Oslo Airport, Norway, 2017. Euro Surveill. 2019 Aug;24(34):1900207.

Simon S, Trost E, Bender J, Fuchs S, Malorny B, Rabsch W, Prager R, Tietze E, Flieger A (2018) Evaluation of WGS based approaches for investigating a food-borne outbreak caused by *Salmonella* enterica serovar Derby in Germany. Food Microbiol 71:46–54

Sintchenko V, Wang Q, Howard P, Ha CW, Kardamanidis K, Musto J, Gilbert GL (2012) Improving resolution of public health surveillance for human *Salmonella enterica* serovar Typhimurium infection: 3 years of prospective multiple-locus variable-number tandem-repeat analysis (MLVA). BMC Infect Dis 12:78

Sivapalasingam S, Barrett E, Kimura A, Van Duyne S, De Witt W, Ying M, Frisch A, Phan Q, Gould E, Shillam P, Reddy V, Cooper T, Hoekstra M, Higgins C, Sanders JP, Tauxe RV, Slutsker L (2003) A multistate outbreak of *Salmonella* enterica Serotype Newport infection linked to mango consumption: impact of water-dip disinfestation technology. Clin Infect Dis 37:1585–1590

Skov MN, Madsen JJ, Rahbek C, Lodal J, Jespersen JB, Jorgensen JC, Dietz HH, Chriel M, Baggesen DL (2008) Transmission of *Salmonella* between wildlife and meat-production animals in Denmark. J Appl Microbiol 105:1558–1568

Sojka WJ, Wray C, Shreeve J, Benson AJ (1977) Incidence of salmonella infection in animals in England and Wales 1968–1974. J Hyg (Lond) 78:43–56

Stam F, Romkens TE, Hekker TA, Smulders YM (2003) Turtle-associated human salmonellosis. Clin Infect Dis 37:e167–e169

Statistisches Bundesamt, S (1993) Meldepflichtige Krankheiten 1992, Stuttgart

Stocker P, Rosner B, Werber D, Kirchner M, Reinecke A, Wichmann-Schauer H, Prager R, Rabsch W, Frank C (2011) Outbreak of Salmonella Montevideo associated with a dietary food supplement flagged in the Rapid Alert System for Food and Feed (RASFF) in Germany, 2010. Euro Surveill 16:20040

Suzuki S (1994) Pathogenicity of *Salmonella* enteritidis in poultry. Int J Food Microbiol 21:89–105

Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, Rolando S, Gutierrez EP, Binsztein N (2006) Building PulseNet international: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. Foodborne Pathog Dis 3:36–50

Tabarani CM, Bennett NJ, Kiska DL, Riddell SW, Botash AS, Domachowske JB (2010) Empyema of preexisting subdural hemorrhage caused by a rare *Salmonella* species after exposure to bearded dragons in a foster home. J Pediatr 156:322–323

Taboada EN, Graham MR, Carrico JA, Van Domselaar G (2017) Food safety in the age of next generation sequencing, bioinformatics, and open data access. Front Microbiol 8:909

Thomas KM, De Glanville WA, Barker GC, Benschop J, Buza JJ, Cleaveland S, Davis MA, French NP, Mmbaga BT, Prinsen G, Swai ES, Zadoks RN, Crump JA (2020) Prevalence of *Campylobacter* and *Salmonella* in African food animals and meat: A systematic review and meta-analysis. Int J Food Microbiol 315:108382

Thomson RM, Henderson HJ, Smith-Palmer A (2019) An outbreak of *Salmonella* Saintpaul in a Scottish childcare facility: the influence of parental under-reporting. BMC Infect Dis 19:847

Timme RE, Sanchez Leon M, Allard MW (2019) Utilizing the public GenomeTrakr database for foodborne pathogen traceback. In: Bridier A (ed) Foodborne bacterial pathogens: methods and protocols. Springer New York, New York

Tindall BJ, Grimont PAD, Garrity GM, Euzéby JP (2005) Nomenclature and taxonomy of the genus *Salmonella*. Int J Syst Evol Microbiol 55:521–524

U.S. Food and Drug Administration (FDA) (2022) NARMS now. In: SERVICES, U. S. D. O. H. A. H. (ed.). Rockville. Available from https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/narms-now-integrated-data. Accessed 14 Mar 2022

Uelze L, Becker N, Borowiak M, Busch U, Dangel A, Deneke C, Fischer J, Flieger A, Hepner S, Huber I, Methner U, Linde J, Pietsch M, Simon S, Sing A, Tausch SH, Szabo I, Malorny B (2021a) Toward an integrated genome-based surveillance of *Salmonella enterica* in Germany. Front Microbiol 12:626941

Uelze L, Bloch A, Borowiak M, Grobbel M, Deneke C, Fischer M, Malorny B, Pietsch M, Simon S, Szabo I, Tausch SH, Fischer J (2021b) What WGS reveals about *Salmonella* enterica subsp. enterica in wildlife in Germany. Microorganisms 9:1911

Ung A, Baidjoe AY, Van Cauteren D, Fawal N, Fabre L, Guerrisi C, Danis K, Morand A, Donguy M-P, Lucas E, Rossignol L, Lefèvre S, Vignaud M-L, Cadel-Six S, Lailler R, Jourdan-Da Silva N, Le Hello S (2019) Disentangling a complex nationwide *Salmonella* Dublin outbreak associated with raw-milk cheese consumption, France, 2015 to 2016. Eur Secur 24:1700703

Van Meervenne E, Botteldoorn N, Lokietek S, Vatlet M, Cupa A, Naranjo M, Dierick K, Bertrand S (2009) Turtle-associated *Salmonella* septicaemia and meningitis in a 2-month-old baby. J Med Microbiol 58:1379–1381

Vandeweyer D, De Smet J, Van Looveren N, Van Campenhout L (2021) Biological contaminants in insects as food and feed. J Insects Food Feed 7:807–822

Velge P, Wiedemann A, Rosselin M, Abed N, Boumart Z, Chaussé AM, Grépinet O, Namdari F, Roche SM, Rossignol A, Virlogeux-Payant I (2012) Multiplicity of *Salmonella* entry mechanisms, a new paradigm for *Salmonella* pathogenesis. Microbiology 1:243–258

Wallis TS, Barrow PA (2005) *Salmonella* epidemiology and pathogenesis in food-producing animals. EcoSal Plus 2005 Nov;1(2).

Wan Norhana MN, Poole SE, Deeth HC, Dykes GA (2010) Prevalence, persistence and control of *Salmonella* and *Listeria* in shrimp and shrimp products: a review. Food Control 21:343–361

Wang X, Wang H, Li T, Liu F, Cheng Y, Guo X, Wen G, Luo Q, Shao H, Pan Z, Zhang T (2020) Characterization of *Salmonella* spp. isolated from chickens in Central China. BMC Vet Res 16:299

Wattiau P, Boland C, Bertrand S (2011) Methodologies for *Salmonella* enterica subsp. enterica subtyping: gold standards and alternatives. Appl Environ Microbiol 77:7877–7885

Werber D, Dreesman J, Feil F, Van Treeck U, Fell G, Ethelberg S, Hauri AM, Roggentin P, Prager R, Fisher IS, Behnke SC, Bartelt E, Weise E, Ellis A, Siitonen A, Andersson Y, Tschape H, Kramer MH, Ammon A (2005) International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infect Dis 5:7

Werber D, Hille K, Frank C, Dehnert M, Altmann D, Muller-Nordhorn J, Koch J, Stark K (2013) Years of potential life lost for six major enteric pathogens, Germany, 2004-2008. Epidemiol Infect 141:961–968

Werner S, Boman K, Einemo I, Erntell M, De Jong B, Lindqvist A, Lofdahl M, Lofdahl S, Meeuwisse A, Ohlen G, Olsson M, Stamer U, Sellstrom E, Andersson Y (2007) Outbreak of *Salmonella* Stanley in Sweden associated with alfalfa sprouts, July-August 2007. Euro Surveill 12:E07101.2

Whitney BM, Mc CM, Hassan R, Pomeroy M, Seelman SL, Singleton LN, Blessington T, Hardy C, Blankenship J, Pereira E, Davidson CN, Luo Y, Pettengill J, Curry P, Mc CT, Gieraltowski L, Schwensohn C, Basler C, Fritz K, Mc KC, Nieves K, Oliveira J, Sandoval AL, Crosby A, Williams D, Crocker K, Thomas D, Fulton T, Muetter L, Li L, Omoregie E, Holloman K, Brennan C, Thomas N, Barnes A, Viazis S (2021) A series of papaya-associated *Salmonella* illness outbreak investigations in 2017 and 2019: a focus on traceback, laboratory, and collaborative efforts. J Food Prot 84:2002–2019

Williams S, Patel M, Markey P, Muller R, Benedict S, Ross I, Heuzenroeder M, Davos D, Cameron S, Krause V (2015) *Salmonella* in the tropical household environment – everyday, everywhere. J Infect 71:642–648

World Health Organization (WHO) (2018) Whole genome sequencing for foodborne disease surveillance: landscape paper. CC BY-NC-SA 3.0 IGO, Lizenz

Wynants E, Frooninckx L, Crauwels S, Verreth C, De Smet J, Sandrock C, Wohlfahrt J, Van Schelt J, Depraetere S, Lievens B, Van Miert S, Claes J, Van Campenhout L (2019) Assessing the microbiota of Black Soldier fly larvae (*Hermetia illucens*) reared on organic waste streams on four different locations at laboratory and large scale. Microb Ecol 77:913–930

Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VP, Nash JH, Taboada EN (2016) The Salmonella In Silico Typing Resource (SISTR): an open web-accessible tool for rapidly typing and subtyping draft *Salmonella* genome assemblies. PLoS One 11:e0147101

Young CC, Ross IL, Heuzenroeder MW (2012) A new methodology for differentiation and typing of closely related Salmonella enterica serovar Heidelberg isolates. Curr Microbiol 65:481–487

Zankari E, Allesoe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM (2017) PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. J Antimicrob Chemother 72:2764–2768

Zarkani AA, Schikora A (2021) Mechanisms adopted by *Salmonella* to colonize plant hosts. Food Microbiol 99:103833

Zhang S, Yin Y, Jones MB, Zhang Z, Deatherage Kaiser BL, Dinsmore BA, Fitzgerald C, Fields PI, Deng X (2015) Salmonella serotype determination utilizing high-throughput genome sequencing data. J Clin Microbiol 53:1685–1692

Zhang S, Den Bakker HC, Li S, Chen J, Dinsmore BA, Lane C, Lauer AC, Fields PI, Deng X (2019) SeqSero2: rapid and improved Salmonella serotype determination using whole-genome sequencing data. Appl Environ Microbiol 85:e01746-19

Zweifel C, Stephan R (2012) Spices and herbs as source of *Salmonella*-related foodborne diseases. Food Res Int 45:765–769

Enteropathogenic *Yersinia* spp.

10

Easily Misidentified Species

Maria Fredriksson-Ahomaa

Contents

Abstract

Yersinia enterocolitica and *Yersinia pseudotuberculosis* can cause enteric yersiniosis among humans and animals. The infection is typically acquired through contaminated food or water. Common symptoms among humans are diarrhea, abdominal pain, and fever, but sometimes sequelae such as joint pain and skin rash occur. Yersiniosis is usually self-limiting and no treatment with

M. Fredriksson-Ahomaa (✉)
Faculty of Veterinary Medicine, Department of Food Hygiene and Environmental Health, University of Helsinki, Helsinki, Finland
e-mail: maria.fredriksson-ahomaa@helsinki.fi

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_8

antimicrobials is needed. Animals are often asymptomatic carriers of pathogenic *Yersinia*. Yersiniosis usually occurs in animals under stress. *Y. enterocolitica* and *Y. pseudotuberculosis* differ clearly from each other both phenotypically and genotypically. *Y. enterocolitica* species consists of a very heterogeneous group of bacteria and not all strains are pathogenic. *Y. pseudotuberculosis* strains show only little variation in their biochemical reactions and correctly identified strains are considered pathogenic. Several plasmid and chromosomal encoded virulence factors are needed for *Yersinia* pathogenicity, and all pathogenic strains carry a virulence plasmid, which is essential for the bacteria to multiply and disseminate in the host. Isolation and identification of enteropathogenic *Yersinia*, especially from non-human sources, is challenging and time-consuming.

Keywords

Yersinia enterocolitica · Yersinia pseudotuberculosis · Yersiniosis, virulence, detection, transmission

10.1 Introduction

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are two species belonging to the enteropathogenic *Yersinia* spp. They cause enteric yersiniosis, which was the fourth most frequently reported foodborne bacterial enteritis in the EU in 2019, with a stable trend in 2015–2019 (EFSA and ECDC 2021). Both species have animal reservoirs and a fecal-oral transmission route (Fredriksson-Ahomaa et al. 2018). The infection is usually acquired through contaminated food, especially raw or undercooked meat or vegetables. It can also be acquired through contact with infected humans or animals. Diarrhea, abdominal pain, and fever are common symptoms. Sequelae as joint pain and skin rash occur occasionally (Rosner et al. 2013; Rivas et al. 2021). Isolation and identification of *Yersinia* spp. is challenging and time-consuming (Fredriksson-Ahomaa et al. 2018). The pathogenicity of *Y. enterocolitica* strains vary from nonpathogenic to highly pathogenic, thus the detection of virulence markers is also necessary for determining the clinical significance of isolated strains.

10.2 *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

10.2.1 Characteristics

10.2.1.1 The Genus *Yersinia* Includes Two Enteropathogenic *Yersinia* spp.

The genus *Yersinia* is classified in the *Yersiniaceae* family of the order *Enterobacteriaceales* (Adeolu et al. 2016). The taxonomy is constantly evolving, and 25 *Yersinia* spp. have been reported at the time of writing (Le Guern et al. 2020).

The genus *Yersinia* includes a heterogeneous group of gram-negative, oxidase-negative, and facultative anaerobic rod-shaped bacteria that do not possess a capsule or spores. *Y. enterocolitica* and *Y. pseudotuberculosis* are the two *Yersinia* species that can cause an enteric infection in humans and animals. They are zoonotic bacterial pathogens that can be transmitted from animals to humans through a fecal-oral route (Laukkanen-Ninios et al. 2014). *Y. enterocolitica* and *Y. pseudotuberculosis* differ clearly from each other, especially genotypically (McNally et al. 2016). *Y. pseudotuberculosis* is genetically closely related to the highly virulent agent of plague, *Yersinia pestis,* which is transmitted from animals to humans by fleas or in aerosols.

10.2.1.2 Not All *Y. enterocolitica* Strains Are Considered Pathogenic

The *Y. enterocolitica* species consists of a very heterogeneous group of bacteria: it comprises over 30 serotypes based on variation of the O antigen dependent on cell wall lipopolysaccharides (Wauters et al. 1991) and six biotypes (1A, 1B 2–5) based on biochemical reactions (Wauters et al. 1987). Certain biotype and serotype combinations have been associated with human and animal infections. Bioserotype 4/O:3 (phylogroup 3), which has a worldwide distribution, is the most common type associated with human disease, followed by bioserotypes 2/O:5,27 (phylogroup 4) and 2/O:9 (phylogroup 5) (Reuter et al. 2015; Hunter et al. 2019; Rivas et al. 2021). Infections due to bioserotype 3/O:3 have frequently been reported, especially in China (Duan et al. 2017). Bioserotype 1B/O:8, which is the most highly pathogenic type, has mainly been found in Northern America (Savin et al. 2018). In recent years, this pathogenic type has also been identified in human yersiniosis in Europe, especially in Poland (Radziszewski et al. 2019). Biotype 1B (phylogroup 2) strains have been associated with high pathogenicity and strains of biotypes 2–5 with moderate or low pathogenicity (Reuter et al. 2015). All pathogenic strains carry the virulence plasmid. Strains belonging to biotype 1A (phylogroup 1) are usually regarded as nonpathogenic because they lack the most important virulence genes. Discussions have been raised of the potential pathogenicity of certain biotype 1A strains (Huovinen et al. 2010). However, no clear difference between *Y. enterocolitica* 1A strains isolated from humans with and without diarrhea could be found in Switzerland (Stephan et al. 2013).

10.2.1.3 Two Clear Lineages Exist Among *Y. pseudotuberculosis* Strains

The genetic diversity of *Y. pseudotuberculosis* strains is quite limited (Laukkanen-Ninios et al. 2011). Two lineages with different geographical locations have been observed: European and Asian clades (Seecharran et al. 2017). Only little variation occurs in their biochemical reactions. The strains can be divided into four biotypes using melibiose, raffinose, and citrate (Tsubokura and Aleksić 1995). Biotyping has been rarely used, and it correlates quite poorly with the pathogenicity and geographical distribution of the strains (Reinhardt et al. 2018). *Y. pseudotuberculosis* strains can be classified into 21 serotypes according to their varying lipopolysaccharide O-antigen structure: O:1–O:15 and three subtypes (a–c) in O:1 and O:2, and two subtypes (a and b) in O:4 and O:5 (Seecharran et al. 2017). Most of the strains in humans belong to

only a few serotypes: O:1a, O:1b, and O:3 in Europe and in the Far East, also O:4b and O:5b (Amphlett 2016). Correctly identified *Y. pseudotuberculosis* strains are considered pathogenic (Le Guern et al. 2016; Fredriksson-Ahomaa et al. 2018). High-pathogenic *Y. pseudotuberculosis* strains, mostly originating from the Far East, can cause a systemic infection called Far East scarlet-like fever (FESLF).

10.2.1.4 Enteropathogenic *Yersinia* Strains Can Grow at Low Temperatures

Yersinia bacteria are able to grow at temperatures ranging from 4 °C to 43 °C; however, slow growth has been reported to occur even below 4 °C (Yehualaeshet et al. 2013; Keto-Timonen et al. 2018). The optimal temperature for growth and metabolic activity is around 30°C. The ability to grow at low temperatures allows *Y. enterocolitica* and *Y. pseudotuberculosis* to multiply in refrigerated foods. *Yersinia* strains typically tolerate freezing for a prolonged time and even repeated cycles of freezing and thawing (Bhaduri 2005). However, *Yersinia* is heat-sensitive and can thus be easily destroyed by heat treatment (Bursová et al. 2017). Pasteurization at 72 °C for 15–20 s kills *Yersinia* bacteria. As a facultative anaerobic bacterium, *Yersinia* can multiply under both aerobic and anaerobic conditions, and under a modified atmosphere. Pathogenic *Y. enterocolitica* has been shown to grow well on pig cheek meat in a modified atmosphere with high oxygen (70% O_2) and carbon dioxide (30% CO_2) concentrations during cold storage at 6°C, even in the presence of large numbers of lactic acid bacteria (Fredriksson-Ahomaa et al. 2012). *Yersinia* bacteria are also able to grow over a wide pH range (pH 4–10). Alkalotolerance of *Yersinia* has been used to separate *Yersinia* strains from background organisms by enrichment broth treatment with potassium hydroxide (0.5%) before streaking onto agar plates (Hallanvuo et al. 2019).

10.2.2 Virulence

10.2.2.1 Several Plasmid and Chromosomal Encoded Virulence Factors Are Needed for *Yersinia* Pathogenicity

Several virulence factors have been identified among *Y. enterocolitica* and *Y. pseudotuberculosis* strains, some of which are common in both species (Table 1). The most important factor is the presence of an approximately 70-kb plasmid, which is termed pYV (plasmid for *Yersinia* virulence) and is present in pathogenic *Yersinia* spp., including *Y. enterocolitica* and *Y. pseudotuberculosis* (Moorman and Cohen 2021). However, *Y. enterocolitica* strains belonging to biotype 1A do not carry pYV and are thus considered nonpathogenic (Reuter et al. 2014). This virulence plasmid is essential for bacterial replication in host tissue, and *Yersinia* strains without pYV are rapidly eliminated from the gut. In addition to pYV-encoded virulence factors, chromosomal encoded factors are also needed for pathogenicity.

Table 1 Virulence factors present in *Y. enterocolitica* and *Y. pseudotuberculosis* strains

Species		pYV	Chromosomal							
			Inv	Ail	YstA	YstB	HPI	MyfA	PsaA	YPM
Y. enterocolitica										
Biotype	1A	–	+	–	–	+	–	–	–	–
	1B	+	+	+	+	–	+	+	–	–
	2–5	+	+	+	+	–	–	+	–	–
Y. pseudotuberculosis										
		+	+	+	–	–	+[a]	–	+	+[b]

[a] Serotypes O:1 (complete HPI) and O:3 (truncated HPI)
[b] Found frequently in Far Eastern strains, which also often carry a large plasmid called pVM82

10.2.2.2 *Y. enterocolitica* and *Y. pseudotuberculosis* Can Be Divided into Different Groups Correlating with Pathogenicity

Y. enterocolitica strains can be divided into six phylogroups (PGs), which correlate with the biotypes and pathogenicity (Reuter et al. 2015). High-pathogenic strains belong to PG2 and biotype 1B, and they all carry the high-pathogenicity island (HPI) in their chromosomes (Table 1). Low-pathogenic strains belong to PG3–6, including biotypes 2–5, and nonpathogenic strains belong to PG1and biotype 1A. *Y. pseudotuberculosis* strains, which are considered pathogenic, can be divided into five genetic groups (G1–3, G4–5) based on the presence of three key virulence factors: pYV, HPI, and the *Y. pseudotuberculosis* – derived mitogen (YPM) toxin (Amphlett 2016). The most common clinical genetic group in Europe is G2. These strains carry pYV and a complete HPI (Table 2). High-pathogenic strains, which belong to G3, can cause a systemic infection associated with FESLF, mainly reported in Japan and Russia. Most FESLF strains synthesize the superantigen toxin YPMa, which is seldom detected in European strains. FESLF strains have lost the HPI, and they often carry an additional pVM82 plasmid (Timchenko et al. 2016).

10.2.2.3 Virulence Factors Encoded by the pYV Virulence Plasmid Are Essential for Pathogenicity

The pYV encodes the Ysc-Yop type 3 secretion system (T3SS), which allows pathogenic *Yersinia* to escape phagocytosis (Tan et al. 2016). *Yersinia* outer proteins (Yops), which are necessary for downregulating antibacterial responses, are translocated directly from the bacterial cytosol into the target cell cytosol by the T3SS (Grabowski et al. 2017). The T3SS is a molecular syringe (injectisome) that delivers Yop cytotoxic effector proteins into the host cell (Berger et al. 2021; Moorman and Cohen 2021). The expression of T3SS genes are controlled primarily by temperature and calcium concentration (Bancerz-Kisiel et al. 2018). With this system, *Yersinia* can replicate extracellularly in lymphatic tissue and encounter the immune defenses of the host. Additionally, pYV encodes the non-fimbrial, outer-membrane protein YadA (*Yersinia* adhesin A) (Mühlenkamp et al. 2015). This protein is a multifunctional protein that, e.g., promotes the attachment of bacteria to the intestinal

Table 2 Distribution of pYV, HPI, and YPM among *Y. pseudotuberculosis* strains of different genetic groups

Genetic group	Presence of			Pathogenicity	O-serotype	Geographical distribution
	pYV	HPI	YPM			
G1	+	Complete	YPMa	High	1, 3, 5	Far East
G2	+	Complete	–	Medium	1	Europe
					1, 3, 5, 13, 14	Far East
G3	+	–	YPMa	High, systemic	4	Europe
					1–7, 10	Far East
G5	+	Truncated	YPMc	Low	3	Europe, Far East
G6	+	–	–	Medium	1–3, 5	Europe
					1–7, 10, 11, 13	Far East

brush border. In *Y. enterocolitica*, YadA also confers resistance to human serum (Chung and Bliska 2016). YadA is optimally expressed at 37 °C. The pYV-encoded proteins VirF of *Y. enterocolitica* and LcrF of *Y. pseudotuberculosis*, respectively, are important thermo-activated transcriptional regulators that activate Yops production (Grabowski et al. 2017). Both *yad*A and *vir*F genes are frequently used as targets to detect and identify pathogenic *Yersinia* strains (Petsios et al. 2016).

10.2.2.4 Chromosomally Encoded Virulence Factors Are Also Needed

Two chromosomal genes, *inv* and *ail*, are important for mammalian cell invasion (Tan et al. 2016). The *inv* gene codes for an outer-membrane invasion (Inv) protein, which plays an important role in promoting the entry into epithelial M cells of the ileum during initial stages of infection. All *Yersinia* spp. have *inv* homologs, but they are only functional in pathogenic strains (Tan et al. 2016). Epithelial cell penetration of *Y. enterocolitica* is also enhanced by the outer membrane attachment invasion locus (Ail) protein encoded by the *ail* gene. In *Y. pseudotuberculosis*, Ail is the primary serum resistance factor (Chung and Bliska 2016). The *ail* gene is suggested to be laterally transferred from *Y. pseudotuberculosis* to *Y. enterocolitica* (Tan et al. 2016). It is generally found only in pathogenic *Y. enterocolitica* strains belonging to biotypes 1B and 2–5. Some strains of biotype 1A have been shown to carry *ail* homologs, and these *ail* homologs have also sporadically been detected in *Y. kristensenii* strains (Joutsen et al. 2020). The *yst* gene in the chromosome of *Y. enterocolitica* encodes a heat-stable enterotoxin called *Yersinia* stable toxin (Yst), which may play a role in diarrhea (Bancerz-Kisiel et al. 2018). YstA is produced by pathogenic *Y. enterocolitica* strains belonging to biotypes 1B and 2–5, and most biotype 1A strains produce YstB; however, not all *yst*-positive strains produce enterotoxins (Bancerz-Kisiel et al. 2018). The HPI pathogenicity island, which is a large integrative and conjugative element, can generally only be found in the chromosome of *Y. enterocolitica* biotype 1B strains and *Y. pseudotuberculosis* O:1 and O:3 strains (McNally et al. 2016). However, it is truncated in *Y. pseudotuberculosis* O:3 strains. HPI codes the yersiniabactin, which is a siderophore facilitating iron uptake. Some *Y. pseudotuberculosis* strains can also

synthesize the superantigen toxin YPM, which plays an important role in systemic infections (Amphlett 2016). Additionally, *Y. enterocolitica* strains elaborate a mucoid *Yersinia* factor (Myf) and *Y. pseudotuberculosis* strains elaborate the pH6 antigen (Psa), which are surface (fimbrial) structures with high sequence similarity (Pakharukova et al. 2016). The Psa is an adhesin, which has an important role in the pathogenesis of *Y. pseudotuberculosis* infections by inhibiting the phagocytosis when the function of MyfA in the *Y. enterocolitica* infection is still unknown.

10.3 Yersiniosis – The disease

10.3.1 Pathogenesis

10.3.1.1 *Y. enterocolitica* and *Y. pseudotuberculosis* Are Transmitted Through the Intestinal Tract

Yersiniosis due to *Y. enterocolitica* or *Y. pseudotuberculosis* is usually acquired through oral ingestion of contaminated food or water. Enteropathogenic *Yersinia* strains produce urease, which is vital for bacteria to survive in acidic environments (Righetto et al. 2020). This is important during an infection, where *Yersinia* must survive the low pH of the stomach. *Yersinia* binds to the mucus layer that covers the epithelial cells, preferably in the terminal ileum. It attaches to M cells, which overlay the Peyer's patches and are specialized in the uptake of intestinal antigens (Bancerz-Kisiel et al. 2018). Subsequently, *Yersinia* penetrates the tissue. Attachment and invasion of the M cells are mediated by chromosomal determinants, including the Inv and Ail proteins, and the pYV-encoded YadA. After penetration of the intestinal epithelium, *Yersinia* colonizes the Peyer's patches. Subsequently, *Yersinia* may spread via the lymphatic system or the blood into the mesenteric lymph nodes and to the internal organs, such as the spleen and liver, where they may form micro-abscesses (Drechsler-Hake et al. 2016). The survival ability within these tissues is dependent on the pYV-encoded Yops, which downregulate the antibacterial response (Grabowski et al. 2017). The multiplication of *Yersinia* in the Peyer's patches may cause severe abdominal pain that may be confused with appendicitis (Bottone 2015). *Y. enterocolitica* and *Y. pseudotuberculosis* are resistant to human serum. In *Y. enterocolitica*, serum resistance requires the YadA adhesin when Ail is the primary serum resistance factor in *Y. pseudotuberculosis* (Chung and Bliska 2016). Most infections are usually localized and self-limiting due the host's inflammatory response, which finally leads to elimination of the pathogen.

10.3.1.2 Some Patients Develop Post-Infective Reactive Arthritis (ReA)

ReA is a form of peripheral spondyloarthritis that can develop after an intestinal infection. In *Yersinia*-triggered ReA, the primary infection is in the gut; however, how the intestine-joint connection operates in the disease remains unclear (Silva et al. 2020). The synovial fluid from the affected joints of patients is usually culture-negative but contains bacterial antigens in the joint (Granfors et al. 1989). Most individuals with post-infective ReA are positive for human leukocyte antigen

HLA-B27 (Vasala et al. 2014). It seems that HLA-B27-positive individuals are more prone to develop severe symptoms and to show a more prolonged disease course than HLA-B27-negative patients.

10.3.2 Yersiniosis in Humans

10.3.2.1 Intestinal Symptoms Are Variable

Yersiniosis due to *Y. enterocolitica* and *Y. pseudotuberculosis* is mostly an uncomplicated enteric disease with diarrhea and abdominal pain (Borud et al. 2020; Rivas et al. 2021). Fever and blood in the feces may also occur. The incubation period typically ranges from 3 days to approximately 1 week, and symptoms may persist for weeks (Espenhain et al. 2019; Borud et al. 2020). The minimal infectious dose is unknown. The severity of the infection depends on the age and immunity of the infected person, the virulence of the strain, and the infection dose. Acute diarrhea, which may be bloody, and high fever occur most frequently in infants and children under 5 years of age (Rivas et al. 2021). Abdominal pain in the right lower quadrant due to mesenteric lymphadenitis and terminal ileitis is a common symptom in older children and adolescents. The abdominal pain resembles the symptoms of appendicitis and may lead to unnecessary surgery (Rosner et al. 2013).

10.3.2.2 Extraintestinal Sequels May Occur

In some cases, *Yersinia* infections may lead to extraintestinal complications such as joint pain (ReA), skin rash (erythema nodosum), and conjunctivitis/iritis (Rosner et al. 2013). *Y. pseudotuberculosis* has also been implicated in the etiology of Kawasaki diseases, which is a febrile vasculitis (Horinouchi et al. 2015). ReA is the most common sequelae of enteric yersiniosis, occurring especially among adults (Borud et al. 2020). ReA, which typically develops 1 week to 1 month after primary infection, usually affects the knees, ankles, elbows, and wrists. Symptoms may last for several months. ReA is usually self-limiting and only rarely remains chronic (Leirisalo-Repo and Suoranta 1988). A skin rash with painful red lesions along the trunk and legs may also occur 2 weeks after infection. The symptoms typically resolve themselves spontaneously within 1 month (Rivas et al. 2021).

10.3.2.3 Far East Scarlet-Like Fever (FESLF) Is a Rare and Poorly Investigated Disease

FESLF is an acute disease caused by certain *Y. pseudotuberculosis* strains (Amphlett 2016). Typical symptoms are severe fever and a rash that covers the body, particularly the face, neck, toes, and hands (Timchenko et al. 2016). A raspberry-like tongue is an additional manifestation. Gastrointestinal manifestations are variable, and abdominal pain mimicking appendicitis is common. Many patients also develop hepatic lesions (Amphlett 2016). The disease typically lasts around 2 weeks, after which the rash and jaundice also disappear. FESLF has mainly been reported in the Far East. Incidence is seasonal, increasing in winter. It is mostly associated with

serotype O:1b (ST2 sequence type), which circulates among rodents (Timchenko et al. 2016).

10.3.2.4 Systemic Infections Are Rare

Septicemic and systemic infections are mainly associated with immunocompromised individuals or in patients with underlying disease such as diabetes mellitus, liver cirrhosis, or hemochromatosis (Guinet et al. 2011; Amphlett 2016). During sepsis, *Yersinia* spreads preferentially to the liver, spleen, kidneys, and lungs, where they can form small tuberculosis-like abscesses. Transfusion-associated sepsis due to asymptomatic bacteremia in blood donors has also been reported (Rivas et al. 2021). Severe sepsis and rapid septic shock are typical symptoms, and older age is more often associated with a fatal outcome.

10.3.2.5 Yersiniosis Is Usually Self-Limiting and No Treatment with Antimicrobials Is Needed

Treatment is only warranted in severe cases such as in systemic infections and sepsis, (Guinet et al. 2011). Antimicrobials should also be considered for patients who are immunocompromised and for patients with iron overload. Most *Y. enterocolitica* strains produce β-lactamases and are thus resistant to penicillins, aminopenicillins, and first-generation cephalosporins (Bonardi et al. 2016; Peng et al. 2018). Third-generation cephalosporin, trimethoprim-sulfamethoxazole, tetracyclines, and fluoroquinolones are the antimicrobials of choice for severe infections (Guinet et al. 2011). *Y. pseudotuberculosis* is usually sensitive to all antimicrobials active against gram-negative bacteria; however, multiresistant strains have also been reported despite being rare (Cabanel et al. 2017). Multidrug-resistant strains are shown to be resistant to β-lactams, streptomycin, tetracycline, chloramphenicol, and gentamicin. Also, colistin resistance occurs among *Y. pseudotuberculosis* strains (Rivas et al. 2021).

10.3.3 Yersiniosis in Animals

10.3.3.1 *Yersinia* Infections Are Most Commonly Latent in Animals

Yersiniosis is quite a rare disease in animals. Animals are often asymptomatic carriers of enteropathogenic *Yersinia* strains. Sporadic infections usually occur in animals under stress, e.g., due to suboptimal weather, poor nutrition, host parasitism, and young age (Stanger et al. 2018a; Ceccolini et al. 2020). Most common symptoms are apathy, diarrhea, and weight loss, but also abortion and mastitis, and septicemia with sudden death has been reported (Magistrali et al. 2015; Stoute et al. 2016). Enterocolitis and military necrotic foci in the liver, spleen, kidneys, and lungs are typical postmortem findings (Stoute et al. 2016; Hammerl et al. 2021). *Y. pseudotuberculosis* has been identified in sick animals more often than *Y. enterocolitica,* especially in wild animals with clear symptoms (Le Guern et al. 2016). Zoo animals have also been more frequently infected with *Y. pseudotuberculosis* than *Y. enterocolitica* (Hammerl et al. 2021). However, a clonal spread of *Y. enterocolitica* bioserotype 1B/O:8 in multiple zoo species has recently been reported in the USA (Nebraska) (Hicks et al. 2020). The

symptoms varied from mild clinical signs to death, while some animals were latent without any signs. *Y. enterocolitica* has more commonly been found in asymptomatic farm animals, especially in pigs, than *Y. pseudotuberculosis* (Le Guern et al. 2016; Rivas et al. 2021). *Y. enterocolitica* strains belonging to pathogenic bioserotypes have been isolated from dogs and cats with diarrhea (Stamm et al. 2013).

10.3.3.2 Outbreaks Due to *Y. pseudotuberculosis* Infections Are Common in Zoo Animals

Several *Y. pseudotuberculosis* outbreaks have been reported in captive nonhuman primates, large rodents, bats, and birds (Ceccolini et al. 2020; Hahn et al. 2021; Hammerl et al. 2021). Lethargy and sudden death typically occur after a period of stress, but sometimes clinical signs, such as diarrhea and anorexia, can be seen (Ceccolini et al. 2020). *Y. pseudotuberculosis* can often be isolated from lung, heart, kidney, liver, spleen, and intestine samples. Recently, an outbreak causing osteomyelitis in a male turkey flock in Finland was reported, and *Y. pseudotuberculosis* was isolated from leg bone samples (Blomvall et al. 2021). Recurrent diarrhea outbreaks caused by pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* have been reported among weaned sheep in Australia (Stanger et al. 2018b). In Canada, a *Y. enterocolitica* outbreak with a high mortality rate (19%) has been reported on an alpaca farm (Ragno et al. 2019). In this study, *Y. enterocolitica* was isolated from the intestine, liver, spleen, and kidneys.

10.4 Epidemiology

10.4.1 Incidence in Humans

10.4.1.1 Notification Rates in Europe Have Been Stable from 2015 to 2019

In 2019, yersiniosis was the fourth most common reported bacterial enteric infection after campylobacteriosis, salmonellosis, and STEC infections in Europe (EFSA and ECDC 2021). *Y. enterocolitica* was the most common species (99%) of the confirmed cases with species information. In total, 7048 confirmed human yersiniosis cases (1.7 cases per 100 000 inhabitants) were reported by 20 European countries in 2019. The highest country-specific notification rates were reported in Finland, Lithuania, and Czechia) (> 5 cases per 100 000 inhabitants). Due to inconsistency of the surveillance systems in various countries, a comparison of yersiniosis incidence between countries is only suggestive. In Europe, incidence is highest among infants and children under 5 years of age. The majority of yersiniosis cases are sporadic and domestically acquired, and no seasonal distribution has been reported (EFSA and ECDC 2021).

10.4.1.2 The Incidence of *Yersinia* Infections Is Underestimated if Culture Methods Only Are Used

The incidence of yersiniosis in the USA was 1.4 cases per 100 000 inhabitants in 2019 (Tack et al. 2020). It has increased significantly (158%) in 2019 compared with

2016–2018. However, 57% of the cases were diagnosed using culture-independent diagnostic methods (CIDM) such as PCR and antigen-based methods. One reason for the high incidence rate may be that many laboratories have begun using PCR methods to avoid problems with time-consuming and insensitive isolation methods (Fredriksson-Ahomaa and Korkeala 2003). From 2015, diagnostic laboratories in New Zealand have progressively introduced CIDM for fecal samples. This may have influenced the incidence of human yersiniosis, which significantly increased between 2015 and 2018. The current rate of yersiniosis in New Zealand is 24.1 cases per 100 000 inhabitants, which is very high compared to other industrialized countries (Rivas et al. 2021). Introduction of PCR screening for fecal samples has also significantly increased the annual incidence rate of yersinosis in England (Clarke et al. 2020).

10.4.2 Foodborne Outbreaks

10.4.2.1 Foodborne *Yersinia* Infections Are Mainly Sporadic Infections and Outbreaks Are Rare

Foodborne yersiniosis is mostly caused by *Y. enterocolitica* (EFSA and ECDC 2021). Although *Y. pseudotuberculosis* is a rare cause of sporadic human foodborne infections, it has caused several foodborne outbreaks in Finland, Japan, and Russia. During past years, several foodborne *Y. enterocolitica* outbreaks have been reported in Nordic countries (Table 3).

10.4.2.2 *Y. enterocolitica* O:3 Outbreaks Have Recently Been Linked to Vegetables in Nordic Countries

In Sweden, a *Y. enterocolitica* O:3 (ST18) outbreak with 53 cases was linked to imported iceberg lettuce in 2021 (https://www.foodsafetynews.com/2021/03/yersinia-outbreak-linked-to-imported-iceberg-lettuce/). In 2020, pre-cut lettuce was associated with *Y. enterocolitica* O:3 outbreaks in Norway (Promed, Archive number: 20201227.8050413). The most common symptoms were diarrhea, fever, and abdominal pain, which lasted 1–3 weeks. A *Y. enterocolitica* O:3 cross-border outbreak was identified in Denmark and Sweden in 2019 (Espenhain et al. 2019). This outbreak was confirmed with whole-genome sequencing (WGS) and the sequence type was ST18, which is a typical type found in pigs. Imported fresh spinach was identified as the vehicle by combining epidemiological and trace-back investigations. Symptoms such as fever, diarrhea, and abdominal pain in the right lower part of the abdomen occurred after an incubation period of 3–7 days. Earlier in the same year, another *Y. enterocolitica* O:3 outbreak was linked to imported spinach in Sweden. Outbreaks due to serotype O:9 have also been reported (Table 3). Three *Y. enterocolitica* O:9 outbreaks have been reported in Norway, all linked to fresh produce. The largest *Y. enterocolitica* O:9 outbreak, which was linked to mixed salad, was reported in military camps in 2014 (MacDonald et al. 2016). In total, 133 cases with identical genotypes were found. The same year, sushi was linked to an outbreak caused by *Y. enterocolitica* O:9 (ST12) in New Zealand (Strydom et al. 2019). Outbreaks due to serotype O:8, which is a quite rare serotype and is regarded

Table 3 Reported outbreaks due to *Y. enterocolitica* (YE) and *Y. pseudotuberculosis* (YP) during the past decade

Year of outbreak	Country	Serotype of causing agent		Confirmed cases	Suspected sources
		YE	YP		
2021	Norway	O:3		15	Unknown
2021	Sweden	O:3		53	Iceberg lettuce
2020	Norway	O:3		10	Mixed salad
2020	Norway	O:3		23	Spinach
2019	Sweden	O:3		30	Spinach
2019	Denmark + Sweden	O:3		20+37	Spinach
2019	USA (Pennsylvania)	O:8		48	Milk
2018	Norway	O:9		18	Mixed salad
2016	New Zealand	O:9		24	Sushi
2014	Finland		O:1	55	Raw milk
2014	New Zealand		O:1	220	Vegetables
2014	Norway	O:9		133	Mixed salad
2013	Japan	O:8		52	Salad
2012	Japan	O:8		39	Unknown
2011	Norway	O:9		21	Mixed salad
2011	USA (Pennsylvania)	O:8		109	Milk

highly pathogenic, have been reported in Japan and the USA (Table 3). The two US outbreaks were associated with pasteurized milk, which was most probably contaminated after pasteurization (Longenberger et al. 2014; Gruber et al. 2021).

10.4.2.3 *Y. pseudotuberculosis* Outbreaks Have Been Associated with Vegetables and Raw Milk

A large *Y. pseudotuberculosis* outbreak was reported in New Zealand in 2014. In total, 220 laboratory-confirmed cases were infected by a novel *Y. pseudotuberculosis* O:1 clone belonging to sequence type ST42 (Williamson et al. 2016). The case-control study implicated contaminated carrots and lettuce as the most probable infection source. In Finland, an outbreak of *Y. pseudotuberculosis* O:1 infection with 55 cases was reported in 2014 (Pärn et al. 2015). The same strain was found in humans and raw milk, which was also associated with the outbreak using a case-control study. In Finland, *Y. pseudotuberculosis* infection is a notifiable disease, and outbreaks cause large variation in the annual incidence. Most cases are sporadic, but ten outbreaks were reported between 1997 and 2008 (Rimhanen-Finne et al. 2009). These outbreaks were caused by serotypes O:1 and O:3 and were linked to contaminated carrots and iceberg lettuce. Several large *Y. pseudotuberculosis* outbreaks linked to raw vegetables (cabbage, onions, and carrots) have been reported in Russia (Tseneva et al. 2012). Outbreaks have been registered mostly in schools and day-care

centers. The number of children hospitalized has been large. Multiple FESLF outbreaks due to serotypes O:1b and O:3 have also been linked to contaminated vegetable (Timchenko et al. 2016). The vegetables have probably been contaminated by rodents. Incidences are highest in winter, possibly due to the need to store vegetables for a prolonged period, with a subsequent higher risk of being contaminated by rodents (Amphlett 2016). In Japan, surface water has also been linked to outbreaks (Tsubokura et al. 1989).

10.4.3 Prevalence in Animals

10.4.3.1 Nonpathogenic *Y. enterocolitica* Are Common in Animals

Numerous works have been carried out to study the presence of enteropathogenic *Yersinia* in a variety of animals, including farm, pet, wild, and zoo animals (Fredriksson-Ahomaa et al. 2018). *Y. enterocolitica* strains have frequently been isolated from animal sources; however, these strains mainly belong to biotype 1A, regarded as nonpathogenic (Joutsen et al. 2017; Lucero-Estrada et al. 2020). Human pathogenic *Y. enterocolitica* strains have mostly been found in slaughtered fattening pigs (Laukkanen-Ninios et al. 2014; Morka et al. 2018).

10.4.3.2 *Y. enterocolitica* of Bioserotype 4/O:3 Is Frequently Isolated from Pigs

Pigs are asymptomatic carriers of human pathogenic *Yersinia* strains. High seroprevalence (60–90%) of enteropathogenic *Yersinia* has been reported in pigs at slaughter (Bonardi et al. 2016; Lorencova et al. 2016; Felin et al. 2019). *Y. enterocolitica* belonging to bioserotype 4/O:3, the most common type associated with human disease, has mainly been identified in pigs (Råsbäck et al. 2018). This pathogenic type has frequently been isolated from the oral cavity of pigs at slaughter in Europe, especially from the tonsils, but also from the submaxillar lymph nodes and intestinal content (Laukkanen-Ninios et al. 2014; Fois et al. 2018). It has also been isolated from surface swabs of pig carcasses and edible offal at the slaughterhouse. *Y. pseudotuberculosis* has also been isolated from the tonsils and feces of pigs at slaughter, but much more rarely than *Y. enterocolitica* (Laukkanen-Ninios et al. 2014; Bonardi et al. 2016).

10.4.3.3 *Y. enterocolitica* O:9 Has Sporadically Been Found in Ruminants

Y. enterocolitica O:9, which is the second most frequently reported serotype among human *Yersinia* infections in many countries, has mainly been isolated from ruminants, cattle, and deer but also from sheep and goats (Le Guern et al. 2016; Rivas et al. 2021). *Y. enterocolitica* O:9-positive animals can give false-positive results for *Brucella* when using serological tests (O'Grady et al. 2016). *Y. enterocolitica* bioserotype 5/O:3, which is a rare pathogenic type mainly associated with hares, has quite recently been isolated from Finnish sheep at slaughter (Joutsen et al. 2016). High seroprevalences of *Y. enterocolitica* and *Y. pseudotuberculosis* in sheep and *Y. enterocolitica* in goats were recently reported in Pakistan (Ullah et al. 2019).

10.4.3.4 Dogs and Cats Are a Source of Human Pathogenic *Y. enterocolitica* Strains

Human pathogenic *Y. enterocolitica* strains have sporadically been isolated from dogs and cats in Europe (Stamm et al. 2013). Dogs were shown to excrete pathogenic *Y. enterocolitica* more frequently in their feces than cats. The most dominant *Y. enterocolitica* type in dogs in Europe is biotype 4, followed by biotypes 2 and 3 (Stamm et al. 2013; Le Guern et al. 2016). Bioserotype 3/O:3, which is the dominant type among human infections in China, is the dominant type found in the tonsils and feces of dogs in China (Wang et al. 2014). *Y. pseudotuberculosis* has more frequently been detected in cats than in dogs in France (Le Guern et al. 2016). In China, *Y. pseudotuberculosis* strains of various serotypes have been found in dog tonsils and feces.

10.4.3.5 *Y. pseudotuberculosis* Is a Common Finding in Wildlife

Y. pseudotuberculosis has mostly been isolated from wildlife, especially from birds, rodents, and lagomorphs, which are therefore considered the most important reservoirs of this pathogen (Le Guern et al. 2016). *Y. pseudotuberculosis* of various serotypes has also been isolated from wild boar tonsils (Reinhardt et al. 2018; Sannö et al. 2018). Pathogenic *Y. enterocolitica* has quite rarely been found in wild animals (Joutsen et al. 2017). However, in Sweden, pathogenic *Y. enterocolitica* was isolated from the tonsils of hunted wild boars (Sannö et al. 2018). A high seroprevalence (>50%) of enteropathogenic *Yersinia* has also been reported in wild boars in Europe, indicating that wild boars are an important reservoir of *Y. enterocolitica* and *Y. pseudotuberculosis* (Arrausi-Subiza et al. 2015; Lorencova et al. 2016; Fredriksson-Ahomaa et al. 2020). High pathogenic *Y. enterocolitica* 1B/O:8 and *Y. pseudotuberculosis* have recently been reported in deer in Japan (Takahashi et al. 2020). In Belgium, *Y. enterocolitica* bioserotypes, 2/O:5,27 and 3/O:1,2,3, which are very seldom identified in human infection, have been reported in brown rats (Rouffaer et al. 2017). Bioserotype 4/O:3, the most common *Y. enterocolitica* type found in human infections and pigs, has been isolated from brown rats near pig farms in Sweden (Backhans et al. 2011). The genotypes of rat and pig strains were indistinguishable, indicating that rats may spread this pathogen within a farm.

10.4.4 Prevalence in Food and Water

10.4.4.1 A High Prevalence of *Y. enterocolitica* Has Been Detected in Pork Products by PCR

Pathogenic *Y. enterocolitica* strains have frequently been detected by PCR in pork products, but also sporadically in game meat, dairy products, vegetables, and surface water (Cheyne et al. 2010; Fredriksson-Ahomaa 2017; Bonardi et al. 2018; Verbikova et al. 2018). Using culture methods, most of the *Y. enterocolitica* isolates found in food and water have belonged to nonpathogenic biotype 1A. Pathogenic *Y. enterocolitica,* mainly belonging to bioserotype 4/O:3, has been isolated from pork, especially from pig head meat and tongues in Europe (Laukkanen-Ninios et al. 2014). In China, pathogenic *Y. enterocolitica* bioserotype 3/O:3 (ST135), which is a

typical bioserotype in pigs in China, was quite recently isolated for the first time from raw chicken (Peng et al. 2018). *Y. pseudotuberculosis* has only rarely been found in meat. It has more commonly been isolated from vegetables (Tseneva et al. 2012) but also from surface water (Fukushima et al. 1995; Le Guern et al. 2016). Recently, *Y. pseudotuberculosis* was isolated from raw milk associated in a *Y. pseudotuberculosis* outbreak in Finland (Castro et al. 2019).

10.4.5 Transmission Routes

10.4.5.1 Several Transmission Routes Exist for Enteropathogenic *Yersinia*

Both *Y. enterocolitica* and *Y. pseudotuberculosis* are primarily transmitted fecal-orally from animal reservoirs to humans through contaminated food and water, but direct animal contact is also a possible transmission route (Fig. 1). Transmission of enteropathogenic *Yersinia* may also occur directly from human to human or indirectly by blood transfusion.

10.4.5.2 Consumption of Pork Is a Significant Risk Factor for Sporadic *Y. enterocolitica* Infections

In a recent review, pork consumption and occupational contact with pigs were significantly associated with sporadic *Y. enterocolitica* infections (Guillier et al. 2020). In the same study, untreated water was also a risk factor for sporadic yersiniosis. Consumption of raw minced pork has been identified as the main risk factor for sporadic *Y. enterocolitica* in Germany using a population-based case-control study (Rosner et al. 2012). Raw pork consumption may play an important role in countries such as Belgium, Germany, and the Netherlands, where raw minced pork with pepper and onion is a delicacy that can be purchased as ready-to-eat food from butcher shops. However, in most countries, transmission is more likely to occur via cross-contamination of cooked pork or foods not normally harboring *Y. enterocolitica*.

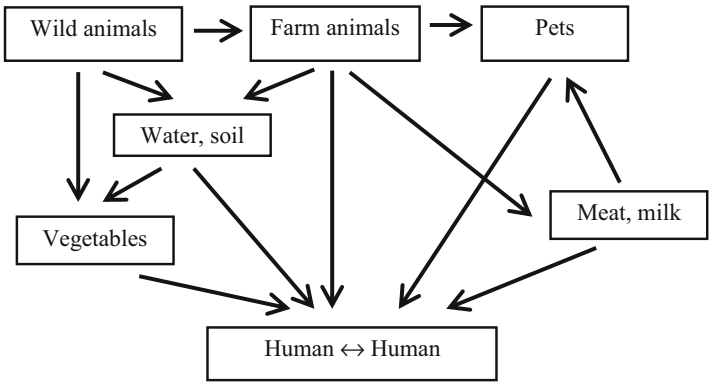

Fig. 1 Transmission routes of enteropathogenic *Yersinia*

10.4.5.3 *Y. pseudotuberculosis* Is Transmitted by Contaminated Vegetables

Both fresh produce and untreated surface water are important infection sources for *Y. pseudotuberculosis* infections. Wildlife feces may have contaminated the vegetables during storage, or the vegetables have already been contaminated at the farm through contaminated irrigation water or soil (Rimhanen-Finne et al. 2009). Untreated drinking water from wells, springs, and streams contaminated with wildlife feces has been associated with *Y. pseudotuberculosis* infections in Japan (Tsubokura et al. 1989).

10.4.5.4 Enteropathogenic *Yersinia* Can Be Transmitted to Humans via Pets

Companion animals, such as dogs and cats, are suspected sources of human yersiniosis through close contact, especially for young children (Boqvist et al. 2009). In China, indistinguishable *Y. enterocolitica* bioserotype 3/O:3 strains have been found in humans, dogs, and pigs, indicating a link between human infections, dogs, and pigs (Wang et al. 2014). Pathogenic *Y. enterocolitica* 4/O:3 may be transmitted to dogs and cats via raw pork and offal (Fredriksson-Ahomaa et al. 2001).

10.4.5.5 Human-to-Human Transmission Has Rarely Been Reported

Enteropathogenic *Yersinia* can be transmitted from human-to-human either by direct contact or indirectly through blood transfusion. Direct transmission may occur when basic hygiene and handwashing habits are inadequate (Ong et al. 2012). Transmission of *Y. enterocolitica* from chitterlings (prepared pig intestine, which is a traditional dish in African-American households) to infants occurs through contact with their caretakers preparing chitterlings (Jones et al. 2003). Indirect transmission may occur by transfusion of contaminated blood products (Guinet et al. 2011). A blood donor with subclinical bacteremia is the most likely source of *Yersinia* contamination (Rivas et al. 2021).

10.5 Detection and Typing

10.5.1 Detection Methods

10.5.1.1 Culturing Is Still Commonly Used to Find Enteropathogenic *Yersinia* from Various Sample Types

The isolation of enteropathogenic *Yersinia* is time-consuming, especially when samples with low levels of *Yersinia* and high levels of other bacteria are studied (Fredriksson-Ahomaa and Korkeala 2003; Petsios et al. 2016). Isolation methods for food and environmental samples are based on (1) direct culturing on selective agar media, (2) a short selective enrichment step before culturing on agar plates, and/or (3) cold enrichment at 4°C for 2–3 weeks (Hallanvuo et al. 2019; Weagant and Feng 2017). Direct culturing on selective agar plates without any pre-enrichment is

usually successful only for samples containing a large number of *Yersinia,* such as clinical samples from humans and animals with acute yersiniosis. The most widely used commercially available selective agar plate is CIN (cefsulodin-igasan-novobiocin) agar (Petsios et al. 2016). In parallel to CIN agar, commercially available chromogenic agar is used for *Y. enterocolitica* isolation (Hallanvuo et al. 2019). No chromogenic agar for *Y. pseudotuberculosis* is currently available commercially, and a low-selective MacConkey agar is therefore used along with CIN (Weagant and Feng 2017). The isolation of *Y. pseudotuberculosis* is very challenging because it grows slowly, thus being easily overgrown by other bacterial species present in the sample.

10.5.1.2 PCR Is Increasingly Used to Screen for the Presence of Pathogenic *Yersinia* in Various Sample Types

PCR is a very practical method that is both cost- and time-effective to screen for the presence of pathogenic *Yersinia* before culturing. There is still no sensitive and accurate isolation method available for detecting enteropathogenic *Yersinia* in food and environmental samples, thus several PCR methods have been designed for this purpose (Petsios et al. 2016). Currently, PCR is also used to screen for the presence of enteropathogenic *Yersinia* in the fecal samples of humans (Clarke et al. 2020). The *ail* gene located in the chromosome of *Y. enterocolitica* and *Y. pseudotuberculosis* is the most commonly used target in the PCR methods. The chromosomal *ail* gene is preferred over the virulence genes located on the pYV plasmid because pYV may be lost during culturing, giving a false-negative result. However, false-positive results are possible using *ail* because it has also been detected in nonpathogenic *Y. enterocolitica* and *Y. kristensenii* strains (Joutsen et al. 2020). PCR methods are rapid and have a superior sensitivity compared to culture methods; however, they may also detect nonviable bacteria and do not yield bacterial isolates that are essential for further epidemiological studies. Therefore, isolation methods should be used together with PCR.

10.5.1.3 MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) Is Used for Rapid Identification of *Yersinia*

Commercially available identification kits, especially the API 20E test, have been widely used for identifying *Y. enterocolitica* and *Y. pseudotuberculosis* (Petsios et al. 2016). However, tests based on biochemical reactions easily misidentify *Yersinia* spp. (Fredriksson-Ahomaa et al. 2018). Identification of *Yersinia* spp. using MALDI-TOF has increased recently. It is a rapid and accurate method that provides protein profiles for identifying *Yersinia* at the species and subspecies levels (Stephan et al. 2011). However, rare and untypical strains cannot be identified if the protein profiles are missing from the database (Morka et al. 2018). Also, PCR methods using species- and virulence-specific genes are commonly used for rapid and accurate identification of enteropathogenic *Yersinia*.

10.5.1.4 Serological Tests Are Used to Screen the Prevalence of Yersiniosis in Animal Reservoirs

There are several commercial serological tests available to detect anti-*Yersinia* antibodies in humans and animals (Dalby et al. 2017; Wielkoszynski et al. 2018; Ullah et al. 2019). Serological tests are very practical for monitoring yersiniosis in animal reservoirs and for estimating the prevalence of pathogenic *Yersinia* in livestock at the herd level, as these tests are not as expensive and time-consuming as traditional culture methods (Vanantwerpen et al. 2017; Felin et al. 2019). Serological testing can be performed from the serum or muscle fluid, which can conveniently be collected at the slaughterhouse. Serological diagnosis is not equivalent to classical microbiological detection of the organism, as the serological response is delayed with respect to the time of infection. Serological tests are also valuable when *Yersinia* has not been isolated from an asymptomatic patient and when clinical symptoms indicate a previous infection. The diagnosis is especially important for diagnosing sequelae after gastroenteritis, such as arthritis.

10.5.2 Typing Methods

10.5.2.1 Biotyping Is Still Used to Assess the Potential Pathogenicity of the *Y. enterocolitica* Isolates

Phenotyping methods based on biotyping and serotyping are still used for characterization of *Y. enterocolitica* isolates (Fredriksson-Ahomaa et al. 2018). The potentially pathogenic *Y. enterocolitica* isolates can be identified by the pyrazinamidase test, which is one of the key tests included in the biotyping scheme (Hallanvuo et al. 2019). Information of biotype and/or serotype alone often lacks the discriminatory power needed to differentiate *Y. enterocolitica* isolates that belong to the same bioserotype. Therefore, in addition to bio- and serotyping, more discriminatory typing methods are needed. Correctly identified *Y. pseudotuberculosis* isolates are considered pathogenic. However, *Y. similis* and *Y. wautersii* are impossible to differentiate from *Y. pseudotuberculosis* using biochemical tests, and they may also share the same serotypes (Savin et al. 2014). Therefore, the presence of certain virulence genes in presumptive *Y. pseudotuberculosis* isolates should be studied.

10.5.2.2 Whole-Genome Sequencing (WGS) Is Replacing Other Typing Methods

Bacterial WGS can now be obtained rapidly and affordably. Nowadays, WGS is increasingly used for identifying *Yersinia* strains and in epidemiological studies, including outbreak studies (Williamson et al. 2016; Fredriksson-Ahomaa et al. 2018; Inns et al. 2018). Currently, two seven-gene multilocus sequence typing (MLST) schemes are available for enteropathogenic *Yersinia*. The first one was designed for identifying and typing *Y. pseudotuberculosis* isolates (Laukkanen-Ninios et al. 2011). The newer scheme was a pan-*Yersinia* scheme for accurate identification of *Yersinia* spp. (Hall et al. 2015). Increasingly, MLST based on core genes (cgMLST) is used for more discriminatory typing (Inns et al. 2018; Hunter et al. 2019; Hammerl et al. 2021). Currently, there are two databases for MLST and cgMLST typing of *Yersinia* isolates

available online: EnteroBase (https://enterobase.warwick.ac.uk/species/index/yersinia) (Zhou et al. 2020) and BIGSdb-Pasteur (https://bigsdb.pasteur.fr/yersinia/) (Savin et al. 2019). cgMLST is an accurate and reliable high-throughput typing method that is suited for surveillance, outbreak studies and other epidemiological studies. In addition to sequence types, i.e., serotypes, virulence and resistance genes can be derived from the WGS data (Hammerl et al. 2021). Pulsed-field gel electrophoresis (PFGE) and multiple locus variable-number tandem repeat analysis (MLVA), which have shown to be quite discriminatory methods for *Yersinia* typing, are also used in epidemiological studies (Saraka et al. 2017; Raymond et al. 2019; Strydom et al. 2019; Hammerl et al. 2021). However, they may be replaced by WGS in the future.

10.6 Conclusions

Y. enterocolitica and *Y. pseudotuberculosis* are important pathogens that cause enteric yersiniosis in humans and animals. These pathogens differ phenotypically and genotypically from each other. However, while they cause a disease with similar symptoms, their animal reservoirs and transmission routes may be different. PCR is increasingly used to screen the presence of enteropathogenic *Yersinia* in human and nonhuman samples usually combined with culture methods. Efforts should be made to develop better and standardized isolation methods for these pathogens. Identification of enteropathogenic *Yersinia* is challenging because *Yersinia* spp. are easily misidentified. This can be overcome by using methods based on WGS, which enables accurate identification of pathogenic *Yersinia* spp.

10.7 Cross-References

▶ Zoonotic Diseases of Swine: Food-Borne and Occupational Aspects of Infection

References

Adeolu M, Alnajar S, Naushad S, Gupta RS (2016) Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families *Enterobacteriaceae, Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int J Syst Evol Microbiol 66:5575–5599

Amphlett A (2016) Far East Scarlet-Like Fever: a Review of the epidemiology, symptomatology, and role of superantigenic toxin: *Yersinia pseudotuberculosis*-derived mitogen A. Open Forum Infect Dis 3. https://doi.org/10.1093/ofid/ofv202

Arrausi-Subiza M, Gerrikagoitia X, Alvarez V et al (2015) Prevalence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in wild boars in the Basque country, northern Spain. Acta Vet Scand 58:1–7

Backhans A, Fellström C, Lambertz ST (2011) Occurrence of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in small wild rodents. Epidemiol Infect 139:1230–1238

Bancerz-Kisiel A, Pieczywek M, Łada P, Szweda W (2018) The most important virulence markers of *Yersinia enterocolitica* and their role during infection. Gene 9:235

Berger C, Ravelli RB, López-Iglesias C et al (2021) Structure of the *Yersinia* injectisome in intracellular host cell phagosomes revealed by cryo FIB electron tomography. J Struct Biol 213:107701

Bhaduri S (2005) Survival, injury, and virulence of freeze-stressed plasmid-bearing virulent *Yersinia enterocolitica* in ground pork. Foodborne Pathog Dis 2:353–356

Blomvall L, Pelkola K, Lienemann T, Lehtoniemi S, Pohjola L, Fredriksson-Ahomaa M (2021) Osteomyelitis in slaughter turkeys caused by *Yersinia pseudotuberculosis* sequence type ST42. Vet Microbiol. Submitted

Bonardi S, Bruini I, D'Incau M et al (2016) Detection, seroprevalence and antimicrobial resistance of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in pig tonsils in Northern Italy. Int J Food Microbiol 235:125–132

Bonardi S, Le Guern A, Savin C et al (2018) Detection, virulence and antimicrobial resistance of *Yersinia enterocolitica* in bulk tank milk in Italy. Int Dairy J 84:46–53

Boqvist S, Pettersson H, Svensson Å, Andersson Y (2009) Sources of sporadic *Yersinia enterocolitica* infection in children in Sweden, 2004: A case-control study. Epidemiol Infect 137:897–905. https://doi.org/10.1017/S0950268808001209

Borud EK, Håberg SE, Norheim AJ et al (2020) *Yersinia enterocolitica*: A follow-up of the outbreak in the Norwegian armed forces in 2014. Military Med USA 518

Bottone EJ (2015) *Yersinia enterocolitica*: revisitation of an enduring human pathogen. Clin Microbiol Newsl 37:1–8

Bursová Š, Necidová L, Haruštiaková D, Janštová B (2017) Growth potential of *Yersinia enterocolitica* in pasteurised cow's and goat's milk stored at 8° C and 24° C. Food Control 73:1415–1419

Cabanel N, Galimand M, Bouchier C et al (2017) Molecular bases for multidrug resistance in *Yersinia pseudotuberculosis*. Int J Med Microbiol 307:371–381

Castro H, Jaakkonen A, Hakakorpi A et al (2019) Genomic epidemiology and phenotyping reveal on-farm persistence and cold adaptation of raw milk outbreak-associated *Yersinia pseudotuberculosis*. Front Microbiol 10:1049

Ceccolini ME, Macgregor SK, Spiro S et al (2020) *Yersinia pseudotuberculosis* infections in primates, artiodactyls, and birds within a zoological facility in the United Kingdom. J Zoo Wildlife Med 51:527–538. https://doi.org/10.1638/2019-0205

Cheyne BM, Van Dyke MI, Anderson WB, Huck PM (2010) The detection of *Yersinia enterocolitica* in surface water by quantitative PCR amplification of the ail and yadA genes. J Water Health 8:487–499

Chung LK, Bliska JB (2016) *Yersinia* versus host immunity: how a pathogen evades or triggers a protective response. Curr Opin Microbiol 29:56–62

Clarke M, Dabke G, Strakova L et al (2020) Introduction of PCR testing reveals a previously unrecognized burden of yersiniosis in Hampshire, UK. J Med Microbiol 69:419–426

Dalby T, Rasmussen E, Schiellerup P, Krogfelt KA (2017) Development of an LPS-based ELISA for diagnosis of *Yersinia enterocolitica* O:3 infections in Danish patients: a follow-up study. BMC Microbiol 17:1–7

Drechsler-Hake D, Alamir H, Hahn J et al (2016) Mononuclear phagocytes contribute to intestinal invasion and dissemination of *Yersinia enterocolitica*. Int J Med Microbiol 306:357–366

Duan R, Liang J, Zhang J et al (2017) Prevalence of *Yersinia enterocolitica* bioserotype 3/O:3 among children with diarrhea, China, 2010–2015. Emerg Infect Dis 23:1502

Espenhain L, Riess M, Müller L et al (2019) Cross-border outbreak of *Yersinia enterocolitica* O3 associated with imported fresh spinach, Sweden and Denmark, March 2019. Eurosurveillance 24:1900368

EFSA and ECDC (European Food Safety Authority, European Centre for Disease Prevention and Control) (2021) The European Union One Health 2019 Zoonoses Report. EFSA J 19:e06406

Felin E, Hälli O, Heinonen M et al (2019) Assessment of the feasibility of serological monitoring and on-farm information about health status for the future meat inspection of fattening pigs. Prev Vet Med 162:76–82

Fois F, Piras F, Torpdahl M et al (2018) Prevalence, bioserotyping and antibiotic resistance of pathogenic *Yersinia enterocolitica* detected in pigs at slaughter in Sardinia. Int J Food Microbiol 283:1–6

Fredriksson-Ahomaa M (2017) *Yersinia enterocolitica*. In: Foodborne diseases. Elsevier, pp 223–233

Fredriksson-Ahomaa M, Joutsen S, Laukkanen-Ninios R (2018) Identification of *Yersinia* at the species and subspecies levels is challenging. Curr Clin Microbiol Rep 5:135–142

Fredriksson-Ahomaa M, Korkeala H (2003) Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. Clin Microbiol Rev 16: 220–229

Fredriksson-Ahomaa M, Korte T, Korkeala H (2001) Transmission of *Yersinia enterocolitica* 4/O:3 to pets via contaminated pork. Lett Appl Microbiol 32:375–378. https://doi.org/10.1046/j.1472-765X.2001.00922.x

Fredriksson-Ahomaa M, London L, Skrzypczak T et al (2020) Foodborne zoonoses common in hunted wild boars. EcoHealth:1–11

Fredriksson-Ahomaa M, Murros-Kontiainen A, Säde E et al (2012) High number of *Yersinia enterocolitica* 4/O:3 in cold-stored modified atmosphere-packed pig cheek meat. Int J Food Microbiol 155:69–72

Fukushima H, Gomyoda M, Tsubokura M, Aleksić S (1995) Isolation of *Yersinia pseudotuberculosis* from river waters in Japan and Germany using direct KOH and HeLa cell treatments. Zentralblatt für Bakteriologie 282:40–49

Grabowski B, Schmidt MA, Rüter C (2017) Immunomodulatory *Yersinia* outer proteins (Yops)–useful tools for bacteria and humans alike. Virulence 8:1124–1147

Granfors K, Jalkanen S, von Essen R et al (1989) *Yersinia* antigens in synovial-fluid cells from patients with reactive arthritis. N Engl J Med 320:216–221

Gruber JF, Morris S, Warren KA et al (2021) *Yersinia enterocolitica* outbreak associated with pasteurized milk. Foodborne Pathog Dis

Guillier L, Fravalo P, Leclercq A et al (2020) Risk factors for sporadic *Yersinia enterocolitica* infections: a systematic review and meta-analysis. Microbial. Risk Anal 100141

Guinet F, Carniel E, Leclercq A (2011) Transfusion-transmitted *Yersinia enterocolitica* sepsis. Clin Infect Dis 53:583–591

Hahn K, Veiga IB, Schediwy M et al (2021) *Yersinia pseudotuberculosis* serotype O:1 infection in a captive Seba's short tailed-fruit bat (Carollia perspicillata) colony in Switzerland. BMC Vet Res 17. https://doi.org/10.1186/s12917-021-02796-y

Hall M, Chattaway MA, Reuter S et al (2015) Use of whole-genus genome sequence data to develop a multilocus sequence typing tool that accurately identifies *Yersinia* isolates to the species and subspecies levels. J Clin Microbiol 53:35–42

Hallanvuo S, Herranen M, Jaakkonen A et al (2019) Validation of EN ISO method 10273-Detection of pathogenic *Yersinia enterocolitica* in foods. Int J Food Microbiol 288:66–74

Hammerl J, Vom Ort N, Barac A et al (2021) Analysis of *Yersinia pseudotuberculosis* isolates recovered from deceased mammals of a German zoo animal collection. J Clin. Microbiology

Hicks CL, Napier JE, Armstrong DL et al (2020) Clonal spread of *Yersinia enterocolitic*a 1B/O:8 in multiple zoo species. J Zoo Wildlife Med 51:170–176. https://doi.org/10.1638/2019-0062

Horinouchi T, Nozu K, Hamahira K et al (2015) *Yersinia pseudotuberculosis* infection in Kawasaki disease and its clinical characteristics. BMC Pediatr 15:1–9

Hunter E, Greig DR, Schaefer U et al (2019) Identification and typing of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* isolated from human clinical specimens in England between 2004 and 2018. J Med Microbiol 68:538–548

Huovinen E, Sihvonen LM, Virtanen MJ et al (2010) Symptoms and sources of *Yersinia enterocolitica*-infection: a case-control study. BMC Infect Dis 10:1–9

Inns T, Flanagan S, Greig DR et al (2018) First use of whole-genome sequencing to investigate a cluster of *Yersinia enterocolitica*, Liverpool, United Kingdom, 2017. J Med Microbiol 67: 1747–1752

Jones R, Fernandez J, Gerber S, Paul W (2003) *Yersinia enterocolitica* gastroenteritis among infants exposed to chitterlings-Chicago, Illinois, 2002. MMWR Morbidity Mortal Week Rep 52: 956–956

Joutsen S, Eklund K-M, Laukkanen-Ninios R et al (2016) Sheep carrying pathogenic *Yersinia enterocolitica* bioserotypes 2/O:9 and 5/O:3 in the feces at slaughter. Vet Microbiol 197:78–82

Joutsen S, Johansson P, Laukkanen-Ninios R et al (2020) Two copies of the ail gene found in *Yersinia enterocolitica* and *Yersinia kristensenii*. Vet Microbiol 247:108798

Joutsen S, Laukkanen-Ninios R, Henttonen H et al (2017) *Yersinia* spp. in wild rodents and shrews in Finland. Vector-Borne Zoono Dis 17:303–311

Keto-Timonen R, Pöntinen A, Aalto-Araneda M, Korkeala H (2018) Growth of *Yersinia pseudotuberculosis* strains at different temperatures, pH values, and NaCl and ethanol concentrations. J Food Prot 81:142–149

Laukkanen-Ninios R, Didelot X, Jolley KA et al (2011) Population structure of the *Yersinia pseudotuberculosis* complex according to multilocus sequence typing. Environ Microbiol 13: 3114–3127

Laukkanen-Ninios R, Fredriksson-Ahomaa M, Korkeala H (2014) Enteropathogenic *Yersinia* in the pork production chain: challenges for control. Compr Rev Food Sci Food Saf 13:1165–1191

Le Guern A-S, Martin L, Savin C, Carniel E (2016) Yersiniosis in France: overview and potential sources of infection. Int J Infect Dis 46:1–7. https://doi.org/10.1016/j.ijid.2016.03.008

Le Guern A-S, Savin C, Angermeier H et al (2020) *Yersinia artesiana* sp. nov., *Yersinia proxima* sp. nov., *Yersinia alsatica* sp. nov., *Yersina vastinensis* sp. nov., *Yersinia thracica* sp. nov. and *Yersinia occitanica* sp. nov., isolated from humans and animals. International Journal of Systematic and Evolutionary Microbiology 70:5363

Leirisalo-Repo M, Suoranta H (1988) Ten-year followup study of patients with *Yersinia* arthritis. Arthritis Rheum 31:533–537

Longenberger A, Gronostaj M, Yee G et al (2014) *Yersinia enterocolitica* infections associated with improperly pasteurized milk products: southwest Pennsylvania, March–August, 2011. Epidemiol Infect 142:1640–1650

Lorencova A, Babak V, Lamka J (2016) Serological prevalence of enteropathogenic *Yersinia* spp. in pigs and wild boars from different production systems in the Moravian region. Czech Rep Foodborne Pathogen Dis 13:275–279

Lucero-Estrada C, Favier GI, Escudero ME (2020) An overview of *Yersinia enterocolitica* and related species in samples of different origin from San Luis, Argentina. Food Microbiol 86:103345

MacDonald E, Einöder-Moreno M, Borgen K et al (2016) National outbreak of *Yersinia enterocolitica* infections in military and civilian populations associated with consumption of mixed salad, Norway, 2014. Eurosurveillance 21:30321

Magistrali C, Cucco L, Pezzotti G et al (2015) Characterisation of *Yersinia pseudotuberculosis* isolated from animals with yersiniosis during 1996–2013 indicates the presence of pathogenic and Far Eastern strains in Italy. Vet Microbiol 180:161–166

McNally A, Thomson NR, Reuter S, Wren BW (2016) "Add, stir and reduce": *Yersinia* spp. as model bacteria for pathogen evolution. Nat Rev Microbiol 14(177)

Moorman VR, Cohen JI (2021) Insights into the individual evolutionary origins of *Yersinia* virulence factor effector proteins. Plasmid 114:102562

Morka K, Bystroń J, Bania J et al (2018) Identification of *Yersinia enterocolitica* isolates from humans, pigs and wild boars by MALDI TOF MS. BMC Microbiol 18:1–10

Mühlenkamp M, Oberhettinger P, Leo JC et al (2015) *Yersinia* adhesin A (YadA)–beauty & beast. Int J Med Microbiol 305:252–258

O'Grady D, Kenny K, Power S et al (2016) Detection of *Yersinia enterocolitica* serotype O:9 in the faeces of cattle with false positive reactions in serological tests for brucellosis in Ireland. Vet J 216:133–135

Ong KL, Gould LH, Chen DL et al (2012) Changing epidemiology of *Yersinia enterocolitica* infections: markedly decreased rates in young black children, Foodborne Diseases Active Surveillance Network (FoodNet), 1996–2009. Clin Infect Dis 54:S385–S390

Pakharukova N, Roy S, Tuittila M et al (2016) Structural basis for Myf and Psa fimbriae-mediated tropism of pathogenic strains of *Yersinia* for host tissues. Mol Microbiol 102:593–610
Pärn T, Hallanvuo S, Salmenlinna S et al (2015) Outbreak of *Yersinia pseudotuberculosis* O:1 infection associated with raw milk consumption, Finland, spring 2014. Eurosurveillance 20:30033
Peng Z, Zou M, Li M et al (2018) Prevalence, antimicrobial resistance and phylogenetic characterization of *Yersinia enterocolitica* in retail poultry meat and swine feces in parts of China. Food Control 93:121–128
Petsios S, Fredriksson-Ahomaa M, Sakkas H, Papadopoulou C (2016) Conventional and molecular methods used in the detection and subtyping of *Yersinia enterocolitica* in food. Int J Food Microbiol 237:55–72
Radziszewski F, Kucharczyk B, Sadkowska-Todys M (2019) Yersiniosis in Poland in 2017. Przegl Epidemiol 73:429–436
Ragno VM, Uehlinger FD, Gabadage K et al (2019) Investigation of a *Yersinia enterocolitica* outbreak in a commercial alpaca farm in Saskatchewan. Can Vet J 60:877–882
Råsbäck T, Rosendal T, Stampe M et al (2018) Prevalence of human pathogenic *Yersinia enterocolitica* in Swedish pig farms. Acta Vet Scand 60:1–8
Raymond P, Houard E, Denis M, Esnault E (2019) Diversity of *Yersinia enterocolitica* isolated from pigs in a French slaughterhouse over 2 years. MicrobiologyOpen 8:e00751
Reinhardt M, Hammerl JA, Kunz K et al (2018) *Yersinia pseudotuberculosis* prevalence and diversity in wild boars in northeast Germany. Appl Environ Microbiol 84
Reuter S, Connor TR, Barquist L et al (2014) Parallel independent evolution of pathogenicity within the genus *Yersinia*. Proc Natl Acad Sci 111:6768–6773
Reuter S, Corander J, de Been M et al (2015) Directional gene flow and ecological separation in *Yersinia enterocolitica*. Microbial. Genomics 1
Righetto RD, Anton L, Adaixo R et al (2020) High-resolution cryo-EM structure of urease from the pathogen *Yersinia enterocolitica*. Nat Commun 11:1–10
Rimhanen-Finne R, Niskanen T, Hallanvuo S et al (2009) *Yersinia pseudotuberculosis* causing a large outbreak associated with carrots in Finland, 2006. Epidemiol Infect 137:342–347
Rivas L, Strydom H, Paine S et al (2021) Yersiniosis in New Zealand. Pathogens 10:191
Rosner B, Stark K, Höhle M, Werber D (2012) Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009–2010. Epidemiol Infect 140:1738–1747
Rosner BM, Werber D, Höhle M, Stark K (2013) Clinical aspects and self-reported symptoms of sequelae of *Yersinia enterocolitica* infections in a population-based study, Germany 2009–2010. BMC Infect Dis 13:1–8
Rouffaer LO, Baert K, Van den Abeele A-M et al (2017) Low prevalence of human enteropathogenic *Yersinia* spp. in brown rats (*Rattus norvegicus*) in Flanders. PLoS One 12:e0175648
Sannö A, Rosendal T, Aspán A et al (2018) Distribution of enteropathogenic *Yersinia* spp. and Salmonella spp. in the Swedish wild boar population, and assessment of risk factors that may affect their prevalence. Acta Vet Scand 60:1–9
Saraka D, Savin C, Kouassi S et al (2017) *Yersinia enterocolitica*, a neglected cause of human enteric infections in Cote d'Ivoire. PLoS Negl Trop Dis 11:e0005216
Savin C, Criscuolo A, Guglielmini J et al (2019) Genus-wide *Yersinia* core-genome multilocus sequence typing for species identification and strain characterization. Microbial. Genomics:5
Savin C, Le Guern A-S, Lefranc M et al (2018) Isolation of a *Yersinia enterocolitica* biotype 1B strain in France, and evaluation of its genetic relatedness to other European and North American biotype 1B strains. Emerging Microbes Infect 7:1–3
Savin C, Martin L, Bouchier C et al (2014) The *Yersinia pseudotuberculosis* complex: characterization and delineation of a new species, Yersinia wautersii. Int J Med Microbiol 304:452–463
Seecharran T, Kalin-Manttari L, Koskela K et al (2017) Phylogeographic separation and formation of sexually discrete lineages in a global population of *Yersinia pseudotuberculosis*. Microbial. Genomics 3
Silva JE, Mayordomo AC, Dave MN et al (2020) Dendritic cells of mesenteric and regional lymph nodes contribute to *Yersinia enterocolitica* O:3–induced reactive arthritis in TNFRp55−/− mice. J Immunol 204:1859–1868

Stamm I, Hailer M, Depner B et al (2013) *Yersinia enterocolitica* in diagnostic fecal samples from European dogs and cats: identification by Fourier transform infrared spectroscopy and matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 51: 887–893

Stanger K, McGregor H, Marenda M et al (2018a) A longitudinal study of faecal shedding of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by Merino lambs in south-eastern Australia. Prev Vet Med 153:30–41

Stanger KJ, McGregor H, Larsen JWA (2018b) Outbreaks of diarrhoea ('winter scours') in weaned Merino sheep in south-eastern Australia. Aust Vet J 96:176–183. https://doi.org/10.1111/avj.12696

Stephan R, Cernela N, Ziegler D et al (2011) Rapid species specific identification and subtyping of *Yersinia enterocolitica* by MALDI-TOF mass spectrometry. J Microbiol Methods 87:150–153

Stephan R, Joutsen S, Hofer E et al (2013) Characteristics of *Yersinia enterocolitica* biotype 1A strains isolated from patients and asymptomatic carriers. Eur J Clin Microbiol Infect Dis 32: 869–875

Stoute ST, Cooper GL, Bickford AA et al (2016) Retrospective study of avian yersiniosis at the California animal health and food safety laboratory system (1990–2015). Bus Manag 60:82–86

Strydom H, Wang J, Paine S et al (2019) Evaluating sub-typing methods for pathogenic *Yersinia enterocolitica* to support outbreak investigations in New Zealand. Epidemiol Inf 147

Tack DM, Ray L, Griffin PM et al (2020) Preliminary incidence and trends of infections with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 US Sites, 2016–2019. Morb Mortal Wkly Rep 69:509

Takahashi T, Kabeya H, Sato S et al (2020) Prevalence of *Yersinia* among wild sika deer (*Cervus nippon*) and boars (*Sus scrofa*) in japan. J Wildlife Dis 56:270–277

Tan SY, Tan IKP, Tan MF et al (2016) Evolutionary study of *Yersinia* genomes deciphers emergence of human pathogenic species. Sci Rep 6:1–10

Timchenko NF, Adgamov RR, Popov AF et al (2016) Far East Scarlet-like fever caused by a few related genotypes of *Yersinia pseudotuberculosis,* Russia. Emerg Infect Dis 22:503

Tseneva GY, Chesnokova MV, Timofeevich KV et al (2012) Pseudotuberculosis in the Russian federation. In: Advances in Yersinia Research. Springer, pp 63–68

Tsubokura M, Aleksić S (1995) A simplified antigenic scheme for serotyping of *Yersinia pseudotuberculosis*: phenotypic characterization of reference strains and preparation of O and H factor sera. Contrib Microbiol Immunol 13:99–105

Tsubokura M, Otsuki K, Sato K et al (1989) Special features of distribution of Yersinia pseudotuberculosis in Japan. J Clin Microbiol 27:790–791

Ullah Q, Jamil T, Hussain MH et al (2019) High seroprevalence of pathogenic *Yersinia* spp. in sheep and goats across nine government farms in the Pakistani Punjab. J Inf Dev Countries 13: 843–846

Vanantwerpen G, Berkvens D, De Zutter L, Houf K (2017) Assessment of factors influencing the within-batch seroprevalence of human enteropathogenic *Yersinia* spp. of pigs at slaughter age and the analogy with microbiology. Prev Vet Med 137:93–96

Vasala M, Hallanvuo S, Ruuska P et al (2014) High frequency of reactive arthritis in adults after *Yersinia pseudotuberculosis* O: 1 outbreak caused by contaminated grated carrots. Ann Rheum Dis 73:1793–1796

Verbikova V, Borilova G, Babak V, Moravkova M (2018) Prevalence, characterization and antimicrobial susceptibility of *Yersinia enterocolitica* and other *Yersinia* species found in fruits and vegetables from the European Union. Food Control 85:161–167

Wang X, Liang J, Xi J et al (2014) *Canis lupus* familiaris involved in the transmission of pathogenic *Yersinia* spp. in China. Vet Microbiol 172:339–344. https://doi.org/10.1016/j.vetmic.2014.04.015

Wauters G, Aleksić S, Charlier J, Schulze G (1991) Somatic and flagellar antigens of *Yersinia enterocolitica* and related species. Contrib Microbiol Immunol 12:239–243

Wauters G, Kandolo K, Janssens M (1987) Revised biogrouping scheme of *Yersinia enterocolitica*. Contrib Microbiol Immunol 9:14–21

Weagant SD, Feng P (2017) BAM Chapter 8: *Yersinia enterocolitica.* https://www.fda.gov/food/laboratory-methods-food/bam-chapter-8-yersinia-enterocolitica

Wielkoszynski T, Moghaddam A, Bäckman A et al (2018) Novel diagnostic ELISA test for discrimination between infections with *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Eur J Clin Microbiol Infect Dis 37:2301–2306

Williamson DA, Baines SL, Carter GP et al (2016) Genomic insights into a sustained national outbreak of *Yersinia pseudotuberculosis*. Genome Biol Evol 8:3806–3814

Yehualaeshet T, Graham M, Montgomery M et al (2013) Effects of temperature on the viability, growth and gene profile of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* inoculated in milk. Food Control 34:589–595

Zhou Z, Alikhan N-F, Mohamed K et al (2020) The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. Genome Res 30:138–152

Enterohemorrhagic *E. coli* (EHEC): Environmental-Vehicle-Human Interface

11

Carlos L. Correa-Martinez, Shana R. Leopold, Robin Köck, Annelene Kossow, Andreas Bauwens, and Alexander Mellmann

Contents

Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) are a pathogenic subgroup of Shiga toxin-producing *E. coli* (STEC) that can cause severe intestinal disease and hemolytic uremic syndrome (HUS) in humans, the latter of which is of significant clinical concern. Cattle are the major reservoir of EHEC, where host-related factors allow bacteria to persist asymptomatically for years. Of particular concern are a small percentage of animals in herds that shed extremely high numbers of

Current addresses: Carlos L. Correa-Martinez: Zentrum für Internationalen Gesundheitsschutz (ZIG), Robert Koch-Institut, Berlin, Germany; Robin Köck: DRK Kliniken Berlin, Berlin, Germany; Annelene Kossow: Gesundheitsamt Köln, Köln, Germany; Andreas Bauwens: Westfälische Wasser- und Umweltanalytik, Gelsenkirchen, Germany

C. L. Correa-Martinez · R. Köck · A. Kossow · A. Bauwens · A. Mellmann (✉)
National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS), Institute of Hygiene, University Hospital Münster, Münster, Germany
e-mail: correa-martinezc@rki.de; kockr@uni-muenster.de; r.koeck@drk-kliniken-berlin.de; annelene.kossow@stadt-koeln.de; andreas.bauwens@wwu-labor.de; mellmann@uni-muenster.de; Alexander.Mellmann@ukmuenster.de

S. R. Leopold
Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA
e-mail: shana.leopold@jax.org

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_9

EHEC ($\geq 10^4$ CFU/g of feces), termed "supershedders," and are responsible for the majority of EHEC spread and contamination in a farm environment. Another transmission route is through the environment, where EHEC can express genetically encoded factors of bacterial fitness, enabling the bacteria to remain viable in bovine feces, soil, and water for weeks, up to several months. Contamination of meat during slaughter or processing and contamination of plants via EHEC-containing water or manure are major routes of entry into the food chain. The predominant EHEC-serotype O157 as well as non-O157 strains caused several hundred outbreaks in industrialized countries worldwide. Current and future research efforts are focused on rapid outbreak identification, development of therapeutics to treat HUS, and implementation of measures to reduce colonization of herds as well as prevent spread from animal reservoirs.

Keywords

Enterohemorrhagic Escherichia coli (EHEC) · Shiga toxin-producing E. coli (STEC) · Food-bornd outbreak · Transmission · Supershedder

11.1 Introduction

Most members of the species *E. coli* are part of the physiological flora in the gastrointestinal tracts of humans and animals. In addition to these commensal bacteria, there are pathogenic *E. coli* that cause extraintestinal and intestinal disease. Intestinal pathogenic *E. coli* presently include seven pathogroups: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), adherent invasive *E. coli* (AIEC), diffusely adherent *E. coli* (DAEC), and enterohemorrhagic *E. coli* (EHEC) (Croxen et al. 2013). Each pathotype is associated with unique epidemiological characteristics and specific forms of disease that cause significant morbidity and mortality. Zoonotic *E. coli*, of which EHEC are the prototype, are a major cause of foodborne disease, thus posing complex challenges to the food industry. Due to their public health relevance, EHEC are intensively studied in human and veterinary medicine. Ongoing investigations are addressing ecology of EHEC in animals, persistence and survival in the environment, and how these factors affect entry into, or dissemination along, the food chain. Other areas of research include the epidemiology of EHEC infections in humans, diagnostics, pathogenic mechanisms of these bacteria, and treatment as there is currently no specific therapy.

EHEC can cause a broad clinical spectrum of disease which includes watery or bloody diarrhea and hemolytic uremic syndrome (HUS), a leading cause of acute renal failure in children (Tarr et al. 2005). Since the first isolation of an EHEC outbreak strain in the USA in 1982 (Riley et al. 1983), which was identified as *E. coli* serotype O157:H7, EHEC has emerged as an important public health concern worldwide. The large EHEC O104:H4 outbreak in Germany in 2011 with 3842 cases, 855 HUS patients, and 53 deaths demonstrates the significant impact of an

EHEC outbreak on human health (RKI 2011) and also the challenges in source tracing due to growing globalization of food production (Karch et al. 2012; Kampmeier et al. 2018).

11.2 Expression of Shiga Toxins in EHEC

A key characteristic of the EHEC pathotype is the presence of bacteriophage-encoded Shiga toxins (Stx). Stx, also known as verocytotoxins (VTs), are members of a large family of cytotoxins that are characterized by a high degree of sequence diversity. The Stx family is divided into two major branches, Stx1 and Stx2, and many toxin subtypes and variants have been described (Karch et al. 2009; Bergan et al. 2012; Scheutz et al. 2012). Classification of Stx subtypes is used not only for taxonomic purposes, but also serves as an important clinical predictor, because Stx found in strains associated with HUS differ from those Stx subtypes that are carried by strains causing a milder course of disease (Scheutz et al. 2012). More specifically, a significant association has been established between strains producing Stx2 and the development of hemorrhagic colitis and HUS. The subtypes Stx2a, Stx2c, and Stx2d are most relevant in human infection (Joseph et al. 2020), while Stx2e is associated with edema disease of pigs, one of the rare veterinary clinical manifestations of EHEC infections (Moxley 2000).

A sequence-based protocol for characterization of the Stx genes has been described (Scheutz et al. 2012) and includes three levels of classification: types, subtypes, and variants (see Table 1).

1. Types: The two major branches Stx1 and Stx2 share structure and function but are not cross-neutralized with heterologous antibodies. The terms Stx1 and Stx2 should only be used when the subtype is unknown.
2. Subtypes: Currently the antigenically related members of Stx1 (Stx1a, Stx1c, and Stx1d) and Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g) are distinguished.

Table 1 Types, subtypes, and variants of Shiga toxins according to Scheutz et al. (2012)

Types	Subtypes	Variants (examples)
Stx1	Stx1a	Stx1a-O157-EDL933
	Stx1c	Stx1c-O174-DG131-3
	Stx1d	Stx1d-ONT-MHI813
Stx2	Stx2a	Stx2a-O104-G5506
	Stx2b	Stx2b-O111-S-3
	Stx2c	Stx2c-O157-A75
	Stx2d	Stx2d-O91-B2F1
	Stx2e	Stx2e-O26-R107
	Stx2f	Stx2f-O128-T4-97
	Stx2g	Stx2g-O2-S86

3. Variants: Variants include the subtype-specific prototypic toxins or related toxins within a subtype (that differ by one or more amino acids from the prototype). The variants are designated by toxin subtype, O-antigen group of the host *E. coli* strain, followed by the strain name or number from which that toxin was initially described, for example, Stx1a-O157-EDL933 or Stx2a-O104-G5506 (Scheutz et al. 2012) (see Table 1). Nucleotide variants within a given Stx subtype are italicized.

All Stx consist of a single A subunit and five identical B subunits (AB_5). The A subunit is the enzymatically active component, and the Stx B pentamer binds to endothelial cellular ligand glycosphingolipids (GSLs): with high affinity to globotriaosylceramide (Gb3Cer) and less effective binding to globotetraosylceramide (Gb4Cer) (Müthing et al. 2009). Stx1 and Stx2 share identical binding specificity (Müthing et al. 2009). After binding to the cell surface, the AB_5-Gb3Cer complex is internalized by various endocytic mechanisms and routed from the early endosomes through the *trans*-Golgi-network and the Golgi stacks to the endoplasmic reticulum (Sandvig et al. 2010; Bauwens et al. 2013). Moreover, evidence suggests that Stx (like other ribosome-inactivating proteins) not only remove adenine moieties from rRNA, but also efficiently depurinate DNA. Stx genes are found within the genomes of temperate bacteriophages, which are mobile elements that can easily integrate at specific sites in the bacterial chromosome. In vitro and in vivo studies have demonstrated that most EHEC can lose the Stx-encoding gene by bacteriophage excision during infection, isolation, or subculture, resulting in *stx*-negative isolates (Mellmann et al. 2009). This finding is also important for the diagnostics of EHEC infections: If stool samples from patients, especially those suffering from HUS, which usually develops >7 days after the onset of infection, lack the detection of *stx*, EHEC-associated HUS still cannot be ruled out.

A close genetic relationship between *stx*-positive and *stx*-negative EHEC O157:H7 isolates has been recently described using next-generation sequencing (NGS). The loss of *stx* genes poses an additional diagnostic challenge, since routine detection of EHEC relies on the identification of *stx* genes. This could potentially lead to misidentification, with the therapeutic and epidemiologic consequences that this entails (Ferdous et al. 2015; Bielaszewska et al. 2007; Mellmann et al. 2005).

11.3 Epidemiology of EHEC in Animals

Several studies have demonstrated that cattle are the main reservoir of human pathogenic EHEC O157:H7, in addition to many pathogenic non-O157 EHEC serotypes (Naylor et al. 2005a). These bacteria have adapted to an oral-fecal cycle in cattle, where EHEC colonization begins with ingestion and subsequent entrance to the rumen and gastrointestinal tract, but they generally do not have a pathogenic effect on adult animals. Systemic disease similar to that observed in humans does not occur in cattle due to the absence of Stx receptors in bovine endothelial cells; however, EHEC may cause diarrhea and enterocolitis in calves (Dean-Nystrom et al. 1997). Although

certain non-O157 serogroups (O26, O111, O118) (Naylor et al. 2005a) have been reported to cause disease in young calves, their role in diarrheal disease among these animals remains controversial, as EHEC has been more commonly detected in healthy calves than in symptomatic ones (Kolenda et al. 2015). Prevalence among cattle varies widely and may be due to several circumstances including the geographical region, animal age, or the specific farm conditions (Ferens and Hovde 2011). Published prevalence rates vary dramatically, from 0% to 36% among animals studied in different countries and farm types (Naylor et al. 2005a), with highest prevalence values described in Africa and North America (Islam et al. 2014). Studies have also shown that EHEC prevalence is related to the type of farm (i.e., beef, dairy, or mixed) and may be influenced by factors such as cattle movement, hygiene management, diet, husbandry, as well as the type of specimen analyzed (Menrath et al. 2010; Cobbaut et al. 2009; Ferens and Hovde 2011; Islam et al. 2014). While cattle are the major known reservoir of EHEC, other minor reservoirs include sheep, goats, pigs, horses, dogs, poultry, and deer (Naylor et al. 2005a).

The persistence of EHEC O157:H7 in cattle may be due to its ability to colonize a particular niche within the lower gastrointestinal tract (Grauke et al. 2002). Tissue tropism for the colon has been demonstrated by immunofluorescent detection of microcolonies on the lymphoid follicle-dense mucosa at the terminal rectum within 3–5 cm proximal to the recto-anal junction (Grauke et al. 2002; Naylor et al. 2003, 2005b). A correlation between recto-anal junction colonization and supershedder status has been described (Cobbold et al. 2007; Low et al. 2005). Supershedding is thought to be caused, in part, by an impaired innate and adaptive immune response in rectal tissue due to the downregulation of relevant genes. EHEC's ability to form a biofilm on the bovine intestinal epithelium may be an additional factor contributing to the supershedding phenomenon as portions of the biofilm may detach during defecation, allowing for the sporadic shedding of high numbers of bacteria (Munns et al. 2015). Furthermore, several fimbrial and afimbrial proteins expressed by EHEC O157 and non-O157 strains likely play a role in ruminant reservoir persistence (Farfan and Torres 2012). In studies that used bovine terminal rectal primary epithelial cells, the H7 flagellum was demonstrated to act as an adhesin to bovine intestinal epithelium, supporting its involvement in initiating colonization of the cattle reservoir (Mahajan et al. 2009). Additionally, proteins such as EhaB, ELF, HCP, and UpaG have been shown to enhance intestinal adhesion by binding to laminin, an extracellular matrix protein (Segura et al. 2018). Stx may also play a role in colonization and persistence by blocking bovine lymphocyte activation and thus suppressing the bovine host's immune response to the intestinal colonization (Moussay et al. 2006).

11.4 EHEC in the Environment

EHEC can survive in bovine feces for a long time making feces (or manure) a likely vehicle for transmission to cattle, food, and the environment. Survival in feces can range from 1 to 18 weeks depending on the temperature (5–25 °C) (Fukushima et al. 1999). Several factors contributing to a prolonged survival of specific EHEC O157:

H7 strains have been identified, including biofilm-formation and the ability to activate genes promoting bacterial fitness in adverse environmental conditions (Segura et al. 2018). Entry of EHEC to the environment may occur directly through deposit of feces onto land or through drainage runoff of fecal material in soil, especially after heavy rainfalls (Thurston-Enriquez et al. 2005). Moreover, under experimental conditions, EHEC can even survive for more than 1 year in various manure-amended soils at different temperatures (Fremaux et al. 2008). Long-term survival of EHEC in lake water (13 weeks) and in cold river water (27 days) has also been demonstrated (Wang and Doyle 1998; Maule 2000). This extended persistence in the environment likely plays a significant role in the colonization of cattle and subsequent human infection (Fremaux et al. 2008).

EHEC O157:H7 is also able to colonize various types of plants and fruits. For example, EHEC O157:H7 has been shown to form bacterial aggregates on apples (Janes et al. 2005) as well as on the surface of lettuce leaves (Seo and Frank 1999; Auty et al. 2005). Furthermore, studies have found EHEC in the internal inner tissues of plants, including radishes, carrots, and lettuce (Itoh et al. 1998; Solomon et al. 2002). These subsurface localizations may be protective to the bacteria as they are inaccessible to other competitive bacteria as well as surface treatments and washing. The growth rate of EHEC in leafy vegetables, sprouts, and soil has been found to depend on the type of plant; however, growth was detected irrespective of the serotype or the Stx carriage (Merget et al. 2020). Once attached to food items, EHEC can remain viable for long periods of time, such as up to 9 months at room temperature in wheat flour (Forghani et al. 2018) and over 6 months in meat products frozen at −18 °C, even surviving freeze-thaw cycles (Ro et al. 2015).

11.5 EHEC Infections in Humans

After ingestion of EHEC, a 3- to 12-day incubation period is typically followed by development of watery diarrhea accompanied with abdominal cramping and pain. Approximately 90% of patients with culture-positive infection will subsequently suffer from bloody diarrhea resulting from a hemorrhagic colitis caused by EHEC (Karch et al. 2005). About 1 week after the initial onset of diarrhea, HUS develops in a variable proportion of cases, depending on the causative EHEC strain serotype and Stx subtype (Tarr et al. 2005). HUS patients present with widespread thrombotic microvascular lesions in the kidneys, the gastrointestinal tract, and other organs (Richardson et al. 1988). Since EHEC infections are rarely bacteremic, that is, bacteria do not penetrate the circulatory system and are not found in patient blood cultures (Bielaszewska and Karch 2005), it is hypothesized that HUS results from vascular endothelial injury by circulating Stx. According to the generally accepted model of HUS pathogenesis, Stx is released by EHEC in the intestine, absorbed across the gut epithelium into the circulation (Hurley et al. 2001; Müthing et al. 2009), and transported to small vessel endothelial cells.

HUS is the most common cause of acute renal failure in children, with a 1–4% mortality rate (Spinale et al. 2013; Karch et al. 2005). While 70% of EHEC-infected

patients were fully recovered within 5 years after diagnosis, the remaining 30% still experienced hypertension (9%), neurological symptoms (4%), decreased glomerular filtration rate (7%), and/or proteinuria (18%) (Rosales et al. 2012). The occurrence of extra-renal sequelae has also been reported, including colonic strictures, diabetes mellitus, cholelithiasis, and neurological disorders (Spinale et al. 2013). There is currently no effective therapy, and antibiotic treatment is mostly discouraged, as in vitro data suggested that antibiotics used in an early phase of the infection might increase the release of Stx by EHEC bacteria and thus induce HUS (Wong et al. 2000; Davis et al. 2013). In children, the risk of HUS development associated with antibiotic treatment has been shown to reach up to 25% (Wong et al. 2012). In a first round of a meta-analysis including all available studies, the association between antibiotics and the development of HUS was initially questioned with a pooled Odds Ratio (OR) of 1.33 (95% confidence interval [CI], 0.89–1.99). The re-analysis, however, focusing only on studies with less bias risks and appropriate HUS-definitions, yielded a significant association with an OR of 2.24 (95% CI, 1.45–3.46) (Freedman et al. 2016). In contrast to cattle, EHEC O157:H7 colonizes humans only for a limited time of about 4 weeks (Karch 1996) (Fig. 1). Moreover, in cattle many different EHEC O157:H7 subtypes can co-exist in a single animal (Jacob et al. 2011), while human patients are infected mostly by a distinct O157:H7 subtype.

EHEC O157:H7 is the most prevalent EHEC serotype identified as causative of sporadic HUS cases (Tarr et al. 2005; Karch et al. 2005), though non-O157:H7 EHEC (especially O26:H11, O103:H2, O111:H8, O145:H28/H25 and sorbitol-fermenting (SF) O157:H$^-$) represent a significant portion of EHEC infections leading to HUS complications (Karch et al. 2005; Mellmann et al. 2008; Bielaszewska et al. 2013). In Europe, O26 is the serotype most commonly associated with HUS development, accounting for 39% of all cases, followed by O157 with 23% (EFSA/ECDC 2021). Although EHEC strains are often considered as a pathogroup, there may be important differences between serotypes (CDC 2006).

SF EHEC O157:H$^-$ represent a significant serotype in Europe which has not yet been detected in North America. These strains are characterized by a specific

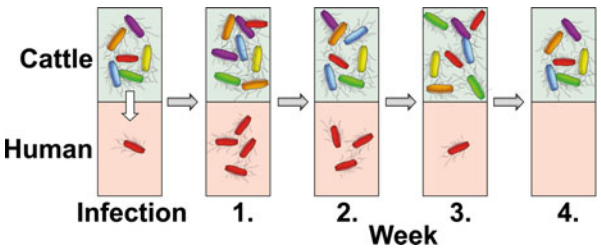

Fig. 1 Schematic illustration of EHEC O157:H7 infection in cattle and humans. In contrast to cattle, EHEC O157:H7 colonizes humans only for a limited time of about 4 weeks. Moreover, whereas in cattle many different EHEC O157:H7 subtypes can co-exist in a single animal, human patients are infected mostly by a distinct O157:H7 subtype. Different EHEC O157:H7 subtypes are indicated by different colors

combination of their phenotypic and virulence characteristics that differentiates them from classical non-SF EHEC O157:H7, including the ability to ferment sorbitol overnight and produce β-D-glucuronidase (Karch and Bielaszewska 2001; Bauwens et al. 2017). The *sfp* gene cluster, which encodes fimbriae and mediates mannose-resistant hemagglutination, has been identified on the large plasmid of SF STEC O157:H$^-$ and is notably absent in EHEC O157:H7 (Brunder et al. 2001).

The minimum infectious dose of EHEC in humans is extremely low, with approximately 10–50 bacteria needed for colonization (Teunis et al. 2004). In meat implicated as an outbreak source in the USA in 1993, there were less than 700 EHEC O157:H7 bacterial cells per hamburger patty prior to cooking (Tuttle et al. 1999). Moreover, a high degree of tolerance to acid and drying enables EHEC to survive even in food items that rarely cause foodborne illness (e.g., apple cider, semi-dry fermented sausage). Nonmeat products such as cookie dough, flour, and soy nut butter have also been identified as sources of EHEC outbreaks, demonstrating the complexity of transmission chains between animals and humans (Neil et al. 2012; Hassan et al. 2019; Crowe et al. 2017). Three principal routes of transmission of EHEC infection have been identified: (1) contaminated food and contaminated water used for drinking or swimming, (2) person-to-person transmission, and (3) direct animal contact. The latter was described for contact in petting zoos, on farms, and within homes (e.g., housing domesticated sheep, goats, and other small animals like cats and dogs) (Crump et al. 2002; Karch et al. 2005; Neil et al. 2012).

11.6 EHEC Outbreaks

EHEC is the cause of hundreds of outbreaks worldwide (Griffin et al. 1988; Michino et al. 1999; Karch et al. 1999; Tack et al. 2021). Examples of large outbreaks caused by EHEC O157:H7 and non-O157 are described in Tables 2 and 3, respectively.

After its discovery in 1982 as the causative agent of two unprecedented outbreaks related to consumption of undercooked meat in the United States (Riley et al. 1983), EHEC O157:H7 increasingly became acknowledged as a public health threat. As observed in these early outbreaks, consumption of raw or undercooked food items of bovine origin, particularly ground beef (hamburger), is a common mode of EHEC O157:H7 transmission. Moreover, contaminated sprouted seeds, fruits, and leafy greens, such as radish sprouts, lettuce, spinach, strawberries, as well as contaminated water have been implicated in transmitting EHEC O157:H7 (Kintz et al. 2019; Saxena et al. 2015).

One of the largest outbreaks occurred in 1996 in Sakai City, Japan (Watanabe et al. 1996; Michino et al. 1999), where thousands were affected, mostly school children. White radish sprouts served during school lunches were the most probable vehicle of the infection. In the winter of 1992–1993, the largest outbreak of EHEC O157:H7 in the United States affected 501 persons in four western states including Washington, Idaho, Nevada, and California (Bell et al. 1994) where 45 persons, mostly children, developed HUS and three children died. Hamburgers from a single fast-food restaurant chain were identified as the vehicle of the infection (Bell et al.

Table 2 Example of outbreaks caused by EHEC O157:H7

Year	Country	Cases/HUS/deaths[a]	Source	Reference
1982	USA	47/0/0	Hamburger[b]	Riley et al. (1983)
1992–1993	USA	501/45/3	Hamburger[b]	Bell et al. (1994)
1996	Scotland	345/34/16	Meat[b]	Dundas et al. (2001)
1996	Japan	>6000/n.a./2	Radish sprouts	Watanabe et al. (1996)
2000	Canada	~2300/28/7	Drinking water[b]	Hrudey et al. (2003)
2005	Sweden	135/11/0	Lettuce	Söderström et al. (2008)
2006	USA	199/31/3	Spinach[b]	CDC (2006)
2006	USA	77/7/0	Iceberg lettuce	Sodha et al. (2011)
2011	USA	15/4/2	Strawberries[b]	Laidler et al. (2013)
2012	Japan	107/2/5	Pickled cabbage	Tabuchi et al. (2015)
2015	USA	19/2/0	Rotisserie chicken salad[b]	CDC (2015)
2017	USA	32/9/0	SoyNut Butter[b]	CDC (2017)
2020	USA	40/4/0	Leafy greens	CDC (2020)

n.a. not available
[a]Total number of cases identified/number of HUS cases/number of deaths
[b]Strain isolated from the source

1994). The largest outbreak caused by EHEC O157:H7 contaminated drinking water occurred in Canada in 2000. Approximately 2300 people became seriously ill and seven died. In Europe, a large EHEC O157:H7 outbreak occurred in Central Scotland in 1996; 345 people contracted an infection after consuming meat from a single butcher's shop and 16 died (Dundas et al. 2001). Foodborne transmission of EHEC O157:H7 also plays an important role in healthcare facilities, as shown by an outbreak in Japan associated with consumption of contaminated pickles from one manufacturer, affecting a total of 94 residents in 10 different nursing homes (Tabuchi et al. 2015). Similarly, outbreaks in childcare facilities have also been reported (Kanayama et al. 2015).

Non-O157 EHEC strains representing a wide range of serotypes have caused numerous large outbreaks, with the largest to-date occurring in Germany in 2011 associated with EHEC O104:H4 contaminated fenugreek sprouts (RKI 2011; Karch et al. 2012). Here, the proportion of patients who developed HUS was significantly higher than in O157-associated outbreaks (Tables 2 and 3). Similarly, 40% of 86 individuals affected by an EHEC O111 outbreak in 2015 in Japan involving raw meat consumption became severely ill with HUS (Yahata et al. 2015).

11.7 Future Strategies and Unresolved Issues

Advances in rapid alert systems for the early detection of EHEC outbreaks have created greater awareness for both the public and the clinical community. Moreover, an increasing number of clinical microbiological laboratories routinely screen for EHEC by detection of Stx-encoding genes and/or toxin production in cases of

Table 3 Examples of outbreaks caused by non-O157 EHEC

Year	Serotype	Country	Cases/HUS/deaths[a]	Source	Reference
1994	O104:H21	USA	18/0/0	Past. cow milk	CDC (1995)
1995	O111:H$^-$	Australia	n.a./20/1	Sausage[b,c]	Paton et al. (1996)
1999	O111:H8	USA	55/2/0	Salad bar	Brooks et al. (2004)
2001	O26:H11	Germany	11/0/0	Beef	Werber et al. (2002)
2004	O111:H$^-$	USA	27/0/0	Apple cider	Schaffzin et al. (2012)
2006	O103:H25	Norway	17/10/1	Mutton sausage[b]	Schimmer et al. (2008)
2007	O26:H11	Denmark	20/0/0	Beef sausage[b]	Ethelberg et al. (2009)
2007	O145 and O26	Belgium	12/5/0	Ice cream[b]	De Schrijver et al. (2008)
2009	O145:H28	Norway	16/0/0	Sheep	Wahl et al. (2011)
2010	O145:H$^-$	USA	33/3/0	Romanian lettuce[b]	CDC (2010)
2011	O104:H4	Germany	3842/855/53	Fenugreek sprouts	RKI (2011)
2011	O111	Japan	86/34/5	Raw beef[d]	Yahata et al. (2015)
2016	O26	USA	60/0/0	Mexican grill restaurants, ingredient not identified	CDC (2016)
2019	O26	USA	21/0/0	Flour[b]	CDC (2019)
2019	O26	France	16/14/0	Raw cow milk cheese	Jones et al. (2019)
2021	O121	USA	16/1/0	Cake mix[b]	CDC (2021)

n.a. not available
[a]Total number of cases identified/number of HUS cases/number of deaths
[b]Strain isolated from the source
[c]"Mettwurst," German sausage made from raw minced pork
[d]"Yukhoe," Korean raw beef dish

bloody diarrhea or clinical HUS. Diagnosed cases are now legally required to be reported in nearly every country. New high-resolution techniques including NGS are becoming more accessible, which enable the rapid identification of outbreaks at the earliest stages (Mellmann et al. 2011). In recent years, new approaches that integrate NGS data and epidemiological information to establish national genomic surveillance networks have been implemented in countries such as the USA, England, and Germany to keep track of EHEC cases, enhancing outbreak detection (FDA 2021; Jenkins et al. 2019; WWU/RKI/FZB 2021). With strain linkage analyses, common sources of infection can be identified accurately and rapidly. Moreover, microevolutionary models of important non-O157 EHEC could already be developed (Eichhorn et al. 2018). This is especially important nowadays when foodborne outbreaks less frequently follow the "church picnic" model, in which small isolated

clusters of illness can easily be identified with case interviews and more frequently result from the dissemination of (industrially produced) vehicles that are contaminated by relatively low levels of pathogens. In a globalized world, food safety has also gained a global dimension. Livestock and food production crosses regional and national borders, which facilitates large-scale EHEC outbreaks across vast geographical areas. In this context, NGS is a valuable tool to understand complex transmission chains (Fung et al. 2018; Jagadeesan et al. 2019).

Another area where considerable efforts are being expended is improvement of farming practices and environmental factors that affect colonization of animals with EHEC. EHEC transmits readily between ruminants in the farm setting and wild animals can represent important vectors. For many years, the cattle industry and researchers have focused on improving the safety of meat products after slaughter. Post-slaughter antiseptic treatments of carcasses and HACCP policies in slaughter plants have been shown to significantly reduce meat contamination (Elder et al. 2000). Alternatively, probiotic bacteria could contribute to colonization control in cattle by producing metabolites that are inhibitory to EHEC. The addition of probiotics, such as *Lactobacillus acidophilus* (Sargeant et al. 2007; Stephens et al. 2007; Peterson et al. 2007; Younts-Dahl et al. 2005) and *Propionibacterium freudenreichii* (Sargeant et al. 2007), to animal feeds has been shown to effectively reduce fecal EHEC O157 shedding. Environmental contamination has been attained by applying soil solarization, with studies describing a >3.0-log reduction of EHEC O157 after 6 weeks of soil treatment (Berry and Wells 2012).

Due to the widespread distribution of EHEC O157 and non-O157 in cattle, its control will require intervention at the individual farm level. Vaccination approaches could be useful in controlling EHEC colonization among cattle, thus reducing the risk of human infection. Statistical models estimate that a vaccine efficacy of 60% would be required to effectively reduce infection rates in a herd (Wood et al. 2006). While use of these vaccines could reduce the risk of EHEC in cattle by 50%, this translates to approximately 85% reduction in human cases (Matthews et al. 2013). Different vaccine candidates have been developed, including compounds based on antibodies against Stx, as well as bacterial components such as proteins, peptides, DNA, and polysaccharides (Rojas-Lopez et al. 2018). Furthermore, plant-based vaccines have shown to effectively reduce EHEC shedding in animal models (Miletic et al. 2017). Two vaccines against EHEC O157:H7, which have been approved for use in cattle in Canada and the USA, have demonstrated a significant decrease in EHEC colonization and fecal load (O'Ryan et al. 2015). These vaccines have not yet been widely accepted by farmers, however, due to several factors including burden of responsibility and lack of economic return as vaccinated cattle has no added value over unvaccinated animals in the market (Matthews et al. 2013; Smith 2014). Still, more research is needed to develop viable strategies targeting the different factors that contribute to EHEC transmission (cattle, food, person-to-person spread, etc.) to work towards better control of EHEC.

Further research is also needed to address effective therapies for humans upon EHEC infection. Experimental investigations have focused on the use of Stx receptor analogs and Stx-specific antibodies. Pharmacokinetic and clinical factors,

limitations with extrapolation of results from research in animal models to human cases, and the low availability of patients for phase III clinical trials continue to significantly hinder the development of EHEC-specific therapeutic agents (Mühlen and Dersch 2020).

References

Auty M, Duffy G, O'Beirne D, McGovern A, Gleeson E, Jordan K (2005) In situ localization of *Escherichia coli* O157:H7 in food by confocal scanning laser microscopy. J Food Prot 68:482–486

Bauwens A, Betz J, Meisen I, Kemper B, Karch H, Müthing J (2013) Facing glycosphingolipid-Shiga toxin interaction: dire straits for endothelial cells of the human vasculature. Cell Mol Life Sci 70:425–457

Bauwens A, Marejková M, Middendorf-Bauchart B, Prager R, Kossow A, Zhang W, Karch H, Mellmann A, Bielaszewska M (2017) Sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:H(−) isolates from Czech patients with novel plasmid composition not previously seen in German isolates. Appl Environ Microbiol 16:83

Bell BP, Goldoft M, Griffin PM, Davis MA, Gordon DC, Tarr PI, Bartleson CA, Lewis JH, Barrett TJ, Wells JG et al (1994) A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. JAMA 272:1349–1353

Bergan J, Dyve Lingelem AB, Simm R, Skotland T, Sandvig K (2012) Shiga toxins. Toxicon 60: 1085–1107

Berry ED, Wells JE (2012) Soil solarization reduces *Escherichia coli* O157:H7 and total *Escherichia coli* on cattle feedlot pen surfaces. J Food Prot 75:7–13

Bielaszewska M, Karch H (2005) Consequences of enterohaemorrhagic *Escherichia coli* infection for the vascular endothelium. Thromb Haemost 94:312–318

Bielaszewska M, Köck R, Friedrich AW, von Eiff C, Zimmerhackl LB, Karch H, Mellmann A (2007) Shiga toxin-mediated hemolytic uremic syndrome: time to change the diagnostic paradigm? PLoS One 2:e1024

Bielaszewska M, Mellmann A, Bletz S, Zhang W, Köck R, Kossow A, Prager R, Fruth A, Orth-Höller D, Marejková M, Morabito S, Caprioli A, Piérard D, Smith G, Jenkins C, Curová K, Karch H (2013) Enterohemorrhagic *Escherichia coli* O26:H11/H−: a new virulent clone emerges in Europe. Clin Infect Dis 56:1373–1381

Brooks JT, Bergmire-Sweat D, Kennedy M, Hendricks K, Garcia M, Marengo L, Wells J, Ying M, Bibb W, Griffin PM, Hoekstra RM, Friedman CR (2004) Outbreak of Shiga toxin-producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis 38:190–198

Brunder W, Khan AS, Hacker J, Karch H (2001) Novel type of fimbriae encoded by the large plasmid of sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:H(−). Infect Immun 69:4447–4457

CDC (1995) Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21 – Helena, Montana, 1994. MMWR Morb Mortal Wkly Rep 44:501–503

CDC (2006) Update on multi-state outbreak of *E. coli* O157:H7 infections from Fresh spinach [online]. Available: http://www.cdc.gov/ecoli/2006/september/updates/100606.htm. Accessed 6 Oct 2006

CDC (2010) Investigation update: multistate outbreak of human *E. coli* O145 infections linked to shredded Romaine lettuce from a single processing facility [online]. Available: http://www.cdc.gov/ecoli/2010/ecoli_o145/index.html?s_cid=ccu051010_006. Accessed 4 Oct 2014

CDC (2015) Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to Costco Rotisserie Chicken Salad (final update) [online]. Available: https://www.cdc.gov/ecoli/2015/o157h7-11-15/index.html. Accessed 9 Sept 2021

CDC (2016) Multistate outbreaks of Shiga toxin-producing *Escherichia coli* O26 infections linked to Chipotle Mexican Grill restaurants (final update) [online]. Available: https://www.cdc.gov/ecoli/2015/o26-11-15/index.html. Accessed 9 Sept 2021

CDC (2017) Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to I.M. Healthy Brand SoyNut Butter (final update) [online]. Available: https://www.cdc.gov/ecoli/2017/o157h7-03-17/index.html. Accessed 9 Sept 2021

CDC (2019) Outbreak of *E. coli* infections linked to flour [online]. Available: https://www.cdc.gov/ecoli/2019/flour-05-19/index.html. Accessed 9 Sept 2021

CDC (2020) Outbreak of *E. coli* infections linked to leafy greens [online]. Available: https://www.cdc.gov/ecoli/2020/o157h7-10-20b/index.html. Accessed 9 Sept 2021

CDC (2021) *E. coli* outbreak linked to cake mix [online]. Available: https://www.cdc.gov/ecoli/2021/o121-07-21/index.html. Accessed 9 Sept 2021

Cobbaut K, Berkvens D, Houf K, De Deken R, De Zutter L (2009) *Escherichia coli* O157 prevalence in different cattle farm types and identification of potential risk factors. J Food Prot 72:1848–1853

Cobbold RN, Hancock DD, Rice DH, Berg J, Stilborn R, Hovde CJ, Besser TE (2007) Rectoanal junction colonization of feedlot cattle by *Escherichia coli* O157:H7 and its association with supershedders and excretion dynamics. Appl Environ Microbiol 73:1563–1568

Crowe SJ, Bottichio L, Shade LN, Whitney BM, Corral N, Melius B, Arends KD, Donovan D, Stone J, Allen K, Rosner J, Beal J, Whitlock L, Blackstock A, Wetherington J, Newberry LA, Schroeder MN, Wagner D, Trees E, Viazis S, Wise ME, Neil KP (2017) Shiga toxin-producing *E. coli* infections associated with flour. N Engl J Med 377:2036–2043

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013) Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev 26:822–880

Crump JA, Sulka AC, Langer AJ, Schaben C, Crielly AS, Gage R, Baysinger M, Moll M, Withers G, Toney DM, Hunter SB, Hoekstra RM, Wong SK, Griffin PM, Van Gilder TJ (2002) An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med 347:555–560

Davis TK, McKee R, Schnadower D, Tarr PI (2013) Treatment of Shiga toxin-producing *Escherichia coli* infections. Infect Dis Clin N Am 27:577–597

De Schrijver K, Buvens G, Possé B, Van den Branden D, Oosterlynck O, De Zutter L, Eilers K, Piérard D, Dierick K, Van Damme-Lombaerts R, Lauwers C, Jacobs R (2008) Outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with the consumption of ice cream produced at a farm, Belgium, 2007. Euro Surveill 13:8041

Dean-Nystrom EA, Bosworth BT, Cray WC Jr, Moon HW (1997) Pathogenicity of *Escherichia coli* O157:H7 in the intestines of neonatal calves. Infect Immun 65:1842–1848

Dundas S, Todd WT, Stewart AI, Murdoch PS, Chaudhuri AK, Hutchinson SJ (2001) The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. Clin Infect Dis 33:923–931

EFSA/ECDC (2021) The European Union One Health 2019 zoonoses report. EFSA J 19:e06406

Eichhorn I, Semmler T, Mellmann A, Pickard D, Anjum MF, Fruth A, Karch H, Wieler LH (2018) Microevolution of epidemiological highly relevant non-O157 enterohemorrhagic *Escherichia coli* of serogroups O26 and O111. Int J Med Microbiol 308:1085–1095

Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW (2000) Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Natl Acad Sci U S A 97:2999–3003

Ethelberg S, Smith B, Torpdahl M, Lisby M, Boel J, Jensen T, Nielsen EM, Mølbak K (2009) Outbreak of non-O157 Shiga toxin-producing *Escherichia coli* infection from consumption of beef sausage. Clin Infect Dis 48:e78–e81

Farfan MJ, Torres AG (2012) Molecular mechanisms that mediate colonization of Shiga toxin-producing *Escherichia coli* strains. Infect Immun 80:903–913

FDA (2021) Whole genome sequencing (WGS) program [online]. Available: https://www.fda.gov/food/science-research-food/whole-genome-sequencing-wgs-program. Accessed 1 Mar 2022

Ferdous M, Zhou K, Mellmann A, Morabito S, Croughs PD, de Boer RF, Kooistra-Smid AM, Rossen JW, Friedrich AW (2015) Is Shiga toxin-negative *Escherichia coli* O157:H7

enteropathogenic or enterohemorrhagic *Escherichia coli*? Comprehensive molecular analysis using whole-genome sequencing. J Clin Microbiol 53:3530–3538

Ferens WA, Hovde CJ (2011) *Escherichia coli* O157:H7: animal reservoir and sources of human infection. Foodborne Pathog Dis 8:465–487

Forghani F, den Bakker M, Futral AN, Diez-Gonzalez F (2018) Long-term survival and thermal death kinetics of enterohemorrhagic *Escherichia coli* serogroups O26, O103, O111, and O157 in wheat flour. Appl Environ Microbiol 84:e00283-18

Freedman SB, Xie J, Neufeld MS, Hamilton WL, Hartling L, Tarr PI, Nettel-Aguirre A, Chuck A, Lee B, Johnson D, Currie G, Talbot J, Jiang J, Dickinson J, Kellner J, MacDonald J, Svenson L, Chui L, Louie M, Lavoie M, Eltorki M, Vanderkooi O, Tellier R, Ali S, Drews S, Graham T, Pang XL (2016) Shiga toxin-producing *Escherichia coli* infection, antibiotics, and risk of developing hemolytic uremic syndrome: a meta-analysis. Clin Infect Dis 62:1251–1258

Fremaux B, Prigent-Combaret C, Vernozy-Rozand C (2008) Long-term survival of Shiga toxin-producing *Escherichia coli* in cattle effluents and environment: an updated review. Vet Microbiol 132:1–18

Fukushima H, Hoshina K, Gomyoda M (1999) Long-term survival of Shiga toxin-producing *Escherichia coli* O26, O111, and O157 in bovine feces. Appl Environ Microbiol 65:5177–5181

Fung F, Wang HS, Menon S (2018) Food safety in the 21st century. Biomed J 41:88–95

Grauke LJ, Kudva IT, Yoon JW, Hunt CW, Williams CJ, Hovde CJ (2002) Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. Appl Environ Microbiol 68:2269–2277

Griffin PM, Ostroff SM, Tauxe RV, Greene KD, Wells JG, Lewis JH, Blake PA (1988) Illnesses associated with *Escherichia coli* O157:H7 infections. A broad clinical spectrum. Ann Intern Med 109:705–712

Hassan R, Seelman S, Peralta V, Booth H, Tewell M, Melius B, Whitney B, Sexton R, Dwarka A, Vugia D, Vidanes J, Kiang D, Gonzales E, Dowell N, Olson SM, Gladney LM, Jhung MA, Neil KP (2019) A multistate outbreak of *E. coli* O157:H7 infections linked to soy nut butter. Pediatrics 144:e20183978

Hrudey SE, Payment P, Huck PM, Gillham RW, Hrudey EJ (2003) A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. Water Sci Technol 47:7–14

Hurley BP, Thorpe CM, Acheson DW (2001) Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. Infect Immun 69:6148–6155

Islam MZ, Musekiwa A, Islam K, Ahmed S, Chowdhury S, Ahad A, Biswas PK (2014) Regional variation in the prevalence of *E. coli* O157 in cattle: a meta-analysis and meta-regression. PLoS One 9:e93299

Itoh Y, Sugita-Konishi Y, Kasuga F, Iwaki M, Hara-Kudo Y, Saito N, Noguchi Y, Konuma H, Kumagai S (1998) Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl Environ Microbiol 64:1532–1535

Jacob ME, Almes KM, Shi X, Sargeant JM, Nagaraja TG (2011) *Escherichia coli* O157:H7 genetic diversity in bovine fecal samples. J Food Prot 74:1186–1188

Jagadeesan B, Gerner-Smidt P, Allard MW, Leuillet S, Winkler A, Xiao Y, Chaffron S, Van Der Vossen J, Tang S, Katase M, McClure P, Kimura B, Ching Chai L, Chapman J, Grant K (2019) The use of next generation sequencing for improving food safety: translation into practice. Food Microbiol 79:96–115

Janes ME, Kim KS, Johnson MG (2005) Transmission electron microscopy study of enterohemorrhagic *Escherichia coli* O157:H7 in apple tissue. J Food Prot 68:216–224

Jenkins C, Dallman TJ, Grant KA (2019) Impact of whole genome sequencing on the investigation of food-borne outbreaks of Shiga toxin-producing *Escherichia coli* serogroup O157:H7, England, 2013 to 2017. Euro Surveill 24:1800346

Jones G, Lefèvre S, Donguy MP, Nisavanh A, Terpant G, Fougère E, Vaissière E, Guinard A, Mailles A, de Valk H, Fila M, Tanné C, Le Borgne C, Weill FX, Bonacorsi S, Jourdan-Da Silva N, Mariani-Kurkdjian P (2019) Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O26 paediatric haemolytic uraemic syndrome (HUS) cases associated with the consumption of soft raw cow's milk cheeses, France, March to May 2019. Euro Surveill 24: 1900305

Joseph A, Cointe A, Mariani Kurkdjian P, Rafat C, Hertig A (2020) Shiga toxin-associated hemolytic uremic syndrome: a narrative review. Toxins 12:67

Kampmeier S, Berger M, Mellmann A, Karch H, Berger P (2018) The 2011 German enterohemorrhagic *Escherichia coli* O104:H4 outbreak-the danger is still out there. Curr Top Microbiol Immunol 416:117–148

Kanayama A, Yahata Y, Arima Y, Takahashi T, Saitoh T, Kanou K, Kawabata K, Sunagawa T, Matsui T, Oishi K (2015) Enterohemorrhagic *Escherichia coli* outbreaks related to childcare facilities in Japan, 2010–2013. BMC Infect Dis 15:539

Karch H (1996) Control of enterohaemorrhagic *Escherichia coli* infection: the need for a network involving microbiological laboratories and clinical and public health institutions. Eur J Clin Microbiol Infect Dis 15:276–280

Karch H, Bielaszewska M (2001) Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(−) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. J Clin Microbiol 39:2043–2049

Karch H, Bielaszewska M, Bitzan M, Schmidt H (1999) Epidemiology and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Diagn Microbiol Infect Dis 34:229–243

Karch H, Tarr PI, Bielaszewska M (2005) Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol 295:405–418

Karch H, Mellmann A, Bielaszewska M (2009) Epidemiology and pathogenesis of enterohaemorrhagic *Escherichia coli*. Berl Munch Tierarztl Wochenschr 122:417–424

Karch H, Denamur E, Dobrindt U, Finlay BB, Hengge R, Johannes L, Ron EZ, Tønjum T, Sansonetti PJ, Vicente M (2012) The enemy within us: lessons from the 2011 European *Escherichia coli* O104:H4 outbreak. EMBO Mol Med 4:841–848

Kintz E, Byrne L, Jenkins C, Mc CN, Vivancos R, Hunter P (2019) Outbreaks of Shiga toxin-producing *Escherichia coli* linked to sprouted seeds, salad, and leafy greens: a systematic review. J Food Prot 82:1950–1958

Kolenda R, Burdukiewicz M, Schierack P (2015) A systematic review and meta-analysis of the epidemiology of pathogenic *Escherichia coli* of calves and the role of calves as reservoirs for human pathogenic *E. coli*. Front Cell Infect Microbiol 5:23

Laidler MR, Tourdjman M, Buser GL, Hostetler T, Repp KK, Leman R, Samadpour M, Keene WE (2013) *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. Clin Infect Dis 57:1129–1134

Low JC, McKendrick IJ, McKechnie C, Fenlon D, Naylor SW, Currie C, Smith DG, Allison L, Gally DL (2005) Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. Appl Environ Microbiol 71:93–97

Mahajan A, Currie CG, Mackie S, Tree J, McAteer S, McKendrick I, McNeilly TN, Roe A, La Ragione RM, Woodward MJ, Gally DL, Smith DG (2009) An investigation of the expression and adhesin function of H7 flagella in the interaction of *Escherichia coli* O157:H7 with bovine intestinal epithelium. Cell Microbiol 11:121–137

Matthews L, Reeve R, Gally DL, Low JC, Woolhouse ME, McAteer SP, Locking ME, Chase-Topping ME, Haydon DT, Allison LJ, Hanson MF, Gunn GJ, Reid SW (2013) Predicting the public health benefit of vaccinating cattle against *Escherichia coli* O157. Proc Natl Acad Sci U S A 110:16265–16270

Maule A (2000) Survival of verocytotoxigenic *Escherichia coli* O157 in soil, water and on surfaces. Symp Ser Soc Appl Microbiol 2000:71s–78s

Mellmann A, Bielaszewska M, Zimmerhackl LB, Prager R, Harmsen D, Tschäpe H, Karch H (2005) Enterohemorrhagic *Escherichia coli* in human infection: in vivo evolution of a bacterial pathogen. Clin Infect Dis 41:785–792

Mellmann A, Bielaszewska M, Köck R, Friedrich AW, Fruth A, Middendorf B, Harmsen D, Schmidt MA, Karch H (2008) Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli*. Emerg Infect Dis 14:1287–1290

Mellmann A, Bielaszewska M, Karch H (2009) Intrahost genome alterations in enterohemorrhagic *Escherichia coli*. Gastroenterology 136:1925–1938

Mellmann A, Harmsen D, Cummings CA, Zentz EB, Leopold SR, Rico A, Prior K, Szczepanowski R, Ji Y, Zhang W, McLaughlin SF, Henkhaus JK, Leopold B,

Bielaszewska M, Prager R, Brzoska PM, Moore RL, Guenther S, Rothberg JM, Karch H (2011) Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104: H4 outbreak by rapid next generation sequencing technology. PLoS One 6:e22751

Menrath A, Wieler LH, Heidemanns K, Semmler T, Fruth A, Kemper N (2010) Shiga toxin producing *Escherichia coli*: identification of non-O157:H7-Super-Shedding cows and related risk factors. Gut Pathog 2:7

Merget B, Dobrindt U, Forbes KJ, Strachan NJC, Brennan F, Holden NJ (2020) Variability in growth responses of non-O157 EHEC isolates in leafy vegetables, sprouted seeds and soil extracts occurs at the isolate level. FEMS Microbiol Lett 367:30

Michino H, Araki K, Minami S, Takaya S, Sakai N, Miyazaki M, Ono A, Yanagawa H (1999) Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. Am J Epidemiol 150:787–796

Miletic S, Hünerberg M, Kaldis A, MacDonald J, Leuthreau A, McAllister T, Menassa R (2017) A plant-produced candidate subunit vaccine reduces shedding of enterohemorrhagic *Escherichia coli* in ruminants. Biotechnol J 12:405

Moussay E, Stamm I, Taubert A, Baljer G, Menge C (2006) *Escherichia coli* Shiga toxin 1 enhances il-4 transcripts in bovine ileal intraepithelial lymphocytes. Vet Immunol Immunopathol 113: 367–382

Moxley RA (2000) Edema disease. Vet Clin North Am Food Anim Pract 16:175–185

Mühlen S, Dersch P (2020) Treatment strategies for infections with Shiga toxin-producing *Escherichia coli*. Front Cell Infect Microbiol 10:169

Munns KD, Selinger LB, Stanford K, Guan L, Callaway TR, McAllister TA (2015) Perspectives on super-shedding of *Escherichia coli* O157:H7 by cattle. Foodborne Pathog Dis 12:89–103

Müthing J, Schweppe CH, Karch H, Friedrich AW (2009) Shiga toxins, glycosphingolipid diversity, and endothelial cell injury. Thromb Haemost 101:252–264

Naylor SW, Low JC, Besser TE, Mahajan A, Gunn GJ, Pearce MC, McKendrick IJ, Smith DG, Gally DL (2003) Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. Infect Immun 71:1505–1512

Naylor SW, Gally DL, Low JC (2005a) Enterohaemorrhagic *E. coli* in veterinary medicine. Int J Med Microbiol 295:419–441

Naylor SW, Roe AJ, Nart P, Spears K, Smith DGE, Low JC, Gally DL (2005b) *Escherichia coli* O157:H7 forms attaching and effacing lesions at the terminal rectum of cattle and colonization requires the LEE4 operon. Microbiology 151:2773–2781

Neil KP, Biggerstaff G, MacDonald JK, Trees E, Medus C, Musser KA, Stroika SG, Zink D, Sotir MJ (2012) A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough – United States, 2009. Clin Infect Dis 54:511–518

O'Ryan M, Vidal R, del Canto F, Carlos Salazar J, Montero D (2015) Vaccines for viral and bacterial pathogens causing acute gastroenteritis: part II: vaccines for Shigella, Salmonella, enterotoxigenic *E. coli* (ETEC) enterohemorragic *E. coli* (EHEC) and *Campylobacter jejuni*. Hum Vaccin Immunother 11:601–619

Paton AW, Ratcliff RM, Doyle RM, Seymour-Murray J, Davos D, Lanser JA, Paton JC (1996) Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. J Clin Microbiol 34:1622–1627

Peterson RE, Klopfenstein TJ, Erickson GE, Folmer J, Hinkley S, Moxley RA, Smith DR (2007) Effect of *Lactobacillus acidophilus* strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. J Food Prot 70:287–291

Richardson SE, Karmali MA, Becker LE, Smith CR (1988) The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. Hum Pathol 19:1102–1108

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML (1983) Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 308:681–685

RKI (2011) Abschlussbericht zum EHEC/HUS-Ausbruch [online]. Available: https://www.rki.de/DE/Content/InfAZ/E/EHEC/EHEC_O104/EHEC-Abschlussbericht.pdf?__blob=publicationFile. Accessed 9 Sept 2011

Ro EY, Ko YM, Yoon KS (2015) Survival of pathogenic enterohemorrhagic *Escherichia coli* (EHEC) and control with calcium oxide in frozen meat products. Food Microbiol 49:203–210

Rojas-Lopez M, Monterio R, Pizza M, Desvaux M, Rosini R (2018) Intestinal pathogenic *Escherichia coli*: insights for vaccine development. Front Microbiol 9:440

Rosales A, Hofer J, Zimmerhackl LB, Jungraithmayr TC, Riedl M, Giner T, Strasak A, Orth-Höller D, Würzner R, Karch H (2012) Need for long-term follow-up in enterohemorrhagic *Escherichia coli*-associated hemolytic uremic syndrome due to late-emerging sequelae. Clin Infect Dis 54:1413–1421

Sandvig K, Bergan J, Dyve AB, Skotland T, Torgersen ML (2010) Endocytosis and retrograde transport of Shiga toxin. Toxicon 56:1181–1185

Sargeant JM, Amezcua MR, Rajic A, Waddell L (2007) Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of weaned domestic ruminants: a systematic review. Zoonoses Public Health 54:260–277

Saxena T, Kaushik P, Krishna Mohan M (2015) Prevalence of *E. coli* O157:H7 in water sources: an overview on associated diseases, outbreaks and detection methods. Diagn Microbiol Infect Dis 82:249–264

Schaffzin JK, Coronado F, Dumas NB, Root TP, Halse TA, Schoonmaker-Bopp DJ, Lurie MM, Nicholas D, Gerzonich B, Johnson GS, Wallace BJ, Musser KA (2012) Public health approach to detection of non-O157 Shiga toxin-producing *Escherichia coli*: summary of two outbreaks and laboratory procedures. Epidemiol Infect 140:283–289

Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD (2012) Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J Clin Microbiol 50:2951–2963

Schimmer B, Nygard K, Eriksen HM, Lassen J, Lindstedt BA, Brandal LT, Kapperud G, Aavitsland P (2008) Outbreak of haemolytic uraemic syndrome in Norway caused by stx2-positive *Escherichia coli* O103:H25 traced to cured mutton sausages. BMC Infect Dis 8:41

Segura A, Auffret P, Bibbal D, Bertoni M, Durand A, Jubelin G, Kérourédan M, Brugère H, Bertin Y, Forano E (2018) Factors involved in the persistence of a Shiga toxin-producing *Escherichia coli* O157:H7 strain in bovine feces and gastro-intestinal content. Front Microbiol 9:375

Seo KH, Frank JF (1999) Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. J Food Prot 62:3–9

Smith DR (2014) Vaccination of cattle against *Escherichia coli* O157:H7. Microbiol Spectr 2:6

Söderström A, Osterberg P, Lindqvist A, Jönsson B, Lindberg A, Blide Ulander S, Welinder-Olsson C, Löfdahl S, Kaijser B, De Jong B, Kühlmann-Berenzon S, Boqvist S, Eriksson E, Szanto E, Andersson S, Allestam G, Hedenström I, Ledet Muller L, Andersson Y (2008) A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce. Foodborne Pathog Dis 5:339–349

Sodha SV, Lynch M, Wannemuehler K, Leeper M, Malavet M, Schaffzin J, Chen T, Langer A, Glenshaw M, Hoefer D, Dumas N, Lind L, Iwamoto M, Ayers T, Nguyen T, Biggerstaff M, Olson C, Sheth A, Braden C (2011) Multistate outbreak of *Escherichia coli* O157:H7 infections associated with a national fast-food chain, 2006: a study incorporating epidemiological and food source traceback results. Epidemiol Infect 139:309–316

Solomon EB, Yaron S, Matthews KR (2002) Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Appl Environ Microbiol 68:397–400

Spinale JM, Ruebner RL, Copelovitch L, Kaplan BS (2013) Long-term outcomes of Shiga toxin hemolytic uremic syndrome. Pediatr Nephrol 28:2097–2105

Stephens TP, Loneragan GH, Karunasena E, Brashears MM (2007) Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. J Food Prot 70:2386–2391

Tabuchi A, Wakui T, Yahata Y, Yano K, Azuma K, Yamagishi T, Nakashima K, Sunagawa T, Matsui T, Oishi K (2015) A large outbreak of enterohaemorrhagic *Escherichia coli* O157, caused by low-salt pickled Napa cabbage in nursing homes, Japan, 2012. Western Pac Surveill Response J 6:7–11

Tack DM, Kisselburgh HM, Richardson LC, Geissler A, Griffin PM, Payne DC, Gleason BL (2021) Shiga toxin-producing *Escherichia coli* outbreaks in the United States, 2010–2017. Microorganisms 9:1529

Tarr PI, Gordon CA, Chandler WL (2005) Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 365:1073–1086

Teunis P, Takumi K, Shinagawa K (2004) Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. Risk Anal 24:401–407

Thurston-Enriquez JA, Gilley JE, Eghball B (2005) Microbial quality of runoff following land application of cattle manure and swine slurry. J Water Health 3:157–171

Tuttle J, Gomez T, Doyle MP, Wells JG, Zhao T, Tauxe RV, Griffin PM (1999) Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiol Infect 122:185–192

Wahl E, Vold L, Lindstedt BA, Bruheim T, Afset JE (2011) Investigation of an *Escherichia coli* O145 outbreak in a child day-care centre – extensive sampling and characterization of eae- and stx1-positive *E. coli* yields epidemiological and socioeconomic insight. BMC Infect Dis 11:238

Wang G, Doyle MP (1998) Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. J Food Prot 61:662–667

Watanabe H, Wada A, Inagaki Y, Itoh K, Tamura K (1996) Outbreaks of enterohaemorrhagic *Escherichia coli* O157:H7 infection by two different genotype strains in Japan, 1996. Lancet 348:831–832

Werber D, Fruth A, Liesegang A, Littmann M, Buchholz U, Prager R, Karch H, Breuer T, Tschäpe H, Ammon A (2002) A multistate outbreak of Shiga toxin-producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. J Infect Dis 186:419–422

Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI (2000) The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. N Engl J Med 342: 1930–1936

Wong CS, Mooney JC, Brandt JR, Staples AO, Jelacic S, Boster DR, Watkins SL, Tarr PI (2012) Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157: H7: a multivariable analysis. Clin Infect Dis 55:33–41

Wood JC, McKendrick IJ, Gettinby G (2006) A simulation model for the study of the within-animal infection dynamics of *E. coli* O157. Prev Vet Med 74:180–193

WWU/RKI/FZB (2021) Microbial genomic surveillance (miGenomeSurv) [online]. Available: https://www.medizin.uni-muenster.de/migenomesurv/home.html. Accessed 1 Mar 2022

Yahata Y, Misaki T, Ishida Y, Nagira M, Watahiki M, Isobe J, Terajima J, Iyoda S, Mitobe J, Ohnishi M, Sata T, Taniguchi K, Tada Y, Okabe N (2015) Epidemiological analysis of a large enterohaemorrhagic *Escherichia coli* O111 outbreak in Japan associated with haemolytic uraemic syndrome and acute encephalopathy. Epidemiol Infect 143:2721–2732

Younts-Dahl SM, Osborn GD, Galyean ML, Rivera JD, Loneragan GH, Brashears MM (2005) Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. J Food Prot 68:6–10

Listeriosis: The Dark Side of Refrigeration and Ensiling

12

Franz Allerberger, Zoltán Bagó, Steliana Huhulescu, Ariane Pietzka, and Sonja Pleininger

Contents

Abstract

In contrast to most pathogenic bacteria, *Listeria monocytogenes* is *psychrotrophic,* capable of multiplying at low temperatures. In an era when food production and food storage heavily rely on refrigeration, this ability to grow (albeit slowly) in a cold environment has opened a new ecological niche for *L. monocytogenes*. Because of the severity of certain clinical manifestations (infections of the central nervous system, septicemia, and abortion), the high case-fatality rate (approximately 20% of cases), and the long incubation time, human listeriosis is now a zoonosis of major public health concern. *L. monocytogenes* causes invasive illness mainly in certain well-defined high-risk groups, including immunocompromised persons, pregnant women, neonates, and the elderly. However, listeriosis can occur in otherwise healthy individuals, particularly during an outbreak. The evolvement of silage as a dominant

F. Allerberger · Z. Bagó · S. Huhulescu · A. Pietzka · S. Pleininger (✉)
National Reference Laboratory/Centre for Listeria, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria
e-mail: Sonja.Pleininger@ages.at

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_10

feed for ruminants constitutes another key factor, responsible for the emergence of listeriosis as a relevant animal disease. *L. monocytogenes* has been isolated from numerous species of mammals, birds, fish, crustaceans, and insects. Nevertheless, the primary habitats of *L. monocytogenes* are considered to be the soil and decaying vegetable matter, in which it survives and grows saprophytically.

Keywords

Listeria monocytogenes · Epidemiology of Listeriosis · Listeriosis in Humans and Animals

12.1 Introduction

The genus *Listeria* is presently composed of 21 species: *L. monocytogenes, L. ivanovii, L. seeligeri, L. welshimeri, L. grayi, L. innocua, L. marthii, L. rocourtiae, L. weihenstephaniensis, L. fleishmanii, L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis, L. booriae, L. newyorkensis, L. costaricensis, L. goaensis, L. thailandensis,* and *L. valentina* (Leclercq et al. 2010; Bertsch et al. 2012; Den Bakker et al. 2014; Weller et al. 2015; Núñez-Montero et al. 2018; Doijad et al. 2018; Leclercq et al. 2019; Nwaiwu 2020; Quereda et al. 2020). Although *Listeria* spp. are basically environmental bacteria, two species—*L. monocytogenes* and *L. ivanovii*—are pathogenic for animals and humans. In the genus, *L. monocytogenes* is the most commonly isolated member responsible for listeriosis in humans and animals. Occasional human infections are also due to *L. ivanovii*, which is mainly responsible for abortion in sheep. In contrast to most pathogenic bacteria, listeria are *psychrotrophic,* capable of multiplying at low temperatures as applied in refrigeration. In an era when food production and food storage heavily rely on refrigeration, this ability to grow (albeit slowly) at low temperatures has opened a new ecological niche to a pathogen that previously had mediocre relevance only. Industrialized food manufacturing also constitutes an ecological niche due to the ability of *L. monocytogenes* to form biofilms for colonization of surfaces (Jemmi and Stephan 2006). Not only surfaces but also raw materials seem to play a role in contamination of finished products. Molecular analyses have shown that some clonal types are stable over time and are persisting in food and food-related environment (Andrade et al. 2020). The evolvement of silage as a dominant feed for ruminants during the mid-twentieth century constitutes another key factor, responsible for the emergence of listeriosis as a relevant animal disease. H.P.R. Seeliger even dubbed this zoonosis a "man-made disease" (Allerberger et al. 1997).

Listeria monocytogenes is a facultative anaerobic, rod-shaped Gram-positive bacterium that is motile when cultured at 20 °C and immotile when grown at 36 °C (Figs. 1 and 2). It is able to produce severe sepsis, meningoencephalitis, and a wide variety of focal infections in animals and in humans. *L. monocytogenes*, the causative agent of listeriosis, was discovered in 1927 by Murray and Pirie, working

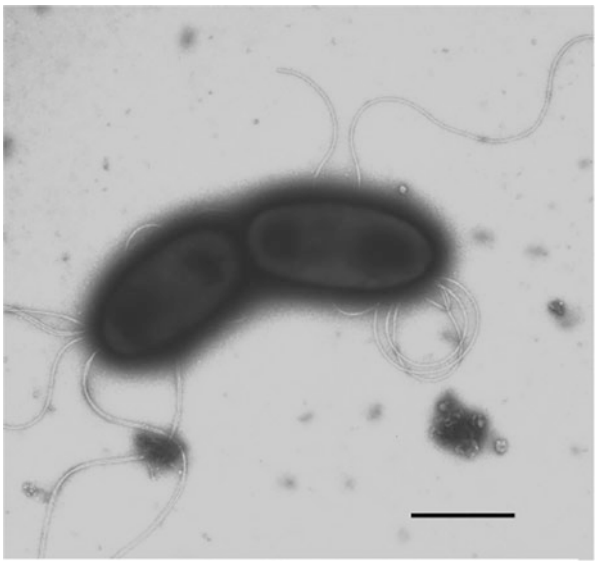

Fig. 1 Transmission electron microscopy image of *L. monocytogenes* grown in liquid culture at 20 °C showing flagellated bacteria. The bar is 1 μm in size. (Image gratuity: S. Richter)

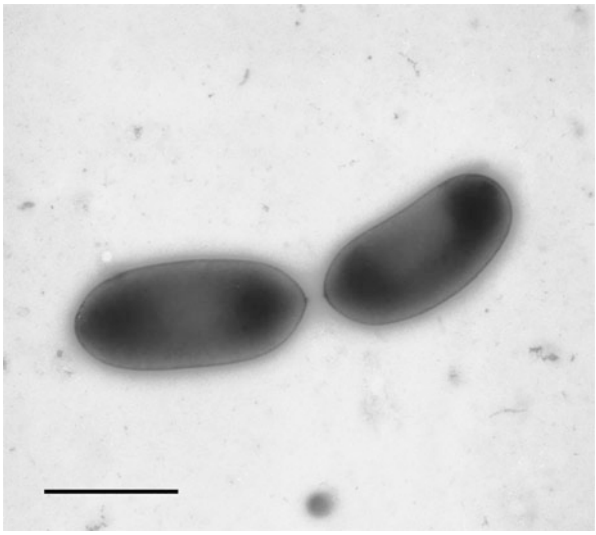

Fig. 2 Transmission electron microscopy image of *L. monocytogenes* grown in liquid culture at 36 °C showing bacteria without flagellae. The bar is 1 μm in size. (Image gratuity: S. Richter)

independently of each other on outbreaks among laboratory rabbits and guinea pigs (Rocourt 1999). The first-documented case on human listeriosis involved a soldier who suffered from meningitis at the end of World War I (McLauchlin 1997). *L. monocytogenes* was not considered a significant animal pathogen until the late 1970s and early 1980s when it was recognized as a major foodborne pathogen (Paoli et al. 2005). Because of the severity of certain clinical manifestations (infections of

the central nervous system, septicemia, and abortion), the high case-fatality rate (up to 30% of cases), and the long incubation time, human listeriosis is now a zoonosis of major public health concern (LeMonnier and Natas 2012; Halbedel et al. 2020). Outbreaks in humans were associated with the consumption of a wide range of foods such as contaminated coleslaw, soft cheese, ready-to-eat meat products like jellied pork or cold cuts, fish and seafood products, chocolate milk, ice cream, rice salad, corn salad, (frozen) vegetables, sprouts, precut celery and melons (Allerberger 2007; Pichler et al. 2011; Szymczak et al. 2020). It is now recognized that most cases of listeriosis, both sporadic cases and common-source outbreak cases, are caused by *L. monocytogenes*-contaminated food or feed. Although rare, infection can also be transmitted directly from infected animals to humans as well as between humans and between animals.

12.2 Pathogenicity

Although Murray recognized the oral route of infection in his original isolation of *L. monocytogenes* in the 1930s, the key to recognizing the organism as a food-borne pathogen came nearly 60 years later, when an outbreak of listeriosis was epidemiologically linked to the consumption of contaminated coleslaw (Paoli et al. 2005).

L. monocytogenes has the capacity to cross three important barriers in humans: intestinal epithelium, blood–brain barrier, and placenta (Chen et al. 2009). After ingestion of *L. monocytogenes*-contaminated food or feed, bacteria pass through the stomach and cross the intestinal barrier, presumably via M cells. Entry into mammalian cells is mediated by surface invasion proteins such as internalin A (InlA), internalin B (InlB), and internalin C (InlC) (Lee et al. 2012b). The listerial protein internalin (InlA) mediates bacterial adhesion and invasion of enterocytes in the human intestine through specific interaction with its host cell receptor E-cadherin, an adhesion molecule located at adherens junctions between epithelial cells (Lecuit et al. 2001). E-cadherin was identified as InlA receptor in 1996 (Mengaud et al. 1996). The importance of InlA for the entry of *L. monocytogenes* into nonphagocytic cells was demonstrated in 1991, when *L. monocytogenes* InlA was shown to confer to *L. innocua* the ability to enter human Caco-2 cells, cells originating from a human epithelial colorectal adenocarcinoma (Cossart et al. 2003). Internalin B (InlB) is another surface protein of *L. monocytogenes*. It contributes to invasion into a wider range of cell types such as endothelial cells, hepatocytes, and fibroblasts owing to the ubiquitous nature of its receptor, the hepatocyte growth factor receptor Met (Lee et al. 2012b). InlB is responsible for internalization into Vero cells (originating from African green monkey kidneys), HeLa cells (originating from a human cervical adenocarcinoma), and CHO cells (originating from Chinese hamster ovary) (Lecuit et al. 1997). Internalin C (InlC) contributes to cell-to-cell dissemination between polarized epithelial cells by decreasing cortical tension at apical junctions (Rajabian et al. 2009) and damps the host's immune system in preventing the proinflammatory pathway via NF-kB (Matle et al. 2020).

The intestine is the primary port of entry for *L. monocytogenes*, but questions about the exact mechanisms by which *L. monocytogenes* transgresses the intestinal barrier remain, and clear differences among host species exist (Hoelzer et al. 2012). In host species deficient of functional E-cadherin such as mice, *L. monocytogenes* is thought to translocate through the intestinal wall by gaining access into M cells, phagocytic cells in the Peyer's patches of the ileum. In species such as humans or guinea pigs that possess functional E-cadherin, *L. monocytogenes* is thought primarily to invade the epithelium of the intestinal villi, followed by bacterial replication in the underlying lamina propria. *L. monocytogenes* then rapidly translocates across the intestinal barrier, without a need for bacterial replication in the intestinal wall (Hoelzer et al. 2012).

After crossing the intestinal barrier, *Listeria* spp. are—within minutes of oral inoculation—transported by lymph or blood to the mesenteric lymph nodes, the spleen, and the liver. *L. monocytogenes* and *L. ivanovii* are facultative intracellular pathogens, which are able to replicate in macrophages and a variety of non-phagocytic cells, such as epithelial and endothelial cells, and in hepatocytes. After entering these cells, listeria escape early from the phagocytic vacuole, multiply in the host cell cytosol, and then move through the cell by induction of actin polymerization. The bacteria then protrude into cytoplasmic evaginations, and these pseudopod-like structures are phagocytosed by the neighboring cells (Schmid and Hensel 2004). When listeria enter cells, they not only trigger actin and membrane rearrangements, but they also use clathrin (Lebreton et al. 2011).

All major virulence factors of *L. monocytogenes* and *L. ivanovii* are involved in a single process: the cell-to-cell spread. By this function, the pathogen can avoid extracellular environments and can escape humoral efforts of the immune system during their dissemination in the host. In *Listeria* species, four virulence gene clusters have been identified to date and termed *Listeria* pathogenicity islands 1, 2, 3, and 4 (LIPI-1, LIPI-2, LIPI-3 and LIPI-4). *Listeria* pathogenicity island 1 (LIPI-1) refers to a genomic region flanked by *prs* and *ldh* and harboring several well-known virulence genes (*prfA*, *plcA*, *hly*, *mpl*, *actA*, *plcB*) in a 9-kb gene cluster (Vazquez-Boland et al. 2001). LIPI-1 was identified in *L. monocytogenes*, *L. seeligeri*, and *L. ivanovii*. The *hly* gene encodes the pore-forming listeriolysin O (LLO), a thiol-activated hemolysin, which is able to lyse erythrocytes and other cells in a cholesterol-dependent manner. LLO is an essential virulence factor of *L. monocytogenes*, and its inactivation leads to avirulence. It has been shown that LLO has several modes of action-promoting infection and evasion of the immune system, including the support of internalization of *L. monocytogenes* into phagosomes as well as disruption of the phagocytic vacuole for release of bacteria into the cytoplasm, which enables intracellular replication (Matereke and Okoh 2020, Maury et al. 2017). Ribet et al. showed that *L. monocytogenes* is able to dampen the host response by decreasing the SUMOylation level of proteins critical for infection (Ribet et al. 2010). Also this event is triggered by the bacterial virulence factor LLO.

A second island of 22 kb was termed LIPI-2. LIPI-2 is specific for *L. ivanovii* and may play a role in the tropism of this pathogen for ruminants (Gonzalez-Zorn et al. 2000). LIPI-3 contains the eight-gene cluster (*llsA*, *llsG*, *llsH*, *llsX*, *llsB*, *llsY*, *llsD*,

and *llsP*) encoding listeriolysin S (LLS), a bacteriocin-like modified peptide exhibiting hemolytic and cytotoxic activities (Clayton et al. 2011). In contrast to LIPI-1, which is found in all strains of *L. monocytogenes* as well as in other *Listeria* species, LIPI-3 is present only in a subset of *L. monocytogenes* lineage I (Lee et al. 2012b). LIPI-4, a cluster of six genes annotated as cellobiose family phosphotransferase system (PTS)—identified through whole-genome sequencing (Matle et al. 2020)—confers hypervirulence (Hurley et al. 2019). Selective tropism of *L. monocytogenes* for CNS and fetal-placental infections is associated with LIPI-4 that enhances invasion, leading to CNS and maternal-neonatal (MN) listeriosis (Maury et al. 2016).

European-wide cluster analyses of food and human isolates demonstrated a high degree of EU-wide dissemination of certain strains, but not all widespread food isolates match with any human cases, indicating a virulence variation among *L. monocytogenes* strains (ECDC, ELiTE. 2021).

12.3 Epidemiology of Listeriosis in Animals

L. monocytogenes has been isolated from numerous species of mammals, birds, fish, crustaceans, and insects. Nevertheless, the primary habitats of *L. monocytogenes* are considered to be the soil and decaying vegetable matter, in which it survives and grows saprophytically (Bille 2007).

Animal models have played fundamental roles in elucidating the pathophysiology and immunology of human listeriosis (Lecuit 2007). Such tests include intraperitoneal inoculation of mice, inoculation of the chorioallantoic membranes of embryonated eggs, and inoculation of the conjunctivae of rabbits (Anton test). Data derived from animal studies helped to characterize the importance of cell-mediated immunity in controlling infection, allowed evaluation of antimicrobial treatments for listeriosis, and contributed to quantitative assessments of the public health risk associated with *L. monocytogenes* contaminated food commodities (Hoelzer et al. 2012). However, data about species-specific differences have raised severe concern about the validity of most traditional animal models of listeriosis (Disson et al. 2008).

Even though *L. monocytogenes* can infect a wide variety of animal species, listeriosis is primarily a clinical disease of ruminants. Sheep appear to be particularly susceptible to infection, but listeriosis is also common in a variety of other polygastric species, and *L. monocytogenes* has for instance been isolated from cattle, goats, llamas, alpacas, deer, reindeer, antelopes, water buffalos, and moose (Hoelzer et al. 2012). Listeriosis represents one of the most common etiologies for encephalitis among adult ruminants. Ruminants affected by encephalitis generally show marked neurological symptoms including ataxia, circling, opisthotonus, and paralysis of cranial nerves, combined with hyperthermia, anorexia, and depression. Large epidemics of third trimester abortions, typically manifested as stillbirth, as well as atypical manifestations such as conjunctivitis have also repeatedly been described (Ryser and Marth 2007). With the exception of neonates and young ruminants, septicemia is unusual, but can result in mastitis, gastroenteritis, hepatitis, or

pneumonitis. Notably, in a given affected herd, listeriosis usually exhibits a single clinical manifestation (Ryser and Marth 2007).

Clinical listeriosis is relatively rare in most monogastric mammals such as dogs, cats, horses, and pigs, but appears more common in rodents and lagomorpha. Listeriosis in monogastric mammals is predominantly manifested as septicemia. Abortion, meningoencephalitis, and other manifestations such as conjunctivitis are also possible, but their relative frequency differs by animal species. Large outbreaks of listeriosis have been reported among colonies of captive rodents and lagomorpha, including chinchillas, rabbits, rats, and guinea pigs (Ryser and Marth 2007). Contaminated feed such as silage or sugar beets was implicated as the outbreak vehicle in many of these outbreaks, and coprophagy may have contributed to some of these events (Ryser and Marth 2007). In a pregnant primate model, oral administration of *L. monocytogenes* resulted in stillbirth with isolation of the bacterium from placental and fetal tissues (Smith et al. 2003).

Among ruminants, listeriosis occurs seasonally with the highest incidence in winter and spring, and appears strongly associated with ingestion of spoiled silage, although cases do occur where silage feeding has not been used (Ryser and Marth 2007; Sanaa et al. 1993). Silage is high-moisture fodder that can be fed to ruminants. It is fermented and stored in a process called ensiling, and is usually made from grass crops, using the entire green plant (not just the grain) [http://en.wikipedia.org/wiki/Silage]. The ensiled product retains a much larger proportion of its nutrients than if the crop had been dried and stored as hay. Silage undergoes anaerobic fermentation, which starts about 48 hours after the silo is filled. While properly produced silage is largely free of listeria, spoiled silage, often at the end of the silage-feeding period, can harbor high numbers of *L. monocytogenes*. Poor quality is often due to insufficient herbage quality or to contamination by soil or feces. The change to production of silage in polythene bales ("big bales") corresponded to increases in ovine listeriosis in the United Kingdom (McLauchlin 2011). According to McLauchlin, the "big bale" method is more prone to spoilage and growth of *L. monocytogenes*: High numbers are often associated with sites where the damage to the bags has occurred or at the tied end. Figure 3 gives the number of listeriosis cases diagnosed in farm ruminants submitted for necropsy to the Institutes for Veterinary Disease Control of the Austrian Agency for Health and Food Safety, based on data from 2007 to 2020 by month of reporting, again showing strong seasonality for animal listeriosis. The peak in the numbers of animal listeriosis in winter (goats) and in spring (cattle and sheep) may reflect a seasonal decrease in the quality of silage used for feed.

Animals may also be asymptomatic intestinal carriers and can shed the organism in significant numbers, contaminating the environment (Ho et al. 2007). The European Union summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2010 lists the following rates of fecal carriage of *L. monocytogenes*: cattle 5.5%; pigs 0.0%; sheep 7.0%; goats 10.5%; fowl (*Gallus gallus*) 0.4%; water buffalo 3.7%; and wild rodents 5.3% (EFSA 2012). As observed in 2009, the highest proportions of positive findings were found in decreasing order in goats, sheep, and cattle. In comparison to 76 cattle- and 39 sheep isolates of *L. monocytogenes, L. ivanovii* was cultured from only one cattle and two sheep

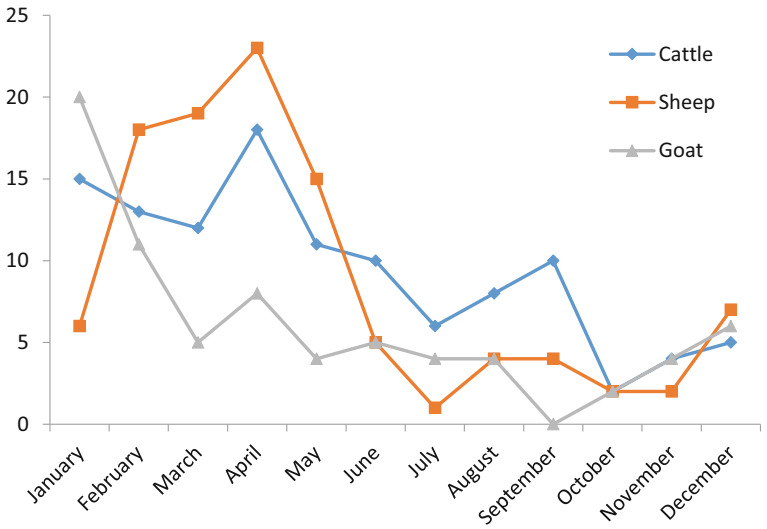

Fig. 3 Number of listeriosis cases in farm ruminants (n = 434), diagnosed by bacterial isolation and/or morphologically at the Austrian Agency for Health and Food Safety from 2007 till 2012 stratified by month of reporting

(Allerberger 2007). The 2001 report also reported rates of fecal carriage of *L. monocytogenes* for horses (fecal carriage rate: 2.1%), red deer (8.3%); farmed rabbit (0.7%), cats (0.7%), and dogs (0.0%) (Allerberger 2007).

In most countries, listeriosis in animals is not a notifiable disease. In Europe, listeriosis in animals is notifiable only in Germany, Finland, Sweden, and Norway. Usually, surveillance in animals is based on clinicopathological observations.

12.4 Epidemiology of Listeriosis in Humans

The large majority of listeriosis cases (sporadic and outbreak-related) are caused by food-borne transmission, which—according to Scallan et al. (2011)—accounts for 99% of human cases. In neonatal infections, *L. monocytogenes* can be transmitted from mother to child in utero, during passage through the infected birth canal or possibly via ascending infections from vaginal colonization. There are rare reports of nosocomial transmission in the nursery attributed to contaminated material or patient-to-patient transmission via healthcare workers (Hof and Lampidis 2001; Roberts et al. 1994; Heymann 2015). Hospital cross-infection between newborn infants occurs, usually originating form an infant born with congenital listeriosis. There is little evidence for cross-infection or person-to-person transmission outside the neonatal period. Rarely listeriosis may be transmitted by direct contact with infected animals or animal material. Usually, such local infections present as cutaneous lesions on the upper arms or wrists of farmers or veterinarian one to four days

after attending bovine abortions but not in association with sheep (McLauchlin 2011).

Invasive illness mainly manifests in certain well-defined high-risk groups, including immunocompromised persons, pregnant women, neonates, and the elderly. However, listeriosis can occur in otherwise healthy individuals, particularly during an outbreak.

Investigation of several outbreaks has demonstrated that all epidemic listeriosis was caused by food-borne transmission of *L. monocytogenes.* Outbreaks of listeriosis have been associated with the ingestion of raw milk, soft cheeses, contaminated vegetables, and ready-to-eat meat products such as paté. Also the sporadic cases of listeriosis mostly result from food-borne transmission. In several cases, by tracing a strain of *L. monocytogenes* isolated from a patient to a food item in the patient's refrigerator, and then to the retail source, public health officials were even able to provide microbiological confirmation of foodborne transmission of sporadic listeriosis (Pinner et al. 1992; Huhulescu 2012). Eating soft cheeses or food purchased from store delicatessen counters and eating undercooked chicken have been shown to increase the risk of sporadic listeriosis (Schuchat et al. 1992; Pinner et al. 1992).

Figure 4 presents the number of human invasive *L. monocytogenes* isolates registered at the Austrian National Reference Centre from 2015 to 2020, showing up- and downturns during the year. The European Union One Health Zoonoses Report 2019 observed high summer peaks followed by smaller winter peaks over a five-year period (2010–2019) (EFSA 2021), whereas only one clear slight seasonal peak during the late summer into the fall was described elsewhere (Wagner and MacLauchlin 2008, McLauchlin 2011; Feng et al. 2013).

In 2019, 28 member states reported 2621 confirmed invasive human cases of listeriosis. The overall EU notification rate was 0.46 cases per 100,000 population,

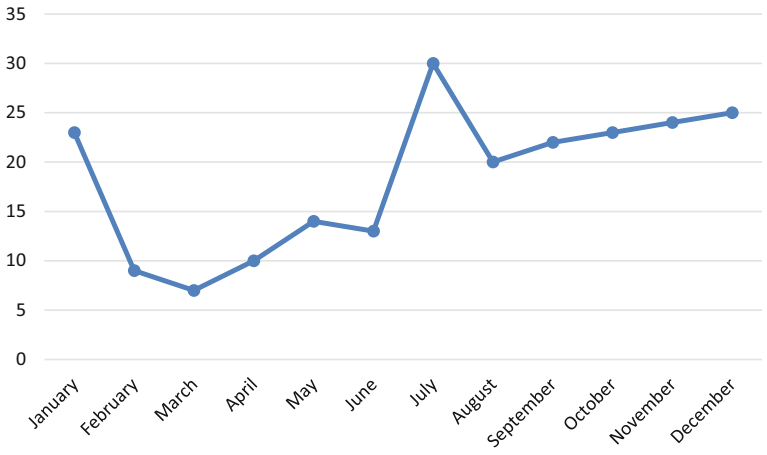

Fig. 4 Number of *L. monocytogenes* isolates (n = 220) from invasive cases registered at the Austrian National Reference Centre, based on data from the years 2015 to 2020 (by month of receipt of isolate)

with the highest country-specific notification rates observed in Estonia (1.59 cases per 100,000 population) followed by Sweden and Denmark (1.10 and 1.05 cases per 100,000 population, respectively). After an increasing trend over a long period, the EU trend of confirmed listeriosis cases remained stable from 2015 on. Particularly, the proportion of elderly has steadily increased over the last ten years until 2017, especially in those aged over 84. The proportion of cases in this age group slightly decreased in 2018 till 2019. Nevertheless, according to the European Union One Health Zoonoses Report, the notification rate in 2019 was highest in those aged over 65, covering 64.5% of all reported cases. Looking at the data of each reporting member state, ongoing statistically significant increasing trends in listeriosis notification rates from 2015 to 2019 were noted in Estonia, Poland, and Portugal. Statistically significant decreasing trends from 2015 to 2019 were noted in Greece (EFSA 2021).

The reason for the increased incidence and the upsurge in septicemia cases remain unknown. It has been hypothesized, however, that the higher incidence of listeriosis might be related to higher exposure to *L. monocytogenes* (Gillespie et al. 2006; Goulet et al. 2008). Increasing use of acid inhibitors (H_2 receptor antagonists) was also postulated to contribute to increased vulnerability to *L. monocytogenes* infection (Gillespie et al. 2009).

Sisó et al. studied the incidence of listeriosis during pregnancy over a 25-year period based on data compiled in a tertiary referral hospital in Spain. Whereas between 1985 and 2000 the incidence remained almost constant at 0.24%, an increasing incidence was observed from then on, reaching 0.86% during the last years until 2010 (Sisó et al. 2012). According to their findings, a four-fold increase in listeriosis rate during pregnancy has occurred in recent years in Spain. No such increase has been reported for pregnancy-associated cases from other countries. In Austria in 2020, listeriosis occurred in 3 of approximately 83,000 deliveries (0.04%).

Despite the high contamination rates of certain food, listeriosis is a relatively rare disease compared to other common food-borne illnesses such as campylobacteriosis or salmonellosis. However, because of its high case fatality rate of approximately 20%, human listeriosis ranks among the most frequent causes of food-borne death (Allerberger and Wagner 2010). Listeriosis has the highest number of fatal cases among food-borne diseases in the EU, with a continuous increase in reported deaths among cases and has, with more than 90%, the highest proportion of hospitalization among zoonoses under EU surveillance (EFSA 2021). Therefore, besides the economic consequences, listeriosis remains of great public health concern. The substantial burden of listeriosis in Europe is anticipated to increase in line with the projected growth of the elderly population. The proportion of people aged at least 65 is 20% by 2016 and expected to reach almost 30% by 2070 (European Commission 2018).

Control of listeriosis requires action from public health agencies and from the food industry. Important control strategies from public health agencies include developing and maintaining timely and effective disease surveillance programs, as well as promptly investigating clusters of listeriosis cases. Routine characterization of human, animal, food, and environmental isolates, and utilization of large-scale subtype databases facilitate Europe-wide outbreak detection and control. Outbreak

investigations provide a unique source of information to improve our understanding of transmission of listeria and to identify gaps in industry and regulatory measures to safeguard against contamination of the food and feed supply. In this respect, the importance of isolating the pathogen as a prerequisite for an accurate epidemiological investigation and ultimately stopping transmission cannot be overemphasized (Cartwright et al. 2013; Lachmann et al. 2021).

12.5 Epidemiology of *Listeria monocytogenes* in Food

Listeria monocytogenes is a particularly important cause of illness, mainly found in foods that are packaged and prepared commercially, rather than those prepared in the home (Carpentier and Cerf 2011). During the last decades, consumer lifestyles have changed with less time for food preparation and more ready-to-eat (RTE) and take-away foods. Changes in food production and technology have led to the production of foods with a long shelf life that are typical "listeria risk foods," because the bacteria have time to multiply, and the food does not undergo a listericidal process such as cooking before consumption. Main factors propagating the incidence of listeriosis are the high degree of centralization and consolidation of food production and processing, the increased use of refrigerators as the primary means of preserving food, and the above-mentioned changes in food consumption habits (increased consumer demand for convenient food) (Swaminathan et al. 2007). Gillespie et al. studied the food exposures of listeriosis cases aged $\geq$60 years reported in England from 2005 to 2008 and compared them to those of market research panel members representing the same population (i.e., residents of England aged $\geq$60 years) and time period. Cases were more likely than panel members to report the consumption of cooked meats (beef and ham/pork, but not poultry), cooked fish (specifically smoked salmon) and shellfish (prawns), dairy products (most noticeably milk, but also certain cheeses), and mixed salads. They were less likely to report the consumption of other forms of seafood, dairy spreads, other dairy products, sandwiches, and fresh vegetables. The diversity of high-risk food exposures reflects the ubiquity of the microorganism in the environment and the susceptibility of those at risk, and suggests that a wide variety of foods can give rise to listeriosis (Gillespie et al. 2010a, 2010b). In the United States, two case–control studies on risk factors for sporadic listeriosis found that cases were most likely to have eaten melons, hummus prepared in a commercial establishment, and soft cheeses or food purchases from store delicatessen counters (Schuchat et al. 1992; Varma et al. 2007).

L. monocytogenes is widespread in nature and has been isolated from soil, dust, food products for humans (both of animal and vegetable origin), feed, water, and sewage, and it can be carried by almost any animal species, including asymptomatic humans. The principal reservoir of the organism is said to be in soil, forage, water, mud, livestock food, and silage (Heymann 2015). Due to this environmental ubiquity, listeria strains are also frequently detected in food products. In addition, growth and survival of these psychrotrophic bacteria are favored particularly due to the above-mentioned increasing use of refrigeration in food production, food distribution, and

food storage. Unlike most other food-borne pathogens, *L. monocytogenes* tends to multiply in refrigerated foods that are contaminated. To understand why *L. monocytogenes* may persist in food industry equipment and premises, notably at low temperature, scientific studies have so far focused on adhesion potential, biofilm-forming ability, resistance to desiccation, acid and heat, tolerance to increased sublethal concentration of disinfectants, or resistance to lethal concentrations. Carpentier and Cerf postulated that the main factor associated with the presence of *L. monocytogenes* in production plants is growth promotion (Carpentier and Cerf 2011). Good growth conditions can be found in so-called harborage sites, i.e., shelters due to unhygienic design of equipment and premises or unhygienic or damaged materials. These sites are hard to eliminate. Carpentier and Cerf stipulated that there are no strains of *L. monocytogenes* with unique properties that lead to persistence, but harborage sites in food industry premises and equipment where *L. monocytogenes* can persist (Carpentier and Cerf 2011). In the European Union, foods that contain less than 100 colony-forming-units (cfu)/g are considered to pose a negligible risk for a healthy human population (EFSA 2012). EU legislation defines different criteria for three categories of foods (25 g samples) (European Commission 2005). In Europe, only food products for vulnerable populations (e.g., infants) have to be entirely free from *L. monocytogenes*. For food products enabling growth of *L. monocytogenes*, total absence is required for products sampled at the production plant, and a level of 100 cfu *L. monocytogenes* per gram food is tolerated at the consumption stage. For food products unable to support the growth (e.g., "hard cheese" or "fermented sausages") of *L. monocytogenes,* a limit of 100 cfu *L. monocytogenes* per gram food is accepted, when sampled on the market during their shelf life. Foods were defined as unable to support the growth of *L. monocytogenes* by pH $\leq$ 4.4; $a_w \leq 0.92$; pH $\leq$5.0 and $a_w \leq 0.94$; and shelf life less than 5 days.

In the United States, regulations require food companies to guarantee zero *L. monocytogenes* levels in all ready-to-eat products. The achievement of this objective is probably impractical, and it is clearly unattainable for raw foods or those which have not undergone a listericidal process (McLauchlin 2011).

Microbiological surveys have documented that *L. monocytogenes* may be present in a wide range of retail foods (Schuchat et al. 1992; Pinner et al. 1992). Wagner et al. studied samples of ready-to-eat (RTE) foodstuffs in Vienna, Austria, in 2007. They found 4.8% of 946 samples collected from 103 supermarkets positive for *L. monocytogenes*, with 5 smoked fish samples exceeding the tolerated limit of 100 cfu/g food (Wagner et al. 2007). Products showing the highest contamination rates were fish and seafood (19.4%), followed by raw meat sausages (6.3%), soft cheese (5.5%), and cooked meat products or patés (4.5%). Pulsed field gel electrophoresis (PFGE) typing of the collected *L. monocytogenes* isolates revealed a high degree of diversity between the isolates. Also evidence from EU-wide routine food safety investigations indicates that a substantial proportion of RTE products is contaminated by *L. monocytogenes* (EFSA 2021). No major changes compared with previous years were recently detected in the proportions of RTE foods not in compliance with the EU microbiological criteria. Once again the highest proportions exceeding the limit were observed in RTE fish products and RTE meat products. *L. monocytogenes* was detected in 2.8% of RTE meat products and meat preparations

of beef tested in 2019, in 2.1% of RTE products and meat preparations of pork, in 0.9% respectively 1.6% of RTE products and meat preparations of meat from broilers and turkeys, in 1.2% of soft and semisoft cheeses made from raw or low heat-treated milk from cows, in 1.5% respectively 0.0% of soft and semisoft cheeses made from raw or low heat-treated milk from sheep and goats, in 0.8% of hard cheeses made from raw or low heat-treated milk from cows, in 2.7% respectively 3.5% of hard cheeses made from raw or low heat-treated milk from sheep and goats, and in 4.4% respectively 4.3% of RTE fish products and fishery products (EFSA 2021).

Painter et al. studied the attribution of deaths from food-borne diseases to food commodities by using outbreak data (United States, 1998–2008) and found that more deaths were attributed to poultry (19%) than to any other commodity, and that most poultry-associated deaths were caused by listeria (Painter et al. 2013).

From 1998 to 2002, three large listeriosis outbreaks were linked to turkey delicatessen meat contaminated in the processing plant after cooking (Gottlieb et al. 2006; Mead et al. 2006; Olsen et al. 2005). However, pork paté ("rillettes"), a beef meat dish and horse minced meat, were also involved in outbreaks (Goulet et al. 1998; Smith et al. 2011; Goulet et al. 2013). A risk-ranking model for listeriosis among RTE foods identified delicatessen meat as the highest risk food (FDA 2003).

Soft cheeses, especially soft cheeses made with unpasteurized milk (Mexican-style), red smeared cheeses, brie, camembert, and sour milk curd cheese "Quargel" were responsible for large outbreaks in Europe and in the United States (Pichler et al. 2011; Goulet et al. 1995; Johnson et al. 2010). In Australia, 29 cases (including three fatal cases) were linked to brie, blue cheese, and camembert from one company in 2013 (Anonymous 2013).

Agricultural commodities are increasingly identified as a source of listeriosis outbreaks: In the United States, sprouts caused an outbreak in 2009, and precut celery caused an outbreak in 2010 (CDC 2012). A total of 147 persons infected with any of five subtypes of *L. monocytogenes* were reported to the Centers for Disease Control and Prevention from 28 states in connection within an outbreak associated with cantaloupes (melons) in 2011, including 33 outbreak-associated deaths (CDC 2012).

Although fish and seafood are very often found to be contaminated with *L. monocytogenes*, this food only occasionally was involved in outbreaks. Five cases with gastroenteritis related to consumption of cold smoked trout were reported from Finland (Miettinen et al. 1999). Shrimp was implicated as food source in two pregnancy-associated cases with bacteremia in the United States in 1989 (Riedo et al. 1994).

In China, an outbreak of gastroenteritis involving 82 cases was traced back to a vacuum-sealed product of cooked, unshelled eggs served to children as a meal during school break (Feng et al. 2013). In Italy, 1566 cases of listeria gastroenteritis were traced to consumption of corn salad in 1997, and 14 cases to rice salad in 1993 (Salamina et al. 1996; Aureli et al. 2000).

The discovery of *L. monocytogenes* mainly in raw and RTE meat, poultry, seafood, and dairy products has prompted numerous product recalls leading to large financial losses for the industry and to numerous health scares. In the European Union, a rapid alert system for food and feed (RASFF) was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation

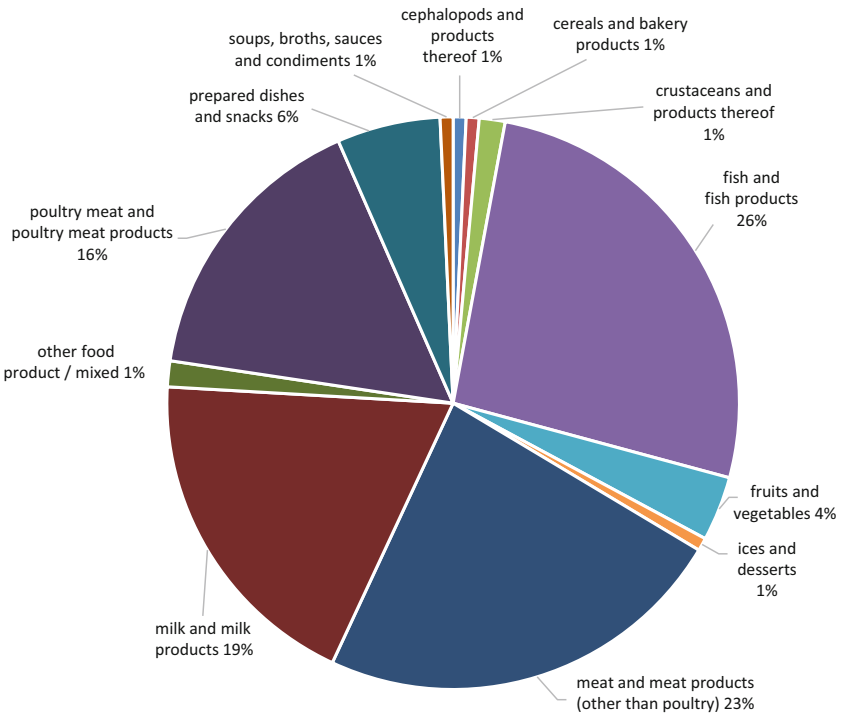

Fig. 5 Rapid Alert System for Food and Feed (RASFF) notifications concerning *Listeria monocytogenes*, 2020

to food or feed. This exchange of information helps member states to act more rapidly and to respond to a health threat caused by food or feed in a coordinated manner. During the last two decades, RASFF messages with information concerning listeria have increased by the factor of four. While in 2020, 137 notifications concerned *L. monocytogenes* (2019:138, 2018:122, 2017: 92, 2016: 81, 2015: 99, 2014: 98, 2013: 72, 2012: 90, 2011: 107, 2010: 108, 2009: 85, 2008: 51, 2007: 37, 2006: 26, 2005: 117, 2004: 122, 2003: 58, 2002: 51, 2001: 36), only 33 alerts were issued in the year 2000. This increase seems to reflect increased awareness about listeria contamination as a potential public health risk. In 2020, fish, meat, and milk products accounted for 85% (n = 116) of the 137 RASFF notifications (Fig. 5).

12.6 Molecular Typing and Interpretation of Typing Results

12.6.1 Typing Methods

Typing of bacterial isolates is essential for active surveillance and outbreak investigation. If a typing method shows isolates to be very similar to each other, transmission is likely, and we speak of "making" a cluster ("rule in"). If a typing method

shows isolates to be different, transmission is unlikely, and we speak of "breaking" a cluster ("rule out"). However, there are various caveats like occurrence of genetic recombinations (falsely ruling out) and occurrence of epidemiologically independent sources (falsely ruling in). Typing results can miss epidemiological relations due to underdiscrimination and due to overdiscrimination. The resolution can be too crude and too fine, so transmission chains can be overlooked. The challenge is to find a typing method with a resolution that correlates with epidemiological events. In the last years, molecular typing methods have become more and more important and have displaced classical methods step by step.

Molecular typing refers to any technique and method that is used to characterize microorganisms at the nucleotide level. It supports studies to trace back the source of an outbreak and to identify new risk factors as the strains can be linked more accurately to epidemiological and clinical data. All of this information can be applied toward improving and better targeting existing infectious disease prevention and control measures and thus presents a clear and immense benefit for the public health and public health policies (Allerberger 2012).

Early studies identified two genomic divisions ("lineages") by means of a variety of genotyping tools (Lee et al. 2012b). An additional lineage, lineage III, was first discovered in 1995 (Rasmussen et al. 1995). More recently, four lineages of *L. monocytogenes* were proposed by dividing lineage III into two separate evolutionary groups (den Bakker et al. 2010). Lineage I includes strains of serotypes 4b, 4d, 4e, 1/2b, and 3b. Serotype 4b is implicated in many outbreaks and sporadic cases resulting in lineage I being overrepresented among clinical samples (Lee et al. 2012b). Lineage II encompasses serotypes 1/2a, 1/2c, 3a, and 3c. Isolates belonging to this lineage are frequently found in foods and natural environments, although serotype 1/2a is also frequently identified among clinical isolates and sometimes implicated in outbreaks. Lineages III originally consisted of serotypes 4a and 4c. It was divided into two subgroups, one subgroup with strains of serotype 4a and the other with strains of serotype 4c (Doumith et al. 2004b). Even though these "new" lineages III and IV have been implicated in occasional sporadic cases, they are markedly less common in human listeriosis than strains of lineage I or II.

Molecular typing of *L. monocytogenes* splendidly complements traditional epidemiological surveillance by providing appropriate discriminatory analyses to foster rapid and early detection of dispersed clusters or outbreaks and to facilitate detection and investigation of transmission chains.

In the following, we would like to give an overview of the typing methods used so far.

12.6.1.1 Serotyping

Serotyping was the first method available for subtyping *L. monocytogenes* isolates. Serotyping is based on somatic (O) and flagellar (H) antigens. Published references define 13 *L. monocytogenes* serotypes: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7 (Allerberger 2003; Chen 2012). Serotypes are determined by reactivity with antisera. Commercially available serotyping sera (available from BD Diagnostics, USA, and Denka Seiken Co. Ltd., Japan) include a serotyping scheme for these 13 serotypes. Listeria handbooks do not agree on this issue; for example, the latest

edition of "Listeria, Listeriosis, and Food Safety" (Graves et al. 2007) does not list the *L. monocytogenes* serotype 4ab, whereas the "Handbook of *Listeria monocytogenes*" (Chen and Knabel 2008) does. In light of our experience, we question the existence of *L. monocytogenes* serotype 4ab. Taking into consideration the significance of reliable and unambiguous serotyping, especially in epidemiological tracking, an official revision of the *L. monocytogenes* serotyping scheme might be advised. In epidemiological investigations, bacterial serotyping usually is unable to estimate the relatedness of different isolates, as both invasive listeriosis and febrile gastroenteritis are caused mostly by serotypes 1/2a, 1/2b, and 4b strains. Serotyping of *L. monocytogenes* therefore has only limited practical value for investigating chains of transmission.

Doumith et al. developed a PCR-based serotyping method. Their multiplex PCR divides *L. monocytogenes* isolates into four groups employing primers annealing to *Listeria* genus-specific *prs* and genes specific to serotype-associated phylogenetic lineages of *L. monocytogenes*; this method can differentiate between strains of serotype 1/2a or 3a, 1/2c or 3 c, 1/2b or 3b, and the serotype 4b complex (4b, 4d, 4e) (Doumith et al. 2004a). The *prs* primers are specific for the putative phosphoribosyl pyrophosphate synthetase (*prs*) gene of *Listeria* spp.

Using whole-genome sequence (WGS) based methods, serogroup information can be extracted directly from the sequence data (Hyden et al. 2016).

12.6.1.2 High-Resolution Melting Curve-PCR (HRM-PCR) Analysis

High-resolution melting (HRM) curve-PCR analysis for *L. monocytogenes* was developed by Pietzka et al. (2011). Genomic bacterial DNA (gDNA) is extracted from bacterial cells grown overnight at 37 °C on blood agar plates, and a 500-bp fragment located in the virulence gene internalin B (*inlB*) is amplified for subsequent HRM analysis. HRM curve analysis constitutes an inexpensive assay and represents an improvement in typing relative to classical serotyping. This method provides a rapid and powerful screening tool for simultaneous preliminary typing of up to 384 samples in approximately 2 hours.

12.6.1.3 Pulsed-Field Gel Electrophoresis (PFGE)

DNA macrorestriction analysis by pulsed field gel electrophoresis (PFGE) has been the gold standard for typing of food-borne pathogens like Listeria, Salmonella, Campylobacter, and *Bacillus cereus* for many years (Allerberger 2012).

Electrophoresis as an analytical method separates macromolecules such as nucleic acid by their size, charge, conformation, and reactivity (Lee et al. 2012a). Classical electrophoresis employs a steady electric field orientated in one direction. This procedure, relying on a single paired electrode, permits the movement of DNA molecules only to a maximal size of 50 kb (Fangman 1978). PFGE is able to separate molecules as large as 12 Mb in size (Orbach et al. 1988). D.C. Schwartz and C.R. Cantor developed this variation of agarose gel electrophoresis, which revolutionized precise separation of DNA fragments greater than 40 to 50 kb. In PFGE, the orientation of the electric field across the gel is changed periodically ("pulsed") rather than kept constant as it was in conventional agarose gel electrophoresis

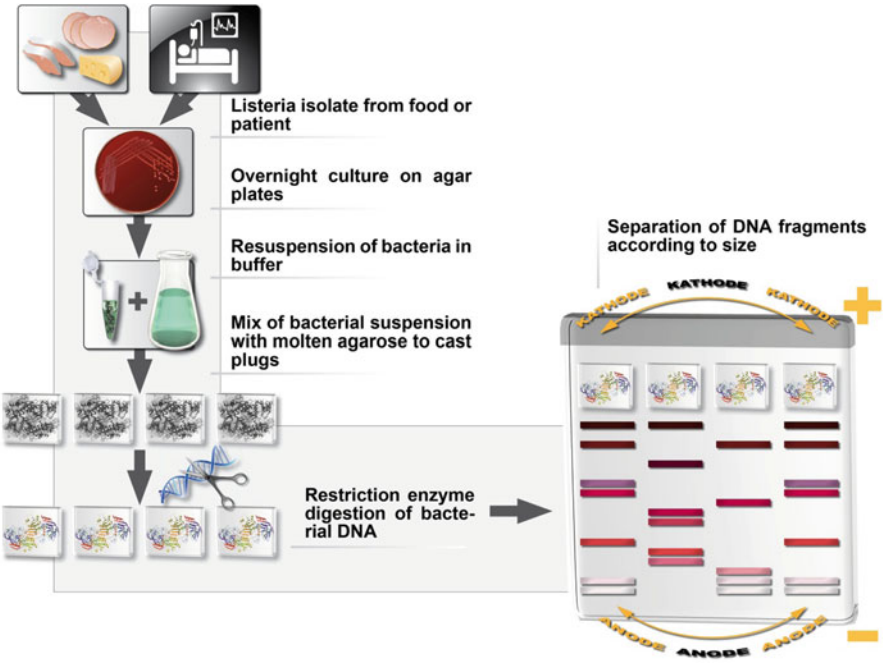

Fig. 6 Schematic principles of pulsed field gel electrophoresis (PFGE)

(Schwartz and Cantor 1984). This technology separates large fragments of unsheared microbial chromosomal DNA obtained by embedding intact bacteria in agarose gel plugs, enzymatically lysing the cell wall, and digesting the cellular proteins. The intact DNA is digested with an infrequently cutting restriction enzyme. Subsequent restriction fragment length polymorphism analysis allows differentiation of clonal isolates from unrelated ones. PFGE relies on a direct current electric field that changes orientation and intensity relative to the agarose gel. The rate of the changes is called pulse time, and its duration is an important factor to determine what molecular size range can be separated. The DNA molecules migrate through the agarose gel in a zigzag way, responding to the changes of the electric field. For larger molecules, the reorientation takes more time. They become trapped in the matrix if the pulse time is too short (Birren et al. 1988). Figure 6 depicts the basic principles of pulsed field gel electrophoresis (PFGE).

12.6.1.4 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) analysis was developed in the early 1990s by Vos et al. and is a registered trademark of KeyGene (Vos et al. 1995). It is based on PCR amplification of restriction fragments of a complete genomic digest. AFLP represents a relatively simple, low-cost, rapid, and highly discriminatory method which covers a larger portion of the genome than other typing techniques. In principle, the method scans the genome for sequence polymorphisms

producing DNA fragments, mainly between 50 base pairs and 700 base pairs in size. The fragments are separated on a denaturing acrylamide gel. The presence and absence of fragments produce a band pattern or AFLP profile comparable to bar codes used for product identification in commerce (Rupptisch 2013). Here, it determines a genetic fingerprint. For subsequent data analysis, the resulting AFLP profile can be converted into a binary presence–absence (1/0) code, a process known as "scoring" (Kück et al. 2012).

The national *Listeria*-reference laboratory for England and Wales had used fluorescent Amplified Fragment Length Polymorphism (fAFLP) analysis with *Hind*III/*Hha*I as subtyping method for *L. monocytogenes* since 2008. In fAFLP, the *L. monocytogenes* genome is digested with a rare cutting restriction enzyme such as *Hind*III and a more frequently cutting enzyme like *Hha*I. In a second step, adapters containing about 15 bp are ligated to the restriction fragments. These adapters serve as targets for two different primers, one of which has a fluorescent label. Usually, a FAM-labeled *Hind*III and a nonlabeled *Hha*I primer are used. Both primers contain the adapter sequence, the specific restriction site sequence, and one additional base pair on the 3′ end which extend into the restriction fragment. PCR amplifies only one fourth of the fragments containing the corresponding additional base pair and only hybrid fragments with two different restriction sites. Electrophoretic separation of amplicons on a gel matrix is followed by visualization of the band pattern. While PFGE is known to be a very time-consuming and work-intensive method (starting with a pure culture, first results can be achieved within 3 to 4 days), fAFLP can be completed within 48 h and is easier to perform. Roussel et al. analyzed 109 different *L. monocytogenes* isolates by both methods (Roussel et al. 2013). The isolates were divided by fAFLP and PFGE into three distinguished lineages. Strains known to be epidemiologically associated with one another were found to have unique PFGE and fAFLP types. fAFLP and PFGE divided the strains into 76 and 82 distinct profiles, or types, respectively. The discriminatory index calculated was 0.993 and 0.996 for fAFLP and PFGE, respectively. The authors concluded that the discriminatory ability of fAFLP was similar to that of PFGE for the subtyping of *L. monocytogenes* isolates and that, "as a less labour intensive technique, fAFLP may be a better method to use than PFGE in investigating outbreaks of human listeriosis and tracking the source of contamination in food processing facilities in real time."

12.6.1.5 Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA)

The availability of whole-genome sequences had facilitated the discovery of variable number tandem repeats (VNTRs), loci that contain short strings of nucleotides that are repeated, from a few to many times, and which are scattered throughout the bacterial genome. This has led to the birth of Multi-Locus Variable Number Tandem Repeat (VNTR) Analysis (MLVA) (Lindstedt 2005). Different organisms show variations in the number of these tandem repeated DNA sequences stashed in different loci. These loci can be multiplexed via PCR and separated by capillary gel electrophoresis. The size of the fragments varies according to the number of repeats in each locus. Results are depicted by numerical codes, giving the number of

alleles at each locus tested. These numbers consisting of integers are the MVLA profile and can be compared to those in an existing profile database (Sperry et al. 2008; Jadhav et al. 2012). Murphy et al. called this approach "a valuable tool, which has the capability to provide comparable results when compared with other more established typing methods, including pulsed-field gel electrophoresis" (Murphy et al. 2007). We consider MLVA less discriminatory than PFGE or AFLP.

12.6.1.6 Whole-Genome Sequencing

The "endpoint" of typing techniques is sequencing the whole genome, a method to determine the complete DNA sequence of a single organism. To construct the complete nucleotide sequence of a genome, multiple short sequence reads must be assembled based on overlapping regions (de novo assembly), or comparisons with previously sequenced "reference" genomes (resequencing). The emergence of benchtop sequencers using next-generation sequencing (NGS) technology makes bacterial whole-genome sequencing (WGS) feasible even in small research and clinical laboratories.

The progress in technology from automated Sanger sequencing (first-generation sequencing) to next-generation sequencing has revolutionized the field of molecular epidemiology. Sequencing methods still become faster and more affordable from year to year Ng and Kirkness 2010). According to this development, genome sequencing has become the method of choice for characterization of *Listeria monocytogenes*.

Regardless of the respective sequencing method, NGS can be divided into the process of DNA extraction, library preparation, sequencing, and data interpretation using bioinformatics tools (Hess et al. 2020).

Sequencing requires a high quality of DNA; therefore, high-molecular-weight DNA has to be prepared. The first step after DNA extraction is the preparation of a so-called library. Sequencing libraries are typically created by fragmenting DNA followed by ligation of adapter sequences and index sequences on both ends (Giani et al. 2020). During the sequencing steps, the library is loaded onto a flow cell, where an amplification process takes place. Chemically modified nucleotides (containing fluorescent tags) are used to bind to the DNA template strand. The fluorescent tag indicates which nucleotide has been added. Short sequences with a defined range of length, the so-called reads, are generated. These reads are overlapping and were set together in an assembly process (de novo genome assembly). The added index sequences allow a correct assembly of the generated reads. After sequencing, the data can be analyzed using bioinformatics tools (Hess et al. 2020). Figure 7 depicts the basic principles of the whole-genome sequencing process.

For the characterization of *L. monocytogenes* strains, high-resolution typing schemes using WGS technology have been developed (Ruppitsch et al. 2015a; Pightling et al. 2015; Kwong et al. 2016; Moura et al. 2016). Core genome (cg) MLST scheme-based typing of *L. monocytogenes* represents an expansion of the classical seven-gene MLST scheme (Salcedo et al. 2003) and is in all aspects superior for tracking and source identification, as compared to PFGE and fAFLP

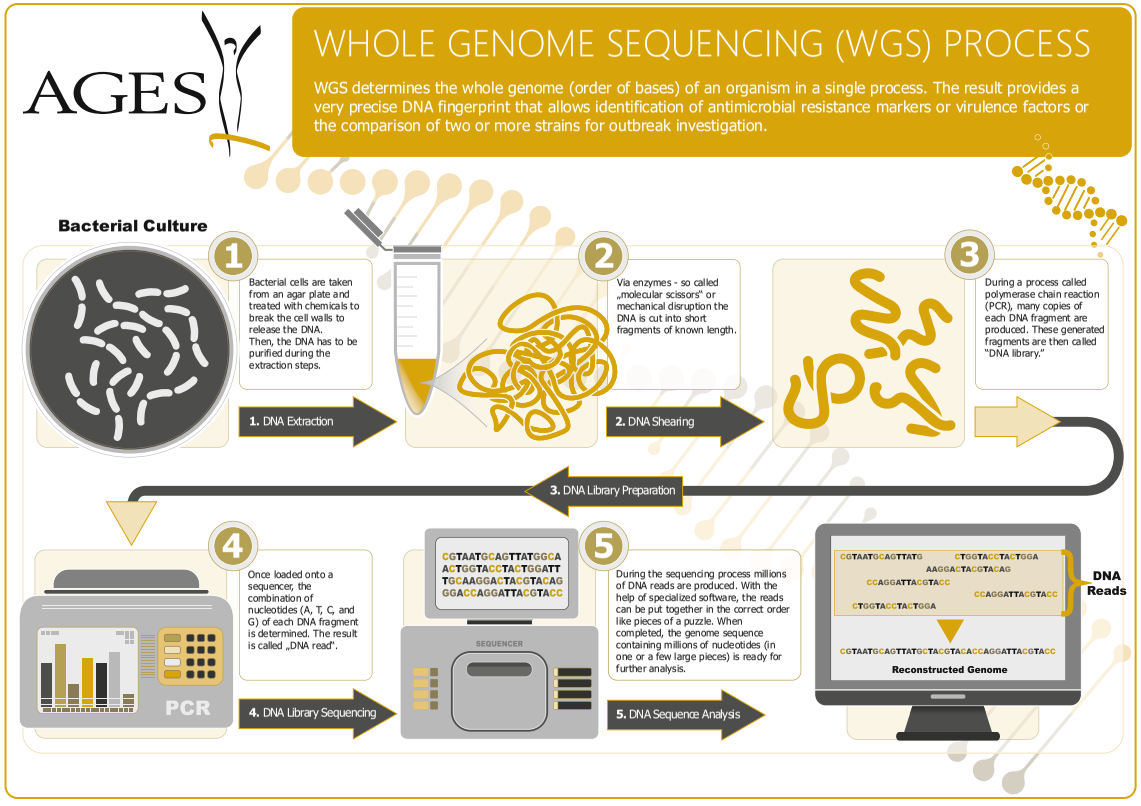

Fig. 7 Schematic principles of whole-genome sequencing (WGS)

(Schmid and Hensel 2004; Ruppitsch et al. 2015b). cgMLST-based typing results can be easily shared between different laboratories all over the world.

Due to the high informative value and the good comparability of the results, the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) have recommended to introduce routine WGS-based typing of human and nonhuman *L. monocytogenes* isolates across Europe (ECDC-EFSA 2019).

12.6.2 Interpretation of Typing Results

Adequate molecular typing enables linking pathogen data from human, food, animal, and environmental sources (provided the discrimination correlates epidemiologically). If applied routinely in real time, molecular typing allows for an early detection of national and international clusters and thus facilitates the early identification of potential sources of outbreaks (ECDC 2013). However, the use of analytical studies to investigate outbreaks of listeriosis has had only mixed success (McLauchlin 2011).

According to the World Health Organization (WHO), "foodborne illness is almost 100 % preventable" (WHO 2003). In order to achieve that position, an overview of the sources of infection and transmission routes is essential. If thoroughly investigated, food-borne outbreaks of listeriosis provide an opportunity to identify the food vehicle involved and the factors in the food preparation and handling that contributed to the outbreak. Within the European Union, reporting on food-borne outbreaks is mandatory under the framework of DIRECTIVE 2003/99/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of November 17, 2003, on the monitoring of zoonoses and zoonotic agents. According to this directive, a food-borne outbreak is defined as an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source.

While it is clear what constitutes a food-borne outbreak, there is no consensus on criteria for defining a *L. monocytogenes* cluster as a possible food-borne outbreak. Even the term cluster is still under controversial discussion: Within the European Centre for Disease Prevention and Control, a cluster is considered for *Listeria monocytogenes* having $\leq$7 cgMLST allelic differences from another or an outbreak reference isolate. The cgMLST scheme should be either that of Moura et al. (2016), Ruppitsch et al. (2015a), or a country respective cgMLST scheme.

In Austria (unlike in Germany or in Switzerland), medical laboratories have a legal obligation to forward human *L. monocytogenes* isolates to the National Reference Centre. From 2009 to 2016, when PFGE was the gold-standard method routinely used in Austria, more than 50% of human isolates yielded PFGE patterns that fell into clusters (more than one isolate showing a unique PFGE pattern).

Lay persons often urge to define a possible food-borne outbreak by the occurrence of just two indistinguishable human isolates, ignoring the fact that many

two-isolate clusters are due to chance and ignoring that any pregnancy-related pair of isolates (mother/child isolates yield identical DNA fingerprinting patterns) would incorrectly be deemed a possible food-borne outbreak. In contrast to the situation in forensic human genetics, where—with the exception of monozygotic twins—indistinguishable DNA sequences prove epidemiological relatedness, in bacteriology, epidemiologically unrelated *L. monocytogenes* isolates can yield DNA fingerprinting patterns or sequences indistinguishable from each other. Therefore, pure chance yields pairs of epidemiologically unrelated human *L. monocytogenes* isolates with PFGE patterns indistinguishable from each other or closely related DNA sequences and pairs of contemporary isolates of human and of food origin, without any causal relation. Figure 8 shows PFGE patterns of seven food isolates received by the Austrian reference center for *L. monocytogenes* in 2012, isolates not linked to one food or food producer. The occurrence of indistinguishable DNA sequences in different food products—epidemiologically unrelated to each other or to human cases—also hampers attribution of human illness to certain food. Since 2017, WGS-based typing methods are routinely used in Austria, only a few human isolates were assigned to a single cluster. This is mainly due to the superiority of WGS in contrast to the former used PFGE fingerprinting technique.

While much has been learned about food sources for epidemic listeriosis, little is known about food sources of sporadic listeriosis, which, in fact, represents the majority of cases. In Austria, when $\geq$2 *L. monocytogenes* isolates show closely related DNA sequences ($\leq$7 allelic differences, using the cgMLST by Ruppitsch et al. 2015a) within the actual year, this cluster is evaluated. If these isolates were detected within the last three to six months, the cluster is deemed a possible food-borne outbreak. If already typed *L. monocytogenes* isolates from food or food-associated sources cluster with patient isolates within a similar timeframe, the national Public Health (PH) authority is informed. At the discretion of the national authority, the local authorities are requested to check the food safety measures at the site of the related producers. The occurrence of a phylogenetically related case of listeriosis within 3 months prompts epidemiological investigations including food exposure analysis, tracing analyses, and targeted sampling of the incriminated food processor(s) to test the hypothesis on the outbreak source. Clusters containing only nonhuman isolates are reported quarterly to the national PH authority (Cabal et al. 2019).

An EFSA-ECDC collaboration on typing of listeria in RTE products and clinical cases of human listeriosis started in 2010 called the European Listeria typing exercise (ELiTE) (ECDC-EFSA 2021). This was a joint study of ECDC, EFSA, and the European Union Reference Laboratory for *Listeria monocytogenes* hosted by the French Agency for Food, Environmental and Occupational Health & Safety—ANSES. This collaborative study was initiated in 2010 as a multisectoral, multicenter exercise and was based on a dataset of certain RTE food isolates from the EU base line study (BLS) and a dataset of human isolates collected from clinical cases around the same time period as the food BLS. The molecular typing method mainly used in this study was PFGE, which has been the gold-standard molecular typing method for several food-borne pathogens for many years, with an improved discriminatory power compared to phenotyping methods. At the time of the study, this method was still the

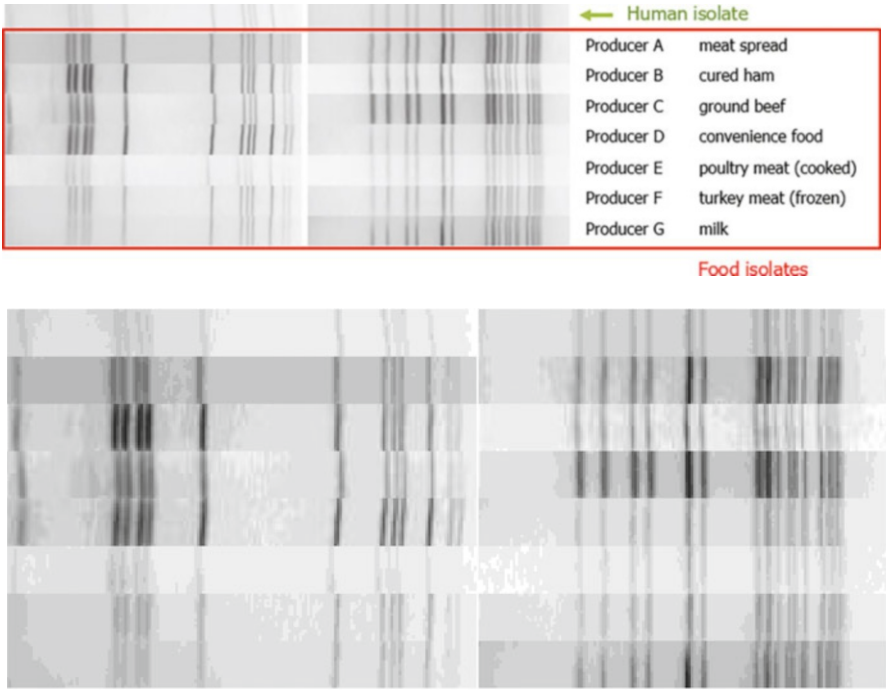

Fig. 8 Indistinguishable pulsed field gel electrophoresis (PFGE) patterns from seven different food items from seven individual (geographically and economically unrelated) food producers in comparison with a contemporary isolate from a sporadic human case (source of infection unknown), Austria 2012 (RTE = ready to eat)

best available standard method for *L. monocytogenes* genotyping. While the WGS technique is currently the recommended method for real-time surveillance of listeriosis and is increasingly used by reference laboratories, the EU public health and food safety value of PFGE has been bridging the historical national and EU-wide PFGE databases to WGS typing for the investigation of multicountry *L. monocytogenes* outbreaks during the transition period. The results provided by this study contributed to a better understanding of listeriosis epidemiology in the EU and helped to target effective

control and preventive measures within both food safety and public health as the presence of commonly circulating *L. monocytogenes* strains in food and humans has been demonstrated. The study has been a good example of a successful joint scientific exercise in the spirit of One Health.

12.7 Disease Symptoms in Both Animals and Humans

12.7.1 Disease Symptoms in Animals

In general, listeriosis in animals can be classified as six different forms (Selbitz 2011): (a) latent intestinal colonization with shedding via feces, (b) encephalitic listeriosis, (c) septicemic form, (d) metrogenic form, (e) mastitis, and (f) ocular manifestation. Table 1 summarizes the listeriosis cases in animals, submitted for necropsy and diagnosed at the Institutes for Veterinary Disease Control of the Austrian Agency for Health and Food Safety from 2007 to 2020 (total: 450). The simultaneous incidence of several distinct forms (b–f) in one animal is possible, but constitutes a rare event. Several factors influence the development of these manifestations: site of bacterial entry (oral or nasal mucosa, eye, gastrointestinal mucosa, navel), microbial virulence, host immune competence, and way of dissemination (lympho-hematogenous or neurogenic) (Selbitz 2011). Therefore, incubation periods can vary between a few days and 4 weeks.

The most frequent encephalitic form (and other organic manifestations as well) can readily be diagnosed by histological examination with subsequent immunohistochemical detection (i.e., avidin–biotin–peroxidase complex (ABC) technique) of the pathogen. Suspicion of listerial infection is usually raised by typical morphological features, in a high number of cases upon sole examination of H&E sections. In a retrospective study of 178 encephalitis cases in ruminants, Bagó et al. (2001) found morphological characteristics of listerial encephalitis in 52 cases, which were all confirmed by immunohistochemistry. Moreover, immunohistochemical examination

Table 1 Cases of listeriosis in animals submitted for necropsy and diagnosed at the Institutes for Veterinary Disease Control (Austrian Agency for Health and Food Safety) from 2007 to 2020 (total: 450)

Species	Encephalitic form	Abortion	Septicemic form	Mastitis
Cattle	137	39		2
Sheep	156	4	1	
Goat	86	8	1	
Alpaca			1	
Wild-ruminants	7			
Swine		1	2	
Brown hare			2	
Horse	1			
Poultry	1		1	
Total	388	52	8	2

revealed two additional cases of listeriosis, formerly classified as nonpurulent encephalitis of unknown etiology. These findings underline the value of immunohistochemistry in the diagnosis of encephalitic listeriosis. Figures 9 and 10 display two histological features of listerial rhombencephalitis in ovine brain with different detection intensity of *L. monocytogenes* (Bagó et al. 2001).

Ruminants are most frequently affected by the encephalitic form (Selbitz 2011) with histological features of a characteristic, predominantly purulent rhombencephalitis and nonpurulent leptomeningitis. Symptoms include elevated temperature (initially), depression, exaggerated forward or sideward stance, vestibular ataxia, circling, paresis of cranial nerves (nervus facialis, nervus glossopharyngeus, nervus trigeminus), salivation, strabismus, nystagmus, nasal and ocular discharge, reduced pupillary reflex, reduced tongue movement, and head tilt (Schweizer et al. 2006; Selbitz 2011; Weiss and Amtsberg 1995; Braun et al. 2002). Young animals during dentition are particularly susceptible for neurogenic dissemination via the trigeminal and glossopharyngeal nerves due to the increased vulnerability of the oral mucosa. The metrogenic form is characterized by late abortions, premature delivery, and frailty of the offspring displaying liver necrosis, abomasal erosions, and multifocal accumulations of *Listeria* in parenchymatous organs of the fetus/neonates with or without inflammatory reaction. Metritis or placentitis of the dame is rarely encountered (Dennis 1975; Wagner et al. 2005; Weiss and Amtsberg 1995). The septicemic form primarily affects juveniles (predominantly lambs) and is characterized by fever,

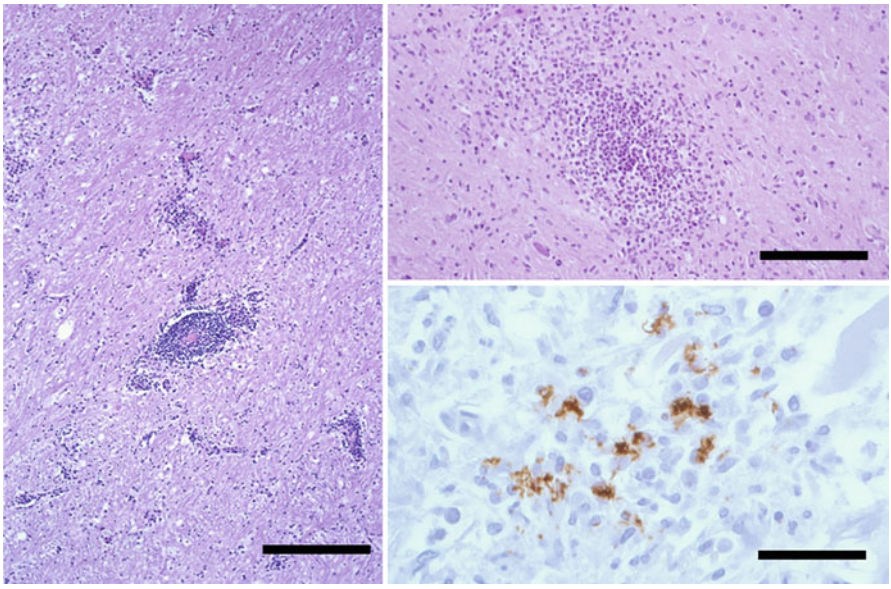

Fig. 9 Listeriosis in sheep, "purulent type inflammation." Left: mononuclear perivascular cuffing; Hematoxylin and eosin (H&E) staining; bar = 250 μm; Right top: microabscess-like cluster of neutrophils; H&E staining; bar = 150 μm; Right below: immunohistochemical staining of *L. monocytogenes* (brown signals) in an microabscess; bar = 25 μm. (Reprint by permission of Österreichische Gesellschaft der Tierärzte (ÖGT))

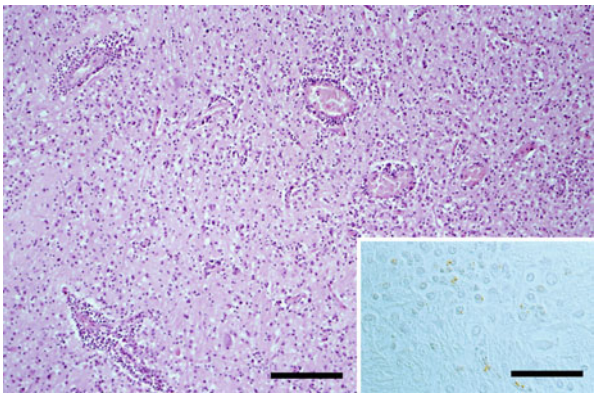

Fig. 10 Listeriosis in sheep, "nonpurulent type inflammation." Mononuclear perivascular cuffing and diffuse microgliosis; Hematoxylin and eosin (H&E) staining; bar = 170 μm; Inset: immunhistochemical detection of sporadic *L. monocytogenes* organisms (brown signals) inside an area of microgliosis; interference contrast microscopy; bar = 70 μm. (Reprint by permission of Österreichische Gesellschaft der Tierärzte (ÖGT))

general discomfort, and diarrhea (Weiss and Amtsberg 1995). The pathomorphological findings are dominated by multifocal necrosis in parenchymatous organs and occasionally fibrinopurulent meningitis. Listerial mastitis is a rare form that usually presents with subclinical chronic interstitial mastitis resulting in parenchymal atrophy (Winter et al. 2004). The ocular manifestation of listeriosis is characterized by a granulomatous to purulent keratoconjunctivitis and uveitis/iridocyclitis (Evans et al. 2004; Selbitz 2011). The so-called exposition keratitis is interpreted as a result of cranial nerve paresis and cannot be attributed to the infection itself. Special manifestations like listerial gastroenteritis and granulomatous-suppurative lymphadenitis have also been described (Fairley et al. 2012; Otter et al. 2004; Thompson et al. 2009).

In **swine**, listeriosis usually presents as abortion, septicemia of suckling and fattening pigs as well as encephalitis. Fattening pigs frequently develop hemorrhagic enteritis in the septicemic phase. Animals suffering from cerebral affection display incoordination, torticollis, circling, tremor, and paresis of hind legs (Wendt and Bickhardt 2001).

Listeria infection is occasionally detected in **horses**. Few cases of encephalitic (Rütten et al. 2006), abortive (Welsh 1983), ocular (Evans et al. 2004), and septicemic (Jose-Cunilleras and Hinchcliff 2001; Warner et al. 2012) forms have been reported. The septicemic form is characterized by necrotizing hepatitis and typhlocolitis.

Listeriosis of **dogs** and **cats** is exceptionally rare: Septicemic form (Schroeder and van Rensburg 1993; Weiss 2005), tonsillitis (Läikkö et al. 2004), cutaneous manifestation (Weiss 2005), and abortion (Sturgess 1989) have been described.

In **poultry**, septicemic listeriosis leading to acute death and encephalitic listeriosis with central nervous system disturbances such as torticollis and drowsiness has been described. Pathomorphologically, granulomatous, and purulent (heterophilic)

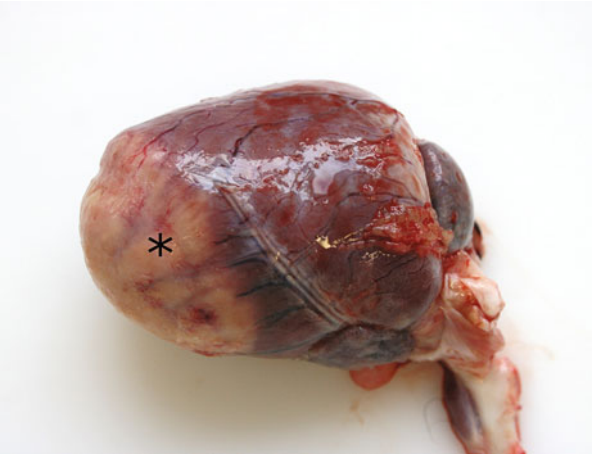

Fig. 11 Myocardial listeriosis in a red panda. Note the acute myocardial necrosis (*). In this case, we detected listeria intralesionally by immunohistochemistry. (Image courtesy Dr. S. Merbach)

inflammation of the central nervous system could be demonstrated (Kurazono et al. 2003; Ramos et al. 1988).

Lagomorpha and **rodents** are susceptible to listeriosis as well, usually developing septicemic, metritic, and ocular forms (Hoelzer et al. 2012; Peters and Scheele 1996; Selbitz 2011).

In addition, listeriosis has been reported in a multitude of **wild mammalian species**, whereas the above-described courses of disease and lesions can be transmitted to related genera of domesticated species (Hoelzer et al. 2012).

Recently, a very rare event of myocardial listeriosis in a red panda (Fig. 11) could be detected by bacteriological examination and immunohistochemistry (Merbach et al. 2019).

The increased frequency of raising/holding **reptiles and amphibians** as pets draws additional attention to the zoonotic potential of listeria, as these animals provide good examples for latent intestinal colonization of listeria with shedding via feces (Weber et al. 1993). Nevertheless, some case reports about fatal clinical disease, e.g., myocarditis in these species exist (Matt et al. 2019).

12.7.2 Disease Symptoms in Humans

L. monocytogenes is prevalent in many different foods for human consumption, and exposure to this pathogen by the consumption of contaminated food would be considered fairly common. However, clinical disease is rare and mainly occurs among the immunocompromised, the pregnant, and the elderly (age $\geq$ 60 years). Clinical manifestations range from febrile gastroenteritis to more severe invasive forms including sepsis, meningitis, rhombencephalitis, abortion, and perinatal infections.

The median incubation period is estimated to be 3 weeks. Outbreak cases have occurred 3 to 70 days following a single exposure to an implicated product (Heymann 2015). Noninvasive listeriosis (commonly manifested as febrile self-limited gastroenteritis) usually has a shorter incubation period, which ranges between six hours and ten days (American Medical Association et al. 2001). Invasive listeriosis generally needs a much longer incubation period, usually about 20 to 30 days (Linnan et al. 1988). Goulet et al. (2013) calculated the incubation period of listeriosis by reviewing published literature on patients with a single exposure to a confirmed food source contaminated by *L. monocytogenes*. For gastroenteritis cases, the median incubation period was 24 hours with variation from 6 hours to 30 days. For invasive listeriosis, the overall median incubation period was 8 days (range: 1–67 days); it differed significantly by clinical form of the disease: pregnancy-related cases showed a median incubation period of 27.5 days (range: 17–67 days), cases with central nervous system (CNS) manifestation a median of 9 days (range: 1–14 days) and for bacteremia cases a median of 2 days (range: 1–12 days).

Ten to twenty percent of clinical cases are pregnancy-associated (including neonates within the first 3 weeks after birth), and the majority of the rest occurs in nonpregnant immunocompromised individuals or in the elderly. While listeriosis during pregnancy usually presents with flu-like symptoms, which can lead to infection of the fetus causing abortion, premature birth, or stillbirth, in nonpregnancy-associated cases, it mainly manifests as meningoencephalitis or septicemia. The onset of meningoencephalitis (rare in pregnant women) can be sudden, with fever, intense headache, nausea, vomiting, and signs of meningeal irritation, or may be subacute, particularly in an immunocompromised or an elderly host (Heymann 2015; Tunkel et al. 2004).

Rhombencephalitis is an unusual form of listeriosis (Armstrong and Fung 1993). This brain stem encephalitis occurs in previously healthy adults. It is analogous to "circling disease" in sheep. Clinical features are biphasic: fever, headache, nausea, and vomiting, lasting several days and then cerebellar signs: cranial nerve deficits and hemiparesis. Cerebrospinal fluid shows increased protein and white blood cell counts; culture is positive in only 50%.

Kasper et al. studied a total of 150 human cases of listeriosis reported in Austria between 1997 and 2007 and found 9.3% to be pregnancy-associated (mother/child illness considered as a single case) (Kasper et al. 2009). Among the 136 nonpregnancy-associated cases, 55.2% were male and 44.9% female. Overall, 131 of 150 human cases (87%) had some type of risk factor or underlying disease associated with contracting listeriosis. The majority of cases (90.7%) were caused by systemic infections, only 9.3% of cases were local infections. Among nonpregnancy-associated cases, the 30-day all-cause fatality rate was 28.7% (39/136) and among the pregnancy-associated cases 35.7% (5/14; miscarriage x3, stillbirth x1, death in a newborn within 15 days of birth x1).

The widespread use of immunosuppressive medications for treatment of malignancy and management of organ transplantation has expanded the immunocompromised population at increased risk of listeriosis. The estimated risk for contracting listeriosis is 300 to 1000 times higher for AIDS patients than for the

general population. However, relatively few cases have been reported worldwide among HIV-positive or AIDS patients; in Austria, listeriosis in HIV-positive patients is a rare event (da Silva et al. 1992; Kasper et al. 2009). The preventive dietary recommendations to avoid the high-risk foods in HIV-positive persons and the repeated antimicrobial therapy for opportunistic infections may explain the relatively low incidence rate of listeriosis in AIDS patients (Vazquez-Boland et al. 2001).

L. monocytogenes can produce a wide variety of focal infections: conjunctivitis, skin infection, lymphadenitis, hepatic abscess, cholecystitis, endocarditis, peritonitis, splenic abscess, pleuropulmonary infection, joint infection, osteomyelitis, pericarditis, myocarditis, arteritis, tonsillitis, and endophthalmitis (Lorber 2010; Guerrero et al. 2004, Allerberger et al. 1989; Allerberger et al. 1992; unpublished data). Cutaneous listeriosis usually presents as papular or pustular lesions on the arms or hands (of veterinarians). Cutaneous listeriosis is observed extremely rarely and is most often acquired as an occupational hazard from infected animals (McLauchlin and Low 1994; Regan et al. 2005; McLauchlin 2011).

In healthy adults, exposure to *L. monocytogenes*-contaminated food usually causes only a short period of fecal shedding without illness. Foodborne transmission of *L. monocytogenes* can also cause a self-limiting acute gastroenteritis (in immunocompetent persons). From the data available in normal hosts in Italy, Illinois (USA), and Austria, it appears that febrile gastroenteritis in normal hosts requires the ingestion of a high dose of several million bacteria (Aureli et al. 2000; Dalton et al. 1977; Pichler et al. 2009). Majority of invasive listeriosis cases are caused by consumption of food containing more than 2000 CFU (colony-forming units)/g. Growth of *L. monocytogenes* during storage at costumer's home is responsible for diseases in about one third of cases (EFSA BIOHAZ Panel 2018). Grif et al. studied the incidence of fecal carriage of *L. monocytogenes* in healthy volunteers (Grif et al. 2003). The PCR results of the subjects indicated an incidence of five to nine exposures to *L. monocytogenes* per person/year. On an average, the incidence of culture-confirmed fecal carriage in healthy adults was two episodes of *L. monocytogenes* carriage per person/year. Fecal shedding was of short duration (maximum four days). The discrepancy between PCR results and the results from conventional culture could be explained by protective host effects. In particular, secretion of gastric acid provides an important protective factor against the passage of potentially pathogenic organisms. Cobb et al. have shown a drastically increased prevalence of *L. monocytogenes* in the feces of patients receiving long-term H_2-antagonists compared to the prevalence in patients with normal gastric secretion (Cobb et al. 1996).

12.8 Conclusion

Although invasive listeriosis occurs primarily in patients with underlying diseases, there are also reports on a rise of listeriosis in previously health persons (Goulet et al. 1998). The mechanism of this appearance has not yet been elucidated. Mainly affected from invasive listeriosis are elderly, immunocompromised individuals and

pregnant women. After a long period of increasing rate of invasive listeriosis, cases remained stable since 2015 but this trend placed listeriosis on place five of foodborne diseases, still with the highest rate of hospitalization and death (EFSA 2021). Goulet et al. hypothesized that the reduced salt content in ready-to-eat food (RTE) products may contribute to the growth of the organism, if present as a contaminant, and increase the likelihood of infection when these products are consumed by susceptible individuals (Goulet et al. 2008). The food industry reduced the salt content of selected products, such as RTE meat products, in response to recommendations in 2002 from food safety agencies, asking for a 20 % reduction in average salt intake, spread over five years, in order to prevent disease attributable to hypertension-related conditions. The influence of salt content in food on the general incidence of human listeriosis is unclear.

Changes in the way food is produced and distributed have increased the potential for widespread outbreaks involving many countries as a result of contamination of widely distributed commercial food products. Results of the European Listeria typing exercise (ELiTE) demonstrated a high degree of dissemination of certain *L. monocytogenes* strains (ECDC-EFSA 2021).

In 2008, ECDC (European Centre for Disease Prevention and Control) established a surveillance of invasive listeriosis and reporting to the European Surveillance System (ECDC-EFSA 2021). A rapid alert system (RAFFS System) allows prompt action and recall of contaminated products, and rapid exchange of whole-genome sequencing data is an important mechanism for collaborative outbreak investigation.

The dose–response relationship of *L. monocytogenes*, which represents an essential component of risk assessment, is still a pivotal question (Hoelzer et al. 2012). Presumably, it depends upon the serotype, concentration, virulence, and pathogenicity of the involved strain and also on host risk factors (Vázquez-Boland et al. 2001). The infectious dose for systemic listeriosis has not yet been determined. Recently, EFSA published a scientific opinion on *L. monocytogenes* contamination of RTE foods and the risks for human health: The majority of invasive listeriosis cases are attributable to ingestion of $>$10,000 cfu (EFSA BIOHAZ Panel 2018).

The emergence of human listeriosis is the result of complex interactions between various factors that may reflect changes in social patterns. Gillespie et al. (2010a, 2010b) studied the association of human listeriosis with neighborhood deprivation and found that listeriosis incidence was highest in the most deprived areas of England when compared with the most affluent, and those affected were more likely to purchase foods from small convenience stores or from local services (bakers, butchers, fishmongers, and greengrocers) than were the general population. They hypothesized that small businesses do not have access to the same level of food safety expertise as do larger retail companies, that increased deprivation could be associated with conditions where refrigeration may be inadequate or unavailable, and that financial pressures may encourage individuals to store food longer than the food product's safe shelf-life. The exact role of changes in social patterns is still an unresolved issue.

Morvan et al. have analyzed the evolution of susceptibility to antibiotics in 4186 clinical isolates of *L. monocytogenes* through several decades and found the prevalence of resistant strains in humans at a stable low level of 1.3% (Morvan et al. 2010). Marco et al. tested the in vitro activity of 22 antimicrobial agents against *L. monocytogenes* isolated in Spain (Marco et al. 2000) and found no increase in resistance with sequential analysis over the study period. Yan et al. studied 2862 food-borne *L. monocytogenes* isolates and detected 28 resistant isolates, 11 of them multiresistant, demonstrating the ability of *L. monocytogenes* to acquire antimicrobial resistance (2019). The question why *L. monocytogenes*, in contrast to all other zoonotic agents, is not showing an increase in antimicrobial resistance to drugs widely used in animal production and in human medicine remains unresolved. Baquero et al. stated that rare exposure of *L. monocytogenes* population to antibiotics as well as the large core genome and therefore limited need for acquisition of foreign genes might play a role (2020).

References

Allerberger F (2003) Listeria: growth, phenotypic differentiation and molecular microbiology. FEMS Immunol Med Microbiol 35:183–189

Allerberger F (2007) Listeria. In: Simjee S (ed) Foodborne diseases. Humana Press, Totowa, pp 27–39

Allerberger F (2012) Molecular typing in public health laboratories: from an academic indulgence to an infection Control imperative. J Prev Med Public Health 45:1–10

Allerberger F, Wagner M (2010) Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 16:16–23

Allerberger F, Langer B, Hirsch O et al (1989) *Listeria monocytogenes* cholecystitis. Z Gastroenterol 27:145–147

Allerberger F, Kasten MJ, Cockerill FR et al (1992) *Listeria monocytogenes* infection in prosthetic joints. Int Orthop 16:237–239

Allerberger F, Dierich MP, Grundmann H et al (1997) Typing of Austrian *Listeria monocytogenes* isolates by automated laser fluorescence analysis of randomly amplified polymorphic DNA. Zbl Bakt 286:33–40

American Medical Association, American Nurses Association–American Nurses Foundation, Centers for Disease Control and Prevention et al (2001) Diagnosis and management of foodborne illnesses: a primer for physicians and other health care professionals MMWR 50[No. RR-2

Andrade JC, João AL, Alonso CS et al (2020) Genetic subtyping, biofilm-forming ability and biocide susceptibility of *Listeria monocytogenes* strains isolated from a ready-to-eat Food industry. Antibiotics (Basel) 9:416

Anonymous (2013) Third listeria death linked to Jindi cheese. ABS News [cited 2013 March 29]. http://www.abc.net.au/news/2013-02-10/third-death-linked-to-cheese/4510440

Armstrong RW, Fung PC (1993) Brainstem encephalitis (rhombencephalitis) due to *Listeria monocytogenes*: case report and review. Clin Infect Dis 16:689–702

Aureli P, Fiorucci GC, Caroli D et al (2000) An outbreak of febrile gastroenteritis associated with corn contaminated with *Listeria monocytogenes*. N Engl J Med 342:1236–1241

Bagó Z, Bauder B, Baumgartner W, Weissenböck H (2001) Zur Ätiologie von Enzephalitiden bei Wiederkäuern: eine retrospektive Analyse. Wien Tierärztl Mschr 88:289–303

Baquero F, Lanza FV, Duval M et al (2020) Ecogenetics of antibiotic resistance in *Listeria monocytogenes*. Mol Microbiol 113:570–579

Bertsch D, Rau J, Eugster MR et al (2012) *Listeria fleischmannii* sp. nov., isolated from cheese. Int J Syst Evol Microbiol 63:526–532

Bille J (2007) Listeria and Erysipelothrix. In: Murray PR, Baron EJ, Jorgensen JH, Landry MC, Pfaller MA (eds) Manual of clinical microbiology, 9th edn. ASM Press, Washington DC, pp 474–484

Birren BW, Lai E, Clark SM et al (1988) Optimized conditions for pulsed field gel electrophoretic separations of DNA. Nucleic Acids Res 16:7563–7582

Braun U, Stehle C, Ehrensperger F (2002) Clinical findings and treatment of listeriosis in 67 sheep and goats. Vet Rec 150:38–42

Cabal A, Pietzka A, Huhulescu S, Allerberger F (2019) Isolate-based surveillance of *Listeria monocytogenes* by whole genome sequencing in Austria. Front Microbiol 10:2282

Carpentier B, Cerf O (2011) Review – resistance of *Listeria monocytogenes* in food industry equipment and premises. Int J Food Microbiol 145:1–8

Cartwright EJ, Jackson KA, Johnson SD et al (2013) Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. Emerg infect dis Emerg infect dis [internet]. 2013 Jan. https://doi.org/10.3201/eid1901.120393. https://doi.org/10.3201/eid1901.120393

CDC, Centers for Disease Control and Prevention (2012) Multistate outbreak of Listeriosis linked to whole cantaloupes from Jensen farms, Colorado. August 27, 2012, Accessed: March 29, 2013; http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/082712/index.html

Chen Y (2012) Listeria monocytogenes. In: Lampel KA, Al-Khaldi S, Cahill SM (eds) Bad bug book - foodborne pathogenic microorganisms and natural toxins, 2nd edn. Food and Drug Administration, Washington, pp 99–102. http://www.fda.gov/downloads/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/UCM297627.pdf

Chen Y, Knabel SJ (2008) Strain typing. In: Dongyou L (ed) Handbook of *Listeria monocytogenes*. CRC Press, Boca Raton, pp 305–356

Chen J, Luo X, Jiang L et al (2009) Molecular characteristics and virulence potential of *Listeria monocytogenes* isolates from Chinese food systems. Food Microbiol 26:103–111

Clayton EM, Hill C, Cotter PD, Ross RP (2011) Real-time PCR assay to differentiate Listeriolysin S-positive and –negative strains of L. monocytogenes. Appl Environ Microbiol 77:163–171

Cobb CA, Curtis GD, Bansi DS et al (1996) Increased prevalence of *Listeria monocytogenes* in the feces of patients receiving long-term H2-antagonistes. Eur J Gastroenterol Hepatol 8:1071–1074

Cossart P, Pizarro-Cerdá J, Lecuit M (2003) Invasion of mammalian cells by *Listeria monocytogenes*: functional mimicry to subvert cellular functions. Trends Cell Biol 13:23–31

da Silva LJ, Resende MR, de Abreu WB et al (1992) Listeriosis and AIDS: case report and literature review. Rev Inst Med Trop Sao Paulo 34:475–478

Dalton CB, Austin CC, Sobel J et al (1977) An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N Engl J Med 336:100–105

den Bakker HC, Bundrant BN, Fortes ED et al (2010) A population genetics-based and phylogenetic approach to understanding the evolution of virulence in the genus *Listeria*. Appl Environ Microbiol 76:6085–6100

Den Bakker HC, Warchocki S, Wright EM et al (2014) *Listeria floridensis* sp. nov., *Listeria aquatica* sp. nov., *Listeria cornellensis* sp. nov., *Listeria riparia* sp. nov. and *Listeria grandensis* sp. nov., from agricultural and natural environments. Int J Syst Evol Microbiol 64:1882–1889

Dennis SM (1975) Perinatal lamb mortality in Western Australia. 6. Listeric infection Aust Vet J 51: 75–79

Disson O, Grayo S, Huillet E et al (2008) Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. Nature 455:1114–1118

Doijad SP, Poharkar KV, Kale SB et al (2018) *Listeria goaensis* sp. nov. Int J Syst Evol Microbiol 68:3285–3291

Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P (2004a) Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol 42:3819–3822

Doumith M, Cazalet C, Simoes N et al (2004b) New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. Infect Immun 72: 1072–1083

ECDC, European Centre for Disease Prevention and Control (2013) Surveillance of communicable diseases in Europe – a concept to integrate molecular typing data into EU-level surveillance. Stockholm: ECDC; 2013. Stockholm, March 2013 ISBN 978-92-9193-451-5. https://doi.org/10.2900/7881

EFSA BIOHAZ Panel (2018) Scientific opinion on the *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA J 16. Accessed 20 July 2021. https://www.efsa.europa.eu/en/efsajournal/pub/5134

European Centre for Disease Prevention and Control [ECDC], European Food Safety Authority [EFSA] (2012) The European Union summary report on trends and sources of Zoonoses, zoonotic agents and foodborne outbreaks in 2010. EFSA J 10:2597

European Centre for Disease Prevention and Control [ECDC], European Food Safety Authority [EFSA] (2021) The European Union one health 2019 Zoonoses report. EFSA J 19:e06406

European Centre for Disease Prevention and Control [ECDC], European Food Safety Authority [EFSA], French Agency for Food, Environmental and Occupational Health & Safety (2021) European listeria typing exercise (ELiTE). ECDC, Stockholm. ISBN 978-92-9498-531-6

European Centre for Disease Prevention and Control [ECDC], European Food Safety Authority [EFSA], Van Walle I, Guerra B, Borges V, Carriço JA et al (2019) EFSA and ECDC technical report on the collection and analysis of whole genome sequencing data from foodborne pathogens and other relevant microorganisms isolated from human, animal, food, feed and food/feed environmental samples in the joint ECDC–EFSA molecular typing database. EFSA Support Publ 16:1337E

European Commission. Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Luxembourg: Publications Office of the European Union. 22.12.2005:L 338/1. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF

European Commission (2018) The 2018 ageing report: economic and budgetary projections for the EU member states (2016–2070) 2018, Brussels

Evans K, Smith M, McDonough P, Wiedmann M (2004) Eye infections due to *Listeria monocytogenes* in three cows and one horse. J Vet Diagn Investig 16:464–469

Fairley RA, Pesavento PA, Clark RG (2012) *Listeria monocytogenes* infection of the alimentary tract (enteric listeriosis) of sheep in New Zealand. J Comp Pathol 146:308–313

Fangman WL (1978) Separation of very large DNA molecules by gel electrophoresis. Nucleic Acids Res 5:653–665

FDA, Food and Drug Administration (2003) Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. FDA/Center for Food Safety and Applied Nutrition USDA/Food Safety and Inspection Service, Washington [cited 2013 March 12]. http://www.fda.gov/food/scienceresearch/researchareas/riskassessmentsafetyassessment/ucm183966.htm

Feng Y, Wu S, Varma JK et al (2013) A systematic review of human listeriosis in China, 1964-2010. Tropical Med Int Health. in press

Giani AM, Gallo GR, Gianfranceschi L, Formenti G (2020) Long walk to genomics: history and current approaches to genome sequencing and assembly. Comput Struct Biotechnol J 18:9–19

Gillespie IA, McLauchlin J, Grant KA et al (2006) Changing pattern of human listeriosis, England and Wales, 2001-2004. Emerg Infect Dis 12:1361–1366

Gillespie IA, McLauchlin J, Little CL et al (2009) Disease presentation in relation to infection foci for non-pregnancy-associated human listeriosis in England and Wales, 2001 to 2007. J Clin Microbiol 47:3301–3307

Gillespie IA, Mook P, Little CL et al (2010a) Human listeriosis in England, 2001-2007: association with neighbourhood deprivation. Euro Surveill 15:7–16

Gillespie IA, Mook P, Little CL et al (2010b) *Listeria monocytogenes* infection in the over-60s in England between 2005 and 2008: a retrospective case-control study utilizing market research panel data. Foodborne Pathog Dis 7:1373–1379

Gonzalez-Zorn B, Dominguez-Bernal G, Suarez M et al (2000) SmcL, a novel membrane-damaging virulence factor in *Listeria*. Int J Med Microbiol 290:369–374

Gottlieb SL, Newbern EC, Griffin PM et al (2006) Multistate outbreak of listeriosis linked to Turkey deli meat and subsequent changes in US regulatory policy. Clin Infect Dis 42:29–36
Goulet V, Jacquet C, Vaillant V et al (1995) Listeriosis from consumption of raw-milk cheese. Lancet 345:1581–1582
Goulet V, Rocourt J, Rebiere I et al (1998) Listeriosis outbreak associated with the consumption of rillettes in France in 1993. J Infect Dis 177:155–160
Goulet V, Hedberg C, Le Monnier A, De Valk H (2008) Increasing incidence of listeriosis in France and other European countries. Emerg Infect Dis 14:734–740
Goulet V, King LA, Vaillant V, deValk H (2013) What is the incubation period of listeriosis? BMC Infect Dis 13:11
Graves LM, Swaminathan B, Hunter SB (2007) Subtyping *Listeria monocytogenes*. In: Ryser ET, Marth EH (eds) Listeria, Listeriosis, and Food Safety. CRC Press, Boca Raton, pp 85–109
Grif K, Patscheider G, Dierich MP, Allerberger F (2003) Incidence of fecal carriage of *Listeria monocytogenes* in three healthy volunteers: A one-year prospective stool survey. Eur J Clin Microbiol Infect Dis 22:16–20
Guerrero MLF, Rivas P, Ràbago R et al (2004) Prosthetic valve endocarditis due to *Listeria monocytogenes*. Report of two cases and reviews. Int J Infect Dis 8:97–102
Halbedel S, Wilking H, Holzer A et al (2020) Large Nationwide Outbreak of Invasive Listeriosis Associated with Blood Sausage, Germany, 2018-2019. Emerg Infect Dis 26:1456–1464
Hess JF, Kohl TA, Kotrová M et al (2020) Library preparation for next generation sequencing: A review of automation strategies. Biotechnol Adv 41:107537
Heymann D (ed) (2015) Control of communicable diseases manual, 20th edn. American Public Health Association, Washington, DC
Ho AJ, Ivanek R, Grohn YT et al (2007) *Listeria monocytogenes* fecal shedding in dairy cattle shows high levels of day-to-day variation and includes outbreaks and sporadic cases of shedding of specific L. monocytogenes subtypes. Prev Vet Med 80:287–305
Hoelzer K, Pouillot R, Dennis S (2012) Animal models of listeriosis: a comparative review of the current state of the art and lessons learned. Vet Res 43:18. https://doi.org/10.1186/1297-9716-43-18
Hof H, Lampidis R (2001) Retrospective evidence for nosocomial listeria infection. J Hosp Infect 48:421
Huhulescu S (2012) Nationale Referenzzentrale für Listeriose Jahresbericht 2011. Newsletter. http://www.bmg.gv.at/cms/home/attachments/4/8/0/CH1338/CMS1339606416820/listeriose-jb-2011.2.pdf
Hurley D, Luque-Sastre L, Parker CT (2019) Whole-Genome Sequencing-Based Characterization of 100 *Listeria monocytogenes* Isolates Collected from Food Processing Environments over a Four-Year Period. mSphere 4:e00252–e00219
Hyden P, Pietzka A, Lennkh A et al (2016) Whole genome sequence based serogrouping of *Listeria monocytogenes* isolates. J Biotechnol 235:181–186
Jadhav S, Bhave M, Palombo EA (2012) Methods used for the detection and subtyping of *Listeria monocytogenes*. J Microbiol Meth 88:327–341
Jemmi T, Stephan R (2006) *Listeria monocytogenes*: foodborne pathogen and hygiene indicator. Rev Sci Tech 25:571–580
Johnson BO, Lingaas E, Torfoss D et al (2010) A large outbreak of *Listeria monocytogenes* infection with short incubation period in a tertiary care hospital. J Infect 61:465–470
Jose-Cunilleras E, Hinchcliff KW (2001) *Listeria monocytogenes* septicaemia in foals. Equine Vet J 33:519–522
Kasper S, Huhulescu S, Auer B et al (2009) Epidemiology of listeriosis in Austria. Wien Klin Wochenschr 121:113–119
Kück P, Greve C, Misof B, Gimnich F (2012) Automated masking of AFLP markers improves reliability of phylogenetic analyses. PLoS One 7:e49119
Kurazono M, Nakamura K, Yamada M et al (2003) Pathology of listerial encephalitis in chickens in Japan. Avian Dis 47:1496–1502
Kwong JC, Mercoulia K, Tomita T et al (2016) Prospective whole-genome sequencing enhances national surveillance of *Listeria monocytogenes*. J Clin Microbiol 54:333–342. https://doi.org/10.1128/JCM.02344-15

Lachmann R, Halbedel S, Adler M et al (2021) Nationwide outbreak of invasive listeriosis associated with consumption of meat products in health care facilities, Germany, 2014-2019. Clin Microbiol Infect 27:1035.e1–1035.e5
Läikkö T, Båverud V, Danielsson-Tham ML et al (2004) Canine tonsillitis associated with *Listeria monocytogenes*. Vet Rec 154:732
Lebreton A, Lakisic G, Job V et al (2011) A bacterial protein targets the BAHD1 chromatin complex to stimulate type III interferon response. Science 331:1319–1321
Leclercq A, Clermont D, Bizet C et al (2010) *Listeria rocourtiae* sp. nov. Int J Syst Evol Microbiol 60:2210–2214
Leclercq A, Moura A, Vales G, Tessaud-Rita N, Aguilhon C, Lecuit M (2019) *Listeria thailandensis* sp. nov. Int J Syst Evol Microbiol 69(1):74–81
Lecuit M (2007) Human listeriosis and animal models. Microbes Infect 9:1216–1225
Lecuit M, Ohayon H, Braun L et al (1997) Internalin of *Listeria monocytogenes* with an intact leucine-rich repeat region is sufficient to promote internalization. Infect Immun 65:5309–5319
Lecuit M, Vandormael-Pournin S, Lefort J et al (2001) A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science 292:1722–1725
Lee PY, Costumbrado J, Hsu CY, Kim YH (2012a) Agarose gel electrophoresis for the separation of DNA fragments. J Vis Exp 62. https://doi.org/10.3791/3923
Lee S, Siletzky RM, Kathariou S (2012b) Epidemiology, pathogenesis, ecology and Genetics of *Listeria monocytogenes*. In: Faruque SM (ed) Foodborne and waterborne bacterial pathogens. Caister Academic Press, Norfolk, pp 251–268
LeMonnier A, Natas O (2012) *Listeria monocytogenes*. In: Cornaglia G, Courcol R, Herrmann J-L, Kahlmeter G, Peigue-Lafeuille H, Vila J (eds) European manual of clinical microbiology, 1st edn. European Society for Clinical Microbiology and Infectious Disease, Basel, pp 291–295
Lindstedt BA (2005) Multiple-locus variable number tandem repeats analysis for genetic fingerprinting of pathogenic bacteria. Electrophoresis 26:2567–2582
Linnan MJ, Mascola L, Lou XD et al (1988) Epidemic listeriosis associated with Mexican-style cheese. N Engl J Med 319:823–828
Lorber B (2010) Listeria monocytogenes. In: Mandell GL, Bennett JE, Dolin R (eds) Principles and practice of infectious diseases, 7th edn. Churchill Livingstone Elsevier, Philadelphia, pp 2707–2714
Marco F, Almela M, Nolla-Salas J et al (2000) In vitro activities of 22 antimicrobial agents against *Listeria monocytogenes* strains isolated in Barcelona, Spain. Diagn Microbiol Infect 38:259–261
Matereke LT, Okoh AI (2020) *Listeria monocytogenes* virulence, antimicrobial resistance and environmental persistence: A review. Pathogens 9(7):528. https://doi.org/10.3390/pathogens9070528. PMID: 32629911; PMCID: PMC7400505
Matle I, Mbatha KR, Madoroba E (2020) A review of *Listeria monocytogenes* from meat and meat products: epidemiology, virulence factors, antimicrobial resistance and diagnosis. Onderstepoort J Vet Res 87(1):e1–e20
Matt CL, Ramachandran A, Allison RW et al (2019) *Listeria monocytogenes* in an inland bearded dragon (Pogona vitticeps). J Exot Pet Med 30:76–81
Maury M, Tsai YH, Charlier C et al (2016) Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat Genet 48:308–313
Maury MM, Chenal-Francisque V, Bracq-Dieye H et al (2017) Spontaneous loss of virulence in natural populations of *Listeria monocytogenes*. Infect Immun 85:e00541–e00517
McLauchlin J (1997) The discovery of *Listeria*. PHLS Microbiol Dig 31:76–78
McLauchlin J (2011) Listeriosis. In: Palmer SR, Soulsby L, Torgerson PR, Brown DWG (eds) Oxford textbook of Zoonoses, 2nd edn. Oxford University Press, Oxford, pp 117–127
McLauchlin J, Low JC (1994) Primary cutaneous listeriosis in adults: an occupational disease of veterinarians and farmers. Vet Rec 135:615–617
Mead PS, Dunne F, Graves L et al (2006) Nationwide outbreak of listeriosis due to contaminated meat. Epidemiol Infect 134:744–751
Mengaud J, Ohayon H, Gounon P et al (1996) E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. Cell 84:923–932

Merbach S, Osmann C, Bagó Z et al (2019) Listeriose bei einem Kleinen Panda (*Ailurus fulgens*) mit Lungenwurmpneumonie. In Tagungsbericht: 39. Arbeitstagung Verband der Zootieräzte (VZT). Schüling Verl 2021:207–208

Miettinen MK, Siitonen A, Heiskanen P et al (1999) Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J Clin Microbiol 37:2358–2360

Morvan A, Moubareck C, Leclercq A et al (2010) Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. Antimicrob Agents Chemother 54: 2728–2731

Moura A, Criscuolo A, Pouseele H et al (2016) Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nat Microbiol 2:16185

Murphy M, Corcoran D, Buckley JF et al (2007) Development and application of multiple-locus variable number of tandem repeat analysis (MLVA) to subtype a collection of *Listeria monocytogenes*. Int J Food Microb 115:187–194

Ng PC, Kirkness EF (2010) Whole genome sequencing. Methods Mol Biol 628:215–226

Núñez-Montero K, Leclercq A, Moura A et al (2018) *Listeria costaricensis* sp. nov. Int J Syst Evol Microbiol 68:844–850

Nwaiwu O (2020) What are the recognized species of the genus *Listeria*? Access Microbiol 2(9)

Olsen SJ, Patrick M, Hunter SB et al (2005) Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen Turkey meat. Clin Infect Dis 40:962–967

Orbach MJ, Vollrath D, Davis RW, Yanofsky C (1988) An electrophoretic karyotype of *Neurospora crassa*. Mol Cell Biol 8:1469–1473

Otter A, Houlihan MG, Daniel RG et al (2004) Ovine gastrointestinal listeriosis. Vet Rec 154:479

Painter JA, Hoekstra RM, Ayers T et al (2013) Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg Infect Dis 19:407–415

Paoli GC, Bhunia AK, Bayles DO (2005) *Listeria monocytogenes*. In: Fratamico PM, Bhunia AK, Smith JL (eds) Foodborne Pathogenes: microbiology and molecular biology. Caister Academic Press, Norfolk, pp 296–325

Peters M, Scheele G (1996) Listeriose in einem Kaninchenbestand. Dtsch Tierarztl Wochenschr 103:460–462

Pichler J, Much P, Kasper S et al (2009) An outbreak of febrile gastroenteritis associated with jellied pork contaminated with listeria monocytogenes. Wien Klin Wochenschr 121:81–88

Pichler J, Appl G, Pietzka A et al (2011) Lessons to be learned from an outbreak of foodborne listeriosis, Austria 2009-2010. Food Prot Trends 31:268–273

Pietzka AT, Stöger A, Huhulescu S et al (2011) Gene scanning of an internalin B gene fragment using high-resolution melting curve analysis as a tool for rapid typing of *Listeria monocytogenes*. J Mol Diagn 13:57–63

Pightling AW, Petronella N, Pagotto F (2015) The *Listeria monocytogenes* Core-Genome Sequence Typer (LmCGST): a bioinformatic pipeline for molecular characterization with next-generation sequence data. BMC Microbiol 15:224

Pinner RW, Schuchat A, Swaminathan B et al (1992) Role of foods in sporadic listeriosis: II. Microbiol Epidemiol Investig JAMA 267:2046–2050

Quereda JJ, Leclercq A, Moura A et al (2020) *Listeria valentina* sp. nov., isolated from a water trough and the faeces of healthy sheep. Int J Syst Evol Microbiol 70:5868–5879

Rajabian T, Gavicherla B, Heisig M et al (2009) The bacterial virulence factor InlC perturbs apical cell junctions and promotes cell-to-cell spread of listeria. Nat Cell Biol 11:1212–1218

Ramos JA, Domingo M, Dominguez L et al (1988) Immunohistologic diagnosis of avian listeriosis. Avian Pathol 17:227–233

Rasmussen OF, Skouboe P, Dons L et al (1995) *Listeria monocytogenes* exists in at least three evolutionary lines: evidence from flagellin, invasive associated protein and listeriolysin O genes. Microbiology 141:2053–2061

Regan EJ, Harrison GA, Butler S et al (2005) Primary cutaneous listeriosis in a veterinarian. Vet Rec 157:207

Ribet D, Hamon M, Gouin E et al (2010) *Listeria monocytogenes* impairs SUMOylation for efficient infection. Nature 164:1192–1195

Riedo FX, Pinner RW, Tosca ML et al (1994) A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. J Infect Dis 170:693–696

Roberts JR, Quoraishi A, Evans M (1994) Neonatal listeriosis in twins due to crossinfection in theatre recovery room. Lancet 344:1572

Rocourt J (1999) The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. In: Ryer ET, Marth EH (eds) Listeria, Listeriosis, and Food Safety, 2nd edn. Marcel Dekker, New York, pp 1–20

Roussel S, Félix B, Grant K et al (2013) Fluorescence amplified fragment length polymorphism compared to pulsed field gel electrophoresis for *Listeria monocytogenes* subtyping. BMC Microbiol 13:14. https://doi.org/10.1186/1471-2180-13-14

Ruppitsch W, Pietzka A, Prior K et al (2015a) Defining and evaluating a Core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. J Clin Microbiol 53:2869–2876

Ruppitsch W, Prager R, Halbedel S et al (2015b) Ongoing outbreak of invasive listeriosis, Germany, 2012 to 2015. Euro Surveill 20(50). https://doi.org/10.2807/1560-7917

Rupptisch W (2013) Molecular typing for epidemiological surveillance and outbreak investigation. Habilitationsschrift zur Erlangung der venia legendi für das Fach Molekulare Epidemiologie am Institut für Angewandte Mikrobiologe der Universität für Bodenkultur, Wien

Rütten M, Lehner A, Pospischil A, Sydler T (2006) Cerebral listeriosis in an adult Freiberger gelding. J Comp Pathol 134:249–253

Ryser ET, Marth EM (2007) Listeria, Listeriosis and Food Safety, 3rd edn. Taylor & Francis Group, Boca Raton, pp 474–484

Salamina G, Dalle Donne E, Niccolini A et al (1996) A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. Epidemiol Infect 117:429–436

Salcedo C, Arreaza L, Alcalá B et al (2003) Development of a multilocus sequence typing method for analysis of *Listeria monocytogenes* clones. J Clin Microbiol 41:757–762

Sanaa M, Poutrel B, Menard JL, Serieys F (1993) Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. J Dairy Sci 76:2891–2898

Scallan E, Hoekstra RM, Angulo FJ et al (2011) Foodborne illness acquired in the United States – major pathogens. Emerg Infect Dis 17:7–15

Schmid H, Hensel M (2004) Pathogenicity islands in bacterial pathogenesis. Clin Microbiol Rev 17: 14–56

Schroeder H, van Rensburg IB (1993) Generalised *Listeria monocytogenes* infection in a dog. J S Afr Vet Assoc 64:133–136

Schuchat A, Deaver KA, Wenger JD et al (1992) Role of foods in sporadic listeriosis: I. case-control study of dietary risk factors. JAMA 267:2041–2045

Schwartz DC, Cantor CR (1984) Separation of yeast chromosome-sized DNAs by pulsed-field gradient gel electrophoresis. Cell 37:67–75

Schweizer G, Ehrensperger F, Torgerson PR, Braun U (2006) Clinical findings and treatment of 94 cattle presumptively diagnosed with listeriosis. Vet Rec 158:588–592

Selbitz HJ (2011) Gattung Listeria. In: Selbitz HJ, Truyen U, Valentin-Weigand P (eds) Tiermedizinische Mikrobiologie, Infektions- und Seuchenlehre, 9th edn. Enke Verlag, Stuttgart, pp 289–293

Sisó C, Goncé A, Bosch J et al (2012) Listeriosis in pregnancy: a secular trend in a tertiary referral hospital in Barcelona. Eur J Clin Microbiol Infect Dis 31:2125–2132

Smith MA, Takeuchi K, Brackett RE et al (2003) Nonhuman primate model for *Listeria monocytogenes*-induced stillbirths. Infect Immun 71:1574–1579

Smith B, Larrson JT, Lisby M et al (2011) Outbreak of listeriosis caused by infected beef meat from a meals-on-wheels delivery in Denmark 2009. Clin Microbiol Infect 17:50–52

Sperry KE, Kathariou S, Edwards JS, Wolf LA (2008) Multiple-locus variable-number tandem-repeat analysis as a tool for subtyping *Listeria monocytogenes* strains. J Clin Microbiol 46: 1435–1450

Sturgess CP (1989) Listerial abortion in the bitch. Vet Rec 124:177

Swaminathan B, Cabanes D, Zhang W, Cossart (2007) *Listeria monocytogenes*. In: Doyle MP, Beuchat LR (eds) Food microbiology: fundamentals and Frontiers, 3. American Society for Microbiology, Washington, DC, p 457–491

Szymczak B, Szymczak M, Trafiałek J (2020) Prevalence of *Listeria* species and *L. monocytogenes* in ready-to-eat foods in the west Pomeranian region of Poland: correlations between the contamination level, serogroups, ingredients, and producers. Food Microbiol 91(103532)

Thompson H, Taylor DJ, Philbey AW (2009) Fatal mesenteric lymphadenitis in cattle caused by listeria monocytogenes. Vet Rec 164:17–18

Tunkel AR, Hartmann BJ, Kaplan SL et al (2004) Practice guidelines for the management of bacterial meningitis. Clin Infect Dis 39:1267–1284

Varma JK, Samuel MC, Marcus R et al (2007) *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. Clin Infect Dis 44:521–528

Vazquez-Boland JA, Kuhn M, Berche P et al (2001) *Listeria* pathogenesis and molecular virulence determinants. Clin Microbiol Rev 14:586–640

Vos P, Hogers R, Bleeker M et al (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wagner M, MacLauchlin J (2008) Biology. In: Liu D (ed) Handbook of *Listeria monocytogenes*. CRC Press, Boca Raton, pp 3–25

Wagner M, Melzer D, Bagó Z et al (2005) Outbreak of clinical listeriosis in sheep: evaluation from possible contamination routes from feed to raw produce and humans. J Veterinary Med Ser B 52:278–283

Wagner M, Auer B, Trittremmel C et al (2007) Survey on the listeria contamination of ready-to-eat food products and household environments in Vienna. Austria Zoonoses Public Health 54: 16–22

Warner SL, Boggs J, Lee JK et al (2012) Clinical, pathological, and genetic characterization of *Listeria monocytogenes* causing sepsis and necrotizing typhlocolitis and hepatitis in a foal. J Vet Diagn Investig 24:581–586

Weber A, Prell A, Potel J et al (1993) Occurrence of *Listeria monocytogenes* in snakes, tortoises, lizards and amphibians raised as pets. Berl Munch Tierarztl Wochenschr 106:293–295

Weiss R (2005) Listeriose. In: Horzinek M, Schmidt V, Lutz H (eds) Krankheiten der Katze, 4th edn. Enke Verlag, Stuttgart, pp 172–173

Weiss R, Amtsberg G (1995) Listeriose. In: Blobel H, Schließer T (eds) Handbuch der bakteriellen Infektionen bei Tieren, Band II/3, 2nd edn. Gustav Fischer Verlag, Jena/Stuttgart, pp 13–154

Weller D, Andrus A, Wiedmann M, den Bakker HC (2015) *Listeria booriae* sp. nov. and *Listeria newyorkensis* sp. nov., from food processing environments in the USA. Int J Syst Evol Microbiol 65:286–292

Welsh RD (1983) Equine abortion caused by *Listeria monocytogenes* serotype 4. J Am Vet Med Assoc 182:291

Wendt M, Bickhardt K (2001) Listeriose (Listeriosis). In: Waldmann KH, Wendt M (eds) Lehrbuch der Schweinekrankheiten, 3rd edn. Blackwell Wissenschafts-Verlag, Berlin/Wien, p 219

WHO, World Health Organization (2003) Several foodborne diseases are increasing in Europe. Press Release EURO/16/03

Winter P, Schilcher F, Bagó Z et al (2004) Clinical and histopathological aspects of naturally occurring mastitis caused by *Listeria monocytogenes* in cattle and ewes. J Veterinary Med Ser B 51:176–179

Brucellosis

13

The Mediterranean Chameleon

Sabine Zange and Holger C. Scholz

Contents

S. Zange (✉)
Bundeswehr Institute of Microbiology, Munich, Germany
e-mail: sabinezange@bundeswehr.org

H. C. Scholz
Robert Koch Institut, Berlin, Germany
e-mail: ScholzH@rki.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_63

Abstract

Human brucellosis belongs to the most common bacterial zoonotic diseases with about 500,000 reported cases per year and is one of the most widespread zoonoses according to the World Health Organization. The disease is caused by *Brucella* species, a highly contagious bacterial agent causing severe infection in humans and economic loss in livestock. It is considered to be a bioterrorism organism due to its low infectious doses of 10 to 100 bacteria and its ease of transmission as an aerosol. Brucellosis is endemic worldwide but the Mediterranean region has been particularly affected. The species of the genus *Brucella* with the greatest importance as zoonotic pathogens are *Brucella* (*B*.) *melitensis*, *B. abortus*, and *B. suis*. Some species are further subdivided into biovars. The species differ in their animal host specificity and epidemiological occurrence depending on the livestock or the prevalence of the corresponding wild animals in the respective country. In addition, the species and, in the case of *B. suis*, even the biovars differ in their pathogenicity und thereby in the clinical picture caused in humans which is complex and often characterized by relapses or chronification and termed by the syndrome Mediterranean fever, among others. Since 2007, the genus *Brucella* has changed fundamentally. A series of new "atypical" species and *Brucella*-like organisms have been described infecting humans, rodents, amphibians, fish, and even reptiles, like the panther chameleon. The significance as a zoonosis of these rare species remains still unclear, but first cases caused by atypical *Brucella* spp. in humans have been reported. Diagnosis is based on the detection of the pathogen by means of cultivation and the detection of genus-specific nucleic acids in patient or animal samples. Unambiguous species differentiation and genotyping in case of outbreaks are challenging due to the close relationship between the species and can be achieved by multiplex PCRs, sequencing of single genes as well as by whole genome sequencing. The serological diagnosis in humans and livestock is based on the detection of antibodies against the *Brucella* lipopolysaccharide. It is hampered by different lipopolysaccharide types among the species and by cross-reactivities to other zoonotic Gram-negative bacteria. Prevention and control in livestock are achieved by regular testing and vaccination with live vaccines and in humans by avoiding contaminated food products and protection from infected animals and their secretions.

Keywords

Brucellosis · Zoonosis · Mediterranean fever · Bioterrorism

13.1 Introduction

Brucellosis is recognized as a significant public health challenge, with a major economic and financial burden in countries where the disease remains endemic like the Mediterranean region, the Middle East, parts of Central and South America, Africa, and Asia. Over the last 10 years, the infection has re-emerged, with high prevalence in sheep and goats in particular, in Eastern Europe, Central Asia, and Eurasia and single autochthonous cases from countries officially free of bovine brucellosis (Beauvais et al. 2017; Kracalik et al. 2016; Mailles et al. 2012; Gwida et al. 2012a; Schaeffer et al. 2021). Economic losses from *Brucella* infections include decreased productivity as a result of abortion, weak offspring, and decreased milk production, as well as lost trade opportunities. The agent is highly contagious for humans, and the disease, unless diagnosed and treated both promptly and effectively, can become chronic, affecting multiple parts of the body. It is acquired by ingestion of contaminated dairy foods and from occupational exposure to infected live animals or carcasses during slaughter. Furthermore, *B. melitensis*, *B. abortus*, and *B. suis* are on the CDC list of selected agents and toxins (http://www.selectagents.gov) and are considered to be a bioterrorism organism due to its low infectious doses of 10 to 100 bacteria. The latter is also responsible for laboratory infections described for *Brucella*. Brucellosis is the most common bacterial laboratory-acquired infection. Laboratory exposure is regularly reported from both endemic and non-endemic countries, and up to 43% of exposed laboratory workers may develop active infection (Traxler et al. 2013).

The bacteria causing brucellosis, *Brucella*, are small (0.5 to 0.7 × 0.6 to 1.5 μm), nonmotile (with some exceptions among atypical species), facultative intracellular, Gram-negative, nonspore-forming, rod-shaped bacteria. They are catalase positive, oxidase variable, and most species show fast and strong urease activity. The optimal temperature for growth is 37 °C (between 20 °C and 40 °C), and the optimal pH for growth is 6.6 to 7.4, and it occurs within 48 to 72 h (Scholz et al. 2018). The bacteria are named after Sir David Bruce, who first recognized the organism as causing undulant fever in the 1850s in Malta during the Crimean War and was referred to as Malta Fever. Isolates from all *Brucella* species should be regarded as potentially pathogenic for man. In many countries, they are classified as risk group 3 agents, requiring a biosafety level 3 (BSL3) containment for handling of bacterial cultures (Scholz et al. 2018).

Based on the 16S rRNA gene sequence phylogeny the family *Brucellaceae* forms a monophyletic cluster within the *Alphaproteobacteria* including all species of the genera *Brucella*, *Ochrobactrum*, *Paenochrobactrum*, *Pseudochrobactrum*, and *Falsochrobactrum*. The closest phylogenetic neighbour within the family *Brucellaceae* are the members of the genus *Ochrobactrum*, specifically *Ochrobactrum intermedium*, a saprophyte that occasionally infects humans. Notably, *Ochrobactrum* spp. share a wide series of serologically cross-reactive proteins and exhibit 16S rRNA gene similarities of up to 98.6% compared with *Brucella*, indicating the close relationship of both genera (Scholz et al. 2008a, 2018). Because

of this close genetic relationship, the genera *Ochrobactrum* and *Brucella* had been unified to a single genus (Hördt et al. 2020) with *Brucella* as combined genus name. Consequently, all *Ochrobactrum* species now belong taxonomically to the genus *Brucella*.

The genus *Brucella* currently comprises 12 validly published species (30 species together with all formerly *Ochrobactrum* species, see https://lpsn.dsmz.de/genus/brucella) which are genetically highly related to each other. Many of the twelve recognized *Brucella* species are important facultative intracellular human pathogens, infecting a broad spectrum of animal species, mostly isolated from clinical specimen or contaminated food products. Only *B. microti* has been isolated directly from soil (Scholz et al. 2008c). *B. melitensis* is by far the most frequently observed causative agent of human infection, followed by *B. abortus*, *B. suis*, and *B. canis*. Based on DNA-DNA hybridization studies with DNA-DNA similarities of >80%, all species would belong to a single species with several biovars. However, due to historic reasons, predilection for particular animal hosts, biochemical features, etc., the subcommittee on the taxonomy of *Brucella* agreed in 2003 on a return to the pre-1986 taxonomic opinion with a six species concept including the "classical" *Brucella* species (the type species *B. melitensis* and *B. abortus*, *B. canis*, *B. neotomae*, *B. ovis*, *B. suis*) (Osterman 2006). Further *Brucella* species with no or rare associations to human infections were isolated from marine mammals (*B. ceti*, *B. pinnipedalis*), rodents (*B. microti*), primates (*B. papionis*), Canidae (*B. vulpis*), or humans (*B. inopinata*). A number of further atypical strains have been isolated in the past decade from amphibians, fish, and reptiles (see Table 1) and are awaiting their species description. The term "atypical" hereby refers to either a clearly different phenotype when compared to the classical species (*B. microti* and *B. inopinata*) and/or species with a higher degree of genetic diversity (*B. inopinata* and in particular *B. vulpis*). In contrast to classical *Brucella* species, most atypical species or *Brucella* isolates are metabolically very active, fast growing, and biochemically resemble *Ochrobactrum* rather than *Brucella* (Al Dahouk et al. 2017).

The genome of *B. melitensis* consists of two chromosomes, a larger one with 2.1 M bases and a smaller one with 1.2 M bases. The GC content adds up to 57%. Although more than 3,000 genes are predicted, the organism lacks the typical virulence factors, such as exotoxins, capsule, flagella, fimbriae, plasmids, lysogenic phage, antigenic variation, cytolysins, or type I, II, or III secretion systems (Purcell et al. 2007). The most important virulence factors identified in *Brucella* species are, among others, the type IV secretion system (T4SS) and the lipopolysaccharide (LPS). The T4SS, encoded by the *virB* region, is essential for survival and multiplication in macrophages and persistent infection in mice models of infection. The VirB T4SS permits the injection of a large variety of bacterial effectors inside host cell's cytosol, leading to subversion of signaling pathways and favouring bacterial growth and pathogenesis. Studies of the VirB System in different *Brucella* species indicate that the T4SS plays crucial roles in the inhibition of the host innate immune response and in intracellular survival during infection and might be required for chronic persistence during infection (Boschiroli et al. 2002; Lacerda et al. 2013; Ke et al. 2015).

Table 1 Host tropism and zoonotic potential of *Brucella* species and their biovars

Brucella species	Biovar (bv) or strain ID	Animal host	Zoonosis	References
Classical *Brucella* species				
B. melitensis	bv 1, 2, 3	Goat, sheep, camels	Yes	Meyer and Shaw 1920
B. abortus	bv 1–7, 9	Cattle, bison, buffalo, elk	Yes	Meyer and Shaw 1920
B. suis	bv 1	Pig (domestic and feral), dogs	Yes	Huddleson 1924; Meyer and Cameron 1963
	bv 2	European brown hare (*Lepus europaeus)*, wild boar (*Sus scrofa scrofa)*	Yes	Melzer et al. 2007; Mailles et al. 2017
	bv 3	Pig (domestic and feral), wild boar (*Sus scrofa*)	Yes	Jiang et al. 2012
	bv 4	Reindeer (*Rangifer tarandus tarandus*), wild caribou (*Rangifer tarandus groenlandicus*)	Yes	Rausch and Huntley 1978
	bv 5	Rodents (*Apodemus agrarius*, *A. sylvaticus*, *Mus musculus*, *Microtus arvalis*, and *Cricetulus migratorius*)	Unknown	Vershilova et al. 1983
B. canis	Reference strain RM6/66	Dog (*Canis lupus familiaris*), *canidae*	Yes	Carmichael et al. 1968
B. ovis	Reference strain 63/290	Sheep	No	Buddle 1956
B. neotomae	Reference strain 5 K33	Rodents, desert rat (*Neotomae lepida*)	2 cases reported	Stoenner et al. 1957; Villalobos-Vindas et al. 2017
B. ceti	Reference strain B1/94	Cetaceans	1 case report	Jahans et al. 1997; Foster et al. 2007

(continued)

Table 1 (continued)

Brucella species	Biovar (bv) or strain ID	Animal host	Zoonosis	References
B. pinnipedialis	Strain ST27, reference strain B2/94	Seals	3 cases reported with *B. pinnipedialis*-like species	Jahans et al. 1997; Foster et al. 2007; McDonald et al. 2006; Sohn et al. 2003
B. papionis	Strains F8/08-60T, F8/08-61	Baboons (*Papio* spp.)	Unknown	Schlabritz-Loutsevitch et al. 2009; Whatmore et al. 2014
Atypical *Brucella* species				
B. microti	Strain CCM4915T	Common vole (*Microtus arvalis*), wild boar (*Sus scrofa*), red foxes (*Vulpes vulpes*)	Unknown	Hubalek et al. 2007; Scholz et al. 2008b; Rónai et al. 2015; Scholz et al. 2009
B. inopinata	Strain BO1T	Unknown	1 case report	De et al. 2008; Scholz et al. 2010
B. inopinata-like	Strain BO2	Unknown	1 case report	Tiller et al. 2010
B. inopinata-like	Strain BO3	Amphibians are the suspected source	1 case report	Rouzic et al. 2021
B. vulpis	Strains F60T, F965	Red foxes (*Vulpes vulpes*)	Unknown	Scholz et al. 2016a
Brucella sp.	Strain B13-0095	Pac-Man frog (*Ceratophrys ornata*)	Unknown	Soler-Lloréns et al. 2016
Brucella sp.	Strains 09RB8471, 10RB9215, and others	African bullfrog (*Pyxicephalus edulis*)	Unknown	Eisenberg et al. 2012
Brucella sp.	Strains 070194A, 070064E, and others	Cane toad (*Chaunus marinus*)	Unknown	Shilton et al. 2008; Scholz et al. 2016b
Brucella sp.	Strains 10-7-D-02627	Red-eyed tree frog, cb (*Agalychnis callidryas*)	Unknown	Scholz et al. 2016b; Mühldorfer et al. 2016
Brucella sp.	Strain 152	Big-eyed tree frog, (*Leptopelis vermiculatus*)	Unknown	Fischer et al. 2012; Scholz et al. 2016b

(continued)

Table 1 (continued)

Brucella species	Biovar (bv) or strain ID	Animal host	Zoonosis	References
Brucella sp.	Strain UK8/14	White's tree frog, (*Litoria caerulea*)	Unknown	Whatmore et al. 2015; Scholz et al. 2016b
Brucella sp.	Strains 141006639, 141006992, 151-1-2015, 170-7-2016	Amazonian milk frog, (*Trachycephalus resinifictrix*)	Unknown	Scholz et al. 2016b; Mühldorfer et al. 2016
Brucella sp.	Strains 214-1-2015, 236-1-2015, 276-1-2015, 276-5-2015, 319-8-2015, 330-7-2015, 344-1-2015	Tomato frog (*Dyscophus antongilii*)	Unknown	Scholz et al. 2016b; Mühldorfer et al. 2016
Brucella sp.	Strains 161004095-1, 161004095-2	Cranwell's horned frog, (*Ceratophrys cranwelli*)	Unknown	Mühldorfer et al. 2016
Brucella sp.	Strain 141012304	Bluespotted ribbontail ray (*Taeniura lymma*)	Unknown	Eisenberg et al. 2017
Brucella sp.	Strains A105, A141	White's and Denny's tree frogs	Unknown	Kimura et al. 2017
Brucella sp.	Strain 191011898	Panther chameleon (*Furcifer pardalis*)	Unknown	Eisenberg et al. 2020

The *Brucella* cell wall is composed of an outer membrane comprising an external layer of lipopolysaccharide, a range of outer membrane proteins including some with porin activity, lipoproteins, and phospholipids. Colony morphologies have been classified into smooth and rough forms, based on the respective presence or absence of the most external antigen, O-polysaccharide (O-PS), within the LPS of the cell wall, which constitutes the hydrophilic part of the LPS. *B. melitensis*, *B. abortus*, and *B. suis* are examples for smooth species, and the respective LPS occurs as smooth lipopolysaccharide (S-LPS). In rough species, such as *B. canis* and *B. ovis*, the LPS occurs as rough lipopolysaccharide (R-LPS). R-LPS is devoid of the O-chain or O-polysaccharide, which is the most exposed part of S-LPS in the outer membrane of smooth organisms. The LPSs of *Brucella* present biological properties distinct from enterobacterial LPSs, such as low endotoxicity, high resistance to macrophage degradation, and protection against immune responses (Lapaque et al. 2005;

Cardoso et al. 2006). Taken together, these properties may constitute key virulence mechanisms of *Brucella* spp. for intracellular survival and replication and for persistence inside the animal host. The O-PS is critical in this regard for smooth strains, as rough mutants that lack O-PS do not survive as well as their smooth counterparts in macrophages or in animal models of infection. The O-chain of *Brucella* S-LPS carries the major antigenic determinants involved in the humoral immune responses in animals and humans infected by smooth *Brucella* strains (Fernández-Lago and Díaz 1986; Díaz-Aparicio et al. 1993; Alonso-Urmeneta et al. 1998), while immunodominant antigens in rough *Brucella* strains are R-LPS and outer membrane proteins (Riezu-Boj et al. 1986). The genes identified to be involved in O-PS biosynthesis were located in two major separate chromosomal loci termed the *wbk* locus and the *wbo* locus. Part of the *wbk* locus is closely related to that found in *Y. enterocolitica* O:9, which partly explains the common O-PS structure between *Brucella* spp. and *Y. enterocolitica* O:9 and consequent O-PS antigenic relatedness and serological cross-reactions (Godfroid et al. 2000). Recent research suggests that the loss of O-PS results from the spontaneous excision of the *wbkA* glycosyltransferase gene (Mancilla et al. 2012). This phenomenon is referred to as smooth to rough dissociation (Mancilla 2016; Mancilla et al. 2012). The significance of colony morphology and *Brucella* LPS genetics remains controversial, but a potential link to virulence is discussed. Defects in LPS structure that eliminate O-antigen was shown to result in a number of attenuated phenotypes, including sensitivity to complement-mediated killing, increased sensitivity to killing by antimicrobial peptides, increased maturation of dendritic cells, and attenuation in tissue culture and animal infection models.

13.2 Pathogenicity

The genus *Brucella* contains zoonotic pathogens originally associated predominantly with terrestrial livestock and wildlife sources. They are pathogenic for a wide variety of animals, frequently producing generalized infections with a bacteremic phase followed by localization in the reproductive organs and the reticuloendothelial system. Infection in the pregnant animal often results in placental and fetal infection, and this frequently causes abortion at third trimester of gestation. Because fetal membranes and placenta are highly contaminated by bulk of free bacteria, abortion is a major mechanism of *Brucella* transmission in nature (Alexander et al. 1981). The organisms may localize in mammary tissue and can be excreted in the milk. Because all the main species of meat- and milk-producing domesticated animals are susceptible to brucellosis and act as sources of human infection, the economic impact of the disease is enormous. Typically, growth in vivo is intracellular and the organisms can survive within both granulocytes and monocytes. Infections in the natural host are rarely lethal and often mild, with clinical manifestations occurring mainly in the pregnant animal. Nevertheless, localization can occur in a wide range of organs with production of a variety of lesions. All the *Brucella* species may produce infection, but the severity of the infection varies considerably

with the virulence of the infecting *Brucella* species (Braude 1951; Isayama et al. 1977). The more pathogenic strains usually produce a local abscess at the site of inoculation, followed by bacteremia of varying duration. The regional lymph nodes may become enlarged and granulomatous changes develop. Similar changes occur in the liver and spleen and frequently in other organs, particularly the testes and epididymides. *B. melitensis* and *B. suis* biovars sometimes produce fatal infections (Braude 1951). The other species rarely produce severe disease, and infection is usually self-limiting within a period varying from a few weeks to more than six months. Pathogenic effects of these nomenspecies are limited to slight-to-moderate splenic enlargement.

Infection among the nomadic population arises from direct or indirect contact with infected animals. Urban populations mostly contract the disease by consumption of contaminated milk or meat products, establishing cluster infection among family and tribe members, respectively. Person-to-person transmission is extremely rare. It has been described by vertical and sexual route as well as by blood transfusion, but it plays no part in the natural history of the disease (Tuon et al. 2017). Entry of the organisms may be via the respiratory or gastrointestinal mucosa, or the percutaneous route. The subsequent pathogenesis is believed to follow a similar pattern to that observed in experimental animals, with proliferation in lymphoid tissue succeeded followed by a bacteremic phase of variable duration with, in some cases, localization in specific organs. The infection may be completely subclinical, or it can produce a subacute or acute febrile illness. In the absence of adequate antibiotic therapy, this can persist for many months and may be accompanied by the development of severe complications such as endocarditis, meningoencephalitis, arthritis, spondylitis, and orchitis. A postinfectious, chronic, debilitating syndrome may also result. *B. melitensis* accounts for the majority of severe infections (Pappas et al. 2006), followed by *B. suis*. *B. abortus* and *B. canis* are usually associated with milder disease. Infection elicits both antibodies and cell-mediated immunity. The LPS is the dominant antigen in the serological response, but antibodies and delayed hypersensitivity to a variety of proteins can develop.

13.3 Epidemiology of Brucellosis in Animals

Some species and their biovars show distinct host tropism. In general, infection with *Brucella* sp. in animals is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk. In some animal species and for some *Brucella* species, the animals show no symptoms.

13.3.1 *B. melitensis*

B. melitensis is the most significant species in terms of both animal and human disease impact, and this refers to all three phenotypically differentiated biovars (bv 1, 2, 3).

The three biovars of *B. melitensis* can be found globally; however, in some geographical regions, a certain biovar may dominate. As an example, in the Mediterranean region (e.g., Tunisia, Greece, Italy), *B. melitensis* bv 3 occurs most frequently. The main animal hosts of *B. melitensis* are goats and sheep and with much less frequency cattle and camels, as well as yaks (*Bos grunniens*), water buffalo, alpacas, dogs, horses, and pigs. *B. melitensis* is rarely reported from wildlife. However, sporadic cases have been reported in terrestrial wild animals in the alpine ibex (*Capra ibex ibex)*, chamois (*Rupicapra rupicapra*), and wild boar in France (Marreros et al. 2011; Mick et al. 2014; Gennero et al. 2004) and in Arabian oryx (*Oryx leucoryx*) in Saudi Arabia (Ostrowski et al. 2002). Interestingly, *B. melitensis* was also isolated from wild catfish (*Clarias gariepinus*) captured from the Nile Delta region of Egypt. In this case, fish might have been infected due to disposing of animal waste into the canals (El-Tras et al. 2010). Whether fish are a possible reservoir for *B. melitensis* and may have a role in the epidemiology of the disease remains unclear.

The characteristic clinical signs of caprine brucellosis caused by *B. melitensis* are middle- to late-term abortion, stillbirths, the delivery of weak offspring, and association with an extensive negative impact in a flock's productivity leading to economic losses in livestock sector. Historical observations indicate that goats have been the hosts of *B. melitensis* for centuries; but around 1905, the Greek physician Themistokles Zammit was able to build the epidemiological link between "Malta fever" and the consumption of goat milk (Wyat 2005). While the disease has been successfully eradicated in sheep, goats, and cattle in most industrialized countries, it remains a significant burden on animal and human health in the Mediterranean region, the Middle East, Central and Southeast Asia (including India and China), sub-Saharan Africa, and certain areas in Latin America, where approximately 3.5 billion people live at risk (Rossetti et al. 2017). Despite intense joint efforts to eliminate *B. melitensis* from livestock in Europe, the disease still occurs in Portugal, Spain, France, Italy, the Balkans, Bulgaria, and Greece. Northern and Central European countries like the United Kingdom, Belgium, the Netherlands, Denmark, Germany, Austria, Switzerland, the Czech Republic, Hungary, Poland, Romania, Sweden, Norway, and Finland, among others, are officially free of the disease. Case reports from these countries are imported infections or due to imported (contaminated) food. Only single events with only a small number of cases of *B. melitensis* infections among domestic animals occurred. One was, e.g., in Austria, where a small autochthonous outbreak occurred in 2018 with three infections in humans and four in cattle (Schaeffer et al. 2021). Another outbreak in France, where a *B. melitensis* spillover between the alpine ibex and a 21-head dairy herd producing raw milk cheese in the French Alps, occurred in 2012 (Mick et al. 2014).

Camel brucellosis was first reported in 1931 and has since been found in all camel-keeping countries particularly well documented in Africa and the Arabian Peninsula (Gwida et al. 2012b). Infections with *B. melitensis* in camels occur due to the comingling of camels and ruminant livestock (Sprague et al. 2012) and among the highest prevalence rates in camels have been documented when camel herds are intermixed with ruminants (Musa et al. 2008). Prevalence rates of brucellosis in camels vary widely based on several factors, especially animal

husbandry practices (Gwida et al. 2012b). The pathology of brucellosis infection in camels is poorly known as well. Consistent with findings from other livestock, the bacteria appear to localize in reproductive tissues, lymph nodes, and spleen, causing inflammation, edema, and necrosis (Wernery 2014). Infection of pregnant camels can result in placental and fetal pathologies resulting in abortion (Narnaware et al. 2017). As with brucellosis in other animals, these abortion events likely disseminate the bacteria broadly and allow for transmission to other livestock and to animal handlers.

13.3.2 *B. abortus*

B. abortus is worldwide the main cause of brucellosis in cattle and is differentiated into eight biovars (bv 1 to 7 and bv 9). Biovar 3 is further divided into two genetically disparate subgroups 3a and 3b (Whatmore et al. 2016). The predominant biovars in livestock worldwide are bv 1 and bv 2. Biovars 3 and 4 are less frequent, although bv 4 has been reported in South America (Torres Higuera et al. 2019; Matle et al. 2021). Biovar 3 has been isolated from cattle in several European countries and in Africa, whereas the subgroup 3b of *B. abortus* bv 3 is more commonly of European origin than subgroup 3a which is associated with African origins (Ica et al. 2008; Muendo et al. 2012). *B. abortus* is usually transmitted from animal to animal by contact following an abortion. The organisms are mostly acquired by ingestion of contaminated pasture or animal barn. Additionally, inhalation, conjunctival inoculation, skin contamination, and udder inoculation from infected milking cups are other possible routes of infection. Cattle can remain infected for years. They can shed *B. abortus* whether they abort or carry the pregnancy to term, and reinvasion of the uterus can occur during subsequent pregnancies. *B. abortus* is also shed in milk, urine, and semen. Camels can also be infected with *B. abortus*, likely due to the commingling of camel herds with infected cattle (Sprague et al. 2012; Narnaware et al. 2017).

Eradication programs in a number of European nations, Canada, Australia, New Zealand, Japan, and Israel have eliminated this organism from domesticated animals with single exceptions. In terrestrial wildlife, *B. abortus* has been detected in the Greater Yellowstone Ecosystem in the USA and in the Canadian Wood Buffalo National Park from American bison (*Bison bison*) and the Rocky Mountain elk (*Cervus elaphus nelsoni*) (Cross et al. 2010). In Africa, the African buffalo is considered as a reservoir of *B. abortus* (Alexander et al. 2012). In Latin America *B. abortus* was detected, additionally to already listed animals, from foxes, grey weasels, horse capybaras, and ferrets (Lucero et al. 2008). In Europe, it was detected in chamois from large areas of the Western Italian Alps (Ferroglio et al. 2000), and in one case, it was isolated from a single red deer, probably as a spillover from infected cattle. However, in Europe, wild ruminants seem to be occasional victims of brucellosis transmitted from infected livestock, rather than true reservoirs of the infection for livestock (Muñoz et al. 2010).

13.3.3 *B. suis*

B. suis was first isolated from aborted porcine fetuses in Indiana in 1914. Additional isolates obtained from swine fetuses in 1916 were used to demonstrate pathogenicity of the bacterial isolates in swine (Huddleson 1924). Although *B. suis* is the causative agent of brucellosis in pigs, the agent is able to infect several different hosts, including rabbits, reindeer, caribou cattle, dogs, and horses. Swine brucellosis is considered to be one of the main diseases affecting the pig industry. Data from numerous countries indicate widespread distribution of *B. suis* in both domestic livestock and wildlife populations. In some parts of the world, prevalence of swine brucellosis appears to be influenced by religious or cultural preferences that influence consumption of pork and impact populations of the preferred host species. The most common manifestations of the disease are abortion at any stage of gestation, stillbirth, weak piglets in sows, and orchitis in boars. Arthritis with lameness and occasional posterior paralysis may also be seen in both sexes. *B. suis* is currently subdivided into five biovars (bv 1 to bv 5) with different animal hosts and differences in their pathogenicity. *B. suis* bv 1 is the most frequently found biovar in South America isolated from pigs, hares, and dogs (Lucero et al. 2008) and in the People's Republic of China due to high levels of swine production (Deqiu et al. 2002). Furthermore, there are single cases of dogs suffering from *B. suis* bv 1 infections, at which the risk of infection was determined to be a raw meat-based diet of hares from Argentina (van Dijk et al. 2018) or participation in wild boar hunting (Ramamoorthy et al. 2011). In Europe, porcine brucellosis almost exclusively results from transmission of *B. suis* bv 2 from Eurasian wild boar (*Sus scrofa scrofa*) and European brown hare (*Lepus europaeus*) reservoirs (Melzer et al. 2007; Mailles et al. 2017; Muñoz et al. 2010); also *B. suis* bv 1 has been detected in wild boar in the Piedmont region in France (Gennero et al. 2004). Reemergence of swine brucellosis in continental Europe is predominantly related to production systems in which swine are raised outdoors under conditions where contact with wildlife reservoirs may occur (Olsen and Tatum 2017). Venereal transmission is proposed as the main route of transmission of *B. suis* bv 2 from wild boars to domestic swine, whereas transmission from brown hares is probably through oral consumption. Although in the European Union most of the Member States are recognized as "officially free from bovine brucellosis," single cases of *B. suis* bv 2 were reported from cows (Szulowski et al. 2013; Fretin et al. 2013). *B. suis* biovar 3 has been isolated from swine and wild boar in Europe, the USA, Australia, and the People's Democratic Republic of China (Cvetnić et al. 2005, Cvetnić et al. 2009; Cornell et al. 1989; Deqiu et al. 2002). The distribution of *B. suis* bv 4 is exclusively limited to subarctic areas (Alaska, Canada and Russia) where they primarily infect reindeer (*Rangifer tarandus tarandus*) and wild caribou (*Rangifer tarandus groenlandicus*). It was also isolated from wolves, the native relative of dogs, and other carnivores like foxes and wolverines in subarctic areas. (Rausch and Huntley 1978). Isolates of *B. suis* bv 5 also have very limited geographic distribution as they have only been recovered from rodents (*Apodemus agrarius*, *A. sylvaticus*, *Mus musculus*, *Microtus arvalis*, and *Cricetulus migratorius*) in southern Ukraine and southeastern European Russia including the northern Caucasus (Vershilova et al. 1983; Hubalek et al. 2007).

13.3.4 *B. canis*

Brucella canis was discovered in 1966–1967 during an investigation of abortion in beagles, in which the organism was isolated from aborted tissues and vaginal discharge (Carmichael et al. 1968). The host range for *Brucella canis* is predominantly domestic dogs, but other species have been investigated. Serologic studies of wild canids have documented positive antibody titers also in foxes and coyotes (Cosford 2018). Dogs get infected by oronasal contact and ingestion of contaminated tissue or fluid. Transmission between dogs occurs during reproductive, social, and grooming activities. The primary sources of transmission are reproductive fluids: vaginal discharges and semen. Large numbers of infectious bacteria are shed into the environment after abortions or through vaginal or seminal secretions. Clinical signs in dogs range from asymptomatic, lymphadenopathy, orchitis and epididymitis, and embryonic loss to abortion and testicular atrophy (Hollett 2006).

Breeding kennels show high prevalence rates all over the world. In most countries, clinical disease attributed to *B. canis* infection occurs sporadically, but the dog trade leads to spread of the infection. With the unprecedented rates of animals moving across international borders and the lack of federal regulation, canine brucellosis changes its geographical distribution. Reports indicating the infection is endemic by serosurveys or detection of the agent itself are available from Central and South America, southern USA, Canada (Lucero et al. 2008; Lovejoy et al. 1976; Bosu and Prescott 1980); in Asia from Japan, India, the Philippines, Korea, China, Malaysia and Taiwan; and in Africa from Nigeria (Holst et al. 2012). In central Europe, stray dogs in the Mediterranean area are suggested to serve as a reservoir. Published reports of canine brucellosis in Europe are from imported cases due to dog imports or serosurveys from outbreaks in kennels (Holst et al. 2012). Although the literature supports the notion that *B. canis* infection has a worldwide distribution, there are no consistent epidemiological studies assessing the prevalence of canine brucellosis.

13.3.5 *B. ovis*

B. ovis infections in animals affect sheep exclusively, causing genital lesions and overall reproductive failure. It was reported in sheep farming worldwide, but to date, the real distribution of *B. ovis* infection in the world is unknown. It was isolated for the first time in 1952 from a ram in New Zealand (Buddle 1956). Since then, large outbreaks as well as prevalence of the agent are, e.g., described from the Mediterranean region, Australia, and South America (Galuzzo et al. 2021; Sergeant 1994; Costa et al. 2016). The disease is often subclinical and could circulate in the flock without suspicion. The main clinical manifestation in rams is epididymitis (either uni or bilateral), orchitis, resulting in testicular atrophy and infertility. *B. ovis* infection can also induce clinical signs in ewes such as placentitis and abortion in pregnant ewes. The disease is mainly transmitted via mating. Ewes act as a passive reservoir. Rams generally develop a subacute or chronic infection and shed *B. ovis* intermittently with semen, genital secretions, and urine for at least two to four years.

13.3.6 *B. neotomae*

B. neotomae has been isolated from the organs of wood rats in North America (Stoenner and Lackman 1957). It has been shown to display pathogenicity for experimentally infected mice and guinea pigs (Gybby and Gibby 1965; Stoenner 1963) as well as intracellular invasion and survival in macrophages and epithelial cells in vitro (Waldrop and Sriranganathan 2019).

13.3.7 *Brucella* Strains of Marine Origin (*B. ceti* and *B. pinnipedialis*)

Since 1994, *Brucella* strains have been isolated from a wide range of marine mammals. In 2007, the sea mammal *Brucella* isolates were subdivided into two novel *Brucella* species named *B. ceti* and *B. pinnipedialis* with cetaceans and seals as their preferred hosts (Foster et al. 2007). Most marine mammal isolates described have been isolated form the Atlantic, but also from the Pacific (Maquart et al. 2009) as well as from the German North Sea (Prenger-Berninghoff et al. 2008). Transmission of *Brucella* is poorly understood in marine Mammals. *B. ceti* and/or *B. pinnipedialis* has been isolated from the male and female reproductive organs, birth products (including the placenta, fetal fluids, and fetal organs) and the milk or mammary gland. A number of strains has also been detected from lung tissue of marine mammals. The isolation of marine *Brucella* sp. from lungworms (*Parafilaroides* spp.) as well as from marine mammals with bronchopneumonia suggests that transmission of marine *Brucella* strains by lungworms is possible (Prenger-Berninghoff et al. 2008). Experimental infections with marine mammal isolates have been described in cattle, sheep, pigs, and laboratory animals (mice, guinea pigs) with varying outcome (Rhyan et al. 2001; Perrett et al. 2004). In the Arctic, 5 to 10% of polar bears (*Ursus maritimus*) were found to have antibodies against *Brucella*, probably from eating infected seals (Tryland et al. 2001). Whether these infections result in any clinical signs is uncertain.

13.3.8 *B. microti*

Brucella microti was first isolated from systemically diseased common voles (*Microtus arvalis*) in the Czech Republic in Central Europe in 2007 (Hubalek et al. 2007). Natural acute infection was characterized by edema of extremities, arthritis, lymphadenitis, and perforations of the skin resulting from colliquated abscesses, orchitis, and peritoneal granulomas. It has also been isolated from mandibular lymph nodes of red foxes in Lower Austria (Scholz et al. 2009) and even directly from soil (Scholz et al. 2008c). Long-time persistence in the soil suggests that *B. microti* might at least have a transient reservoir in the environment and that common voles (and other animals) become infected by (aerosolized) contaminated soil. Foxes are presumably infected by the ingestion of diseased rodents. In Hungary, *B. microti* was isolated from a wild boar (*Sus scrofa*) in September 2014 (Rónai et al. 2015). It was isolated from lymph nodes. The (female) wild boar did not develop any clinical disease. In

2017, *B. microti* was isolated from domestic marsh frogs (*Pelophylax ridibundus*) of a French farm producing frogs for human consumption (Jaý et al. 2018). Later it was shown that *B. microti* was highly prevalent in these frogs and also in the environment, suggesting long-term persistence (Jaý et al. 2020). It was speculated that its presence could constitute a possible risk for consumers. The prevalence of *B. microti* in other animal species needs further investigation.

13.3.9 Rare *Brucella* Species Recently Described from Animal Hosts

B. vulpis was isolated in 2008 from mandibular lymph nodes of red foxes in Austria and described as a novel *Brucella* species in 2016 (Scholz et al. 2016a). Up to now, only two strains, the type strain F60T and strain F965, both isolated from a single fox, exist. In contrast to most other atypical *Brucella*, *B. vulpis* is slowly growing, similar to *B. melitensis*. Currently, *B. vulpis* is the genetically most diverse *Brucella* species when compared to classical *Brucella* species.

In 2014 *B. papionis*, isolated from baboons (*Papio* spp.), was described as a novel *Brucella* species (Whatmore et al. 2014). The two available strains (F8/08-60T and F8/08-61) were isolated in 2006 and 2007 from two cases of stillbirth and retained placenta that had delivered stillborn offspring at a primate research center in Texas, USA (Schlabritz-Loutsevitch et al. 2009). Phylogenetically *B. papionis* is most closely related to *B. ovis*. Genetically and phenotypically, *B. papionis* belongs to the group of classical *Brucella* species.

In the last few years, many further atypical *Brucella* have been isolated from cold-blooded vertebrates, mainly from exotic frogs kept in zoos and private housings (Table 1). The presence in frog species native to Africa, Asia, America, and Australia indicates that they might be endemic in these regions. The case history of *Brucella* infections in amphibians reveals a variety of pathologies ranging from localized manifestations to systemic infections. Some isolates seem to be capable of causing high mortality in zoological exhibitions putting higher demands on the management of endangered frog species. In 2020, the isolation of an atypical *Brucella* isolate from a reptile, the panther chameleon (*Furcifer pardalis*), was described the first time the (Eisenberg et al. 2020). The detection of *Brucella* in amphibians and reptiles significantly broadens the host range of this medically important genus.

13.4 Epidemiology of Brucellosis in Humans

B. melitensis is the predominant species causing human brucellosis in most endemic regions, but *B. abortus* or *B. suis* might significantly contribute to the number of human infections in areas with extensive cattle or swine livestock farming, and therefore, the occurrence of the different *Brucella* species varies among geographic regions. In the European Union, brucellosis cases stayed stable over the last years. Highest case numbers (between 40 and 200 cases per year, https://www.ecdc.europa.eu/sites/default/files/documents/zoonoses-EU-one-health-2018-report.pdf) are found

in Italy, Greece, Spain, Portugal and Germany. In these countries, cases are linked to imported brucellosis, but also local outbreaks due to unpasteurized dairy products occurred (Control 2018; Nenova et al. 2015).

13.4.1 *B. melitensis*

B. melitensis is the species with highest zoonotic potential causing most human infections among all *Brucella* species. All three recognized biovars (bv1, bv2, bv3) are pathogenic to humans. The live attenuated Rev-1 vaccine for the vaccination of small ruminants is also pathogenic for humans. Although there are single reports of occurrence in wild animals, the main source for *B. melitensis* infections in humans are domestic animals, in particular sheep and goat and with less frequency camels and cattle and their dairy products (Shimol et al. 2012; Garcell et al. 2016). It is the causative agent of Malta fever and the type species of the genus; that was named after the region where the disease has been described for the first time (*melitensis* is the Latin name for the island Malta).

At highest risk for occupational transmission of *B. melitensis* are abattoir workers, rural workers (animal breeders, farmers), and laboratory workers. The latter are at highest risk for *B. melitensis*, especially when miss-identification occurred and/or cultures were processed outside a safety cabinet or due to accidents in the laboratory (Pereira et al. 2020).

13.4.2 *B. abortus*

B. abortus is also zoonotic. It is the etiological agent of bovine brucellosis characterized by abortion in animals and is an occupational disease among people in contact with ruminants or their tissues, such as farmers, butchers, abattoir workers, veterinarians, and laboratory personnel. In addition to wild-type strains of *B. abortus* bv 1 to bv 7 and bv 9, the live attenuated vaccines containing organisms of strain 19 or RB51 are also pathogenic for humans. They must be handled with caution to avoid accidental injection or contamination of mucous membranes or abraded skin. Human exposure can be reduced by controlling brucellosis in livestock and by wearing personal protective equipment when handling infected animals. In milk *B. abortus* is killed off by pasteurization. The consumption of raw milk and dairy products derived from raw milk are among the potential sources of brucellosis. Their persistence in unpasteurized cheese is influenced by factors such as the type of fermentation, temperature, water content, pH, and ripening time.

13.4.3 *B. suis*

Swine brucellosis in humans is most frequently a disease of farm workers, veterinarians, and abattoir workers, but it can also be contracted through other activities

such as hunting or other associations with feral swine. *B. suis* bv 1 and bv 3 are pathogenic in humans, whereas bv 2 appears to be a very rare cause of human infection. The usual route of transmission to humans is the ingestion of contaminated food, e.g., unpasteurized dairy products or undercooked meat from infected animals. The disease may also be acquired by handling infected animals or inhalation of infectious aerosols. High numbers of *B. suis* bv 1 transmissions were described from Argentina in slaughterhouse workers. Affected employees were infected by injuries when processing pork meat, contaminated aerosols, conjunctival splashes, or direct bacterial entry through skin lesions (Wallach et al. 2017; Escobar et al. 2013). Infections from processing meat are described even outside endemic regions (Zange et al. 2019). From other countries (the USA, Australia, Turkey), *B. suis* bv 1 infections have been also reported in humans who hunt and handle feral pigs and are associated with dressing the killed animals or processing their meat (Kutlu et al. 2016; Carrington et al. 2012; Starnes et al. 2004). From China, *B. suis* bv 3 has been reported as the causative pathogen in human brucellosis in Hainan Province (Jiang et al. 2012). Although the current prevalence of *B. suis* bv 4 in people is unknown, case reports from Alaska and Canada's Arctic highlight that rangiferine brucellosis has occurred among northern peoples who consumed caribou (Chan et al. 1989; Forbes 1991).

13.4.4 *B. canis*

The pathogenicity of *B. canis* is considered to be relatively low, making it less of a perceived public health concern than other *Brucella* species. Mild clinical symptoms, such as fever, headache, anorexia, and fatigue, have been reported in patients suffering from *B. canis* infections, but also single severe cases and/or chronic manifestations, such as peritonitis, endocarditis, aneurysms, osteomyelitis, and arthralgia were described (Javeri et al. 2014; Ying et al. 1999; Wallach et al. 2004; Piampiano et al. 2000). Furthermore, infections in patients with underlying disease like HIV infection even with appropriate CD4 counts and negative viral loads have also been diagnosed and successfully treated for *B. canis* infections (Lawaczeck et al. 2011; Dong et al. 2020). The M-strain of *B. canis*, which is used for the serological diagnosis of canine brucellosis and avirulent in dogs, is described to be pathogenic to humans (Wallach et al. 2004). The most common route of infection is through contact with infected dogs, which disseminate the disease with their secretions. Urine and vaginal fluid from canines suffering from brucellosis may contaminate the environment, leading to human disease. Infections occurs via inhalation of aerosols or direct contact with mucosa or non-intact skin, furthermore, after ingestion, either by contaminated hands or by allowing an infected dog to lick around the face and mouth area. Of high risk are people living in close contact with infected dogs especially in overcrowded living conditions, dog breeders, laboratory personnel handling positive samples, and animal technicians (Boeri et al. 2008; Dentinger et al. 2015; Lucero et al. 2005). The true incidence of *B. canis* in humans is unknown, due to a general lack of reliable serological detection methods and as

not in all countries *Brucella* is routinely tracked beyond the genus level as well as due to misidentification as *B. suis* as a consequence of the similar banding pattern in the *Brucella* bruce-ladder PCR. This points out the zoonotic potential of this disease and the importance of pet owner education as well as public health initiatives to ensure appropriate animal treatment and control.

13.4.5 *B. ovis*

B. ovis is not classified as a zoonosis. Although large outbreaks are described from livestock (sheeps), there are no human cases reported so far.

13.4.6 *B. neotomae*

In 2008 and 2011, *B. neotomae* was detected in two patients with neurobrucellosis in Costa Rica. In both cases, the agent was cultured from blood and cerebrospinal fluid. After antibiotic treatment, the patients recovered with normal mental activities. The source of infection could not be identified (Villalobos-Vindas et al. 2017). In Costa Rica, there are no rats of the genus *Neotomae*, but other Neotominae species are endemic. In the described cases, the bacterium was identified as *B. neotomae* by multiple-locus variable number tandem repeat analysis of 16 sequences (MLVA16) and whole genome sequencing (Suárez-Esquivel et al. 2017). Whole genome analysis of *B. neotomae* has also revealed that this bacterium possesses the same virulence arsenal as the classic zoonotic *Brucella* spp. In vitro, invasion and replication in epithelial cells and macrophages by *B. neotomae* has been shown (Waldrop and Sriranganathan 2019). This demonstrates the zoonotic potential of *B. neotomae*.

13.4.7 *Brucella* Strains of Marine Origin (*B. ceti* and *B. pinnipedialis*)

Three human cases with naturally acquired infection by *Brucella* strains presumably of marine origin have been reported, one case of spinal osteomyelitis from a patient in New Zealand (Mc Donald et al. 2006) and two neurobrucellosis cases from Peruvian patients (Sohn et al. 2003). Potential routes of exposure in these three cases included eating raw fish or shellfish, handling raw fish and bait, and swimming in the ocean. None of the people had been directly exposed to marine mammals. Interestingly, these human isolates presented the same genotype as strains from cetaceans from the Pacific (Whatmore et al. 2008). In MLVA-typing, the human isolates share homologies with *B. pinnipedialis* but might also be a third marine mammal *Brucella* species (Maquart et al. 2009). Another clinical case occurred in a person who was working with *B. ceti* in the laboratory (Brew et al. 1999). Altogether, this may point towards a zoonotic potential of these marine mammal *Brucella* species.

13.4.8 *B. microti*

No human infection with *B. microti* has been reported so far. Nevertheless, *B. microti* was shown to replicate in human macrophages and in human and murine macrophage-like cells. It is highly virulent in murine models of infection in which 10^5 colony-forming units of *B. microti* killed 82% of Balb/c mice within seven days (Jiménez de Bagüés et al. 2010). In a mouse model using wild-type and immunodeficient mice that either lacked B, T and B, or T, B, and NK cells, *B. microti* was able to cause disease in the immunocompromised mice but was cleared completely in wild-type mice after three weeks (Jimenez de Bagüés et al. 2011). Histopathology analysis of diseased mice showed extensive areas of necrotic tissue and thrombosis in livers after one week postinfection. *B. microti* was also shown to cause fatal infection in experimentally infected chicken embryos where it provoked marked gross lesions, i.e., hemorrhages and necroses with a killing rate of 100% (Wareth et al. 2015).

13.4.9 The Role of Atypical *Brucella* Species as Human Pathogens

Only little is known about the virulence and zoonotic potential of atypical *Brucella* species. The first atypical *Brucella* isolated from a patient was strain BO1 (De et al. 2008). It was isolated from an inflamed breast implant of a 71-year-old immunocompetent woman without underlying diseases in the USA, presenting with repeating fever attacks and breast pain. Later this strain was described as a novel *Brucella* species, *B. inopinata* (Scholz et al. 2010). Besides fever and local inflammation of the breast, no complications were observed, and the patient responded well to standard antibiotic treatment. The source of infection could not be verified and remained speculative.

The second case was described in a patient from Australia with chronic destructive pneumonia (Tiller et al. 2010). The strain isolated from a lung biopsy was phenotypically and genetically closely related to *B. inopinata* BO1T and therefore was termed BO2. Like for *B. inopinata*, the source of infection could not be determined. The most recent reported infection with atypical *Brucella* species was reported from a 28-year-old patient in France who was hospitalized for an exploration of polyadenopathies associated with multiple pulmonary condensations (Rouzic et al. 2021). The patient had reported a left cervical lymphadenopathy, associated with general degradation in condition and weight loss (8 kg in 3 months). The patient also reported fever and intense night sweats. A cervicothoracic and abdominal computed tomography (CT) scan revealed multiple mediastinal adenopathies, pulmonary parenchymal condensations, and cystic emphysematous lesions in the right upper lobe and splenomegaly (Rouzic et al. 2021). From a cervical lymph node, an atypical *Brucella* strain (BO3) was isolated. In-depth molecular analysis using comparative genome analysis revealed that strain BO3 was identical to strain B13-0095 recently isolated from a Pac-Man frog in Texas (Soler-Lloréns et al. 2016). Indeed, the patient declared having been in close contact with Pac-Man

frogs during his previous activities as exotic animal keeper. The clinical picture was compatible with severe brucellosis, and the patient required prolonged antibiotic therapy of 3 months.

Besides reported human cases, the virulence of atypical *Brucellae* had been investigated in cell culture experiments and by using animal models. In a murine model of infection, some strains caused death (Jiménez de Bagüés et al. 2014). In vitro, strains including isolates from amphibians were found to effectively multiply in different cell lines (Al Dahouk et al. 2017; Soler-Lloréns et al. 2016), and most strains were capable to persist in mammalian hosts over a period of up to three months (Al Dahouk et al. 2017). Furthermore, they share identical virulence genes compared to classical *Brucellae* (Al Dahouk et al. 2017; Soler Lloréns et al. 2016) and have been associated with significant morbidity and mortality in common voles and amphibians (Hubalek et al. 2007; Mühldorfer et al. 2016). On the other hand, one may argue that only weak pathological signs were observed in mice that cleared infections more efficiently and with fewer signs of inflammation compared to classical *Brucella* species (Al Dahouk et al. 2017).

Summarizing, despite the low number of human case**s**, current reports show that atypical *Brucella* are able to infect humans and may induce a clinical picture resembling brucellosis.

The animal reservoirs of *Brucella inopinata*-like strains for human infections still have to be identified, but the high similarity to strains isolated from exotic frogs suggests this as a possibility. Since *B. inopinata*-like strains have also been isolated from fish and reptiles, all cold-blooded vertebrates may serve as a potential source for human infection.

13.5 Laboratory Diagnosis and Typing

The diagnosis of human brucellosis relies on three different modalities: culture, nucleic acid amplification tests, and serology. Bacterial culture is essential for performing strain typing.

13.5.1 Bacterial Culture

Although the diagnosis of brucellosis by bacterial culture is hampered by the slow-growing features of members of the genus *Brucella*, laboratory safety concerns, and reduced sensitivity in prolonged disease and focal infections, isolation of the bacterium is indisputable evidence of the disease. Culture, when positive, provides the definitive diagnosis and is considered to be the gold standard in the laboratory diagnosis of brucellosis. It is essential for determining antimicrobial susceptibility and performing strain typing. Since the pathogenesis of human brucellosis always involves a bacteremic stage, cultures of peripheral blood represent a suitable tool for confirming the disease, although their sensitivity shows a broad range (10–90%) in different reports (Pappas et al. 2005). As bacteremia is low-grade,

sensitivity can be easily improved by drawing two or three separate blood culture sets. In addition to blood samples, *Brucella* can be cultured from clinical samples from affected organs. Automated continuously monitored blood culture systems and the corresponding culture broths are suitable for cultivation of all *Brucella* species from bacteremic patients. The latter could also be applied for puncture fluids from infected tissue or joints. The incubation of liquid culture media, such as blood culture bottles in the absence of growth, should be at least 21 days. However, the cultivation succeeds with blood culture systems from more than 95% of the positive bottles within the first seven days (Yagupsky 1999). Especially on primary isolation, many strains of the classical species require supplementary CO_2 for growth and growth of the classical species is improved by addition of serum or blood to the culture media.

Antigen tests and biochemical or mass spectrometry methods (MALDI-TOF MS) in combination with appropriate databases can be used for preliminary identification (Lista et al. 2011; Ferreira et al. 2010) and on a RUO basis also down to species level (Mesureur et al. 2018). The identification of *Brucella* spp. by MALDI-TOF has proven to be very useful as it is a rapid and accurate identification method (Karger et al. 2013) preventing laboratory infections (Becker et al. 2018). Using biochemical identification, only few reactions are characteristic for *Brucella*, making identification difficult and reliable species determination almost impossible. Specific reactions for differentiation include urease tests, production of hydrogen sulfide (H_2S), catalase, oxidase, growth in the presence of thionin, and basic fuchsin dyes. For the biochemical identification with commercial systems, misidentifications, e.g., as *Moraxella phenylpyruvica* and *Ochrobactrum anthropi*, are described which have even led to laboratory infections (Vila et al. 2016; Batchelor et al. 1992). Therefore, confirmation and reliable species identification should always be carried out by means of molecular biological methods. In contrast, the differentiation of biovars from *B. melitensis* (3 biovars) and *B. abortus* (8 biovars) and *B. suis* (5 biovars) is carried out traditionally by means of phenotypical tests like growth characteristics, biochemical tests in combination with serotyping and determination for resistance or sensitivity to different *Brucella* phages. In recent years, biotyping has increasingly being replaced by the use of frontline molecular tools like PCRs based on genomic deletions or SNPs (Whatmore et al. 2016).

13.5.2 Serology

For the detection of specific antibodies against *Brucella* spp., a variety of in-house and commercial serological tests are available. Currently, all commercial methods are based on the detection of antibodies against the smooth LPS present in *B. melitensis*, *B. abortus*, and *B. suis*. Since *B. canis* lacks the immunodominant O-polysaccharide of the smooth *Brucella* species, the standard serological tests cannot detect anti-*B. canis* antibodies (Sayan et al. 2011). Therefore, in patients with suspicion of infection with a *Brucella* species with rough LPS-type, *B. canis* strains should be used for ELISA antigen preparation (Cosford 2018; Lucero et al. 2005). In particular,

the M-strain of *B. canis* with its less mucoid phenotype is recommended for antigen preparations from whole cells (Carmichael et al. 1984), and PdhB and Tuf proteins are candidates for recombinant antigens in indirect ELISA assays (Sánchez-Jiménez et al. 2020). For atypical strains, like *B. inopinata*-like strains, no serological assay is evaluated yet.

Due to its rapid and inexpensive performance, the serum agglutination test (SAT) or its microtiter plate variant is most widely used, especially in endemic areas, and is traditionally considered the gold standard in the serodiagnosis of brucellosis. The rose bengal (RBT) test can be used to screen patients and contacts and is also commonly used in endemic regions due to its ease of performance (Díaz et al. 2011). However, the result should always be confirmed by one of the followings methods (Corbel 2006). The complement fixation test (CFT) is used as a confirmatory test because of its high specificity, especially in chronic infections as it provides evidence of active brucellosis. Positive CFT titer often persists for years. Incomplete antibodies can be detected by Coombs test or immunocapture agglutination test (BrucellaCapt®) (Bosilkovski et al. 2010; Casanova et al. 2009). Enzyme-linked immunosorbent assays (ELISA) enable the separate determination of individual antibody classes (IgG and IgM antibodies) and a better evaluation of the results with respect to the course of the disease. During the first week after infection with *Brucella* spp., IgM antibodies directed against the LPS of the bacteria predominate. From the second week on, the IgG antibodies also increase. IgM and IgG antibody concentrations reach their highest level about one month after infection. With the start of adequate antibiotic therapy, IgM antibodies slowly fall. Despite successful therapy, IgM antibodies can be detected for over a year (Ariza et al. 1992; Corbel 2006). In case of therapy failure or relapse, IgG antibodies rise again. However, elevated IgG antibodies alone are not evidence of chronic infection, as they can persist for years after acute brucellosis (Ariza et al. 1992). The immunocapture agglutination test (modified Coombs test) provides additional information to distinguish between a past and a chronic infection or a recurrence (Bosilkovski et al. 2010; Casanova et al. 2009). The interpretation of serological test results can sometimes be challenging especially in endemic areas, where people suffer from reinfections due to continuous exposure to the agent. In particular, low antibody titers are typical in chronic courses or in the early phase of infection, and it can be difficult to distinguish them from past infections. An acute infection is confirmed by a fourfold rise of the specific antibodies after repeated testing at intervals of two to three weeks. However, *Brucella* spp. infections have also been described in which a direct pathogen detection was successful, i.e., the culture and/or PCR were positive, but no seroconversion occurred, i.e., the antibody detection was negative. In particular, this has been observed in local infections, even without known immunosuppression (Raptis et al. 2007; Janmohammadi and Roushan 2009; Çelik et al. 2012; Yaghoobi et al. 2013).

In patients with positive serological findings but without detection of the pathogen by culture or PCR and no brucellosis typical or only nonspecific clinical findings, infections caused by other pathogens should be considered which can

lead to false positive results due to cross-reactivities with *Brucella* spp. LPS. Cross-reactivity has been described for *Yersinia enterocolitica* O9, *Escherichia coli* O:157, *Franciella tularensis*, *Salmonella urbana* O:30 and *Vibrio cholera* (Schoerner et al. 1990; Nielsen et al. 2007; Nielsen et al. 2004; Corbel 2006). As all serological methods available on the market are based on the detection of antibodies against the smooth LPS of *Brucella* spp., a differentiation from cross-reacting antigens in such cases is only possible by excluding the cross-reactive agents, as far as specific tests are available for this purpose. The most common disease in which cross-reactive antibodies occur is yersiniosis. Therefore, in non-endemic regions, if antibodies against *Brucella* spp. are detected without the case definition of brucellosis being fulfilled, an infection with *Yersinia enterocolitica* should always be serologically excluded.

13.5.3 Nucleic Acid Amplification Assays

Conventional polymerase chain reaction (PCR) and real-time PCR (RT-PCR) assays have been attempted for the direct detection of *Brucella* from clinical specimens, to monitor treatment response, and for the identification, speciation, and differentiation of recovered *Brucella* species. PCR-based methods to detect *Brucella* DNA use a variety of targets for genus-specific PCRs: *omp2*, *omp2b*, *bcsp*31, IS*711* (IS*6501*), 16S-23S rRNA spacer region, and 16S rRNA (Wang et al. 2014). Among the listed targets the following two are the most frequently used: Amplification of the insertion sequence IS711, which is present with a variable number of copies in all *Brucella* species (Al-Nakkas et al. 2002). The *bcsp31* gene encodes the synthesis of an immunogenic membrane protein of 31 kDa that is specific to the *Brucella* genus (Al Dahouk et al. 2007a). Due to the high sequence homology, a misidentification of *Brucella* sp. as *Ochrobactrum* sp. or vice versa must be excluded when sequencing of the 16S rRNA gene alone was applied (Scholz et al. 2008d).

Species differentiation as well as determination of the biovar is more complicated. One of the first PCR assays to differentiate among *Brucella* species from pure cultures was the so-called Abortus-Melitensis-Ovis-Suis (AMOS) PCR (Bricker and Halling 1994). This PCR uses a single reverse primer, targeting the *Brucella*-specific insertion element IS711, and four different forward primers, each specific for a given species. Species are differentiated on the basis of different PCR fragment sizes. The disadvantage of this PCR was that not all species could be identified (i.e. *B. canis* and *B. neotomae*) and that some biovars within a given species gave negative results. In 2006, a new conventional multiplex PCR (Bruce-ladder), using eight primer pairs in a single reaction, was developed (Garcia-Yoldi et al. 2006). Later, this PCR was enhanced, to cover novel species such as *B. microti* and *B. inopinata* (Mayer-Scholl et al. 2010). However, some *B. canis* strains and *B. microti* can be misidentified as *B. suis* with this PCR due to a very similar banding pattern. To avoid this and for further differentiation down to biovar level of *B. suis* by PCR, a multiplex PCR assay (Suis-ladder) is recommended (Lopez-Goni et al. 2011).

13.5.4 Molecular Typing

MLST: The conventional multi-locus-sequence typing (MLST) approach uses sequence divergence in housekeeping genes. About seven to nine housekeeping genes are commonly analyzed in order to obtain a reasonable balance between the acceptable identification power, time, and cost for the strain typing. From each housekeeping gene, approximately 450 to 500 base pairs (bp) are amplified by PCR, followed by DNA sequencing and subsequent comparative sequence analysis. Each unique sequence is assigned a specific allele number and alleles are combined into an allelic profile and further assigned to a specific sequence type (ST). New alleles result in a new combination and therefore in a novel ST. Because accumulated changes occur slowly and are regarded as selectively neutral, the MLST approach is a reliable tool for the overall characterization of microbial populations and the investigation of phylogenetic relationships. However, the slow molecular clock rate and the limited number of genes do not allow in-depth phylogenetic reconstructions and analysis of local epidemiological studies. Thus, unlike MLVA, MLST is of little value for outbreak investigations. The classical MLST assay for *Brucella* consists of nine gene targets (Whatmore et al. 2007) and was later expanded to a set of 21 loci in order to achieve better resolution (Whatmore et al. 2016). The MLST database can be accessed via PubMLST (https://pubmlst.org/organisms/brucella-spp).

MLVA: Multiple-locus variable-number tandem-repeat analysis (MLVA) has become a major molecular typing method to characterize several pathogenic bacterial species at the strain level. The *Brucella* MLVA-16 scheme as initially described by Le Flèche et al. (2006) and Al Dahouk et al. (2007b) has been proven to be a valuable tool in epidemiological outbreak investigations with high discriminatory power in several studies (Aftab et al. 2011; Jiang et al. 2011; Gyuranecz et al. 2016; Garofolo et al. 2016). Therefore, MLVA can currently be considered to be the gold standard of high-resolution *Brucella* typing (Scholz and Vergnaud 2013) and is actually the most widely used approach for outbreak investigations (De Massis et al. 2015; Dorneles et al. 2014). Furthermore, for strain comparisons a huge publicly accessible database consisting of MLVA profiles from more than 7000 strains is available (http://mlva.i2bc.paris-saclay.fr/brucella/). On the other hand, MLVA requires sophisticated laboratory skills and high standardization in order to get reliable and reproducible results. In recent years, genome-based methods like SNP analysis and core-genome-based MLST (cgMLST) have started to supplant MLVA due to higher robustness and lower cost at equivalent or even higher genetic resolution.

SNP Typing: Single nucleotide polymorphism (SNP) analysis from whole genomes potentially has the highest discriminatory power among the typing methods, as polymorphisms can be discovered in both coding and noncoding regions of the genome. However, the choice of a reference genome and the specific algorithm applied for SNP identification can significantly influence the number of identified SNPs and the accuracy of the reconstructed phylogenetic relationships. Consequently, depending on a given SNP assay, the genetic resolution can vary significantly. Hundreds of *Brucella* isolates have already been sequenced and analyzed at draft level for whole genome SNP discovery that efficiently complement MLVA typing and clustering

analysis when necessary (Foster et al. 2009; O'Callaghan and Whatmore 2011; Georgi et al. 2017). In all cases, spatial clustering obtained by SNP-Typing was comparable to MLVA and in some cases SNP-Typing outperformed MLVA in terms of genetic resolution (Janowicz et al. 2018; Holzer et al. 2021).

cgMLST: Core-genome based multilocus sequence typing (cgMLST) has become a benchmark tool for bacterial outbreak investigations and was successfully applied to several pathogens. It represents a very robust high-resolution typing method and provides highest reproducibility. Like classical MLST, it uses differences in allelic sequence profiles for strain discrimination, in this case of genes belonging to the core genome. In contrast to classical MLST, hundreds to thousands gene targets of the core-genome are analyzed simultaneously resulting in a genetic resolution comparable to SNP and VNTR typing. Compared to the different SNP assays, cgMLST has the advantage of being readily and consistently applied in different laboratories as it uses a consistent set of well-defined conserved loci. For cgMLST of *B. melitensis*, a typing scheme consisting of 2704 gene targets was developed and validated (Janowicz et al. 2018). It can be downloaded from the Ridom cgMLST.org Nomenclature Server (https://www.cgmlst.org/ncs/schema/6398355/). This scheme is also applicable for high-resolution typing of other *Brucella* species, including atypical isolates. In a direct comparison to MLVA, higher phylogenetic distance resolution was achieved with cgMLST particularly for strains belonging to the same lineage. Another cgMLST assay for *Brucella* spp. using a smaller set of 164 loci of the core genome was published by Sankarasubramanian et al. (Sankarasubramanian et al. 2019). Generally, Illumina paired end sequencing with a coverage of 70x is recommended in order to achieve accurate typing results.

13.6 Disease Symptoms in Humans and Treatment

The usual route of transmission to humans is the ingestion of contaminated food, e.g., unpasteurized dairy products or undercooked meat from infected animals. The disease may also be acquired by handling infected animals or inhalation of infectious aerosols. Occupational exposure is described for hunters, farmers, slaughterhouse, and laboratory workers, as well as accidental exposure of veterinarians, preparing or administering the attenuated live vaccine strains (Pereira et al. 2021).

Regardless of the portal of entry of *Brucella* organisms, bacteria penetrate mucosal barriers and enter the bloodstream, which permits their dissemination throughout the body. The bacterium quickly translocates across the epithelial layer and is endocytosed by mucosal macrophages and dendritic cells (Franco et al. 2007). Internalized *Brucellae* initially localize in the regional lymph nodes and then spread through the bloodstream, entering macrophages-rich tissues such as the liver, spleen, lymph nodes, or bone marrow. There they adopt a facultative and stealthy intracellular lifestyle, evading the innate and adaptive immune responses and the action of many antibiotics (Pappas et al. 2005). Disease-specific symptoms occur within an incubation period of 10 to 21 days, in some cases up to several months. The clinical picture of human brucellosis is not specific, often accompanied with flu-like

symptoms such as undulant fever, sweating, asthenia, myalgia, arthralgia, and headache. The agent can be translocated and adapt to almost any tissue or in vivo site, and in case of improper antibiotic treatment, the disease has a high risk of chronification and/or relapses. To avoid the latter, successful brucellosis-treatment requires long-term antibiotic therapy (Franco et al. 2007; Dean et al. 2012). Due to the intracellular growth and replication of the bacteria and the slow-growing nature of *B. melitensis*, a combination therapy including at least one substance with good cellular penetration is required. Monotherapy is reported to have a high relapse rate. The regimen accompanied with lowest rate of treatment failures and, therefore, recommended by most authors comprise of doxycycline and rifampin and in complicated cases added with gentamycin during the first weeks. Alternative treatment regimens include trimethoprim/sulfamethoxazole and fluoroquinolones (mainly ciprofloxacin) (Ariza et al. 2007; Bossi et al. 2004; Solera 2010). In patients suffering from neurobrucellosis, addition of ceftriaxone is suggested (Erdem et al. 2012; Pappas et al. 2007). Up to now, resistance towards therapy-relevant substances is rare, and treatment failures are associated with noncompliance of patients during the long-term oral treatment or poor absorption of the antibiotics at the site of infection, although mutations are described, e.g., in the *rpoB* leading to resistance towards rifampicin (De rautlin de la Roy et al. 1986; Sayan et al. 2008). The *B. melitensis* Rev-1 vaccine strain is resistant to streptomycin, and *B. abortus* (strain RB51) is resistant to rifampicin. As *B. melitensis* is considered as category B agents of bioterrorism, also engineered antimicrobial resistance is a concern.

13.7 Prevention and Control

The prevention in animals in the EU according to the Veterinary control programs (SANCO/6095/2009) include slaughter or stamping out in infected herds, frequent and regular testing of herds, and the use of tests in association with a compensation scheme. The currently available vaccines, which are officially authorized at European level for the prophylaxis of brucellosis, are *B. abortus* S19 and *B. abortus* RB51 strains for cattle and *B. melitensis* strain Rev.1 for sheep and goats (OIE Terrestrial Manual Brucellosis 2018). They are prepared from adequately derived seed cultures and can potentially provoke two types of adverse effects in the vaccinated animals: the induction of abortion in pregnant females and in the case of the smooth strains (S19 and Rev.1), the persistence of residual antibodies to the classical serological tests (RBT and CFT). The latter may cause diagnostic difficulties in certain situations but can be avoided by the use of conjunctival route for vaccine administration and restrict the age of application to three to four months, maximum six months. No suitable vaccines exist for the control of *Brucella* infection in swine and of canine brucellosis. There are strategies employed for experimental vaccines against *B. canis* including the attenuated mutant vaccine strains such as a *B. canis* mutant in SST4 and a mutant version of *B. abortus* RB51 vaccine strain. Newer studies demonstrated that a *B. ovis* mutant protects against experimental challenge with *B. canis* in mice and is safe for dogs (Eckstein et al. 2020).

In general, vaccinations are recommended depending on the prevalence in the respective region and the circumstances. In Germany, however for reasons of animal disease control, vaccinations against brucellosis of cattle, pigs, sheep, and goats and curative experiments are prohibited. Animals with the disease and animals suspected of having the disease must be separated from the rest of the herd and killed.

Human brucellosis is acquired by direct contact with secretions and excretions from infected animals or by ingestion of contaminated food or dairy products like raw milk or undercooked meat. Pasteurization of milk helps to prevent brucellosis. Cheese made from unpasteurized milk may be contaminated if less than three months old. The World Health Organization recommends storing soft cheeses more than six months if they were made from unpasteurized milk. Meat, blood, and internal organs from animals should be handled carefully and cooked thoroughly. People who handle potentially infected animals or carcasses should wear adequate protective clothing including eyewear and rubber gloves and protect skin lesions from exposure. Wounds should be covered. The risk of infection is greatest when dealing with aborting animals. In several countries, control programs to detect infection in animals, eliminate infected animals, and vaccinate seronegative young cattle and swine are mandatory. There is no human vaccine and the use of the animal vaccine (a live vaccine) in humans is not recommended as it can cause infection. Immunity after infection in humans is short-lived, lasting approximately two years. Antibiotic prophylaxis postexposure is recommended for high-risk exposure (e.g., after unprotected contact with infected animals or *Brucella* cultures in the laboratory or who were exposed to the animal vaccine) and/or people with underlying diseases like *Diabetes mellitus*. Recommended for postexposure prophylaxis are doxycycline plus rifampin for three weeks; rifampin is not used for exposure to inoculation with *B. abortus* (strain RB51) that is resistant to rifampicin.

13.8 Discussion

The overall incidence of human brucellosis in Europe has been dramatically decreased during the previous 40 years. This success can be attributed to several combined public health measures including testing and slaughtering of cattle, livestock vaccination, border control, and surveillance programs that largely eradicated the causative agent from the animal reservoir and minimized the risk of transmission to humans. These efforts resulted in a significant decline of the disease. Consequently, a brucellosis-free status (brucellosis of cattle, sheep, sheep, and goat) was granted by the European Union to most countries of Northern and Western Europe. Eradication of brucellosis in both cattle and sheep and goats is achievable, as it has been demonstrated in most countries and regions within the European Union. However, sporadic introduction into brucellosis free countries cannot be fully avoided, as demonstrated by the recent local outbreak of bovine brucellosis caused by *B. melitensis* bv 3 that occurred in Austria in 2018 and in France 2012 (Mailles et al. 2012; Schaeffer et al. 2021). In some countries where national control strategies

are weakening, the number of cases in animals is moreover rising again like in Eastern Europe, Central Asia, and Eurasia (Beauvais et al. 2017; Kracalik et al. 2016) and the disease is re-emerging.

The *Brucella* ecology, or what we know of it, has evolved rapidly in recent years. WGS has contributed to identify new species and to identify misidentifications by conventional methods and to a better differentiation of atypical *Brucella* spp. from *Ochrobactrum* spp. or *Brucella*-like organisms. Several further atypical species have been isolated from different animal hosts with unknown zoonotic potential. Two novel species, *B. ceti* and *B. pinnipedialis*, with the potential for causing human disease have been isolated from marine mammals. Another novel species, *B. microti*, has been isolated from wildlife animals, while single *B. inopinata*-like species have been detected in human cases. The detection of *Brucella* spp. in a chameleon 2020 confirmed that all three cold-blooded vertebrate classes (fish, amphibians, and reptiles) are susceptible to *Brucella* infection. A first human case caused by an amphibian-type *Brucella* has also been described. An active spillover of *Brucella* between domestic animals and wildlife has been recognized, with elk transmitting *B. abortus* to cattle, wild boars transmitting *B. suis* bv 2 to domestic pigs, alpine ibex transmitting *B. melitensis* to cattle and vice versa freshwater fish becoming infected with *B. melitensis* from waste meat. Some species might be underdiagnosed like *B. canis* and atypical *Brucella* species with rough LPS type that can easily be overlooked by conventional tests.

Therefore, the correct identification in particular of atypical *Brucella* spp., the development of biochemical and serological assays for the detection of all rough LPS-type *Brucella* species, and the estimation of their zoonotic potential remain challenging.

References

Aftab H, Dargis R, Christensen JJ, Le Flèche P, Kemp M (2011) Imported brucellosis in Denmark: molecular identification and multiple-locus variable number tandem repeat analysis (MLVA) genotyping of the bacteria. Scand J Infect Dis 43:536–538. https://doi.org/10.3109/00365548.2011.562531

Al Dahouk S, Nöckler K, Scholz HC, Pfeffer M, Neubauer H, Tomaso H (2007a) Evaluation of genus-specific and species-specific real-time PCR assays for the identification of *Brucella* spp. Clin Chem Lab Med 45(11):1464–1470. https://doi.org/10.1515/CCLM.2007.305

Al Dahouk S, Flèche PL, Nöckler K, Jacques I, Grayon M, Scholz HC, Tomaso H, Vergnaud G, Neubauer H (2007b) Evaluation of Brucella MLVA typing for human brucellosis. J Microbiol Methods 69:137–145. https://doi.org/10.1016/j.mimet.2006.12.015

Al Dahouk S, Köhler S, Occhialini A, Jimenez de Bagues MP, Hammerl JA, Eisenberg T, Vergnaud G, Cloeckaert A, Zygmunt MS, Whatmore AM, Melzer F, Drees KP, Foster JT, Wattam AR, Scholz HC (2017) *Brucella* spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts. Sci Rep 7(44420). https://doi.org/10.1038/srep44420

Alexander B, Schnurrenberger PR, Brown RR (1981) Numbers of *Brucella abortus* in the placenta, umbilicus and fetal fluid of two naturally infected cows. Vet Rec 108:500. https://doi.org/10.1136/vr.108.23.500

Alexander KA, Blackburn JK, Vandewalle ME, Pesapane R, Baipoledi EK, Elzer PH (2012) Buffalo, bush meat, and the zoonotic threat of brucellosis in Botswana. PLoS One 7(3): e32842. https://doi.org/10.1371/journal.pone.0032842

Al-Nakkas AF, Wright SG, Mustafa AS, Wilson S (2002) Single-tube, nested PCR for the diagnosis of human brucellosis in Kuwait. Ann Trop Med Parasitol 96:397–403. https://doi.org/10.1179/000349802125001203

Alonso-Urmeneta B, Marín C, Aragón V, Blasco JM, Díaz R, Moriyón I (1998) Evaluation of lipopolysaccharides and polysaccharides of different epitopic structures in the indirect enzyme-linked immunosorbent assay for diagnosis of brucellosis in small ruminants and cattle. Clin Diagn Lab Immunol 5(6):749–754. https://doi.org/10.1128/CDLI.5.6.749-754.1998

Ariza J, Pellicer T, Pallarés R, Foz A, Gudiol F (1992) Specific antibody profile in human brucellosis. Clin Infect Dis 14(1):131–140. https://doi.org/10.1093/clinids/14.1.131

Ariza J, Bosilkovski M, Colmenero JD CA, Corbel MJ, Falagas ME, Memish ZA, Roushan MR, Rubinstein E, Sipsas NV, Solera J, Young EJ, Pappas G, International Society of Chemotherapy, Institute of Continuing Medical Education of Ioannina (2007) Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS Med 4(12):e317. https://doi.org/10.1371/journal.pmed.0040317

Batchelor BI, Brindle RJ, Gilks GF, Selkon JB (1992) Biochemical mis-identification of *Brucella mellitensis* and subsequent laboratory-acquired infections. J Hosp Infect 22:159–162. https://doi.org/10.1016/0195-6701(92)90100-z

Beauvais W, Coker R, Nurtazina G, Guitian J (2017 Jun) Policies and livestock systems driving brucellosis re-emergence in Kazakhstan. EcoHealth 14(2):399–407. https://doi.org/10.1007/s10393-015-1030-7

Becker SL, Zange S, Brockmeyer M, Grün U, Halfmann A (2018) Rapid MALDI-TOF-based identification of *Brucella melitensis* from positive blood culture vials may prevent laboratory-acquired infections. J Hosp Infect 100(1):117–119. https://doi.org/10.1016/j.jhin.2018.04.008

Boeri E, Escobar GI, Ayala SM, Sosa-Estani S, Lucero NE (2008) Canine brucellosis in dogs in the city of Buenos Aires. Medicina (B Aires) 68(4):291–297

Boschiroli ML, Ouahrani-Bettache S, Foulongne V, Michaux-Charachon S, Bourg G, Allardet-Servent A, Cazevieille C, Lavigne JP, Liautard JP, Ramuz M, O'Callaghan D (2002) Type IV secretion and *Brucella* virulence. Vet Microbiol 90:341–348

Bosilkovski M, Katerina S, Zaklina S, Ivan V (2010) The role of Brucellacapt test for follow-up patients with brucellosis. Comp Immunol Microbiol Infect Dis 33(5):435–442. https://doi.org/10.1016/j.cimid.2009.06.001

Bossi P, Tegnell A, Baka A Van Loock F, Hendriks J, Werner A, Maidhof H, Gouvras G, Task Force on Biological and Chemical Agent Threats, Public Health Directorate, European Commission, Luxembourg (2004) Bichat guidelines for the clinical management of brucellosis and bioterrorism-related brucellosis. Euro Surveill 9(12):E15–E16

Bosu WT, Prescott JF (1980) A serological survey of dogs for *Brucella canis* in southwestern Ontario. Can Vet J 21(7):198–200

Braude AI (1951) Studies in the pathology and pathogenesis of experimental brucellosis. I. A comparison of the pathogenicity of *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* for Guinea pigs. J Infect Dis 89:76–86

Brew SD, Perrett LL, Stack JA, MacMillan AP, Staunton NJ (1999) Human exposure to *Brucella* recovered from a sea mammal. Vet Rec 144(17):483

Bricker BJ, Halling SM (1994) Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv. 1 by PCR. J Clin Microbiol 32(11):2660–2666. https://doi.org/10.1128/jcm.32.11.2660-2666.1994

Buddle MB (1956) Studies on *Brucella ovis* (n.sp.), a cause of genital disease of sheep in New Zealand and Australia. J Hyg (Lond) 54(3):351–364.2. https://doi.org/10.1017/s0022172400044612

Cardoso PG, Macedo GC, Azevedo V, Oliveira SC (2006) *Brucella* spp noncanonical LPS: structure, biosynthesis, and interaction with host immune system. Microb Cell Factories 5:13. https://doi.org/10.1186/1475-2859-5-13

Carmichael LE, Kenney RM (1968) Canine abortion caused by Brucella canis J Am Vet Med Assoc 152(6):605–16

Carmichael LE, Zoha SJ, Flores-Castro R (1984) Biological properties and dog response to a variant (M-) strain of *Brucella canis*. Dev Biol Stand 56:649–656

Carrington M, Choe U, Ubillos S, Stanek D, Campbell M, Wansbrough L, Lee P, Churchwell G, Rosas K, Zaki SR, Drew C, Paddock CD, Deleon-Carnes M, Guerra M, Hoffmaster AR, Tiller RV, De BK (2012) Fatal case of brucellosis misdiagnosed in early stages of *Brucella suis* infection in a 46-year-old patient with Marfan syndrome. J Clin Microbiol 50:2173–2175. https://doi.org/10.1128/JCM.00573-12

Casanova A, Ariza J, Rubio M, Masuet C, Díaz R (2009) BrucellaCapt versus classical tests in the serological diagnosis and management of human brucellosis. Clin Vaccine Immunol 16(6):844–851. https://doi.org/10.1128/CVI.00348-08

Çelik AD, Yulugkural Z, Kilincer C, Hamamcioglu MK, Kuloglu F, Akata F (2012) Negative serology: could exclude the diagnosis of brucellosis? Rheumatol Int 32(8):2547–2549. https://doi.org/10.1007/s00296-010-1453-y

Chan J, Baxter C, Wenman WM (1989) Brucellosis in an Inuit child, probably related to caribou meat consumption. Scand J Infect Dis 21:337–338. https://doi.org/10.3109/00365548909035706

Corbel MJ (2006) Food and Agriculture Organization of the United Nations, World Health Organization & World Organisation for Animal Health. (2006). Brucellosis in humans and animals. World Health Organization. https://apps.who.int/iris/handle/10665/43597

Cornell WD, Chengappa MM, Stuart LA, Maddux RL, Hail RI (1989) *Brucella suis* biovar 3 infection in a Kentucky swine herd. J Vet Diagn Investig 1(1):20–21. https://doi.org/10.1177/104063878900100107

Cosford KL (2018) *Brucella canis*: an update on research and clinical management. Can Vet J 59 (1):74–81

Costa LF, Pessoa MS, Guimarães LB, Faria AK, Morão RP, Mol JP, Garcia LN, Almeida AC, Gouveia AM, Silva MX et al (2016) Serologic and molecular evidence of *Brucella ovis* infection in ovine and caprine flocks in the State of Minas Gerais, Brazil. BMC Res Notes 9: 190. https://doi.org/10.1186/s13104-016-1998-2

Cross PC, Heisey DM, Scurlock BM, Edwards WH, Ebinger MR, Brennan A (2010) Mapping brucellosis increases relative to elk density using hierarchical Bayesian models. PLoS One 5(4): e10322. https://doi.org/10.1371/journal.pone.0010322

Cvetnić Z, Spicić S, Curic S, Jukic B, Lojkic M, Albert D, Thiébaud M, Garin-Bastuji B (2005) Isolation of *Brucella suis* biovar 3 from horses in Croatia. Vet Rec 156(18):584–585. https://doi.org/10.1136/vr.156.18.584

Cvetnić Z, Spicić S, Toncić J, Majnarić D, Benić M, Albert D, Thiébaud M, Garin-Bastuji B (2009) *Brucella suis* infection in domestic pigs and wild boar in Croatia. Rev Sci Tech 28(3):1057–1067. https://doi.org/10.20506/rst.28.3.1947

De BK, Stauffer L, Koylass MS, Sharp SE, Gee JE, Helsel LO, Steigerwalt AG, Vega R, Clark TA, Daneshvar MI, Wilkins PP, Whatmore AM (2008) Novel *Brucella* strain (BO1) associated with a prosthetic breast implant infection. J Clin Microbiol 46(1):43–49. https://doi.org/10.1128/JCM.01494-07

De Massis F, Ancora M, Atzeni M, Rolesu S, Bandino E, Danzetta ML, Zilli K, Di Giannatale E, Scacchia M (2015) MLVA as an epidemiological tool to trace back *Brucella melitensis* Biovar 1 re-emergence in Italy. Transbound Emerg Dis 62(5):463–469. https://doi.org/10.1111/tbed.12397

De Rautlin de la Roy YM, Grignon B, Grollier G, Coindreau MF, Becq-Giraudon B (1986) Rifampicin resistance in a strain of Brucella melitensis after treatment with doxycycline and rifampicin. J Antimicrob Chemother 18(5):648–9. https://doi.org/10.1093/jac/18.5.648

Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, Zinsstag J (2012) Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl Trop Dis 6, e1929. https://doi.org/10.1371/journal.pntd.0001929

Dentinger CM, Jacob K, Lee LV, Mendez HA, Chotikanatis K, McDonough PL, Chico DM, De BK, Tiller RV, Traxler RM, Campagnolo ER, Schmitt D, Guerra MA, Slavinski SA (2015) Human *Brucella canis* infection and subsequent laboratory exposures associated with a puppy, New York City, 2012. Zoonoses Public Health 62(5):407–414. https://doi.org/10.1111/zph.12163

Deqiu S, Donglou X, Jiming Y (2002) Epidemiology and control of brucellosis in China. Vet Microbiol 90(1–4):165–182. https://doi.org/10.1016/s0378-1135(02)00252-3

Díaz R, Casanova A, Ariza J, Moriyón I (2011) The Rose Bengal test in human brucellosis: a neglected test for the diagnosis of a neglected disease. PLoS Negl Trop Dis 5(4):e950. https://doi.org/10.1371/journal.pntd.0000950

Díaz-Aparicio E, Aragón V, Marín C, Alonso B, Font M, Moreno E, Pérez-Ortiz S, Blasco JM, Díaz R, Moriyón I (1993) Comparative analysis of *Brucella* serotype A and M and *Yersinia enterocolitica* O:9 polysaccharides for serological diagnosis of brucellosis in cattle, sheep, and goats. J Clin Microbiol 31(12):3136–3141. https://doi.org/10.1128/jcm.31.12.3136-3141.1993

Dong SB, Wang LP, Wu CX, Li F, Yue Y, Piao DR, Zhao HY, Jiang H (2020) A case of brucellosis concomitant with HIV infection in China. Infect Dis Pov 9:6. https://doi.org/10.1186/s40249-020-0624-7

Dorneles EMS, Santana JA, Alves TM, Pauletti RB, Mol JP da S, Heinemann MB, Lage AP (2014) Genetic stability of *Brucella abortus* isolates from an outbreak by multiple-locus variable-number tandem repeat analysis (MLVA16). BMC Microbiol 14:186. http://www.biomedcentral.com/1471-2180/14/186

Eckstein C, Mol JP, Costa FB, Nunes PP, Lima PA, Melo MM, Carvalho TP, Santos DO, Silva MF, Carvalho TF, Costa LF, Melo Júnior OAO, Giunchette RC, Paixão TA, Santos RL (2020) *Brucella ovis* mutant in ABC transporter protects against *Brucella canis* infection in mice and it is safe for dogs. PLoS One 15(4):e0231893. https://doi.org/10.1371/journal.pone.0231893

Eisenberg T, Hamann HP, Kaim U, Schlez K, Seeger H, Schauerte N, Melzer F, Tomaso H, Scholz HC, Koylass MS, Whatmore AM, Zschöck M (2012) Isolation of potentially novel *Brucella* spp. from frogs. Appl Environ Microbiol 78(10):3753–3755

Eisenberg T, Riße K, Schauerte N, Geiger C, Blom J, Scholz HC (2017) Isolation of a novel 'atypical' *Brucella* strain from a bluespotted ribbontail ray (*Taeniura lymma*). Antonie Van Leeuwenhoek 110(2):221–234. https://doi.org/10.1007/s10482-016-0792-4

Eisenberg T, Schlez K, Fawzy A, Völker I, Hechinger S, Curić M, Schauerte N, Geiger C, Blom J, Scholz HC (2020) Expanding the host range: infection of a reptilian host (*Furcifer pardalis*) by an atypical *Brucella* strain. Antonie Van Leeuwenhoek 113(10):1531–1537. https://doi.org/10.1007/s10482-020-01448-9

El-Tras WF, Tayel AA, Eltholth MM, Guitian J (2010) *Brucella* infection in fresh water fish: evidence for natural infection of Nile catfish, *Clarias gariepinus*, with *Brucella melitensis*. Vet Microbiol 141(3–4):321–325. https://doi.org/10.1016/j.vetmic.2009.09.017

Erdem H, Ulu-Kilic A, Kilic S, Karahocagil M, Shehata G, Eren-Tulek N, Yetkin F, Celen MK, Ceran N, Gul HC, Mert G, Tekin-Koruk S, Dizbay M, Inal AS, Nayman-Alpat S, Bosilkovski M, Inan D, Saltoglu N, Abdel-Baky L, Adeva-Bartolome MT, Ceylan B, Sacar S, Turhan V, Yilmaz E, Elaldi N, Kocak-Tufan Z, Ugurlu K, Dokuzoguz B, Yilmaz H, Gundes S, Guner R, Ozgunes N, Ulcay A, Unal S, Dayan S, Gorenek L, Karakas A, Tasova Y, Usluer G, Bayindir Y, Kurtaran B, Sipahi OR, Leblebicioglu H (2012) Efficacy and tolerability of antibiotic combinations in neurobrucellosis: results of the Istanbul study. Antimicrob Agents Chemother 56(3):1523–1528. https://doi.org/10.1128/AAC.05974-11

Escobar GI, Jacob NR, López G, Ayala SM, Whatmore AM, Lucero NE (2013) Human brucellosis at a pig slaughterhouse. Comp Immunol Microbiol Infect Dis 36:575–580. https://doi.org/10.1016/j.cimid.2013.06.001

EUROPEAN COMMISSION HEALTH AND CONSUMERS DIRECTORATE-GENERAL, Unit 04 – Veterinary control programmes. SANCO/6095/2009, Working Document, on Eradication of Bovine, Sheep and Goats Brucellosis in the EU accepted by the "Bovine" and "Sheep and Goats" Brucellosis subgroups of the Task Force on monitoring animal disease eradication

Fernández-Lago L, Díaz R (1986) Demonstration of antibodies against *Brucella melitensis* 16M lipopolysaccharide and native hapten in human sera by enzyme-linked immunosorbent assay. J Clin Microbiol 24:76–80. https://doi.org/10.1128/jcm.24.1.76-80.1986

Ferroglio E, Rossi L, Gennero S (2000) Lung-tissue extract as an alternative to serum for surveillance for brucellosis in chamois. Prev Vet Med 43(2):117–122. https://doi.org/10.1016/s0167-5877(99)00090-2

Fischer D, Lorenz N, Heuser W, Kämpfer P, Scholz HC, Lierz M (2012) Abscesses associated with a *Brucella inopinata*-like bacterium in a big-eyed tree frog (*Leptopelis vermiculatus*). J Zoo Wildl Med 43(3):625–628. https://doi.org/10.1638/2011-0005R2.1

Forbes LB (1991) Isolates of *Brucella suis* biovar 4 from animals and humans in Canada, 1982–1990. Canadian Vet J 32(11):686–688

Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A (2007) *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. Int J Syst Evol Microbiol 57(11):2688–2693. https://doi.org/10.1099/ijs.0.65269-0

Foster JT, Beckstrom-Sternberg SM, Pearson T, Beckstrom-Sternberg JS, Chain PS, Roberto FF, Hnath J, Brettin T, Keim P (2009) Whole-genome-based phylogeny and divergence of the genus *Brucella*. J Bacteriol 191(8):2864–2870. https://doi.org/10.1128/JB.01581-08

Franco MP, Mulder M, Gilman RH, Smits HL (2007) Human brucellosis. Lancet Infect Dis 7:775–786. https://doi.org/10.1016/S1473-3099(07)

Fretin D, Mori M, Czaplicki G, Quinet C, Maquet B, Godfroid J, Saegerman C (2013) Unexpected *Brucella suis* biovar 2 infection in a dairy cow, Belgium. Emerg Infect Dis 19(12):2053–2054. https://doi.org/10.3201/eid1912.130506

Galuzzo P, Migliore S, Cascio S, Barreca S, Alfano M, Tagliarini A, Candela A, Piraino C, Galuppo L, Condorelli L, Hussein HA, Tittarelli M, Chiarenza G (2021) Diagnostic Findings in a Confirmed Outbreak of Brucella ovis Infection in a Traditional Sheep Farm in Sicily (South-Italy). Pathogens 10(11):1472. https://doi.org/10.3390/pathogens10111472

Garcell HG, Garcia EG, Pueyo PV, Martín IR, Arias AV, Alfonso Serrano RN (2016) Outbreaks of brucellosis related to the consumption of unpasteurized camel milk. J Infect Public Health 9:523–527. https://doi.org/10.1016/j.jiph.2015.12.006

García-Yoldi D, Marín CM, de Miguel MJ, Muñoz PM, Vizmanos JL, López-Goñi I (2006) Multiplex PCR assay for the identification and differentiation of all *Brucella* species and the vaccine strains *Brucella abortus* S19 and RB51 and *Brucella melitensis* Rev1. Clin Chem 52(4):779–781. https://doi.org/10.1373/clinchem.2005.062596

Garofolo G, Fasanella A, Di Giannatale E, Platone I, Sacchini L, Persiani T et al (2016) Cases of human brucellosis in Sweden linked to Middle East and Africa. BMC Res Notes 9:277. https://doi.org/10.1186/s13104-016-2074-7

Gennero MS, Grattarola C, Zoppi S, Di Giannatale E, Dondo A (2004) Brucellosis in wild boars in Piedmont region. Epidémiol et santé anim 45:77–79

Georgi E, Walter MC, Pfalzgraf MT, Northoff BH, Holdt LM, Scholz HC, Zoeller L, Zange S, Antwerpen MH (2017) Whole genome sequencing of *Brucella melitensis* isolated from 57 patients in Germany reveals high diversity in strains from Middle East. PLoS One 12(4):e0175425. https://doi.org/10.1371/journal.pone.0175425

Godfroid F, Cloeckaert A, Taminiau B, Danese I, Tibor A, de Bolle X et al (2000) Genetic organisation of the lipopolysaccharide O-antigen biosynthesis region of *Brucella melitensis* 16M (*wbk*). Res Microbiol 151(8):655–668. https://doi.org/10.1016/s0923-2508(00)90130-x

Gwida M, Neubauer H, Ilhan Z, Schmoock G, Melzer F, Nöckler K, Janczyk P, Tomaso H, Rösler U, Al Dahouk S (2012a) Cross-border molecular tracing of brucellosis in Europe. Comp Immunol Microbiol Infect Dis 35(2):181–185. https://doi.org/10.1016/j.cimid.2011.12.012

Gwida M, El-Gohary A, Melzer F, Khan I, Rosler U, Neubauer H (2012b) Brucellosis in camels. Res Vet Sci 92:351–355. https://doi.org/10.1016/j.rvsc.2011.05.002

Gybby IW, Gibby AM (1965) Host-parasite relationships with *Brucella neotomae*. J Bacteriol 89(1):9–16. https://doi.org/10.1128/jb.89.1.9-16.1965

Gyuranecz M, Wernery U, Kreizinger Z, Juhász J, Felde O, Nagy P (2016) Genotyping of *Brucella melitensis* strains from dromedary camels (*Camelus dromedarius*) from the United Arab

Emirates with multiple-locus variable-number tandem repeat analysis. Vet Microbiol 186:8–12. https://doi.org/10.1016/j.vetmic.2016.02.009
Hollett RB (2006) Canine brucellosis: outbreaks and compliance. Theriogenology 66(3):575–587. https://doi.org/10.1016/j.theriogenology.2006.04.011
Holst BC, Löfqvist K, Ernholm L, Eld K, Cedersmyg M, Hallgren G (2012) The first case of *Brucella canis* in Sweden: background, case report and recommendations from a northern European perspective. Acta Vet Scand 54(1):18. https://doi.org/10.1186/1751-0147-54-18
Holzer K, El-Diasty M, Wareth G, Abdel-Hamid NH, Hamdy MER, Moustafa SA, Linde J, Bartusch F, Sayour AE, Elbauomy EM, Elhadidy M, Melzer F, Beyer W (2021) Tracking the distribution of *Brucella abortus* in Egypt based on Core genome SNP analysis and In Silico MLVA-16. Microorganisms 9(9):1942. https://doi.org/10.3390/microorganisms9091942
Hördt A, López MG, Meier-Kolthoff JP, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, Göker M (2020) Analysis of 1,000+ type-strain genomes substantially improves taxonomic classification of alphaproteobacteria. Front Microbiol 11:468. https://doi.org/10.3389/fmicb.2020.00468
Hubalek Z, Scholz HC, Sedlacek I, Melzer F, Sanogo YO, Nesvadbova J (2007) Brucellosis of the common vole (*Microtus arvalis*). Vector Borne Zoonotic Dis (Larchmont, NY) 7:679–687. https://doi.org/10.1089/vbz.2007.0143
Huddleson IF (1924) Tech Bull Mich Agric Exp Sta No. 65
Ica T, Aydin F, Erdenlig S, Guler L, Büyükcangaz E (2008) Characterisation of *Brucella abortus* biovar 3 isolates from Turkey as biovar 3b. Vet Rec 163(22):659–661. https://doi.org/10.1136/vr.163.22.659
Isayama Y, Azuma R, Tanaka S, Suto T (1977) The pathogenicity and antigenicity of *Brucella canis* QE13 for experimental animals. Ann Sclavo 19:89–98
Jahans KL, Foster G, Broughton ES (1997) The characterisation of *Brucella* strains isolated from marine mammals. Vet Microbiol 57(4):373–382. https://doi.org/10.1016/s0378-1135(97)00118-1
Janmohammadi N, Roushan MR (2009) False negative serological tests may lead to misdiagnosis and mismanagement in osteoarticular brucellosis. Trop Dr 39(2):88–90. https://doi.org/10.1258/td.2008.080042
Janowicz A, De Massis F, Ancora M, Cammà C, Patavino C, Battisti A, Prior K, Harmsen D, Scholz H, Zilli K, Sacchini L, Di Giannatale E, Garofolo G (2018) Core genome multilocus sequence typing and single nucleotide polymorphism analysis in the epidemiology of *Brucella melitensis* infections. J Clin Microbiol 56(9):e00517–e00518. https://doi.org/10.1128/JCM.00517-18
Javeri H, Jamieson S, Sehgal R, Cadena J (2014) *Brucella canis* peritonitis. Infection 42:195–197. https://doi.org/10.1007/s15010-013-0505-0
Jaý M, Girault G, Perrot L, Taunay B, Vuilmet T, Rossignol F, Pitel PH, Picard E, Ponsart C, Mick V (2018) Phenotypic and molecular characterization of *Brucella microti*-like bacteria from a domestic marsh frog (*Pelophylax ridibundus*). Front Vet Sci 5:283. https://doi.org/10.3389/fvets.2018.00283
Jaÿ M, Freddi L, Mick V, Durand B, Girault G, Perrot L, Taunay B, Vuilmet T, Azam D, Ponsart C, Zanella G (2020) *Brucella microti*-like prevalence in French farms producing frogs. Transbound Emerg Dis 67(2):617–625. https://doi.org/10.1111/tbed.13377
Jiang H, Fan M, Chen J, Mi J, Yu R, Zhao H et al (2011) MLVA genotyping of Chinese human *Brucella melitensis* biovar 1, 2 and 3 isolates. BMC Microbiol 11:256. https://doi.org/10.1186/1471-2180-11-256
Jiang H, Chen H, Tian G-Z et al (2012) Genetic comparison of *Brucella suis* biovar 3 in clinical cases in China. Vet Microbiol 160(3–4):546–548. https://doi.org/10.1016/j.vetmic.2012.06.030
Jiménez de Bagüés MP, Marín C, Blasco JM (1991) Effect of antibiotic therapy and strain 19 vaccination on the spread of *Brucella melitensis* within an infected dairy herd. Prev Vet Med 11:17–24
Jiménez de Bagüés MP, Ouahrani-Bettache S, Quintana JF, Mitjana O, Hanna N, Bessoles S, Sanchez F, Scholz HC, Lafont V, Köhler S, Occhialini A (2010) The new species *Brucella microti* replicates in macrophages and causes death in murine models of infection. J Infect Dis 202(1):3–10. https://doi.org/10.1086/653084

Jiménez de Bagüés MP, de Martino A, Quintana JF, Alcaraz A, Pardo J (2011) Course of infection with the emergent pathogen *Brucella microti* in immunocompromised mice. Infect Immun 79 (10):3934–3939. https://doi.org/10.1128/IAI.05542-11

Jiménez de Bagüés MP, Iturralde M, Arias MA, Pardo J, Cloeckaert A, Zygmunt MS (2014) The new strains *Brucella inopinata* BO1 and *Brucella* species 83–210 behave biologically like classic infectious *Brucella* species and cause death in murine models of infection. J Infect Dis 210:467–472. https://doi.org/10.1093/infdis/jiu102

Karger A, Melzer F, Timke M, Bettin B, Kostrzewa M, Nöckler K, Hohmann A, Tomaso H, Neubauer H, Al Dahouk S (2013) Interlaboratory comparison of intact-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry results for identification and differentiation of Brucella spp. J Clin Microbiol 51(9):3123–6. https://doi.org/10.1128/JCM.01720-13

Ke Y, Wang Y, Li W, Chen Z (2015) Type IV secretion system of *Brucella* spp. and its effectors. Front Cell Infect Microbiol 5:72. https://doi.org/10.3389/fcimb.2015.00072

Kimura M, Une Y, Suzuki M, Park E-S, Imaoka K, Morikawa S (2017) Isolation of *Brucella inopinata* -like bacteria from White's and Denny's tree frogs. Vector Borne Zoonotic Dis 17: 297–302. https://doi.org/10.1089/vbz.2016.2027

Kracalik IT, Abdullayev R, Asadov K, Ismayilova R, Baghirova M, Ustun N, Shikhiyev M, Talibzade A, Blackburn JK (2016) Human brucellosis trends: re-emergence and prospects for control using a one health approach in Azerbaijan (1983–2009). Zoonoses Public Health 63(4): 294–302. https://doi.org/10.1111/zph.12229

Kutlu M, Cevahir N, Erdenliğ-Gürbilek S, Akalın Ş, Uçar M, Sayın-Kutlu S (2016) The first report of *Brucella suis* biovar 1 isolation in human in Turkey. J Infect Public Health 9:675–678. https://doi.org/10.1016/j.jiph.2016.01.011

Lacerda TL, Salcedo SP, Gorvel JP (2013) *Brucella* T4SS: the VIP pass inside host cells. Curr Opin Microbiol 16(1):45–51. https://doi.org/10.1016/j.mib.2012.11.005

Lapaque N, Moriyón I, Moreno E, Gorvel JP (2005) *Brucella* lipopolysaccharide acts as a virulence factor. Curr Opin Microbiol 8(1):60–66. https://doi.org/10.1016/j.mib.2004.12.003

Lawaczeck E, Toporek J, Cwikla J, Mathison BA (2011) *Brucella canis* in a HIV-infected patient. Zoonoses Public Health 58(2):150–152. https://doi.org/10.1111/j.1863-2378.2010.01334.x

Le Flèche P, Jacques I, Grayon M, Al Dahouk S, Bouchon P, Denoeud F, Nöckler K, Neubauer H, Guilloteau LA, Vergnaud G (2006) Evaluation and selection of tandem repeat loci for a *Brucella* MLVA typing assay. BMC Microbiol 6:9. https://doi.org/10.1186/1471-2180-6-9

Lista et al (2011) Reliable identification at the species level of Brucella isolates with MALDI-TOF-MS. BMC Microbiol 11:267. https://doi.org/10.1186/1471-2180-11-267

López-Goñi I, García-Yoldi D, Marín CM, de Miguel MJ, Barquero-Calvo E, Guzmán-Verri C, Albert D, Garin-Bastuji B (2011) New Bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*. Vet Microbiol 154(1–2, 152):–155. https://doi.org/10.1016/j.vetmic.2011.06.035

Loutsevitch NE (2014) *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). Int J Syst Evol Microbiol 64:4120–4128. https://doi.org/10.1099/ijs.0.065482-0

Lovejoy GS, Carver HD, Moseley IK, Hicks M (1976) Serosurvey of dogs for *Brucella canis* infection in Memphis, Tennessee. Am J Public Health 66(2):175–176. https://doi.org/10.2105/ajph.66.2.175

Lucero NE, Escobar GI, Ayala SM, Jacob N (2005) Diagnosis of human brucellosis caused by *Brucella canis*. J Med Microbiol 54(5):457–461. https://doi.org/10.1099/jmm.0.45927-0

Lucero NE, Ayala SM, Escobar GI, Jacob NR (2008) *Brucella* isolated in humans and animals in Latin America from 1968 to 2006. Epidemiol Infect 136(4):496–503. https://doi.org/10.1017/S0950268807008795

Mailles A, Rautureau S, Le Horgne JM, Poignet-Leroux B, d'Arnoux C, Dennetière G, Faure M, Lavigne JP, Bru JP, Garin-Bastuji B (2012) Re-emergence of brucellosis in cattle in France and risk for human health. Euro Surveill 17(30):20227

Mailles A, Ogielska M, Kemiche F, Garin-Bastuji B, Brieu N, Burnusus Z, Creuwels A, Danjean MP, Guiet P, Nasser V, Tourrand B, Valour F, Maurin M, O'Callaghan D, Mick V, Vaillant V,

Jay M, Lavigne JP, DE Valk H (2017) *Brucella suis* biovar 2 infection in humans in France: emerging infection or better recognition? Epidemiol Infect 145:2711–2716. https://doi.org/10.1017/S0950268817001704

Mancilla M (2016) Smooth to rough dissociation in brucella: the missing link to virulence. Front Cell Infect Microbiol 5:98. https://doi.org/10.3389/fcimb.2015.00098

Mancilla M, Marin CM, Blasco JM, Zarraga AM, Lopez-Goni I, Moriyon I (2012) Spontaneous excision of the O-polysaccharide *wbkA* glycosyltransferase gene is a cause of dissociation of smooth to rough *Brucella* colonies. J Bacteriol 194(8):1860–1867. https://doi.org/10.1128/JB.06561-11

Maquart M, Le Flèche P, Foster G, Tryland M, Ramisse F, Djønne B, Al Dahouk S, Jacques I, Neubauer H, Walravens K, Godfroid J, Cloeckaert A, Vergnaud G (2009) MLVA-16 typing of 295 marine mammal *Brucella* isolates from different animal and geographic origins identifies 7 major groups within *Brucella ceti* and *Brucella pinnipedialis*. BMC Microbiol 9:145. https://doi.org/10.1186/1471-2180-9-145

Marreros N, Hüssy D, Albini S, Frey CF, Abril C, Vogt HR, Holzwarth N, Wirz-Dittus S, Friess M, Engels M, Borel N, Willisch CS, Signer C, Hoelzle LE, Ryser-Degiorgis MP (2011) Epizootiologic investigations of selected abortive agents in free-ranging Alpine ibex (*Capra ibex ibex*) in Switzerland. J Wildl Dis 47(3):530–543. https://doi.org/10.7589/0090-3558-47.3.530

Matle I, Ledwaba B, Madiba K, Makhado L, Jambwa K, Ntushelo N (2021) Characterisation of *Brucella* species and biovars in South Africa between 2008 and 2018 using laboratory diagnostic data. Vet Med Sci 7:1245–1253. https://doi.org/10.1002/vms3.483

Mayer-Scholl A, Draeger A, Gollner C, Scholz HC, Nockler K (2010) Advancement of a multiplex PCR for the differentiation of all currently described *Brucella* species. J Microbiol Methods 80 (1):112–114. https://doi.org/10.1016/j.mimet.2009.10.015

Mc Donald WL, Jamaludin R, Mackereth G, Hansen M, Humphrey S, Short P, Taylor T, Swingler J, Dawson CE, Whatmore AM et al (2006) Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. J Clin Microbiol 44(12):4363–4370. https://doi.org/10.1128/JCM.00680-06

Melzer F, Lohse R, Nieper H, Liebert M, Sachse K (2007) A serological study on brucellosis in wild boars in Germany. Eur J Wildl Res 53:153–157. https://doi.org/10.1007/s10344-006-0072-0

Mesureur J, Arend S, Cellière B, Courault P, Cotte-Pattat PJ, Totty H, Deol P, Mick V, Girard V, Touchberry J, Burrowes V, Lavigne JP, O'Callaghan D, Monnin V, Keriel A (2018) A MALDI-TOF MS database with broad genus coverage for species-level identification of *Brucella*. PLoS Negl Trop Dis 12(10):e0006874. https://doi.org/10.1371/journal.pntd.0006874

Meyer ME, Cameron HS (1963) Identification of the causative agents and the epidemiology of porcine brucellosis. Bull World Health Organ 28(4):499–503

Meyer KF, Shaw EB (1920) A comparison of the morphologic, cultural and biochemical characteristics of *B. abortus* and *B. melitensis* from cattle. Studies on the genus *Brucella* nov. gen. J Infect Dis 27:173–184. https://doi.org/10.1093/infdis/27.3.173

Mick V, Le Carrou G, Corde Y, Game Y, Jay M, Garin-Bastuji B (2014) *Brucella melitensis* in France: persistence in wildlife and probable spillover from Alpine ibex to domestic animals. PLoS One 9(4):e94168. https://doi.org/10.1371/journal.pone.0094168

Muendo EN, Mbatha PM, Macharia J, Abdoel TH, Janszen PV, Pastoor R, Smits HL (2012) Infection of cattle in Kenya with *Brucella abortus* biovar 3 and *Brucella melitensis* biovar 1 genotypes. Trop Anim Health Prod 44:17–20. https://doi.org/10.1007/s11250-011-9899-9

Mühldorfer K, Wibbelt G, Szentiks CA, Fischer D, Scholz HC, Zschock M, Eisenberg T (2016) The role of 'atypical' *Brucella* in amphibians: are we facing novel emerging pathogens? J Appl Microbiol 122(1):40–53. https://doi.org/10.1111/jam.13326

Muñoz PM, Boadella M, Arnal M, de Miguel M, Revilla M, Martínez D, Vicente J, Acevedo P, Oleaga A, Ruiz-Fons F, Marín C, Prieto J, de la Fuente J, Barral M, Barberán M, de Luco D, Blasco J, Gortazar C (2010) Spatial distribution and risk factors of brucellosis in Iberian wild ungulates. BMC Infect Dis 10(1):46. https://doi.org/10.1186/1471-2334-10-46

Narnaware SD, Dahiya SS, Kumar S, Tuteja FC, Nath K, Patil NV (2017) Pathological and diagnostic investigations of abortions and neonatal mortality associated with natural infection

of *Brucella abortus* in dromedary camels. Comp Clin Pathol 26:79–85. https://doi.org/10.1007/s00580-016-2348-4

Nenova R, Tomova I, Saparevska R, Kantardjiev T (2015) A new outbreak of brucellosis in Bulgaria detected in preliminary report. Euro Surveill 20. https://doi.org/10.2807/1560-7917.ES.2015.20.39.30031

Nicoletti P, Jones LM, Berman DT (1978) Comparison of the subcutaneous and conjunctival route of vaccination with *Brucella abortus* strain 19 vaccine in adult cattle. J Am Vet Med Assoc 173:1450–1456

Nielsen K, Smith P, Widdison J, Gall D, Kelly L, Kelly W, Nicoletti P (2004) Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7. Vet Microbiol 100(1–2):25–30. https://doi.org/10.1016/j.vetmic.2003.12.010

Nielsen K, Smith P, Yu WL, Halbert G (2007) *Salmonella enterica* serotype urbana interference with brucellosis serology. J Immunoass Immunochem 28(3):289–296. https://doi.org/10.1080/15321810701454904

O'Callaghan D, Whatmore AM (2011) *Brucella* genomics as we enter the multi-genome era. Brief Funct Genomic Proteomic 10(6):334–341. https://doi.org/10.1093/bfgp/elr026

OIE Terrestrial Manual Brucellosis 2018: Terrestrial Animal Health Code: (Chapter 3.1.4) Infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Olsen SC, Tatum FM (2017) Swine brucellosis: current perspectives. Vet Med Res Rep 8:1–12. https://doi.org/10.2147/VMRR.S91360

Osterman B (2006) International committee on systematics of prokaryotes; subcommittee on the taxonomy of *Brucella*: minutes of the meeting, 17 September 2003, Pamplona, Spain. Int J Syst Evol Microbiol 56:1173–1175

Ostrowski S, Anajariyya S, Kamp EM, Bedin E (2002) Isolation of *Brucella melitensis* from an Arabian oryx (*Oryx leucoryx*). Vet Rec 150(6):186–188. https://doi.org/10.1136/vr.150.6.186

Pappas G, Akritidis N, Bosilkovski M, Tsianos E (2005) Brucellosis. N Engl J Med 352:2325–2336. https://doi.org/10.1056/NEJMra050570

Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV (2006) The new global map of human brucellosis. Lancet Infect Dis 6(2):91–99. https://doi.org/10.1016/S1473-3099(06)70382-6

Pappas G, Akritidis N, Christou L (2007) Treatment of neurobrucellosis: what is known and what remains to be answered. Expert Rev Anti-Infect Ther 5:983–990. https://doi.org/10.1586/14787210.5.6.983

Pereira CR, Cotrim de Almeida JVF, Cardoso de Oliveira IR, Faria de Oliveira L, Pereira LJ, Zangerônimo MG, Lage AP, Dorneles EMS (2020) Occupational exposure to *Brucella* spp.: a systematic review and meta-analysis. PLoS Negl Trop Dis 14(5):e0008164. https://doi.org/10.1371/journal.pntd.0008164

Pereira CR, de Oliveira IRC, de Oliveira LF, de Oliveira CSF, Lage AP, Dorneles EMS (2021) Accidental exposure to *Brucella abortus* vaccines and occupational brucellosis among veterinarians in Minas Gerais state, Brazil. Transbound Emerg Dis 68(3):1363–1376. https://doi.org/10.1111/tbed.13797

Perrett LL, Brew SD, Stack JA, MacMillan AP, Bashiruddin JB (2004) Experimental assessment of the pathogenicity of *Brucella* strains from marine mammals for pregnant sheep. Small Rumin Res 51(3):221–228. https://doi.org/10.1016/S0921-4488(03)00233-5

Piampiano P, McLeary M, Young LW, Janner D (2000) Brucellosis: unusual presentations in two adolescent boys. Pediatr Radiol 30:355–357. https://doi.org/10.1007/s002470050760

Prenger-Berninghoff E, Siebert U, Stede M, König A, Weiss R, Baljer G (2008) Incidence of *Brucella* species in marine mammals of the German North Sea. Dis Aquat Org 81(1):65–71. https://doi.org/10.3354/dao01920

Purcell BK, Hoover DL, Friedlander (2007) Brucellosis. In: Dembek ZF, ed. Med. Asp. Biol. Warf. Washington, DC; Falls Church, Va.; Fort Sam Houston, Tex.: Borden Institute, Walter Reed Army Medical Center ; Office of the Surgeon General, United States Army ; United States Army Medical Dept. Center and School

Ramamoorthy S, Woldemeskel M, Ligett A, Snider R, Cobb R, Rajeev S (2011) *Brucella suis* infection in dogs, Georgia, USA. Emerg Infect Dis 17(12):2386–2387. https://doi.org/10.3201/eid1712.111127

Raptis L, Pappas G, Akritidis N (2007) A cutaneous cyst caused by brucellosis with a negative serological test. Int J Infect Dis 11(1):82–83. https://doi.org/10.1016/j.ijid.2005.10.005

Rausch RL, Huntley BE (1978) Brucellosis in reindeer, *Rangifer tarandus L.*, inoculated experimentally with *Brucella suis*, type 4. Can J Microbiol 24(2):129–135. https://doi.org/10.1139/m78-024

Rhyan JC, Gidlewski T, Ewalt DR, Hennager SG, Lambourne DM, Olsen SC (2001) Seroconversion and abortion in cattle experimentally infected with *Brucella* sp. isolated from a Pacific harbor seal (*Phoca vitulina richardsi*). J Vet Diagn Investig 13:379–382. https://doi.org/10.1177/104063870101300502

Riezu-Boj JI, Moriyón I, Blasco JM, Marín CM, Díaz R (1986) Comparison of lipopolysaccharide and outer membrane protein-lipopolysaccharide extracts in an enzyme-linked immunosorbent assay for the diagnosis of *Brucella ovis* infection. J Clin Microbiol 23(5):938–942. https://doi.org/10.1128/jcm.23.5.938-942.1986

Rónai Z, Kreizinger Z, Dán Á, Drees K, Foster JT, Bányai K, Marton S, Szeredi L, Jánosi S, Gyuranecz M (2015) First isolation and characterization of *Brucella microti* from wild boar. BMC Vet Res 11:147. https://doi.org/10.1186/s12917-015-0456-z

Rossetti CA, Arenas-Gamboa AM, Maurizio E (2017) Caprine brucellosis: a historically neglected disease with significant impact on public health. PLoS Negl Trop Dis 11(8):e0005692. https://doi.org/10.1371/journal.pntd.0005692

Rouzic N, Desmier L, Cariou ME, Gay E, Foster JT, Williamson CHD, Schmitt F, Le Henaff M, Le Coz A, Lorléac'h A, Lavigne JP, O'Callaghan D, Keriel A (2021) First case of brucellosis caused by an amphibian-type *Brucella*. Clin Infect Dis 72(9):404–407. https://doi.org/10.1093/cid/ciaa1082

Sánchez-Jiménez MM, de la Cuesta Zuluaga JJ, Garcia-Montoya GM, Dabral N, Alzate JF, Vemulapalli R, Olivera-Angel M (2020) Diagnosis of human and canine *Brucella canis* infection: development and evaluation of indirect enzyme-linked immunosorbent assays using recombinant *Brucella* proteins. Heliyon 6(7):e04393. https://doi.org/10.1016/j.heliyon.2020.e04393

Sankarasubramanian J, Vishnu US, Gunasekaran P, Rajendhran J (2019) Development and evaluation of a core genome multilocus sequence typing (cgMLST) scheme for *Brucella* spp. Infect Genet Evol 67:38–43. https://doi.org/10.1016/j.meegid.2018.10.021

Santos RL, Souza TD, Mol JPS, Eckstein C, Paíxão TA (2021) Canine brucellosis: an update. Front Vet Sci 8:1–17. https://doi.org/10.3389/fvets.2021.594291

Sayan M, Yumuk Z, Dündar D, Bilenoglu O, Erdenlig S, Yaşar E, Willke A (2008) Rifampicin resistance phenotyping of *Brucella melitensis* by *rpoB* gene analysis in clinical isolates J Chemother 20(4):431–5. https://doi.org/10.1179/joc.2008.20.4.431

Sayan M, Erdenlig S, Stack J, Kilic S, Guducuoglu H, Aksoy Y, Baklan A, Etiler N (2011) A serological diagnostic survey for *Brucella canis* infection in Turkish patients with brucellosis-like symptoms. Jpn J Infect Dis 64(6):516–519

Schaeffer J, Revilla-Fernández S, Hofer E, Posch R, Stoeger A, Leth C, Schmoll F, Djordjevic V, Lakicevic B, Matovic K, Hufnagl P, Indra A, Allerberger F, Ruppitsch W (2021) Tracking the origin of Austrian human brucellosis cases using whole genome sequencing. Front Med (Lausanne) 24(8):635547. https://doi.org/10.3389/fmed.2021.635547

Schlabritz-Loutsevitch NE, Whatmore AM, Quance CR, Koylass MS, Cummins LB, Dick EJ Jr, Snider CL, Cappelli D, Ebersole JL, Nathanielsz PW, Hubbard GB (2009) A novel *Brucella* isolate in association with two cases of stillbirth in non-human primates – first report. J Med Primatol 38(1):70–73. https://doi.org/10.1111/j.1600-0684.2008.00314.x

Schoerner C, Wartenberg K, Röllinghoff M (1990) Differentiation of serological responses to *Yersinia enterocolitica* serotype O9 and *Brucella* species by immunoblot or enzyme-linked immunosorbent assay using whole bacteria and *Yersinia* outer membrane proteins. J Clin Microbiol 28(7):1570–1574. https://doi.org/10.1128/jcm.28.7.1570-1574.1990

Scholz HC, Vergnaud G (2013) Molecular characterisation of *Brucella* species. Rev Sci Tech Int Off Epizoot 32(1):149–162. https://doi.org/10.20506/rst.32.1.2189

Scholz HC, Al Dahouk S, Tomaso H, Neubauer H, Witte A, Schloter M, Kämpfer P, Falsen E, Pfeffer M, Engel M (2008a) Genetic diversity and phylogenetic relationships of bacteria belonging to the *Ochrobactrum-Brucella* group by *recA* and 16S rRNA gene-based comparative sequence analysis. Syst Appl Microbiol 31(1):1–16. https://doi.org/10.1016/j.syapm.2007.10.004

Scholz HC, Hubalek Z, Sedlácek I, Vergnaud G, Tomaso H, Al Dahouk S, Melzer F, Kämpfer P, Neubauer H, Cloeckaert A, Maquart M, Zygmunt MS, Whatmore AM, Falsen E, Bahn P, Göllner C, Pfeffer M, Huber B, Busse HJ, Nöckler K (2008b) *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. Int J Syst Evol Microbiol 58(2):375–382. https://doi.org/10.1099/ijs.0.65356-0

Scholz HC, Hubalek Z, Nesvadbova J, Tomaso H, Vergnaud G, Le Flèche P, Whatmore AM, Al Dahouk S, Krüger M, Lodri C, Pfeffer M (2008c) Isolation of *Brucella microti* from soil. Emerg Infect Dis 14(8):1316–1317. https://doi.org/10.3201/eid1408.080286

Scholz HC, Pfeffer M, Witte A, Neubauer H, Al Dahouk S, Wernery U, Tomaso H (2008d) Specific detection and differentiation of *Ochrobactrum anthropi*, *Ochrobactrum intermedium* and *Brucella* spp. by a multi-primer PCR that targets the *recA* gene. J Med Microbiol 57(1):64–71. https://doi.org/10.1099/jmm.0.47507-0

Scholz HC, Hofer E, Vergnaud G, Le Fleche P, Whatmore AM, Al Dahouk S, Pfeffer M, Krüger M, Cloeckaert A, Tomaso H (2009) Isolation of *Brucella microti* from mandibular lymph nodes of red foxes, *Vulpes vulpes*, in lower Austria. Vector Borne Zoonotic Dis 9(2):153–156. https://doi.org/10.1089/vbz.2008.0036

Scholz HC, Nöckler K, Göllner C, Bahn P, Vergnaud G, Tomaso H, Al Dahouk S, Kämpfer P, Cloeckaert A, Maquart M, Zygmunt MS, Whatmore AM, Pfeffer M, Huber B, Busse HJ, De BK (2010) *Brucella inopinata* sp. nov., isolated from a breast implant infection. Int J Syst Evol Microbiol 60(4):801–808. https://doi.org/10.1099/ijs.0.011148-0

Scholz HC, Revilla-Fernández S, Dahouk SA, Hammerl JA, Zygmunt MS, Cloeckaert A, Koylass M, Whatmore AM, Blom J, Vergnaud G, Witte A, Aistleitner K, Hofer E (2016a) *Brucella vulpis* sp. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*). Int J Syst Evol Microbiol 66(5):2090–2098. https://doi.org/10.1099/ijsem.0.000998

Scholz HC, Mühldorfer K, Shilton C, Benedict S, Whatmore AM, Blom J, Eisenberg T (2016b) The change of a medically important genus: worldwide occurrence of genetically diverse novel *Brucella* species in exotic frogs. PLoS One 11(12):e0168872. https://doi.org/10.1371/journal.pone.0168872

Scholz HC, Banai M, Cloeckaert A, Kämpfer P, Whatmore AMS (2018) In: Whitman WB, Rainey F, Kämpfer P, Trujillo M, Chun J, DeVos P (eds) *Brucella* in Bergey's manual of systematics of archaea and bacteria. Wiley, Hoboken. https://doi.org/10.1002/9781118960608.gbm00807.pub2

Sergeant ES (1994) Seroprevalence of *Brucella ovis* infection in commercial ram flocks in the Tamworth area. New Zealand Vet J42(3):97–100. https://doi.org/10.1080/00480169.1994.35795

Shilton CM, Brown GP, Benedict S, Shine R (2008) Spinal arthropathy associated with *Ochrobactrum anthropi* in free-ranging cane toads (*Chaunus* [*Bufo*] *marinus*) in Australia. Vet Pathol 45(1):85–94. https://doi.org/10.1354/vp.45-1-85

Shimol SB, Dukhan L, Belmaker I, Bardenstein S, Sibirsky D, Barrett C, Greenberg D (2012) Human brucellosis outbreak acquired through camel milk ingestion in southern Israel. Isr Med Assoc J 14(8):475–478

Sohn AH, Probert WS, Glaser CA, Gupta N, Bollen AW, Wong JD, Grace EM, McDonald WC (2003) Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. Emerg Infect Dis 9(4):485–488. https://doi.org/10.3201/eid0904.020576

Solera J (2010) Update on brucellosis: therapeutic challenges. Int J Antimicrob Agents 36(Suppl 1):S18–S20. https://doi.org/10.1016/j.ijantimicag.2010.06.015

Soler-Lloréns PF, Quance CR, Lawhon SD, Stuber TP, Edwards JF, Ficht TA, Robbe-Austerman S, O'Callaghan D, Keriel A (2016) A *Brucella* spp. isolate from a Pac-Man frog (*Ceratophrys ornata*) reveals characteristics departing from classical brucellae. Front Cell Infect Microbiol 6(116). https://doi.org/10.3389/fcimb.2016.00116

Sprague LD, Al-Dahouk S, Neubauer H (2012) A review on camel brucellosis: a zoonosis sustained by ignorance and indifference. Pathog Glob Health 106:144–149. https://doi.org/10.1179/2047773212Y.0000000020

Starnes CT, Talwani R, Horvath JA, Duffus WA, Bryan CS (2004) Brucellosis in two hunt club members in South Carolina. J S C Med Assoc 100(4):113–115

Stoenner HG (1963) The behavior of *Brucella neotomae* and *Brucella suis* in reciprocal superinfection experiments in mice and Guinea pigs. Am J Vet Re 24:376–380

Stoenner HG, Lackman D (1957) A new species of *Brucella* isolated from the desert wood rat, *Neotoma lepida* Thomas. Am J Vet Res 18:947–951

Suárez-Esquivel M, Ruiz-Villalobos N, Jiménez-Rojas C, Barquero-Calvo E, Chacón-Díaz C, Víquez-Ruiz E, Rojas-Campos N, Baker KS, Oviedo-Sánchez G, Amuy E, Chaves-Olarte E, Thomson NR, Moreno E, Guzmán-Verri C (2017) *Brucella neotomae* infection in humans, Costa Rica. Emerg Infect Dis 23(6):997–1000. https://doi.org/10.3201/eid2306.162018

Szulowski K, Iwaniak W, Weiner M, Złotnicka J (2013) *Brucella suis* biovar 2 isolations from cattle in Poland. Ann Agric Environ Med 20(4):672–675

Tan K-K, Tan Y-C, Chang L-Y, Lee KW, Nore SS, Yee WY, Mat Isa MN, Jafar FL, Hoh CC, AbuBakar S (2015) Full genome SNP-based phylogenetic analysis reveals the origin and global spread of *Brucella melitensis*. BMC Genomics 16:93. https://doi.org/10.1186/s12864-015-1294-x

Tiller RV, Gee JE, Lonsway DR, Gribble S, Bell SC, Jennison AV, Bates J, Coulter C, Hoffmaster AR, De BK (2010) Identification of an unusual *Brucella* strain (BO2) from a lung biopsy in a 52 year-old patient with chronic destructive pneumonia. BMC Microbiol 27(10):23. https://doi.org/10.1186/1471-2180-10-23

Torres Higuera LD, Del C. Jiménez Velásquez S, Rodríguez Bautista JL, Patiño Burbano RE (2019) Identification of *Brucella abortus* biovar 4 of bovine origin in Colombia. Rev Argent Microbiol 51(3):221–228. https://doi.org/10.1016/j.ram.2018.08.002

Traxler RM, Lehman MW, Bosserman EA, Guerra MA, Smith TL (2013) A literature review of laboratory-acquired brucellosis. J Clin Microbiol 51:3055–3062. https://doi.org/10.1128/JCM.00135-13

Tryland M, Derocher AE, Wiig Y, Godfroid J (2001) *Brucella* sp. antibodies in polar bears from Svalbard and the Barents Sea. J Wildl Dis 37:523–531. https://doi.org/10.7589/0090-3558-37.3.523

Tuon F, Gondolfo RB, Cerchiari N (2017) Human-to-human transmission of *Brucella* – a systematic review. Tropical Med Int Health 22(5):539–546. https://doi.org/10.1111/tmi.12856

van Dijk MAM, Engelsma MY, Visser VXN, Spierenburg MAH, Holtslag ME, Willemsen PTJ, Wagenaar JA, Broens EM, Roest HIJ (2018) *Brucella suis* infection in dog fed raw meat, the Netherlands. Emerg Infect Dis 24:1127–1129. https://doi.org/10.3201/eid2406.171887

Vega FL, Castaño S, Sánchez-Juanes F, González-Cabrero S, Menegotto F, Orduña-Domingo A, González-Buitrago JM, Muñoz-Bellido JL (2010) Identification of *Brucella* by MALDI-TOF mass spectrometry. Fast and reliable identification from agar plates and blood cultures. PLoS One 5(12):e14235. https://doi.org/10.1371/journal.pone.0014235

Vershilova PA, Lyamkin GI, Malikov VE, Dranovskaia EA (1983) *Brucella* strains from mouse like rodents isolated in the USRR. Int J Syst Bacteriol 33:399–400. https://doi.org/10.1099/00207713-33-2-399

Vila A, Pagella H, Vera Bello G, Vicente A (2016) *Brucella suis* bacteremia misidentified as *Ochrobactrum anthropi* by the VITEK 2 system. J Infect Dev Ctries 10(4):432–436. https://doi.org/10.3855/jidc.7532

Villalobos-Vindas JM, Amuy E, Barquero-Calvo E, Rojas N, Chacón-Díaz C, Chaves-Olarte E, Guzman-Verri C, Moreno E (2017) Brucellosis caused by the wood rat pathogen *Brucella*

neotomae: two case reports. J Med Case Rep 11:352. https://doi.org/10.1186/s13256-017-1496-8

Waldrop SG, Sriranganathan N (2019) Intracellular invasion and survival of *Brucella neotomae*, another possible zoonotic *Brucella* species. PLoS One 14(4):e0213601. https://doi.org/10.1371/journal.pone.0213601

Wallach JC, Giambartolomei GH, Baldi PC, Fossati CA (2004) Human infection with M- strain of *Brucella canis*. Emerg Infect Dis 10(1):146–148. https://doi.org/10.3201/eid1001.020622

Wallach JC, García JL, Cardinali PS, Seijo AP, Benchetrit AG, Echazarreta SE, Garro SL, Deodato B, Baldi PC (2017) High incidence of respiratory involvement in a cluster of *Brucella suis*-infected workers from a pork processing plant in Argentina. Zoonoses Public Health 64: 550–553. https://doi.org/10.1111/zph.12339

Wang Y, Wang Z, Zhang Y, Bai L, Zhao Y, Liu C, Ma A, Yu H (2014) Polymerase chain reaction-based assays for the diagnosis of human brucellosis. Ann Clin Microbiol Antimicrob13:31. https://doi.org/10.1186/s12941-014-0031-7

Wareth G, Böttcher D, Melzer F, Shehata AA, Roesler U, Neubauer H, Schoon HA (2015) Experimental infection of chicken embryos with recently described *Brucella microti*: pathogenicity and pathological findings. Comp Immunol Microbiol Infect Dis 41:28–34. https://doi.org/10.1016/j.cimid.2015.06.002

Wernery U (2014) Camelid brucellosis: a review. Rev Sci Tech 33:839–857. https://doi.org/10.20506/rst.33.3.2322

Whatmore AM, Davison N, Cloeckaert A, Al Dahouk S, Zygmunt MS, Brew SD, Perrett LL, Koylass MS, Vergnaud G, Quance C, Scholz HC, Dick EJ Jr, Hubbard G, Schlabritz-Ying W, Nguyen MQ, Jahre JA (1999) *Brucella canis* endocarditis: case report. Clin Infect Dis 29:1593–1594. https://doi.org/10.1086/313545

Whatmore AM, Perrett LL, MacMillan AP (2007) Characterisation of the genetic diversity of *Brucella* by multilocus sequencing. BMC Microbiol 7:34. https://doi.org/10.1186/1471-2180-7-34

Whatmore AM, Dawson CE, Groussaud P, Koylass MS, King AC, Shankster SJ, Sohn AH, Probert WS, McDonald WL (2008) Marine mammal *Brucella* genotype associated with zoonotic infection. Emerg Infect Dis 14:517–518

Whatmore AM, Dale E-J, Stubberfield E, Muchowski J, Koylass M, Dawson C, Gopaul KK, Perrett LL et al (2015) Isolation of *Brucella* from a White's tree frog (*Litoria caerulea*). JMM Case Reports. https://doi.org/10.1099/jmmcr.0.000017:1-5

Whatmore AM, Koylass MS, Muchowski J, Edwards-Smallbone J, Gopaul KK, Perrett LL (2016) Extended multilocus sequence analysis to describe the global population structure of the genus *Brucella*: Phylogeography and relationship to biovars. Front Microbiol 7:2049. https://doi.org/10.3389/fmicb.2016.02049

World Health Organisation (WHO) (2006) Brucellosis in humans and animals. WHO. Available at: http://www.who.int/csr/resources/publications/deliberate/WHO_CDS_EPR_2006_7/en/. Accessed 14 Feb 2022

Wyatt HV (2005) How Themistocles Zammit found Malta fever (brucellosis) to be transmitted by the milk of goats. J R Soc Med 98(10):451–454. https://doi.org/10.1258/jrsm.98.10.451

Yaghoobi MH, Siroos B, Foroumandi M, Gharabaghi MA (2013) Tricky brucellosis. BMJ Case Rep bcr2013009522. https://doi.org/10.1136/bcr-2013-009522

Yagupsky P (1999) Detection of Brucellae in blood cultures J Clin Microbiol 37(11):3437–42. https://doi.org/10.1128/JCM.37.11.3437-3442.1999

Yagupsky P, Morata P, Colmeneroc JD (2020) Laboratory diagnosis of human brucellosis. Clin Microbiol Rev 33(1):e00073–e00019. https://doi.org/10.1128/CMR.00073-19

Ying W, Nguyen MQ, Jahre JA (1999) Brucella canisendocarditis: case report. Clin Infect Dis 29(6):1593–4. https://doi.org/10.1086/313545

Zange S, Schneider K, Georgi E, Scholz HC, Antwerpen MH, Walter MC, Zoeller L, von Buttlar H, Borde JP (2019) A headache with surprising outcome: first case of brucellosis caused by *Brucella suis* biovar 1 in Germany. Infection 47(5):863–868. https://doi.org/10.1007/s15010-019-01312-7

Q Fever (*Coxiella burnetii*)

14

A Blueprint for Outbreaks That Some Humans will Remember Long

Hendrik I. J. Roest, Chantal P. Rovers, and Dimitrios Frangoulidis

Contents

H. I. J. Roest (✉)
Animal Supply Chain and Animal Welfare, Ministry of Agriculture, Nature and Food Quality, The Hague, The Netherlands

Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
e-mail: h.i.j.roest@minlnv.nl

C. P. Rovers
Department of Internal Medicine, Radboud Q fever Center of Expertise, Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands
e-mail: chantal.rovers@radboudumc.nl

D. Frangoulidis (✉)
Bundeswehr Medical Service Headquarters VI-2, Medical Intelligence & Information (MI2), Munich, Germany
e-mail: DimitriosFrangoulidis@Bundeswehr.org

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_12

Abstract

About 85 years ago, Q fever research began due to human outbreaks of unknown origin, associated with domestic animals. Since then, some but not all characteristics of this "query" disease, caused by the intracellular bacterium *Coxiella burnetii*, were revealed. In this chapter, the bacteriology of the bacterium, clinical presentation, epidemiology, and transmission of the disease in humans and animals are presented. Domestic small ruminants are the main source of human Q fever. Although Q fever is considered to be an occupational disease, outbreaks affect also people without livestock contact and as such have a major public health impact attracting most attention. The Dutch Q fever outbreak, involving 4000 reported human cases over the years 2007–2010 and at least 40,000 cases presumably unrecognized, is an example of how Q fever can reemerge from an endemic state into an outbreak of unforeseen dimension. In this outbreak, the epidemiological link between dairy goats and human cases was confirmed by genotyping for the first time. This was possible due to the previous development of genotyping assays that are applicable on clinical material. Although Q fever seems to be a blue print for outbreaks, it is not known yet what factors are essential to cause outbreaks and how they interact. To prevent outbreaks, a better understanding of these factors and their interaction is necessary and research should therefore focus on this. Only with a One Health approach involving human medicine, veterinary medicine, and environmental factors, coordinated research under this aspect and up-to-date knowledge and information processing, presentation, and dissemination will be able to reduce the significance of this zoonosis in the future.

Keywords

Zoonosis · Q fever · Acute Q fever · Chronic Q fever · *Coxiella burnetii* · Transmission · Excretion · Outbreak · Therapy · Genotyping · MLVA · Discrimination · Q fever fatigue syndrome · One Health · Public health · Epidemiology · Disease burden · Dutch outbreak · Livestock · Prevention

14.1 Introduction → Characteristics of *Coxiella burnetii*

The first awareness of Q fever was raised by severe outbreaks among abattoir workers in Brisbane, Australia. These outbreaks had been occurring periodically since 1933, but remained undiagnosed until Edward Derrick was assigned to investigate the cause of these febrile illnesses (Derrick 1983). In order to reveal the cause of the disease, several experiments were done, but Derrick failed to detect bacteria. This led him to the (wrong) conclusion that the etiologic agent was a virus. Further studies on the virus of this query, "Q," fever were published by Macfarlane Burnet and Mavis Freeman, indicating the rickettsia-like properties of the virus (Burnet and Freeman 1983). At the same time, the causative agent of Q fever was also discovered

independently in Montana, USA, due to research on Rocky Mountain spotted fever (Cox 1938). Later on, a laboratory infection revealed the linkage of the two discoveries (Dyer 1938; McDade 1990). So, from the early identification of Q fever and the discovery of the etiologic agent it was obvious that the Q fever agent was zoonotic and able to cause severe outbreaks.

The rickettsia-like properties of the agent resulted in the initial designation of the Q fever agent as *Rickettsia diaporica* (diaporica is derived from the Greek word for having the property or ability to pass through [a filter]) by the American group and as *Rickettsia burnetii* (after Burnet) by the Australian group (McDade 1990). In 1948, Philip (Philip 1948) proposed a reclassification into *Coxiella burnetii*. In this name both Harold Cox and Frank Burnet are honored for their contribution to the identification of the Q fever agent. At a later time, *C. burnetii* has been phylogenetically reclassified from the order of Rickettsiales to Legionellales, based on the sequence of its 16S rRNA (Weisburg et al. 1989).

C. burnetii is an obligate intracellular gram-negative bacterium. The pleomorphic rods have a diameter of approximately 0.2–0.4 μm and are 0.4–1.0 μm in length (Drancourt and Raoult 2005). In the developmental cycle of *C. burnetii* two distinct variants have been identified: a large cell variant (LCV) and a small cell variant (SCV) (McCaul and Williams 1981). The LCV is the metabolically active and intracellular replicative entity of the bacterium. The LCV transforms into the SCV, which is the more resistant form of *C. burnetii*. In this form the bacterium is highly resistant to environmental stress, such as high temperatures, UV radiation, and osmotic pressure. The resistance allows *C. burnetii* to survive in the environment while keeping its infectivity (McCaul and Williams 1981). The SCV can infect host cells, closing the developmental cycle.

Lipopolysaccharide (LPS) is demonstrated in both *C. burnetii* LCV and SCV, although presence of LPS is mainly associated with the SCV (Coleman et al. 2007). Like several other gram-negative species, *C. burnetii* can display two different LPS phenotypes. The phase 1 phenotype expresses full length LPS which corresponds to the smooth LPS of other gram-negative bacteria (e.g., *Brucella* spp. and *Enterobacteriaceae*), while the phase 2 phenotype carries LPS that resembles the rough LPS. Phase 2 LPS lacks the O-antigenic region (Toman et al. 2009). Phase 1 bacteria are highly virulent and able to replicate in immunocompetent hosts. This is contrary to phase 2 bacteria, which are avirulent and unable to replicate in immunocompetent animals (Moos and Hackstadt 1987; Andoh et al. 2007). During serial passage in cell culture, phase 1 *C. burnetii* can convert into phase 2 (Hotta et al. 2002). Both LPS phenotypes are inducing phase-specific antibodies in infected hosts. Phase 1 antibodies are directed against the full length LPS of phase 1, whereas phase 2 antibodies are assumed to direct against common surface proteins (Marrie and Raoult 1997). The hypothesis is that these surface proteins are also present on the surface of phase 1 *C. burnetii*, but may be shielded by the long phase 1 LPS. This may prevent binding of phase 2 antibodies to surface proteins of intact phase 1 bacteria (Hackstadt 1988).

The first complete genome sequence of *C. burnetii* was published in 2003 (Seshadri et al. 2003). Analysis of the original strain isolated from ticks by Davies

Table 1 Overview of published genotyping techniques for *C. burnetii* and year of first publication of the technique for *C. burnetii* (Roest et al. 2013b)

Abbr.	Stands for	Based on	Publ. year	Ref.
RFLP typing	Restriction fragment length polymorphism typing	Analysis of the fragments after digestion with specific restriction enzymes	1990	Jager et al. (1998), Heinzen et al. (1990)
Com1 typing	Com1 encoding genes sequencing	Sequence analysis of the Com1 encoding genes	1997	Zhang et al. (1997)
Com1/ MucZ typing	Com1 and MucZ encoding genes sequencing	Sequence analysis of the Com1 and MucZ encoding genes	1999	Sekeyova et al. (1999)
MST	Multispacer sequence typing	DNA sequence variation in short intergenic regions in the genome	2005	Glazunova et al. (2005)
MLVA	Multiple locus variable number tandem repeats analysis	Variation in the repeat number in tandemly repeated DNA elements on multiple loci in the genome	2006	Svraka et al. (2006), Arricau-Bouvery et al. (2006)
IS1111 typing	IS1111 repetitive element PCR-based differentiation typing	Identification of different IS1111 insertion elements	2007	Denison et al. (2007)
RAPD	Randomly amplified polymorphic DNA	Analysis of randomly amplified DNA fragments of the genome	2009	Sidi-Boumedine et al. (2009)
SNP typing *ada*A	Single nucleotide polymorphism typing Acute disease Antigen-A	Differentiating a single nucleotide difference on a locus in the genome by probes Polymorphism in gene region	2011 2013	Huijsmans et al. (2011) Frangoulidis et al. (2013)

Abbr.: abbreviation; Publ. year: year of first publication of the technique for *C. burnetii*; Ref.: reference

and Cox in 1938 (called Nine Mile/RSA493) revealed a circular genome of 1,995,275 base pairs. Today roundabout 100 genomes form *C. burnetii* that are sequenced and available on NCBI (https://www.ncbi.nlm.nih.gov/genome/?term=coxiella+burnetii). The genetic heterogeneity of different *C. burnetii* strains can be assessed with a number of molecular techniques. Different genotyping techniques have been described, mainly based on the identification of differences between selected loci on the genome (Table 1, Massung et al. 2012; Frangoulidis et al. 2022). Accurate identification of the agent is important to differentiate between strains and to identify epidemiological relevant markers. These markers are at the basis of molecular epidemiology, which enables the identification of sources of Q fever outbreaks (Arricau-Bouvery et al. 2006).

In the recent years, a further potential benefit of genomic markers was studied to get an enhanced characterization of *C. burnetii*: the correlation between pheno- and

genotype. This issue was seen since the beginning of using molecular techniques in *Coxiella* research. Already in 1985 Samuel et al. identified the plasmid QpRS that should be associated with chronic disease. This was not confirmed in later studies (Stein and Raoult 1993; Thiele and Willems 1994). Later in 2005 a new genomic marker for acute Q fever was discussed (Zhang et al. 2005), the acute disease antigen A (ada A), but again an in-depth analysis could not confirm the hypothesis although characterization of the ada A region offers some new and interesting typing options for discrimination (Frangoulidis et al. 2013). A SNP at position 431 was predominant in isolates from acute Q fever pneumonia and also carried by most of the sheep strain, consistent with the observation that most of human Q fever cases are sheep related. In 2014, a MLVA (Multiple Locus Variable number tandem repeats Analysis)-based analysis of veterinarian *Coxiella* samples of Germany identified a special cluster, associated to cattle and a mixed cluster with most of the studied isolates from goats and sheep (Frangoulidis et al. 2014). This clustering from MLVA genotypes to animal species was confirmed by a French MLVA study in 2017 (Joulié et al. 2017) and recently in Poland, too (Jodełko et al. 2021).

Beside MLVA the MST (multispacers typing) typing method is also widely used, introduced in 2005 (Glazunova et al. 2005). Lacking the discriminatory power of MLVA in *Coxiella* typing, it shows also some species specific groupings, like the MST genotype 61, strongly associated with cattle.

A more established and much more better evaluated and important use of molecular typing systems in Q fever is the discriminatory power, i.e., the ability to distinguish between unrelated strains. This is determined by the number of different types defined by the test method and the relative frequencies of the types. A single numerical index of discrimination is suggested by Hunter and Gaston (Hunter and Gaston 1988). This Hunter-Gaston Diversity Index (HGDI) is based on the probability that two unrelated strains, sampled from a test population, will be placed into different typing groups. By comparing the HGDI of a typing system the discriminatory power of a method can be identified. It is important to note that the calculated HGDI depends on the panel of strains (i.e., relatedness of the strains), so for an unbiased comparison of typing methods preferably the same panel should be used. Despite its importance for the quality of typing systems the discriminatory power is not assessed for most of the typing systems available for *C. burnetii*. For the ones that were assessed, a HGDI of 0.86 was calculated for RFLP typing (Jager et al. 1998). For the MLVA typing panels 1 and 2 a HGDI of 0.92 is calculated, respectively; for the combination of both a HGDI of 0.99 is calculated (Arricau-Bouvery et al. 2006; Roest et al. 2011b; Frangoulidis et al. 2014). It is suggested that a HGDI of >0.90 is desirable to interpret typing results with confidence (Hunter and Gaston 1988), indicating the MLVA typing system as a useful typing tool for *C. burnetii*. However, still the heterogeneity of the studied population influences the quality. In comparison with the published MST and SNP methods MLVA is probably a more discriminatory typing method for *C. burnetii* (Svraka et al. 2006; Chmielewski et al. 2009; Massung et al. 2012; Frangoulidis et al. 2022).

Recently a study analyzed MLVA, MST, and SNP-associated genomic grouping in *C. burnetii* and postulated a rapid screening marker in one MST-Gen loci (Cox51)

(Hemsley et al. 2021). Future studies will show the benefit of this issue for *Coxiella* typing in humans and animals.

Due to virulence of *C. burnetii* genes, no clear associations could be drawn. So, to date, it is not possible to classify the virulence of strains solely based on the available genotyping methods.

Summing up, genomic typing of *C. burnetii* is an important technique in Q fever outbreak management, especially in back-tracing infections in humans and animals, to identify the source of this zoonotic disease in outbreaks.

Nevertheless, although several efforts have been done, till today an accepted standard laboratory method for *C. burnetii* typing is not established. Despite this, genomic typing data was collected in databases like https://ifr48.timone.univ-mrs.fr/mst/coxiella_burnetii/ for MST and http://mlva.i2bc.paris-saclay.fr/MLVAnet/spip.php?rubrique50 for MLVA and recently in a new database CoxBase (https://coxbase.q-gaps.de/) putting all existing genomic typing data of *C. burnetii* of five different methods and whole genome sequencing information of strains in one place, sharing information and providing analytical tools for comparison (Fasemore et al. 2021). This might support in the future the identification of virulence-associated gene regions of this zoonotic pathogen.

14.2 Disease Symptoms in Animals and Humans

14.2.1 Clinical Presentation in Animals

The most important clinical presentations of Q fever in animals that are relevant for the zoonotic properties of Q fever are abortion and stillbirth. Field observations clearly demonstrate *C. burnetii* as a cause of abortion and stillbirth in goats, sheep, cattle, and cats (van Moll et al. 1993; Guatteo et al. 2011; Berri et al. 2001, 2002; Hatchette et al. 2003; Rousset et al. 2009; Lang 1990; Marrie et al. 1988b). Abortion occurs most frequently at the end of gestation, without preceding clinical symptoms (Arricau-Bouvery and Rodolakis 2005). In nonpregnant animals, *C. burnetii* infection is virtually asymptomatic.

In pregnant goats, abortion, still birth, and also the birth of strong and lively kids can occur after Q fever infection of pregnant animals (Arricau-Bouvery et al. 2005; Roest et al. 2012). When lively kids are born the duration of gestation can be up to 14 days shorter than the normal average gestation duration of 154 days. In dairy goat herds where Q fever abortions occurred, metritis can be observed. Weak kids were reported with low body weight and high mortality. Rearing of apparently healthy kids can be complicated by respiratory and digestive tract disorders (Wouda and Dercksen 2007). Experimental infections with *C. burnetii* in pregnant sheep did not result in any abortions (Martinov et al. 1989; Welsh et al. 1958), while in cattle one experimental infection of a pregnant cow resulted in abortion (Lang 1990). The cause of these differences in pregnancy outcome after infection is unknown. In cattle, *C. burnetii* infection is associated with metritis and reproduction problems (Lang 1990; To et al. 1998).

14.2.2 Clinical Presentation in Humans

In humans infection with *C. burnetii* can manifest in three main clinical presentations: acute Q fever, chronic Q fever, and the Q fever fatigue syndrome (QFS). Following exposure to *C. burnetii* almost 60% of the Q fever cases are asymptomatic. Among the 40% symptomatic acute Q fever patients, the majority will present a nonspecific, self-limiting illness. More severe clinical symptoms include fever, headache, chills, atypical pneumonia, and hepatitis (Derrick 1983; Maurin and Raoult 1999; Raoult et al. 2005). In the Netherlands, the large acute Q fever outbreak between 2007 and 2011 showed a mortality rate of 1.2% within approximately 1 month after hospitalization of patients. All lethal cases suffered severe underlying medical conditions (Kampschreur et al. 2010). Acute Q fever is diagnosed in the laboratory following (i) a positive *C. burnetii* specific PCR; (ii) the presence of IgM phase 2 antibodies in serum accompanied by clinical symptoms; or (iii) a fourfold increase of the IgG phase 2 antibody titer. These laboratory findings are also the notification criteria in the Netherlands (Wegdam-Blans et al. 2010). Acute Q fever can be treated with doxycycline 200 mg per day for 2 weeks. Alternative options are quinolones or trimethoprim-sulphametoxazole.

Chronic Q fever can develop after either a symptomatic or an asymptomatic primary infection in 1–5% of the patients. Chronic Q fever can become manifest years after initial infection. The most common presentations are endocarditis and vascular (prothesis) infection. Clinical symptoms include nonspecific fatigue, fever, weight loss, night sweats, and hepato-splenomegaly (Wegdam-Blans et al. 2012; Raoult et al. 2005). Risk factors for developing chronic Q fever include heart valve surgery, abdominal or iliac aneurysm, vascular prosthesis, and older age (Kampschreur et al. 2012). Diagnosis of chronic Q fever is difficult because of the nonspecific symptoms, but also because C-reactive protein can be normal and echocardiography often does not show typical signs of endocarditis. Proven chronic Q fever is defined as phase 1 IgG $\geq$1024 with a definite endocarditis according to the revised Duke criteria or a proven mycotic aneurysm or infected vascular graft and/or positive PCR for *C. burnetii* on blood or infected tissue (Kampschreur et al. 2012). Probable chronic Q fever is defined as phase 1 IgG $\geq$1024 with a risk factor for developing chronic Q fever, symptoms of a chronic infection, or an atypical focus of infection. In possible chronic Q fever only phase 1 IgG $\geq$1024 is present. ^{18}F-FDG-PET/CT has proven its value in diagnosing prosthetic valve endocarditis and vascular infection in Q fever and is recommended at diagnosis or when complications are suspected during treatment (Kouijzer et al. 2018). After the outbreak in the Netherlands, vascular chronic Q fever was more common than endocarditis (Buijs et al. 2021), but in France endocarditis has always been more common. A combination of both is also possible. Complications such as heart failure, aortoenteric fistulas with gastrointestinal bleeding and/or gram-negative sepsis, abcesses, and/or vertebral osteomyelitis occur in 64% of patients with proven chronic Q fever, while Q fever–related mortality is still 27% despite adequate treatment (Buijs et al. 2021). First-choice treatment is doxycycline with hydroxychloroquine, but doxycycline with a quinolone is a reasonable alternative in case of intolerance (Van Roeden

et al. 2018). Treatment duration is at least 18 months in case of native valve endocarditis and at least 24 months when prosthetic material is present and often longer. Treatment can be stopped after this minimum treatment duration when serum PCR has repeatedly been negative and imaging shows no signs of active infection anymore.

Q fever fatigue syndrome (QFS) is another long-term presentation of symptomatic acute Q fever. Contrary to chronic Q fever, *C. burnetii* cannot be detected in QFS patients. Furthermore, antibody levels against the bacteria are low or negligible. Symptoms of QFS include prolonged fatigue, arthralgia, myalgia, headache, concentration problems, and increase in symptoms after physical activity (Keijmel et al. 2015). The cause of the development of chronic Q fever or QFS in certain individuals is still unknown. Even years after the acute infection, quality of life and social functioning of patients with QFS was significantly lower and anxiety significantly higher compared to patients with diabetes and the general population (Reukers et al. 2019). A randomized controlled trial comparing long-term treatment with doxycycline, placebo, or cognitive behavioral therapy (CBT) showed that CBT is effective in reducing fatigue severity in QFS patients. Long-term treatment with doxycycline does not reduce fatigue severity in QFS patients compared to placebo (Keijmel et al. 2017). Unfortunately, the beneficial effect of CBT on fatigue severity was not maintained 1 year thereafter (Raijmakers et al. 2019).

As in animals, it is suggested that also in pregnant women, *C. burnetii* infection may lead to adverse pregnancy outcome, especially when the acute Q fever remains untreated (Langley et al. 2003; Carcopino et al. 2007). Pregnancy outcomes include spontaneous abortion, intrauterine fetal death and premature delivery, or low birth weight. In pregnant women, the risk to develop chronic Q fever is assumed to be high (Maurin and Raoult 1999; Carcopino et al. 2009). Besides clinical Q fever also asymptomatic infections may lead to the same risk for adverse pregnancy outcomes (Parker et al. 2006). As above was known during the Dutch Q fever outbreak, surveys were set up to investigate the relation between *Coxiella* infection and pregnancy outcome. In a population-based study, including 1,174 women, no relation could be detected between presence of antibodies against *C. burnetii* during early pregnancy and adverse pregnancy outcome (van der Hoek et al. 2011b). An additional study was not supportive to imply a preventive program for seropositive pregnant women as such a program, including serological screening and treatment in case of acute or chronic Q fever, seemed not to be associated with a relevant reduction in obstetric complications in seropositive women.

14.3 Epidemiology and Burden of Disease in Animals

14.3.1 Host Range

C. burnetii can infect a wide range of animal species. The original isolation in the USA was from the tick *Dermacentor andersoni* (Davis and Cox 1938). Since then *C. burnetii* has been detected in over 40 tick species. Several bird species can also

become infected with *C. burnetii*, as experimentally shown (Schmatz et al. 1977a, b; Sethi et al. 1978; Babudieri and Moscovici 1952). Natural infections have been reported in domestic birds and in wild birds (To et al. 1998; Astobiza et al. 2011). In terrestrial as well as in marine wildlife the presence of *C. burnetii* has been confirmed in roe deer, wild boars, rodents, European hare, pacific harbor seal, a Steller sea lion, Northern fur seals, and harbor porpoises (Thompson et al. 2012; Astobiza et al. 2011; Lapointe et al. 1999; Kersh et al. 2010, 2012; Duncan et al. 2012). These data indicate a sylvatic cycle for *C. burnetii*, in which ticks probably play a role as vector.

In domestic animals, *C. burnetii* has been detected in cats, dogs, and horses as well as in domestic ruminants. In cats seroprevalences between 19% and 42% are reported. In dogs seroprevalences up to 22% are detected (Higgins and Marrie 1990; Komiya et al. 2003a; Marrie et al. 1988a; Boni et al. 1998). In domestic ruminants *C. burnetii* infections are widespread. Seroprevalence levels are estimated up to 82% in cattle. In sheep and goats average seroprevalences are slightly lower compared to cattle, with values of up to 73% (Guatteo et al. 2011). Prevalence of *C. burnetii* on cattle herd level as measured from bulk tank milk samples ranges between 32% and 94% (Angen et al. 2011; Kim et al. 2005; Astobiza et al. 2012). These data indicate that animals that live in close contact to humans can become infected with *C. burnetii*.

14.3.2 Excretion Routes

Knowledge of the excretion of *C. burnetii* from infected animals is crucial in understanding the transmission routes and risks for human infection. Abortions in *C. burnetii* infected domestic ruminants are accompanied by massive excretion of the bacteria and spread into the environment. This is the most important excretion route of *C. burnetii*, as up to 10^9 organisms per gram placenta tissue are excreted (Arricau Bouvery et al. 2003). However, experiments in goats indicate that comparable numbers of *Coxiella* are also excreted during the birth of lively kids (Roest et al. 2012). Also sheep can excrete numerous Q fever bacteria during normal parturition. This implies that *C. burnetii* can be excreted without clinical signs of Q fever in the herd. This should be taken into account when tracing sources of human Q fever.

C. burnetii has also been detected in feces, vaginal mucus, and milk of infected domestic ruminants (Berri et al. 2001; Arricau Bouvery et al. 2003; Rousset et al. 2009; Guatteo et al. 2006). In goat herds, both in aborting and nonaborting goats, *C. burnetii* DNA has been detected in feces, vaginal mucus, and/or milk (Rousset et al. 2009). In cattle, also variable excretion via feces, vaginal mucus, and milk has been reported, sometimes independent of an abortion history. However, it is suggested that the presence of *C. burnetii* DNA in feces and vaginal mucus is due to contamination of *C. burnetii* DNA from the environment (Welsh et al. 1958; Roest et al. 2012). Thus, while excretion of high numbers of *C. burnetii* with birth products during parturition is evident, the importance of and the correlation between the excretion routes of *C. burnetii* via feces, vaginal mucus, and milk is much less well established.

How a *C. burnetii* infection persists in a ruminant herd is not clear up to now. During parturition of infected animals high numbers of *C. burnetii* are excreted and can persist in the animal's living environment for years (Rustscheff et al. 2000; McCaul and Williams 1981). Pregnant ruminants can be (re)infected by these persisting bacteria. It can also be considered that once animals are infected, they can be persistently infected with *C. burnetii*. The bacterium can persist in the genital tract (Alsaleh et al. 2011) or mammary tissue (van der Brom et al. 2013) and be excreted into the living environment or internally reinfect placental tissue once available after mating. A recent study suggests that nonpregnant ruminants might also play a role in the persistence of *C. burnetii* in a herd (Roest et al. 2020). In an experimental setting, nonpregnant goats were infected with *C. burnetii* before mating. Although the bacterium could not be detected in placentas and kids, one of the ten infected animals excreted *C. burnetii* in the milk and the bacterium was detected in the mammary gland and associated lymph nodes. So, the persistence of *C. burnetii* in pregnant and nonpregnant animals might play a role in the persistence of the infection in a herd.

14.3.3 Burden of Disease in Animals

It is difficult to assess the burden of disease in farm animals. As an alternative, economic losses can be calculated in farm animal holdings. In the Dutch Q fever outbreak the economic losses have been calculated for goat farms (van Asseldonk et al. 2013). In this paper, it is assumed that about 5% of the animals on Q fever positive farm show clinical signs resulting in production and reproduction losses and that on average 40% of the pregnant goats on the farms resulted in abortion. However, goats abort in late pregnancy and aborting animals recover rapidly, so milk production is hardly affected. Furthermore, the economic value of lambs is low. All this results in a relatively limited loss, mainly due to (re)production losses. Costs on farm level increase when mandatory intervention is implemented. In the Dutch Q fever outbreak the intervention costs are estimated at upon 85 Million Euro (van Asseldonk et al. 2013). These costs consisted of costs for organization (57.96%), culling (21.61%), breeding prohibition (14.19%), and vaccination (6.20%). These costs of intervention were much lower than the total losses in the human domain, which were estimated at 222 Million Euro (van Asseldonk et al. 2013).

14.4 Epidemiology and Burden of Disease in Humans

Humans usually acquire Q fever by inhalation of *C. burnetii*. Alveolar macrophages and other mononuclear phagocytes are thought to be the primary target cells of the pathogen (Shannon and Heinzen 2009). In these cells, *C. burnetii* survives intracellular killing and is able to replicate. A bacteremia will lead to systemic infection with involvement of the liver, spleen, lungs, and bone marrow (Maurin and Raoult 1999). It is assumed that higher doses of *C. burnetii* result in an increasing likelihood of infection and shorter incubation periods (Marrie 1990; Van der Hoek 2012). Human Q fever after oral

ingestion has been suggested, but experimental infection of volunteers via contaminated milk did not result in disease although antibody responses were observed (Benson et al. 1963; Fishbein and Raoult 1992). Rare cases of person-to-person transmission, e.g., sexual transmission and blood transfusion, have been reported, but these routes do not seem to play an important role in the epidemiology of Q fever (Marrie 1990; Milazzo et al. 2001). Thus, in humans Q fever is essentially an airborne infection resulting from the inhalation of contaminated aerosols.

14.4.1 Burden of Disease in Humans

Only a few studies have been published on the burden of Q fever in humans. In general, following exposure, almost 60% will remain asymptomatic. About 40% will become symptomatic, although the majority will only present mild symptoms of a self-limiting disease. This is reflected in the number of infected persons compared to the number of registered Q fever cases in several outbreaks. In the outbreak that occurred in the Val de Bagnes, Switzerland, in 1983, it is suggested that for each patient diagnosed 50 persons remain undiagnosed (Dupuis et al. 1987). In the Dutch Q fever outbreaks during the years 2007–2010 it is assumed that for each Q fever patient, 10 additional people were infected (Kampschreur et al. 2013). The hospitalization rate varies and may depend on the clinical experience of the physicians with Q fever. At the start of the Dutch Q fever outbreak hospitalization rates were up to 50%, whereas in the next year the hospitalization rate went down to 21% (Schimmer et al. 2008; van der Hoek et al. 2010).

Long-term effects of infection with *C. burnetii* have been studied as follow-up of the Dutch Q fever outbreak. Twelve to sixteen months after the onset of illness severe subjective symptoms, functional impairment, and impaired quality of life have been measured (Morroy et al. 2011).

The human disease burden can be quantified via disability-adjusted life years (DALY). For the Dutch Q fever outbreak between 2007 and 2011 the total disease burden is calculated as 2462 DALY, comprising of 22 DALY for acute Q fever, 1481 DALY for QFS, 806 DALY of chronic Q fever, and 153 DALY for the 24 people who died (partly) because of Q fever (van Asseldonk et al. 2013). All this indicates that the impact of Q fever can be significant when outbreaks occur.

14.5 Transmission

In humans, Q fever is essentially an airborne infection resulting from the inhalation of contaminated aerosols (Benenson and Tigertt 1956; Tigertt et al. 1961). Aerosols can become contaminated with *C. burnetii* during abortion and parturition of infected pregnant ruminants (Welsh et al. 1958; Benson et al. 1963). Environmental contamination resulting in contaminated aerosols may also follow excretion of *C. burnetii* via feces, vaginal mucus, or possibly milk of animals. This contributes to the occupational hazard of Q fever. However, contaminated aerosols are able to

travel large distances even up to 18 km and infect humans. Highest risk of infections in urban outbreaks occurs in areas 2–4 km from source farms, while in rural areas this risk was identified within 5 km of infected farms (Clark and Soares Magalhães 2018). Wind (speed and direction) and warm and dry weather conditions can facilitate this transport (Van Steenbergen et al. 2007; Tissot-Dupont et al. 2004; Schimmer et al. 2010; Clark and Soares Magalhães 2018). Also other factors, such as vegetation and soil moisture, seem to be relevant in the dispersion of the bacteria (van der Hoek et al. 2011a; Clark and Soares Magalhães 2018). So, direct contact with animals is not a prerequisite for acquiring Q fever, as outbreaks demonstrate (Salmon et al. 1982; Tissot-Dupont et al. 1999, 2004; Gilsdorf et al. 2008; Roest et al. 2011b). Local residents, having indirect *Coxiella* infected livestock contact, are exposed to risks of acquiring Q fever and as such Q fever has a major public health impact attracting most attention. It is assumed that most animals also become infected via inhalation (Berri et al. 2005), although oral uptake in a heavily infected environment cannot be excluded as this oral infection route is experimentally confirmed (Roest et al. 2012).

Domestic ruminants appear to be the main source of infection for human Q fever. As indicated earlier, parturition in infected pregnant goats, sheep, and cattle results in the massive excretion of *C. burnetii* into the environment. Q fever in cattle seems to result in fewer abortions, probably resulting in a lower risk. Companion animals should also be considered as a source for human Q fever, since several human outbreaks were related to parturient cats and dogs (Marrie et al. 1988b; Pinsky et al. 1991; Komiya et al. 2003b; Buhariwalla et al. 1996). The role of horses and wildlife as a source of Q fever for humans is not clear. In overview, the epidemiology of Q fever can be summarized in a transmission model as presented in Fig. 1 (Roest et al. 2009, 2013a).

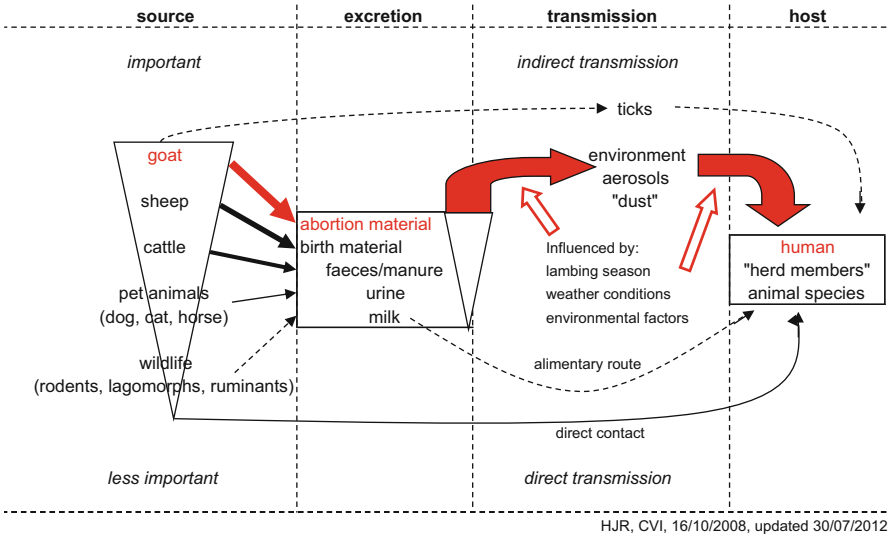

Fig. 1 Transmission model for Q fever

Overview of the possible transmission routes of *C. burnetii* from the animal reservoir to the human (and animal) hosts. The boldness of the arrows indicates the importance of the route, dotted lines indicate possible contributions. The most probable transmission route of *C. burnetii* in the Dutch Q fever outbreak is indicated in red (Roest et al. 2009, 2013a).

14.5.1 Q Fever Outbreaks

Q fever has a major public health impact when outbreaks occur. Outbreaks are reported frequently and worldwide, involving up to 415 laboratory-confirmed human cases per outbreak. Even higher numbers of human cases are reported, but the attribution to Q fever is unclear as cases are not always laboratory confirmed (Van der Hoek 2012; Arricau-Bouvery and Rodolakis 2005; EFSA 2010). Outbreaks are usually geographically localized and restricted to one episode. Sheep are identified as the source in the majority of the outbreaks, with goats as "second best." Only a few outbreaks of Q fever have been related to infected cattle and cats (Van der Hoek 2012; Arricau-Bouvery and Rodolakis 2005; EFSA 2010). Source identification, however, is mainly based on epidemiological examinations. In most outbreaks, confirmation of the identity of the *Coxiella* strain involved in both host and source, for example, by genotyping, is lacking. This is a major drawback in the identification of sources, as sources of Q fever are multiple and *C. burnetii* can be transmitted over larger distances. Thus, identification of the source and confirmation of the relation with human disease is preferably done by genotyping of the involved *C. burnetii* strains.

It should also be remembered that good cooperation and mutual information and communication between the human and veterinary surveillance authorities is essential, otherwise the complete detection and elucidation of a Q fever outbreak is almost impossible!

14.5.2 The Dutch Q Fever Outbreak

Since the 1950s Q fever has been present in the Netherlands. However, only a few human cases were reported until the 1970s. In 1975 Q fever became notifiable for humans (van Vliet 2009) and since that time about 20 cases on average were reported each year. The first human outbreak of Q fever in the Netherlands was reported in 2007: 168 cases were registered in the south of the Netherlands. In 2008 and 2009 the annual number of human cases increased to 1000 and 2355, respectively. Over the years 2007–2010, 4000 human cases were reported making the Dutch Q fever outbreak the largest laboratory-confirmed Q fever outbreak ever seen (Roest et al. 2011b).

Until 2005 Q fever was also known to be present in the animal population by serological investigations, although no clinical symptoms were described. This changed in 2005. Slightly ahead of the human outbreak, Q fever problems started in the dairy goat and dairy sheep population with abortion rates up to 80% per herd (Wouda and Dercksen 2007). Between 2005 and 2009 significant abortions were

registered on 28 dairy goat farms and 2 dairy sheep farms. With goat numbers of 600 up to 7000 per herd huge amounts of *Coxiella burnetii* were spread in the environment during abortion and early birth. These bacteria were transported to the neighboring human population by the prevailing northeast winds in pretty dry spring periods. All this took place in the southeast part of the Netherlands which is highly populated and has a dense dairy goat industry (Roest et al. 2011b). Eventually, the connection between dairy goats and humans was primarily based on epidemiological findings. This connection was confirmed by preliminary genotyping data showing one predominant MLVA type in aborted goats which was also found in infected humans (Roest et al. 2011a; Klaassen et al. 2009). Additional analyses showed also one predominant genotype in humans which was the same as in goats (Tilburg et al. 2012).

As goats were the suspects of the human Q fever outbreaks, increasingly strong measures were imposed to prevent the spreading of *C. burnetii* in the lambing season. The first measures were implemented in 2008, consisting of the notification of abortions in small ruminant holdings, hygiene measure, especially on manure handling and a voluntary vaccination. In 2009 the measures were extended with more tight notification criteria, including positivity of the bulk tank milk for *C. burnetii* DNA, a transport and breeding ban, and a mandatory vaccination. All these measures however did not prevent the increase of human cases in 2009. To ultimately prevent the increase of human cases in 2010 the drastic decision was taken to eliminate all potential high-risk animals. This resulted in the culling of all pregnant goats on Q fever–positive farms. All these measures finally resulted in a decline in human cases in 2010 (Roest et al. 2011b). A number wise summary of the Dutch Q fever outbreak is given in Table 2, based on (van Asseldonk et al. 2013; Roest et al. 2011b; Roest 2013).

The question can be raised at to what the causes of the Dutch Q fever outbreak were. It is clear that dairy goats and sheep were the cause of the human outbreak. Several factors are hypothesized to play a role in the increase in Q fever problems in goats. Firstly, the strong increase in the number of dairy goat herds and goat numbers before 2007. In addition, the partly closed status of some herds to prevent infectious diseases could have made a herd more susceptible for *C. burnetii* or new strains of this bacterium. Finally, the new introduction of a more virulent strain or a genetic shift to a more virulent strain could have contributed to the cause of the outbreak (Roest et al. 2011b).

Although extensive analyses in recent years have shown genomic differences in the Dutch outbreak strains compared to isolates from other countries, these have not provided clear explanations for the unique outbreak in the Netherlands (Kuley et al. 2017).

14.6 Unresolved Issues and Conclusions

An important remaining question is “what are the triggers of an outbreak.” Several factors are known such as *C. burnetii*-infected pregnant small ruminants, abortions in goat herds, probably population density, proximity of small ruminant herds to susceptible humans, and also the virulence of the strain (Georgiev et al. 2013).

Table 2 Overview of the Dutch Q fever outbreak year by year (van Asseldonk et al. 2013; Roest et al. 2011b; Roest 2013, https://www.rivm.nl/q-koorts, vaccination data from 2012 on: personal communication from the Netherlands Food and Consumer Product Safety Authority [NVWA])

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total
Goats																	
Number of confirmed Q fever abortion farms (dairy goats)	2	7	7	8	6	0	0	0	0	0	0	0	0	0	0	0	30
Number of BTM *Coxiella burnetii* DNA positive farms (total number/year)	n.a.	n.a.	n.a.	n.a.	62	74	41	25	14	13	2	0	0	0	0	0	231
Culled animals	0	0	0	0	7,755	50,395	0	0	0	0	0	0	0	0	0	0	58,150
Breeding prohibition	0	0	0	0	0	46,130	n.k.	0	0	0	0	0	0	0	0	0	46,130
Vaccinated animals	0	0	0	0	158,019	346,463	344,424	323,297	375,283	352,343	396,250	462,344	481,076	557,085	637,689	662,409	5,096,687
																	0
Humans																	0
Notified	5	10	168	1000	2354	504	81	53	19	28	22	12	22	17	19	7	4,321
Hospitalized	n.k.	n.k.	83	207	459	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	n.k.	749
Deceased	n.k.	n.k.	0	1	7	11	5	1	0	0	1	0	?	?	?	?	26

BTM bulk tank milk, n.k. not known, n.a. not any

However, which combination of factors triggers an outbreak is unknown. Knowledge on the interaction of these factors and the relative importance of these factors would be very beneficial to come to a (epidemiological) model that can predict the risk for Q fever outbreaks.

However, in addition to the aspects of prevention, monitoring of reservoirs, and prediction of outbreaks, a lot of research is still needed on the immunological processes that trigger this infection in humans and animals. Only in this way will it be possible in the future to identify risk factors for chronification and to improve the therapy of chronic courses such as endocarditis and QFS.

The zoonosis Q fever is a perfect example of the need for a joint approach between human and veterinary medicine, taking into account environmental factors, to contain, control, and prevent the disease. This so-called One Health approach of the WHO has been increasingly in the focus of nations worldwide for years and repeatedly shows its importance in Q fever (e.g., Bond et al. 2016). Despite all efforts, *C. burnetii* has retained many secrets that still require clarification. The challenge here is to bring together the respective institutions from biology, human, and veterinary medicine for joint research. An example of such cooperation is the so-called Q-GAPS (Q fever GermAn Interdisciplinary Program for reSearch) network in Germany, which researched and worked on open questions on the topic of Q fever from 2017–2022 (https://q-gaps.de/en/). An important result of this collaboration will be a guideline with instructions for action, which is intended to serve in the sense of the One Health approach to Q fever control (the guideline will be released and published on the cited homepage in 2023). The global challenge of the future is not only to conduct further research on the characteristics of the pathogen and the consequences of the disease, but also to process new findings and information and make them available in such a way that they can be used by all stakeholders and, if necessary, serve as a basis for new methods and procedures in diagnosis, therapy, and prophylaxis of Q fever – "One-Health at its best"!

References

Alsaleh A, Pellerin JL, Rodolakis A, Larrat M, Cochonneau D, Bruyas JF, Fieni F (2011) Detection of *Coxiella burnetii*, the agent of Q fever, in oviducts and uterine flushing media and in genital tract tissues of the non pregnant goat. Comp Immunol Microbiol Infect Dis 34(4):355–360

Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, Samuel JE (2007) T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in *Coxiella burnetii* infection in mice. Infect Immun 75(7): 3245–3255

Angen O, Stahl M, Agerholm JS, Christoffersen AB, Agger JF (2011) Dynamics of relationship between the presence of *Coxiella burnetii* DNA, antibodies, and intrinsic variables in cow milk and bulk tank milk from Danish dairy cattle. J Dairy Sci 94(12):5750–5759

Arricau Bouvery N, Souriau A, Lechopier P, Rodolakis A (2003) Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. Vet Res 34(4):423–433

Arricau-Bouvery N, Rodolakis A (2005) Is Q fever an emerging or re-emerging zoonosis? Vet Res 36(3):327–349

Arricau-Bouvery N, Souriau A, Bodier C, Dufour P, Rousset E, Rodolakis A (2005) Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. Vaccine 23(35):4392–4402

Arricau-Bouvery N, Hauck Y, Bejaoui A, Frangoulidis D, Bodier CC, Souriau A, Meyer H, Neubauer H, Rodolakis A, Vergnaud G (2006) Molecular characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing. BMC Microbiol 6:38

Astobiza I, Barral M, Ruiz-Fons F, Barandika JF, Gerrikagoitia X, Hurtado A, Garcia-Perez AL (2011) Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. Vet Microbiol 147(1–2):190–194

Astobiza I, Ruiz-Fons F, Pinero A, Barandika JF, Hurtado A, Garcia-Perez AL (2012) Estimation of *Coxiella burnetii* prevalence in dairy cattle in intensive systems by serological and molecular analyses of bulk-tank milk samples. J Dairy Sci 95(4):1632–1638

Babudieri B, Moscovici C (1952) Experimental and natural infection of birds by Coxiella burneti. Nature 169(4292):195–196

Benenson AS, Tigertt WD (1956) Studies on Q fever in man. Trans Assoc Am Phys 69:98–104

Benson WW, Brock DW, Mather J (1963) Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. Public Health Rep 78:707–710

Berri M, Souriau A, Crosby M, Crochet D, Lechopier P, Rodolakis A (2001) Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. Vet Rec 148(16):502–505

Berri M, Souriau A, Crosby M, Rodolakis A (2002) Shedding of *Coxiella burnetii* in ewes in two pregnancies following an episode of Coxiella abortion in a sheep flock. Vet Microbiol 85(1): 55–60

Berri M, Rousset E, Hechard C, Champion JL, Dufour P, Russo P, Rodolakis A (2005) Progression of Q fever and *Coxiella burnetii* shedding in milk after an outbreak of enzootic abortion in a goat herd. Vet Rec 156(17):548–549

Bond KA, Vincent G, Wilks CR, Franklin L, Sutton B, Stenos J, Cowan R, Lim K, Athan E, Harris O, Macfarlane-Berry L, Segal Y, Firestone SM (2016) One Health approach to controlling a Q fever outbreak on an Australian goat farm. Epidemiol Infect 144(6):1129–1141. https://doi.org/10.1017/S0950268815002368. Epub 2015 Oct 23. PMID: 26493615; PMCID: PMC4825098

Boni M, Davoust B, Tissot-Dupont H, Raoult D (1998) Survey of seroprevalence of Q fever in dogs in the southeast of France, French Guyana, Martinique, Senegal and the Ivory Coast. Vet Microbiol 64(1):1–5

Buhariwalla F, Cann B, Marrie TJ (1996) A dog-related outbreak of Q fever. Clin Infect Dis 23(4): 753–755

Buijs SB, Bleeker-Rovers CP, van Roeden SE, Kampschreur LM, Hoepelman AIM, Wever PC, Oosterheert JJ (2021) Still new chronic Q fever cases diagnosed 8 years after a large Q fever outbreak. Clin Infect Dis 73(8):1476–1483

Burnet FM, Freeman M (1983) Experimental studies on the virus of "Q" fever. Rev Infect Dis 5(4): 800–808

Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A (2007) Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy. Clin Infect Dis 45(5):548–555

Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A (2009) Q fever during pregnancy: a cause of poor fetal and maternal outcome. Ann N Y Acad Sci 1166:79–89

Chmielewski T, Sidi-Boumedine K, Duquesne V, Podsiadly E, Thiery R, Tylewska-Wierzbanowska S (2009) Molecular epidemiology of Q fever in Poland. Pol J Microbiol 58(1):9–13

Clark NJ, Soares Magalhães RJ (2018) Airborne geographical dispersal of Q fever from livestock holdings to human communities: a systematic review and critical appraisal of evidence. BMC Infect Dis 18(1):218

Coleman SA, Fischer ER, Cockrell DC, Voth DE, Howe D, Mead DJ, Samuel JE, Heinzen RA (2007) Proteome and antigen profiling of *Coxiella burnetii* developmental forms. Infect Immun 75(1):290–298

Cox HR (1938) A filter-passing infectious agent isolated from ticks III. Description of organism and cultivation experiments. Public Health Rep 53(52):7

Davis GE, Cox HR (1938) A filter-passing infectious agent isolated from ticks I. Isolation from Dermacentor andersoni, reactions in animals, and filtration experiments. Public Health Rep 53(52):9

Denison AM, Thompson HA, Massung RF (2007) IS1111 insertion sequences of *Coxiella burnetii*: characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. BMC Microbiol 7:91

Derrick EH (1983) "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Rev Infect Dis 5(4):790–800

Drancourt M, Raoult D (2005) Family *Coxiellaceae*. In: Garrity GM (ed) Bergey's manual of systematic bacteriology 2nd edition, Part B: the gammaproteobacteria. Springer, New York

Duncan C, Kersh GJ, Spraker T, Patyk KA, Fitzpatrick KA, Massung RF, Gelatt T (2012) *Coxiella burnetii* in northern fur seal (Callorhinus ursinus) placentas from St. Paul Island, Alaska. Vector Borne Zoonotic Dis 12(3):192–195

Dupuis G, Petite J, Peter O, Vouilloz M (1987) An important outbreak of human Q fever in a Swiss Alpine valley. Int J Epidemiol 16(2):282–287

Dyer RE (1938) A filter-passing infectious agent isolated from ticks IV. Human infection. Public Health Rep 53(52):6

EFSA (2010) Scientific opinion on Q fever. EFSA J 8(5). Available: http://www.efsa.europa.eu/en/scdocs/doc/1595.pdf. Accessed

Fasemore AM, Helbich A, Walter MC, Dandekar T, Vergnaud G, Förstner KU, Frangoulidis D (2021) CoxBase: an online platform for epidemiological surveillance, visualization, analysis, and typing of *Coxiella burnetii* genomic sequences. mSystems 6(6):1–10

Fishbein DB, Raoult D (1992) A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. Am J Trop Med Hyg 47(1):35–40

Frangoulidis D, Splettstoesser WD, Landt O, Dehnhardt J, Henning K, Hilbert A, Bauer T, Antwerpen M, Meyer H, Walter MC, Knobloch JK (2013) Microevolution of the chromosomal region of acute disease antigen A (adaA) in the query (Q) fever agent *Coxiella burnetii*. PLoS One 8(1)

Frangoulidis D, Walter MC, Antwerpen M, Zimmermann P, Janowetz B, Alex M, Böttcher J, Henning K, Hilbert A, Ganter M, Runge M, Münsterkötter M, Splettstoesser WD, Hanczaruk M (2014) Molecular analysis of *Coxiella burnetii* in Germany reveals evolution of unique clonal clusters. Int J Med Microbiol 304(7):868–876

Frangoulidis D, Walter MC, Fasemore AM, Cutler SJ (2022) ´*Coxiella burnetii*. In: de Filippis I (ed) Molecular typing in bacterial infections, vol I. Springer, Cham. https://doi.org/10.1007/978-3-030-83217-9_12

Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, Roest H, Stark K, Stegeman J, Vellema P, van der Hoek W, More S (2013) Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro Surveill 18(8):1–13

Gilsdorf A, Kroh C, Grimm S, Jensen E, Wagner-Wiening C, Alpers K (2008) Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. Epidemiol Infect 136(8):1084–1087

Glazunova O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J, Tokarevich N, Kovacava E, Marrie TJ, Raoult D (2005) *Coxiella burnetii* genotyping. Emerg Infect Dis 11(8):1211–1217

Guatteo R, Beaudeau F, Berri M, Rodolakis A, Joly A, Seegers H (2006) Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet Res 37(6):827–833

Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F (2011) Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. Vet Microbiol 149(1–2):1–16

Hackstadt T (1988) Steric hindrance of antibody binding to surface proteins of *Coxiella burnetti* by phase I lipopolysaccharide. Infect Immun 56(4):802–807

Hatchette T, Campbell N, Hudson R, Raoult D, Marrie TJ (2003) Natural history of Q fever in goats. Vector Borne Zoonotic Dis 3(1):11–15

Heinzen R, Stiegler GL, Whiting LL, Schmitt SA, Mallavia LP, Frazier ME (1990) Use of pulsed field gel electrophoresis to differentiate *Coxiella burnetii* strains. Ann N Y Acad Sci 590: 504–513

Hemsley CM, Essex-Lopresti A, Norville IH, Titball RW (2021) Correlating genotyping data of *Coxiella burnetii* with genomic groups´. Pathogens 10(5):604

Higgins D, Marrie TJ (1990) Seroepidemiology of Q fever among cats in New Brunswick and Prince Edward Island. Ann N Y Acad Sci 590:271–274

Hotta A, Kawamura M, To H, Andoh M, Yamaguchi T, Fukushi H, Hirai K (2002) Phase variation analysis of *Coxiella burnetii* during serial passage in cell culture by use of monoclonal antibodies. Infect Immun 70(8):4747–4749

Huijsmans CJ, Schellekens JJ, Wever PC, Toman R, Savelkoul PH, Janse I, Hermans MH (2011) SNP-genotyping of a *Coxiella burnetii* outbreak in The Netherlands. Appl Environ Microbiol 77(6):2051–2057

Hunter PR, Gaston MA (1988) Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 26(11):2465–2466

Jager C, Willems H, Thiele D, Baljer G (1998) Molecular characterization of *Coxiella burnetii* isolates. Epidemiol Infect 120(2):157–164

Jodełko A, Szymańska-Czerwińska M, Rola JG, Niemczuk K (2021) Molecular detection of *Coxiella burnetii* in small ruminants and genotyping of specimens collected from goats in Poland. BMC Vet Res 17(1):341

Joulié A, Sidi-Boumedine K, Bailly X, Gasqui P, Barry S, Jaffrelo L, Poncet C, Abrial D, Yang E, Leblond A, Rousset E, Jourdain E (2017) Molecular epidemiology of *Coxiella burnetii* in French livestock reveals the existence of three main genotype clusters and suggests species-specific associations as well as regional stability´. Infect Genet Evol 48:142–149

Kampschreur LM, Wegdam-Blans MC, Thijsen SF, Groot CA, Schneeberger PM, Hollander AA, Schijen JH, Arents NL, Oosterheert JJ, Wever PC (2010) Acute Q fever related in-hospital mortality in The Netherlands. Neth J Med 68(12):408–413

Kampschreur LM, Hagenaars JC, Wielders CC, Elsman P, Lestrade PJ, Koning OH, Oosterheert JJ, Renders NH, Wever PC (2013) Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 141(4):847–51

Kampschreur LM, Wever PC, Wegdam-Blans MC, Delsing CE, Bleeker-Rovers CP, Sprong T, van Kasteren ME, Coutinho RA, Schneeberger PM, Notermans DW, Bijlmer HA, Koopmans MP, Nabuurs-Franssen MH, Oosterheert JJ (2012) Defining chronic Q fever: a matter of debate. J Infect 65(4):362–363

Keijmel SP, Saxe J, van der Meer JW, Nikolaus S, Netea MG, Bleijenberg G, Bleeker-Rovers CP, Knoop H (2015) A comparison of patients with Q fever fatigue syndrome and patients with chronic fatigue syndrome with a focus on inflammatory markers and possible fatigue perpetuating cognitions and behaviour. J Psychosom Res 79(4):295–302

Keijmel SP, Delsing CE, Bleijenberg G, van der Meer JWM, Donders RT, Leclercq M, Kampschreur LM, van den Berg M, Sprong T, Nabuurs-Franssen MH, Knoop H, Bleeker-Rovers CP (2017) Effectiveness of long-term doxycycline treatment and cognitive-behavioral therapy on fatigue severity in patients with Q fever fatigue syndrome (Qure study): a randomized controlled trial. Clin Infect Dis 64(8):998–1005

Kersh GJ, Lambourn DM, Self JS, Akmajian AM, Stanton JB, Baszler TV, Raverty SA, Massung RF (2010) *Coxiella burnetii* infection of a Steller Sea lion (*Eumetopias jubatus*) found in Washington state. J Clin Microbiol 48(9):3428–3431

Kersh GJ, Lambourn DM, Raverty SA, Fitzpatrick KA, Self JS, Akmajian AM, Jeffries SJ, Huggins J, Drew CP, Zaki SR, Massung RF (2012) *Coxiella burnetii* infection of marine mammals in the Pacific Northwest, 1997–2010. J Wildl Dis 48(1):201–206

Kim SG, Kim EH, Lafferty CJ, Dubovi E (2005) *Coxiella burnetii* in bulk tank milk samples, United States. Emerg Infect Dis 11(4):619–621

Klaassen CH, Nabuurs-Franssen MH, Tilburg JJ, Hamans MA, Horrevorts AM (2009) Multigenotype Q fever outbreak, The Netherlands. Emerg Infect Dis 15(4):613–614

Komiya T, Sadamasu K, Kang MI, Tsuboshima S, Fukushi H, Hirai K (2003a) Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. J Vet Med Sci 65(9): 1047–1048

Komiya T, Sadamasu K, Toriniwa H, Kato K, Arashima Y, Fukushi H, Hirai K, Arakawa Y (2003b) Epidemiological survey on the route of *Coxiella burnetii* infection in an animal hospital. J Infect Chemother 9(2):151–155

Kouijzer IJE, Kampschreur LM, Wever PC, Hoekstra C, van Kasteren MEE, de Jager-Leclercq MGL, Nabuurs-Franssen MH, Wegdam-Blans MCA, Ammerlaan HSM, Buijs J, Geus-Oei LF, Oyen WJG, Bleeker-Rovers CP (2018) The value of ^{18}F-FDG PET/CT in diagnosis and during follow-up in 273 patients with chronic Q fever. J Nucl Med 59(1):127–133

Kuley R, Kuijt E, Smits MA, Roest HIJ, Smith HE, Bossers A (2017) Genome plasticity and polymorphisms in critical genes correlate with increased virulence of Dutch outbreak-related *Coxiella burnetii* strains. Front Microbiol 8:1526

Lang GH (1990) Coxiellosis (Q fever) in animals. In: Marrie TJ (ed) Q fever, volume I: the disease. CRC Press, Boca Raton

Langley JM, Marrie TJ, Leblanc JC, Almudevar A, Resch L, Raoult D (2003) *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. Am J Obstet Gynecol 189(1):228–232

Lapointe JM, Gulland FM, Haines DM, Barr BC, Duignan PJ (1999) Placentitis due to *Coxiella burnetii* in a Pacific harbor seal (Phoca vitulina richardsi). J Vet Diagn Investig 11(6):541–543

Marrie TJ (1990) Q fever. Volume I: The disease. CRC Press, Boca Raton

Marrie TJ, Raoult D (1997) Q fever-a review and issues for the next century. Int J Antimicrob Agents 8(3):145–161

Marrie TJ, Durant H, Williams JC, Mintz E, Waag DM (1988a) Exposure to parturient cats: a risk factor for acquisition of Q fever in maritime Canada. J Infect Dis 158(1):101–108

Marrie TJ, MacDonald A, Durant H, Yates L, McCormick L (1988b) An outbreak of Q fever probably due to contact with a parturient cat. Chest 93(1):98–103

Martinov SP, Neikov P, Popov GV (1989) Experimental Q fever in sheep. Eur J Epidemiol 5(4): 428–431

Massung RF, Cutler SJ, Frangoulidis D (2012) Molecular typing of *Coxiella burnetii* (Q fever). Adv Exp Med Biol 984:381–396

Maurin M, Raoult D (1999) Q fever. Clin Microbiol Rev 12(4):518–553

McCaul TF, Williams JC (1981) Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. J Bacteriol 147(3):1063–1076

McDade JE (1990) Historical aspects of Q fever. In: Marrie TJ (ed) Q fever. Volume I: the disease. CRC Press, Boca Raton

Milazzo A, Hall R, Storm PA, Harris RJ, Winslow W, Marmion BP (2001) Sexually transmitted Q fever. Clin Infect Dis 33(3):399–402

Moos A, Hackstadt T (1987) Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the Guinea pig model. Infect Immun 55(5):1144–1150

Morroy G, Peters JB, van Nieuwenhof M, Bor HH, Hautvast JL, van der Hoek W, Wijkmans CJ, Vercoulen JH (2011) The health status of Q-fever patients after long-term follow-up. BMC Infect Dis 11:97

Parker NR, Barralet JH, Bell AM (2006) Q fever. Lancet 367(9511):679–688

Philip CB (1948) Comments on the name of the Q fever organism. Public Health Rep 63(2):1

Pinsky RL, Fishbein DB, Greene CR, Gensheimer KF (1991) An outbreak of cat-associated Q fever in the United States. J Infect Dis 164(1):202–204

Raijmakers RPH, Keijmel SP, Breukers EMC, Bleijenberg G, van der Meer JWM, Bleeker-Rovers CP, Knoop H (2019) Long-term effect of cognitive behavioural therapy and doxycycline treatment for patients with Q fever fatigue syndrome: one-year follow-up of the Qure study. J Psychosom Res 116:62–67

Raoult D, Marrie TJ, Mege JL (2005) Natural history and pathophysiology of Q fever. Lancet Infect Dis 5(4):219–226

Reukers DFM, van Jaarsveld CHM, Knoop H, Bleeker-Rovers CP, Akkermans R, de Grauw W, van der Velden K, van Loenhout JAF, Hautvast JLA (2019) Explaining the long-term impact of

chronic Q fever and Q fever fatigue syndrome on psychosocial functioning: a comparison with diabetes and the general population. J Psychosom Res 121:37–45

Roest HIJ (2013) *Coxiella burnetii in pregnant goats*, unpublished thesis University Utrecht

Roest HJ, van Steenbergen J, Wijkmans C, van Duijnhoven Y, Stenvers O, Oomen T, Vellema P (2009) Q fever in 2008 in the Netherlands and the expectations of 2009. Tijdschr Diergeneeskd 134(7):300–303

Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, van den Brom R, Dercksen D, Wouda W, Spierenburg MA, van der Spek AN, Buijs R, de Boer AG, Willemsen PT, van Zijderveld FG (2011a) Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, The Netherlands. Emerg Infect Dis 17(4):668–675

Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, Raoult D (2011b) The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol Infect 139(1):1–12

Roest HJ, van Gelderen B, Dinkla A, Frangoulidis D, van Zijderveld FG, Rebel J, van Keulen L (2012) Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. PLoS One 7(11):14

Roest HI, Bossers A, van Zijderveld FG, Rebel JM (2013a) Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis and control of the zoonotic disease Q fever. Vet Q 33(3):148–160

Roest HI, van Solt CB, Tilburg JJ, Klaassen CH, Hovius EK, Roest FT, Vellema P, van den Brom R, van Zijderveld FG (2013b) Search for possible additional reservoirs for human Q fever, the Netherlands. Emerg Infect Dis 19(5):834–835

Roest HIJ, Dinkla A, Koets AP, Post J, van Keulen L (2020) Experimental *Coxiella burnetii* infection in non-pregnant goats and the effect of breeding´. Vet Res 51(1):74

Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A, Rodolakis A (2009) *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl Environ Microbiol 75(2):428–433

Rustscheff S, Norlander L, Macellaro A, Sjöstedt A, Vene S, Carlsson M (2000) A case of Q fever acquired in Sweden and isolation of the probable ethiological agent, *Coxiella burnetii* from an indigenous source`. Scand J Infect Dis 32(6):605–607

Salmon MM, Howells B, Glencross EJ, Evans AD, Palmer SR (1982) Q fever in an urban area. Lancet 1(8279):1002–1004

Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, van der Hoek W (2008) Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro Surveill 13(31)

Schimmer B, Ter Schegget R, Wegdam M, Zuchner L, de Bruin A, Schneeberger PM, Veenstra T, Vellema P, van der Hoek W (2010) The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect Dis 10:69

Schmatz HD, Busche R, Krauss H, Thiel N (1977a) The behavior of the Q-fever agent, *Coxiella burnetii*, in birds. 3. Experimental infection of quail. Dtsch Tierarztl Wochenschr 84(10):375–376

Schmatz HD, Busche R, Thiel N, Sailer J (1977b) Behavior of the Q fever vector *Coxiella burnetii* in birds. 2. Experimental infection of pigeons. Dtsch Tierarztl Wochenschr 84(2):60–63

Sekeyova Z, Roux V, Raoult D (1999) Intraspecies diversity of *Coxiella burnetii* as revealed by com1 and mucZ sequence comparison. FEMS Microbiol Lett 180(1):7

Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, Ward NL, Tettelin H, Davidsen TM, Beanan MJ, Deboy RT, Daugherty SC, Brinkac LM, Madupu R, Dodson RJ, Khouri HM, Lee KH, Carty HA, Scanlan D, Heinzen RA, Thompson HA, Samuel JE, Fraser CM, Heidelberg JF (2003) Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. Proc Natl Acad Sci U S A 100(9):5455–5460

Sethi MS, Singh B, Yadav MP (1978) Experimental infection of *Coxiella burnetii* in chicken: clinical symptoms, serologic response, and transmission through egg. Avian Dis 22(3):391–395

Shannon JG, Heinzen RA (2009) Adaptive immunity to the obligate intracellular pathogen *Coxiella burnetii*. Immunol Res 43(1–3):138–148

Sidi-Boumedine K, Duquesne V, Fernandes I, Marro S, Thiery R (2009) Evaluation of randomly amplified polymorphic DNA (RAPD) for discrimination of *Coxiella burnetii* ruminant strains isolated in France. Clin Microbiol Infect

Stein A, Raoult D (1993) Lack of pathotype specific gene in human *Coxiella bur*netii isolates. Microb Pathog 15(3):177–185

Svraka, S., Toman, R., Skultety, L., Slaba, K. and Homan, W. L. (2006) 'Establishment of a genotyping scheme for *Coxiella burnetii*', FEMS Microbiol Lett, 254(2), 268-274

Thiele D, Willems H (1994) Is plasmid based differentiation of *Coxiella burnetii* in 'acute' and 'chronic' isolates still valid? Eur J Epidemiol 10(4):427–434

Thompson M, Mykytczuk N, Gooderham K, Schulte-Hostedde A (2012) Prevalence of the bacterium *Coxiella burnetii* in wild rodents from a Canadian Natural Environment Park. Zoonoses Public Health

Tigertt WD, Benenson AS, Gochenour WS (1961) Airborne Q fever. Bacteriol Rev 25:285–293

Tilburg JJ, Rossen JW, van Hannen EJ, Melchers WJ, Hermans MH, van de Bovenkamp J, Roest HJ, de Bruin A, Nabuurs-Franssen MH, Horrevorts AM, Klaassen CH (2012) Genotypic diversity of *Coxiella burnetii* in the 2007–2010 Q fever outbreak episodes in The Netherlands. J Clin Microbiol 50(3):1076–1078

Tissot-Dupont H, Torres S, Nezri M, Raoult D (1999) Hyperendemic focus of Q fever related to sheep and wind. Am J Epidemiol 150(1):67–74

Tissot-Dupont H, Amadei MA, Nezri M, Raoult D (2004) Wind in November, Q fever in December. Emerg Infect Dis 10(7):1264–1269

To, H, Sakai R, Shirota K, Kano C, Abe S, Sugimoto T, Takehara K, Morita C, Takashima I, Maruyama T, Yamaguchi T, Fukushi H, Hirai K (1998) Coxiellosis in domestic and wild birds from Japan. J Wildl Dis 34(2):310–316

Toman R, Skultety L, Ihnatko R (2009) *Coxiella burnetii* glycomics and proteomics–tools for linking structure to function. Ann N Y Acad Sci 1166:67–78

van Asseldonk MA, Prins J, Bergevoet RH (2013) Economic assessment of Q fever in The Netherlands. Prev Vet Med 112(1–2):27–34

van der Brom R, van Engelen E, Vos J, Luttikholt SJ, Moll L, Roest HIJ, van der Heijden HMJF, Vellema P (2013) Detection of *Coxiella burnetii* in the bulk tank milk from a farm with vaccinated goats, by using a specific PCR technique. Small Rumin Res 110(2–3):150–154

Van der Hoek W (2012) The 2007–2010 Q fever epidemic in The Netherlands: risk facotrs and risk groups, unpublished thesis University Utrecht

van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, ter Schegget R, Hackert V, van Duynhoven Y (2010) Q fever in The Netherlands: an update on the epidemiology and control measures. Euro Surveill 15(12):1–4

van der Hoek W, Hunink J, Vellema P, Droogers P (2011a) Q fever in The Netherlands: the role of local environmental conditions. Int J Environ Health Res 21(6):441–451

van der Hoek W, Meekelenkamp JC, Leenders AC, Wijers N, Notermans DW, Hukkelhoven CW (2011b) Antibodies against *Coxiella burnetii* and pregnancy outcome during the 2007–2008 Q fever outbreaks in The Netherlands. BMC Infect Dis 11:44

van Moll P, Baumgartner W, Eskens U, Hanichen T (1993) Immunocytochemical demonstration of *Coxiella burnetii* antigen in the fetal placenta of naturally infected sheep and cattle. J Comp Pathol 109(3):295–301

van Roeden SE, Bleeker-Rovers CP, de Regt MJA, Kampschreur LM, Hoepelman AIM, Wever PC, Oosterheert JJ (2018) Treatment of chronic Q fever: clinical efficacy and toxicity of antibiotic regimens. Clin Infect Dis 66(5):719–726

Van Steenbergen JE, Morroy G, Groot CA, Ruikes FG, Marcelis JH, Speelman P (2007) An outbreak of Q fever in The Netherlands–possible link to goats. Ned Tijdschr Geneeskd 151(36):1998–2003

van Vliet JA (2009) History of notification [in Dutch]. Tijdschrift voor Infectieziekten 2(2):51–60

Wegdam-Blans MC, Nabuurs-Franssen MN, Horrevorts AM, Peeters MF, Schneeberger PM, Bijlmer HA (2010) Laboratory diagnosis of acute Q fever. Ned Tijdschr Geneeskd 154:A2388

Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, van Kasteren ME, Notermans DW, Renders NH, Bijlmer HA, Lestrade PJ, Koopmans MP, Nabuurs-Franssen

MH, Oosterheert JJ, Dutch Q, f. C. G. (2012) Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Inf Secur 64(3):247–259

Weisburg WG, Dobson ME, Samuel JE, Dasch GA, Mallavia LP, Baca O, Mandelco L, Sechrest JE, Weiss E, Woese CR (1989) Phylogenetic diversity of the *Rickettsiae*. J Bacteriol 171(8): 4202–4206

Welsh HH, Lennette EH, Abinanti FR, Winn JF (1958) Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. Ann N Y Acad Sci 70(3):528–540

Wouda W, Dercksen DP (2007) Abortion and stillbirth among dairy goats as a consequence of *Coxiella burnetii*. Tijdschr Diergeneeskd 132(23):908–911

Zhang GQ, To, H, Yamaguchi T, Fukushi H, Hirai K (1997) Differentiation of *Coxiella burnetii* by sequence analysis of the gene (com1) encoding a 27-kDa outer membrane protein. Microbiol Immunol 41(11):871–877

Zhang G, To H, Russell KE, Hendrix LR, Yamaguchi T, Fukushi H, Hirai K, Samuel JE (2005) Identification and characterization of an immunodominant 28-kilodalton *Coxiella burnetii* outer membrane protein specific to isolates associated with acute disease. Infect Immun 73(3): 1561–1567

Cysticercosis

15

A Potentially Fatal Neglected Disease Still Present in Developing Countries

Agnès Fleury, Edda Sciutto, Andrea Toledo, Aline S. de Aluja, and Arturo Carpio

Contents

We Have the Knowledge, We Have the Tools, and Although the Epidemiological Situation Is Improving in Some Contexts, This Is Not Generalized, and Certainly Not Fast Enough

A. Fleury (✉)
Instituto de Investigaciones Biomédicas, Departamento de Medicina Genómica y Toxicología Ambiental, Universidad Nacional Autónoma de México, Instituto Nacional de Neurología y Neurocirugía, Secretaría de Salud, México City, Mexico
e-mail: afleury@iibiomedicas.unam.mx

E. Sciutto
Instituto de Investigaciones Biomédicas, Departamento de Inmunología, Universidad Nacional Autónoma de México, Mexico City, Mexico

A. Toledo
Facultad de Medicina, División de Investigación, Universidad Nacional Autónoma de México, Mexico City, Mexico

A. S. de Aluja
Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México City, Mexico
e-mail: aline@servidor.unam.mx

A. Carpio
Escuela de Medicina, Universidad de Cuenca, Cuenca, Ecuador

G.H. Sergievsky Center, Columbia University, New York, NY, USA
e-mail: arturo.carpio@ucuenca.edu.ec

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_13

Abstract

Taenia solium cysticercosis affects both humans and pigs. It has been considered an eradicable disease, and yet its prevalence remains stable in most endemic countries, due to the persistence of risk factors usually associated with the marginalization of an important sector of the population. In this chapter we will review key aspects of its epidemiology, clinical features, diagnosis, treatment, and prevention.

Keywords

Taenia solium · Cysticercosis · Neglected disease

15.1 Introduction

The taeniosis-cysticercosis complex is, in essence, a disease of the poor. Therefore, it is still found in low-income countries where hygiene is lacking, and where people live in close contact with pigs. The adult tapeworm *Taenia solium* lives in the human intestine, its only known definitive host, where it produces thousands of eggs. After ingestion of eggs by humans or pigs, they turn into oncospheres, penetrate the intestinal wall, and become metacestodes (cysticerci) which then can lodge in different tissues including the central nervous system (Sciutto et al. 2000; Larralde and Aluja 2006). Infection of pigs, natural coprophages, occurs if they have access to latrines or if they roam freely in villages and fields to find their food. Humans can also ingest eggs during consumption of contaminated food, through close contact with a carrier or through self-infection if they are themselves carriers of *T. solium*. The cycle is complete when humans ingest undercooked pork containing the parasite larva which develops into a tapeworm in their intestine. In this chapter, we will review several aspects of this potentially very serious zoonosis, including its epidemiology, clinical manifestations, diagnosis, treatment and available preventive measures.

15.1.1 Epidemiology in Humans

15.1.1.1 Epidemiology on a Domestic Level

As the parasite life cycle may suggest, infection with the adult parasite will mainly occur in rural areas where pigs are slaughtered by their owners mostly for local consumption, without previous sanitary inspection. Indeed, in a study conducted in Honduras, where 328 urban and rural inhabitants were examined for intestinal parasites, only one, coming from a rural area, was identified as a *T. solium* carrier (Sánchez et al. 1998). In another study, including 606 rural and urban individuals in northern Vietnam, the only detected *T. solium* carrier was also a rural inhabitant (Somers et al. 2006). The fact that infected pigs are mainly consumed in rural areas has been shown in different studies. Particularly, in a work conducted in rural villages and urban markets of Congo, pigs diagnosed as infected by tongue inspection were found in rural villages only (Praet et al. 2010). Also, it was reported that rural pigs in Mexico are mostly consumed within the locality where they were reared (Morales et al. 2006).

Data on taeniosis epidemiology is scarce, and different factors could explain this fact. One of them is that the infection is frequently asymptomatic. Symptoms and signs such as pain, weight loss, and fatigue have been described, but they are nonspecific, and patients frequently will not attend medical services. Also, laboratory diagnosis is not easy, requiring proglottid or egg identification in stool. The Kato method (Thienpont et al. 1986) is the most used in endemic countries but is not able to distinguish between *Taenia* species. Coproantigen ELISA and copro-PCR exist (Allan et al. 1992, Ng-Nguyen et al. 2017), but the standardization of their exact performances needs additional studies (Praet et al., 2013).

The epidemiology of the infection by *T. solium* larvae (cysticercosis) has different characteristics. The continuous and increasing exchanges between rural and urban areas concerning foods and people explain why cysticercosis infection is not confined to rural areas but can indeed affect all levels of society. A well-known example of this situation was the infection of a New York Jewish family due to the contact with their domestic employee who carried the adult helminth (Schantz et al. 1992). Also, infected urban inhabitants represent a substantial part of patients in several case series (Fleury et al. 2004, Marcin Sierra et al. 2017, Agapejev 2011).

15.1.1.2 Epidemiology on the International Level

As the parasite life cycle shows, endemic regions are those where pigs can be in contact with human feces and where sanitary control is not enforced in all areas.

This explains why almost all non-Muslim underdeveloped countries of Asia, Africa, and Latin America are endemic. The disease distribution map is explicit on this point (Fig. 1; Wertheim et al. 2012).

Interestingly, key differences in the clinical presentation of cysticercosis between continents have been reported. Extra-neurological presentation seems to be very rare in Latin America (e.g., Mexico) compared with Asia and Africa. Likewise, in cases

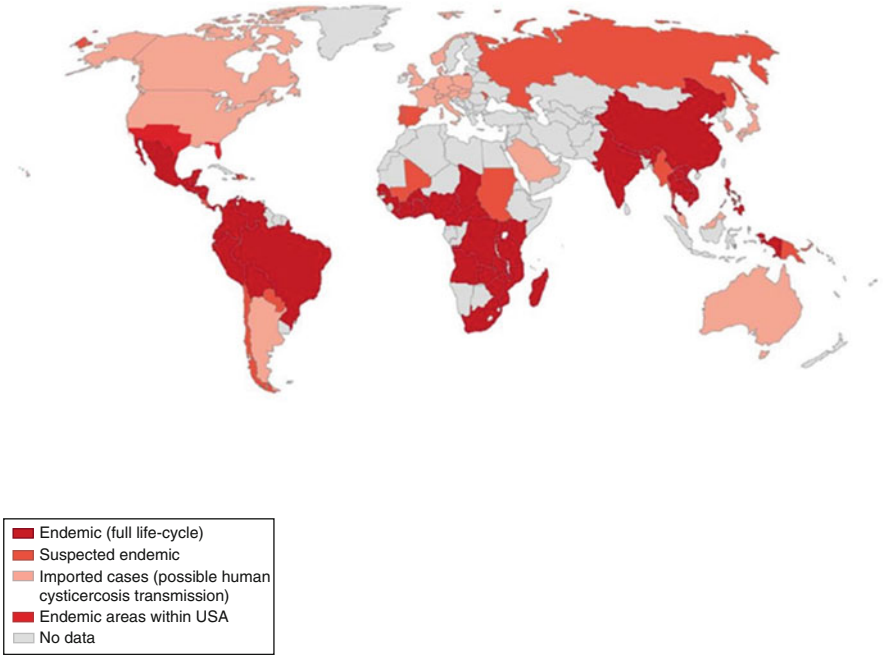

Fig. 1 Global spatial distribution of cysticercosis (updated: January 2010). (From: Wertheim et al. 2012)

of central nervous system (CNS) infection, parasite location in the subarachnoid space and ventricular system seems to be more frequent in Latin America than in India and Africa, where parenchymal location is more frequent (Singh 1997). The reasons for these differences are probably multiple, involving both biological and environmental factors (Fleury et al. 2010). Particularly, although not yet demonstrated, genetic factors, both of the human host and the parasite, might contribute to them. Also, it was recently shown that the intensity of infectious pressure can influence the differences between parenchymal/extra-parenchymal frequencies (Hamamoto Filho et al. 2020). Indeed, time between infection and diagnosis differ between these two groups of patients. The latency seems to be around 5 years in case of parenchymal localization, while it can be around 15–20 years in cases of extra-parenchymal location. So, in case of decrease of infectious pressure and decrease of new cases, we will first observe a reduction of parenchymal NC cases followed by a reduction of extraparenchymal ones (Hamamoto Filho et al. 2020). In this context, the higher ratio of "extraparenchymal NCC cases/parenchymal NCC cases" in Latin America compared, as an example, to India, could be a sign of a current lower incidence of NCC in the former compared to the latter scenario (Hamamoto Filho et al. 2020).

Since around three decades, a concern was raised on the increase of diagnosed cases in non-endemic countries (countries where the life cycle of the parasite did not

take place, i.e., swine cysticercosis is not present), where cases of cysticercosis or taeniasis have been detected (Del Brutto and García 2012). Such cases have been mainly reported in the United States (O'Neal and Flecker 2015; Serpa and White 2012; Croker et al. 2012; O'Neal et al. 2011) and in Spain (Del Brutto 2012; Ruiz et al. 2011; Esquivel et al. 2005), due to the increase of human migration from endemic countries. As migration of *Taenia solium* carriers keeps occurring, autochthonous cysticercosis infections have been reported in these countries due to contact with *T. solium* eggs.

15.1.2 Epidemiology in Pigs

Similar to human cysticercosis, swine cysticercosis is present in most rural areas of Africa, Asia, and Latin America. The results of recent studies evaluating the prevalence of swine cysticercosis are presented in Table 1. Comparison between them is difficult as different diagnostic tests with different sensitivities were used (cf. above). It is clear, however, that swine infection is still present in some rural areas of the three continents. It is also interesting to note that recent studies (>2012) come mostly from Africa and Asia, probably due to the persistence of a higher activity of *Taenia solium*'s life cycle in these continents.

15.1.3 Burden of Disease

The burden of cysticercosis in humans and pigs and of taeniosis in humans is still difficult to assess, as the disease is frequently asymptomatic both in pigs and humans, and since its diagnosis in humans depends on the use of modern radiological tools frequently unavailable for the affected population in endemic countries. However, several estimates have been made (Bhattarai et al. 2013). Current data on DALYs (disability-adjusted life years), mortality, and economic burden are summarized in Tables 2, 3, and 4.

15.2 Clinical Features

15.2.1 Disease in Humans

Clinical manifestations of neurocysticercosis (NC) vary widely and strongly depend on the cyst number and location as well as on the host's immune response to the parasite (Carabin et al. 2006, 2011; Carpio 2002). There is a marked clinical heterogeneity across geographical areas. Most cases from the Indian subcontinent present single lesions, whereas those from Latin America exhibit few viable cysts (Singh 1997; Singh et al. 2010; Vega et al. 2003). These differences are probably due to complex interactions among host, parasite, and environmental factors (Fleury et al. 2010). As previously said, differences in infection pressure could be involved

Table 1 Prevalence of porcine cysticercosis in different countries

Countries (Ref)	Number of animals	Prevalence (methods used) (%)
Latin America		
Ecuador (Rodriguez-Hidalgo et al. 2006)	646 100	3.6 (tongue exam) 74 (EITB)
México (Morales et al. 2008b)	562	13.3 (tongue exam)
Venezuela (Cortez Alcobedes et al. 2010)	52	65.4 (Ab-ELISA) 42.3 (HP10 Ag-ELISA)
Peru (O'Neal et al. 2012)	548	2 (tongue exam) 46.7 (EITB LLGP)
Peru (Jayashi et al. 2012)	1153	45.2 (EITB)
Peru (Jayashi et al. 2014)	107	16,8 (necropsy examination) 57.9 (EITB)
Peru (Lescano et al. 2019)	464	58 (EITB) 1.8–9.5 (necropsy)
Africa		
Eastern/Southern Western Zambia (Sikasunge et al. 2008)	1691	10.8 tongue examination 23.3 Ag-ELISA
Kongwa, Tanzania (Maganira et al. 2019)	447	17 (B158/B60 Ag-ELISA)
Uganda (Waiswa et al. 2009)	480	8.6 (B158/B60 Ag-ELISA)
Tanzania (Ngowi et al. 2010)	784	7.3 (tongue exam)
Mozambique (Pondja et al. 2010)	661	12.7 (tongue exam) 34.9 (B158/B60 Ag-ELISA)
Burkina Faso (Ganaba et al. 2011)	319	35.4 (B158/B60 Ag-ELISA)
Kenya (Eshitera et al. 2012)	392	22 (tongue exam) 32.8 (HP10 Ag-ELISA)
Cameroon (Ngwing et al. 2012)	499	3.6 (tongue exam) 7.6 (B158/B60 Ag-ELISA)
South Africa (Krecek et al. 2012)	261	57 (B158/B60 or HP10 Ag-ELISA)
Katete, Zambia (Bulaya et al. 2015)	379 (104 pre intervention-207 post-intervention)	13.5–16.4 (B158/B60 Ag-ELISA)
Madagascar (Porphyre et al. 2016)	750	2.3 (EITB, B158/B60 and HP10 Ag-ELISAs, meat inspection)
Nyasa District, Tanzania (Shonyela et al. 2017)	698 330	6.3 (tongue examination) 33.3 (B158/B60 Ag-ELISA)
Eastern Province, Zambia (Chembensofu et al. 2017)	68	53 (B158/B60 Ag-ELISA) 56 dissections full carcasses
Cameroon (Assana et al. 2019)	416	8.7 (B158/B60 Ag-ELISA)
Nairobi, Kenia (Maganira et al. 2019)	700	4.4 (B158/B60 Ag-ELISA)
Gauteng, South Africa (Shongwe et al. 2020)	126	7 (carcass inspection)

(continued)

Table 1 (continued)

Countries (Ref)	Number of animals	Prevalence (methods used) (%)
Uganda (Nsadha et al. 2021)	53	15.1 (dissection of full carcasses)
Asia		
India (Prakash et al. 2007)	200 (Brain)	3 (macro and histopathological exam)
India (Sreedevi et al. 2012)	225	11.1 (carcass exam)
Bali, Indonesia (Wandra et al. 2015)	329	13.1 (carcass exam)
Nai Pyi Taw, Myanmar (Khaing et al. 2015)	300 364	23.7 (meat inspection)16 (Ab-ELISA)
Bali, Indonesia (Swastika et al. 2016)	392	6.6 (naked eye ELISA) 9.7 (Ab-ELISA)
South-central, Cambodia (Adenuga et al. 2017)	620	4.7 (B158/B60 Ag-ELISA)
Daklak Province, Vietnam (Ng-Nguyen et al. 2018)	1281	0.94 (recombinant Ag T24H EITB)

in the still high prevalence of parenchymal neurocysticercosis and ocular cysticercosis in countries which have a stable infection pressure and in the high proportion of extraparenchymal neurocysticercosis in other countries, which have had a progressive decrease in infection pressure (Hamamoto Filho et al. 2020).

One of the most intriguing aspects of NC is that presumably a high percentage of individuals with NC remain asymptomatic (Fleury et al. 2010). Some patients develop NC clinical manifestations several years after the parasite lodges in the CNS (Carpio 2002), either by inflammation surrounding the parasite or by a mass effect. Studies carried out in the United States, a non-endemic country, including Latin American migrant patients, and in Great Britain, including British soldiers who worked in India for a period of 5 years, have allowed to specify that this period is around 5 years for parenchymal NC and about 20 years for extraparenchymal NC (Dixon and Hargreaves 1944; Nash et al. 2020). In addition, a recent study shows the ability of parasites to survive for a long time in the extraparenchymal location and explains the chronicity of the disease in some patients (Murrieta et al. 2021).

Current information based on evidence has confirmed that NC is not a singular disease. Location of parasites in the parenchymal or in the extraparenchymal compartments determine two distinct diseases from a clinical, immunological, and pathophysiological perspective (Marcin Sierra et al. 2017, Carpio et al. 2016). Particularly, the clinical signs of parenchymal cysts are usually benign; on the contrary, the clinical presentation of extraparenchymal cyst location is life-threatening and may lead to permanent sequelae (Estañol et al. 1986). The prominent role of the inflammatory reaction associated with the presence of parasites in the two locations is now well defined (Hamamoto Filho et al. 2021). Most symptomatic parenchymal NC patients show seizures as the only clinical manifestation and their neurological status is usually normal (Carabin et al. 2011; Carpio et al. 1998).

Table 2 Studies evaluating DALYs (disability-adjusted life years)

	Total YLL[a]		Total YLD[b]		DALYs per thousand persons-year
	Value	95% CR	Value	95% CR	
Mexico (Bhattarai et al. 2012)	7,062 (28%)	5,509–8,818	18,278 (72%)	5,891–39,238	0.25 (0.12–0.46)
Cameroon (Praet et al. 2009)	39,017	8,195–95,513	6,821	14,108–103,469	9 (2.8–20.4)
Global Burden of Diseases (Murray et al. 2012)					0.07 (0.05–0.1)
Cameroon (Winkler and Richter 2015)					9
Tanzania (Trevisan et al. 2017)	13,076	2,250–37,713	18,788	5,672–40,300	0.7 (0.2–1.6)
India (Singh, et al. 2016)					1.73 (0.82–3.39)
Mozamique (Trevisan et al. 2018)	932	781–1,088	806	415–1,368	6 (4–8)
Burkina Faso, Egypt, Ethiopia, Kenia, Nigeria, Uganda (Herrera -Araujo et al. 2020)	8,542		26,916		35.5

[a]YLL years of life lost due to premature mortality
[b]YLD years of life lost due to time lived in a disability state

Table 3 Mortality due to neurocysticercosis

	Mortality (deaths per million population)			
	Age-adjusted annual mortality rates		Crude mortality rates	
	%	95% CI	%	95% CI
United States (Sorvillo et al. 2007)	0.06	0.05–0.07		
Brazil (Santo 2007)	1.68	1.58–1.78		
California/USA (Sorvillo et al. 2004)			0.33	0.27–0.38
Oregon(USA (Townes et al. 2004)			0.29	0.11–0.64
Tanzania (Trevisan et al. 2017)			0.21	0.037–0.612
Brazil (Martins-Melo et al. 2016)	0.97	0.90–1.33	0.82	0.70–0.96
Angonia, Mozambique (Trevisan et al. 2018)			0.004	0.003–0.005
Burkina Faso, Egypt, Ethiopia, Kenia, Nigeria, Uganda (Herrera -Araujo et al. 2020)			0.160	

Table 4 Economic burden

	Population in the area	Cost (annual)
California/UA (total hospital charges) 2009 (Croker et al. 2012)	39,434,956	> 17 million ($)
West Cameroon (Praet et al. 2009)		
Global cost		10.3 million € (95% CR 6.9–14.7)
Human cysticercosis	5,065,382	95.3%
Porcine cysticercosis	450,000	4.7%
Individual cost of NCC associated epilepsy		194 €
Eastern Cape Province, South Africa (2004) (Carabin et al. 2006)		
Global cost		15–27.5 million €
Human cysticercosis	7,088,000	73.1–85.4%
Porcine cysticercosis		14.6–26.9%
Individual cost of NCC associated epilepsy		US$ $ 632–844
Los Angeles County (hospital charges) 1991–2008 (Croker et al. 2010)		US$ 7.9 million ($)[a]
Peru (Individual total cost of patients during the first 2 years of treatment) (Rajkotia et al. 2007)		US$ 996 ± 80 $[b]
Tanzania (Trevisan et al. 2017)		
Global cost		US$ 7.9 million
Human cysticercosis		US$ 5.1 million
Porcine cysticercosis		US$ 2.7 million
Individual cost of NCC associated epilepsy		US$ 106 (23–281)
Angonia, Mozambique (Trevisan et al. 2018)		
Global cost		US$ 93,370 (39,483–201,463)
Human cysticercosis		US$ 71,088 (27,168–165,816)
Porcine cysticercosis		US$ 22,282 US (12,315–35,647)
Mexico (Bhattarai et al. 2019)		
Global cost	125,236,587	US$ 215,775,056 (109,309,560–361,924,224)
Human cysticercosis		US$ 235 million (128–379 million)
Porcine cysticercosis		US$ 19.5 million (5.7–35.9 million)
Individual cost of NCC associated epilepsy		US$ 436 (296–604)

[a]Average annual charge
[b]Representing 54% of a minimum wage salary during the first year of treatment and 16% during the second

Furthermore, most of the patients with seizures do not evolve to epilepsy (Carpio et al. 2019). Focal neurological deficits, when present, are usually transient over a few days, weeks, or months, with periods of remission and relapse, probably due to different evolutionary stages of the parasite (Carpio 2002). Headache and increased intracranial pressure are frequent in extraparenchymal cyst location patients (Cárdenas et al. 2010; Fleury et al. 2011; Marcin Sierra et al. 2017). This location is found in about one-third of patients. Acute hydrocephalus secondary to intraventricular cysts and chronic hydrocephalus due to subarachnoid cysts, arachnoiditis, or ependymitis are the most frequent causes of this syndrome (Agapejev et al. 2007). Increased intracranial pressure also occurs in patients with cysticercal encephalitis due to the associated high inflammatory reaction (Carpio 2002; Cárdenas et al. 2010; Fleury et al. 2011).

Spinal cord cysticercosis is rare (Alsina et al. 2002). Patients experience nonspecific clinical manifestations, such as nerve root pain or spinal cord compression syndromes, in accordance with the level of the lesion. Massive cysticercal infection of striated muscles occasionally produces a clinical picture of generalized weakness associated with muscle pseudohypertrophy.

Classically, NC predominantly affects adults in the third and fourth decade of life, being relatively uncommon in children (Kelvin et al. 2009; Sáenz et al. 2006). Most pediatric cases show a single transitional cyst that resolves spontaneously over a few months and do not require any treatment apart from symptomatic and anti-seizure drugs (ASD) (Kelvin et al. 2011; Singh et al. 2010). However, severe forms of NC may exceptionally occur in younger patients, including cysticercal encephalitis, which results in permanent neurologic sequels, such as amaurosis. Hydrocephalus and intraventricular NC are extremely rare in children (Agapejev et al. 2007; Carpio 2002).

15.2.2 Disease in Pigs

In our experience, pigs rarely show definite signs of the parasitosis. Signs are inconspicuous even in animals harboring several larvae in the brain, although they may show somnolence and remain inactive during longer periods.

Convulsions occur mostly at night, during sleep. In a study evaluating presence of seizures in infected pigs (Trevisan et al. 2016), 2 of 16 (12.5%) presented severe seizures. The two symptomatic animals were significantly older than the asymptomatic ones. The total number of parasites and their distribution and localization in the brain were not different between symptomatic and asymptomatic animals.

No significant hematological change was detected in a sample group of 17 pigs (Royo Martínez 1996). In one study (Prasad et al. 2006), the following symptoms were found to be very specific for cysticercosis: excessive salivation (dribbling of saliva), excessive blinking (5–10 blinks/min), and tearing (trickling tears from the eye), as well as single subconjunctival nodules. Unfortunately, these symptoms have not been confirmed by other authors, and thus, diagnosis of pig cysticercosis is impossible on clinical ground alone.

15.3 Diagnosis of Cysticercosis

15.3.1 Diagnosis in Humans

NC diagnosis cannot rely on clinical grounds alone, since no typical clinical picture of NC exists. As previously stated, the most common clinical sign of parenchymal NC is epileptic seizure, which occurs in 60–90% of cases, followed by headache, focal deficits, and psychiatric and cognitive symptoms (Carpio 2002; Rodrigues et al. 2012; Marcin Sierra et al. 2017). Diagnosis of extraparenchymal NC is even more difficult, considering that unspecific symptoms and signs of intracranial hypertension and meningitis may occur, either with or without signs of cerebrospinal fluid inflammation (Cárdenas et al. 2010; Fleury et al. 2011; Marcin Sierra et al. 2017).

NC diagnosis is mainly done by neuroimaging. New imaging techniques, including computed tomography (CT) and magnetic resonance imaging (MRI), have improved the detection of scolex, which can be considered pathognomonic of neurocysticercosis (Fig. 2) (Lucato et al. 2007; Mont'Alverne Filho et al. 2011). Imaging procedures allow visualizing the vesicular, colloidal, granularnodular, and calcified phases of the parasite in CNS (Carpio et al. 2013; Escobar 1983) (Fig. 2). MRI is more sensitive than CT in diagnosing viable and degenerating cysticerci, as well as cysts located in the ventricles or the subarachnoid space. In cases of

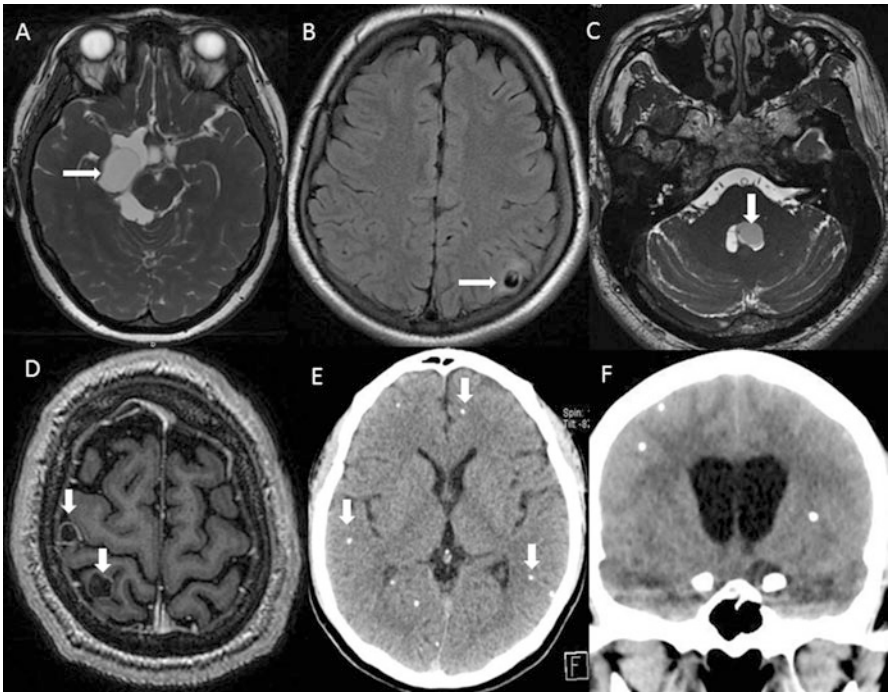

Fig. 2 Neurocysticercosis images

extraparenchymal parasites, 3D MRI sequences increase significantly the sensitivity of the study to diagnose vesicular parasites (Carrillo Mezo et al. 2015). However, CT is more sensitive to detect calcifications (Carpio et al. 2013).

There is no ideal immunological test for diagnosing NC yet. The difficulties of developing a sensitive and specific immunological test for NC diagnosis stem from the characteristics of the disease itself. Different immunological tests have been developed. The most commonly employed methods aiming to detect specific antibodies are enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) assays (Proaño-Narvaez et al. 2002; Tsang et al. 1989). They are useful to identify individuals who have had systemic contact with the parasite at some time. Seropositivity, however, does not necessarily mean an active systemic infection or central nervous system involvement at any time (Carpio 2002). Recently, a study was performed to compare the validity of seven immunodiagnostic tests. The results allow the conclusion that the simple and low-cost ELISA *Taenia solium* antibody instead of EITB is recommended to support NCC diagnosis (Hernández et al. 2019).

Antigen detection by monoclonal or polyclonal antibodies using the ELISA technique has also been developed (Brandt et al. 1992; Fleury et al. 2007, 2013). Detection of specific antigens in serum or CSF by ELISA in patients with parasites located in the subarachnoid space or the ventricular system is a specific sign of parasite viability and may be used to evaluate treatment response (Fleury et al. 2007, 2013).

In spite of the current immunological and imaging advances, NC diagnosis is still challenging in many patients. Diagnostic criteria for NC have been proposed (Del Brutto et al. 2001). These diagnostic criteria may be useful to identify patients with parenchymal NC, but not so for patients with extraparenchymal NC (Machado 2010). Recently, a new set of diagnostic criteria for NC, which allow the detection of NC with a sensitivity of 93.2% and specificity of 81.4%, was proposed (Carpio et al. 2016). Moreover, the new criteria allow to differentiate between the parenchymal and extraparenchymal disease. For parenchymal NC, sensitivity reaches 89.8% and specificity 80.7%, while for extraparenchymal NC, sensitivity is 65.9% and specificity 94.9%.

15.3.2 Diagnosis in Pigs

Different tools are available to diagnose pig cysticercosis, but the results they yield are frequently divergent.

First, and perhaps the most traditional diagnostic method, is the visual inspection of the inferior surface of the tongue (Leuckart 1879). This is the most frequently used procedure, even though it is a risky operation for the person who performs it and a source of stress for the animal, leading to significant changes in cortisol levels (Pérez-Torres et al. 2012). The method is specific, but it fails to detect all infected animals, as only heavily infected pigs will present parasites in the tongue muscles. Studies comparing the results of tongue inspection with MRI or necropsy found that tongue-test sensitivity was between 70% and 84% and its specificity was at 100% (Gonzalez et al. 1990; Phiri et al. 2006; Singh et al. 2013). However, the results are

highly variable between studies; one recent study found that tongue inspection only detects 10% of infected animals (Chembensofu et al. 2017), while other found that it was able to detect 91% of the infected animals (Flecker et al. 2017).

Another method used is the neck muscle examination: a small, 4–5-cm-long skin incision is cut on the lateral side of the neck, and a trained volunteer inserts two fingers to palpate any nodule (Singh et al. 2013). This diagnostic method is very invasive and may lead to infectious complications but has shown a sensitivity of 100% and a specificity of 75% (Singh et al. 2013), as neck muscle seems to be one of the most common sites for cysticercus infection.

Eyelid examination is also used to some extent: the presence of cyst nodules is tested by direct visualization (Singh et al. 2013). This method is very specific (100%), but its sensitivity is very low (25%) (Singh et al. 2013).

A number of serological tests have also been evaluated: detection of specific antibodies using different antigens (whole crude lysate, cyst fluid, scolex, cyst wall) by ELISA and EITB or specific antigen detection. As shown in Table 5, these tests have shown varying performance among studies, probably due in part, to different infection intensity.

Table 5 Swine cysticercosis diagnosis: sensitivity and specificity of different immunodiagnostic tests

Country (Ref)	Type of swine infection	Method	Antigen	Sensitivity (%)	Specificity (%)
India (Singh et al. 2013)					
	Natural infection	ELISA	Crude lysate	85	98
		ELISA	Cyst fluid	70	98
		ELISA	Scolex	65	96
		ELISA	Cyst wall	45	98
Peru (Gonzalez et al. 1990)					
	Natural infection	ELISA	Crude lysate	79.2	76.2
		EITB		100	100
Mexico (Sciutto et al. 1998)					
	Experimental infection	Ag-ELISA		83.7	95.9
		ELISA	Cyst fluid	86	95.7
	Natural infection	Ag-ELISA		44.4	45.8
		ELISA	Cyst fluid	55.5	75
		EITB		64.7	59.1
Brasil (Nunes et al. 2000)					
	Experimental + natural infection	ELISA	Crude lysate	85.7	96.4
			Cyst fluid	67.8	98.2
Tanzania (Kabululu et al. 2020)					
	Natural infection	Ag-ELISA		82.7	86.3
Zambia (Chembensofu et al. 2017)					
	Natural infection	Ag-ELISA		68	67

Mexican researchers developed a diagnostic method using portable ultrasonography equipment to examine pig muscles, with very satisfactory results (Herrera et al. 2007). A more recent study found that ultrasonography was 100% sensitive and 90% specific for swine cysticercosis diagnosis (Flecker et al. 2017). So, in spite of the high cost of the equipment, this could be a recommended diagnostic method in some circumstances.

15.4 Treatment

15.4.1 Treatment of Humans

NC treatment should be individualized, based on the pathogenesis and natural history of the disease in each patient (Fig. 3). Therapy is limited in most cases to symptomatic treatment for patients with seizures. Oral mannitol or glycerol is used in patients with high intracranial pressure, and analgesics should be given for headache. Corticosteroids are strongly recommended, on the premise that they reduce inflammation and edema around dying parenchymal cysts, to treat arachnoiditis and, associated to cysticidal drugs, to prevent inflammatory complications, mostly in cases of extraparenchymal localizations (Carpio et al. 2013; Fleury et al. 2011). However, the dosage, the treatment duration and form, and most significantly the administration timing are not clearly defined (Carpio et al. 2008). Recently, the possibility that corticosteroids might favor parasite survival was stated and research aiming to find the best anti-inflammatory treatment must be pursued (Toledo et al. 2018; Palomares-Alonso et al. 2020).

Surgery is now almost restricted to ventricular shunt placement for hydrocephalus and to cases of intraventricular cysts, which are mainly removed by endoscopic

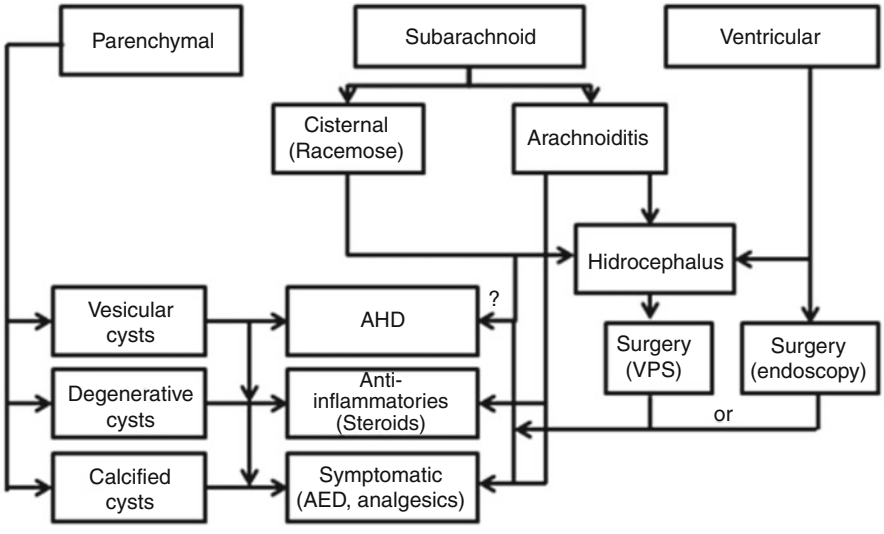

Fig. 3 Neurocysticercosis treatment. (From Carpio et al. 2013)

approach (Torres-Corzo et al. 2010). Transitional or degenerating cysts should not be biopsied or removed if differential diagnosis has been discarded and if mass effect is moderated, since the parasite is dead and will disappear or be calcified spontaneously (Singh et al. 2010).

NC treatment with anti-helminthic drugs (AHD), praziquantel (PZQ) (Robles and Chavarria 1979), and albendazole (ALB) (Xiao et al. 1986) has been available for more than 30 years. PZQ is an acylated isoquinoline-pyrazine with broad anthelminthic activity. Its mechanism of action is not fully understood; however, it is assumed that PZQ changes calcium metabolism and intracellular permeability, with the main effect of inhibiting muscular movements (Garcia-Dominguez et al. 1991). ALB is a benzimidazole with a broad anti-helminthic spectrum, which exerts an anticysticercal effect by inhibiting the glucose uptake through parasitic membranes, thus causing energy depletion (Lacey 1990). There is evidence for the efficacy of AHD treatment for viable parenchymal cysts; however, no controlled clinical trials have so far established definitive doses and treatment duration (Carpio et al. 2013). According to placebo-controlled clinical trials (Carpio et al. 2008; Garcia et al. 2004), AHD are effective in about 30–40% of patients, according to the disappearance of viable parenchymal cysts in imaging studies. The most frequent treatment scheme for PZQ is 50 mg/kg/day for 15 days, and for ALB it is 15 mg/kg/day for 8 days (Carpio et al. 2013). Recently, the combination of the two drugs has been shown to be more effective than ALB alone in the case of multiple parenchymal parasites (Garcia et al. 2016). The more recent Cochrane review (Monk et al. 2021) concluded that ALB probably reduces the recurrence of seizures in people with a single NC cyst (moderate-certainty evidence), while there is no certainty whether ALB reduces seizure recurrence for people with more than one NC cyst (very low-certainty evidence). Also, ALB probably increases the clearance and evolution of cysts in people with NC (moderate-certainty evidence) (Monk et al. 2021).

For extraparenchymal cysts the management is even less clear. While AHD have demonstrated efficacy in some cases, it is clear that not all cases respond to the current treatment (Carpio et al. 2008; Fleury et al. 2011; Osorio et al. 2019). This stresses the urge for looking for new treatment alternatives (Diazgranados-Sánchez et al. 2008).

15.4.2 Treatment of Pigs

Several cysticidal drugs have proven to be effective in destroying the parasite (Gonzalez et al. 2012). Indeed, the treatment of infected pigs has been proposed and successfully tested locally, being oxfendazole the drug most frequently evaluated (Gonzalez et al. 1997; Mkupasi et al. 2013). In spite of their effectiveness, cysticidal drugs have not been extensively used to treat swine cysticercosis, due to practical reasons: treatment is hardly manageable, and the necessity of waiting several months for cysticerci destruction is costly for rural pig breeders. Possible detrimental effects on the environment through the promotion of drug resistance should be considered as well (Domke et al. 2012).

15.5 Preventive Measures

Although this parasitosis has a high impact on human health and on economy, its eradication remains a major challenge.

Different interventions have been proposed and proved effective for control, albeit with varying practical possibilities. The combination of simultaneous actions targeting pigs and humans is probably the approach that achieves the best results.

15.5.1 Focus on Pig-Targeted Actions

Focusing preventive measures on porcine cysticercosis is particularly relevant, since it is the essential step for parasite transmission (Aluja 2008).

In rural communities, pigs become infected because of poverty-related factors, i.e., low hygiene standards and inadequate human feces disposal, which contribute to environment contamination by *T. solium* eggs. In these rural areas, pigs (being natural coprophages) are allowed to freely roam in search of food, favoring them to ingest human feces contaminated with parasite eggs (Copado et al. 2004). This rustic rearing promotes the parasite life cycle, since after ingestion the eggs will evolve to cysticerci and subsequently be delivered to humans via undercooked infected pork meat (Sciutto et al. 2000).

Compulsory meat inspection aiming to avoid infected pork meat consumption seems a reasonable effort. Unfortunately, while this measure is fully operative in official slaughterhouses, it cannot be enforced in rural, hardly accessible small communities where pigs are clandestinely killed and consumed at the occasion of private feasts (Willingham et al. 2010). Educational programs to train the inhabitants of rural communities in good pig sacrificing practices would be a useful intervention to prevent the consumption of undercooked infected meat. Here, too, the challenge will be covering all persons involved in this practice.

Making pig confinement obligatory to avoid contact with human feces would clearly help to interrupt transmission. While this could be a feasible possibility, the poor economic conditions in these rural communities promote the free-roam foraging of pigs to supplement the meager food their owners provide them with (Thys et al. 2016).

Porcine cysticercosis is a vaccine-preventable disease. Thus, pig vaccination may provide an additional tool for taeniosis-cysticercosis control and prevention.

The first report on successful porcine cysticercosis vaccination was established using total extracts from *T. solium* cysticerci, recovered from naturally infected pigs, as a source of vaccine antigens (Molinari et al. 1997). Since then, various native and subunitary vaccine candidates have been identified, but only a few have been found effective under the complex natural field conditions (Huerta et al. 2001; Morales et al. 2008a; Jayashi et al. 2012). Among them figures the vaccine named S3Pvac and the HP6/Tsol18 vaccine. S3Pvac vaccine is composed by three small peptides (KETc1, KETc12, and GK1 [KETc7]), originally isolated from *Taenia crassiceps* and shared by other cestodes including *T. solium.* Both synthetic S3Pvac (Huerta et al. 2001) and S3Pvac recombinantly expressed in filamentous phages (Morales et al. 2008a, 2011) were successfully tested in the field. HP6 antigen, originally isolated from *Taenia*

saginata (Benitez et al. 1996) and reported to induce high protection levels against bovine cysticercosis (Lightowlers et al. 1996), has been found present in *T. solium* (HP6/*Tsol*18) cysticerci and showed a protective effect against porcine cysticercosis (Assana et al. 2010; Poudel et al. 2019; Nsadha et al. 2021).

Finally, infected pigs could be exchanged by vaccinated pigs, better suited to endure the hardship of their lives.

15.5.2 Focus on Human-Targeted Actions

Here, too, different measures have been taken and showed some efficacy. An aggressive hygiene-promotion campaign in rural areas is an obvious start point, since it can eradicate not only *T. solium*, but many other infectious diseases transmitted by human feces (Yap et al. 2012). This type of measures did allow the control of *T. solium*-related diseases in most European countries in the early twentieth century.

However, achieving the same in currently endemic countries would require an economic and social development that does not seem achievable in the near future.

Massively administered human cestocidal treatment to reduce the number of tapeworm carriers is another measure which has been applied in different circumstances (Haby et al. 2020). Human deworming can be achieved using a single oral dose of niclosamide (2 g in adults, 1 g in children), praziquantel (10 mg/kg), or albendazole (400 mg/day) for three consecutive days (Pearson and Guerrant 1983; Pearson and Hewlett 1985; Haby et al. 2020). A priori, niclosamide should be preferred as it is not absorbed by the intestinal mucosa, thus avoiding possible symptoms derived from an occult NC. This strategy has been used in several studies in endemic countries, either administered alone (Cruz et al. 1989; Allan et al. 1997; Sarti et al. 2000; Wu et al. 2012; Ramiandrasoa et al. 2020) or associated with other preventive measures (Garcia et al. 2006, 2010).

Health education programs aiming to promote a better understanding of the parasite transmission mechanisms and to improve the hygienic behavior, pig management, and sanitary conditions which foster transmission is another measure that has been carried out (Keilbach et al. 1989; Sarti et al. 1997; Ngowi et al. 2008; Wohlgemut et al. 2010; Wu et al. 2012).

These interventions, all of which have been implemented on a small to medium scale, have produced generally relevant immediate results, although long-term evaluations are most often lacking.

Finally, it should be noted that the role of national health authorities in these programs is essential and must be promoted. Researchers alone are not able to implement nationwide programs, the only realistic way to eradicate this parasitic disease.

15.6 Conclusions and Unsolved Issues

Cysticercosis is considered a neglected "tools-ready disease" according to WHO (2007), and as a potentially eradicable disease since 1993 (Recommendations of the International Task Force for Disease Eradication). Eradication is feasible, because—as

we have stated earlier—(1) humans and pigs are the only affected species; (2) humans (the definitive host) are the only source of pig *T. solium* infection; and (3) there exist efficient intervention strategies which can interrupt the parasite life cycle.

In spite of this, cysticercosis is still endemic in most countries of Latin America, Asia, and Africa, although its burden is difficult to estimate, and some recent data seem to show a decreasing tendency in some of these countries.

The reasons for this situation are multiple, a major cause being that it is a "forgotten disease of forgotten people" (Hotez 2008), which does not motivate governments to take the necessary measures. As said before, instituting nationwide government control programs is one of the main unresolved issues.

With regard to neurocysticercosis, several items remain unsolved at this point.

Focusing on diagnosis, for instance, neuroradiological studies (currently representing the gold standard), is a strategy not available to all affected population due to its cost; therefore, improving the immunodiagnostic techniques is mandatory, as all existent tools show low sensitivity, particularly in cases of single-cyst infection. At a therapeutic level, further research is required to understand why some patients do not respond to specific cysticidal treatment and to develop alternative treatment approaches for these cases. Additionally, the adequate management of the inflammatory reaction is a pending problem. Corticosteroids are of great utility to avoid inflammatory complications, but they show severe collateral effects, and their possible role in the lack of response to specific treatment forbids their indiscriminate use (Toledo et al. 2018). Further research is much needed with that respect, too.

Finally, it should be stressed that taeniosis/cysticercosis is an eradicable disease and that firm government commitment in affected countries is key to reach this goal.

15.7 Cross-References

- ▶ Cryptosporidium and Cryptosporidiosis: Trickle or Treat?
- ▶ Zoonoses and Poverty: The Multiple Burdens of Zoonoses in Low- and Middle-Income Countries
- ▶ Zoonotic Diseases of Swine: Food-Borne and Occupational Aspects of Infection

References

Adenuga A, Mateus A, Ty C, Borin K, Holl D, San S, Duggan V, Clark M, Smith GJD, Coker R, Vaughn A, Rudge JW (2017) Seroprevalence and awareness of porcine cysticercosis across different pig production systems in south-central Cambodia. Parasite Epidemiol Control 3:1–12

Agapejev S (2011) Neurocysticercosis: the enigmatic disease. Cent Nerv Syst Agents Med Chem 11:261–284

Agapejev S, Pouza AF, Bazan R, Faleiros AT (2007) Clinical and evolutive aspects of hydrocephalus in neurocysticercosis. Arq Neuropsiquiatr 65:674–680

Allan JC, Craig PS, Garcia Noval J, Mencos F, Liu D, Wang Y, Wen H, Zhou P, Stringer R, Rogan M et al (1992) Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology 104:347–356

Allan JC, Velasquez-Tohom M, Fletes C, Torres-Alvarez R, Lopez-Virula G, Yurrita P, Soto de Alfaro H, Rivera A, Garcia-Noval J (1997) Mass chemotherapy for intestinal *Taenia solium* infection: effect on prevalence in humans and pigs. Trans R Soc Trop Med Hyg 91:595–598

Alsina GA, Johnson JP, McBride DQ, Rhoten PRL, Mehringer CM et al (2002) Spinal neurocysticercosis. Neurosurg Focus 12:1–7

Aluja AS (2008) Cysticercosis in pigs. Curr Top Med Chem 8:368–374

Assana E, Kyngdon CT, Gauci CG, Geerts S, Dorny P, De Deken R, Anderson GA, Zoli AP, Lightowlers MW (2010) Elimination of *Taenia solium* transmission to pigs in a field trial of the HP6-TSOL18 vaccine in Cameroon. Int J Parasitol 40:515–519

Assana E, Awah-Ndukum J, Djonmaïla JD, Zoli AP (2019) Prevalence of porcine *Taenia solium* and *Taenia hydatigena* cysticercosis in Cameroon. Prev Vet Med 169:104690. https://doi.org/10.1016/j.prevetmed.2019.104690

Benitez L, Garate T, Harrison LJ, Kirkham P, Brookes SM, Parkhouse RM (1996) Cloning and sequencing of the gene encoding the principal 18 kDa secreted antigen of activated oncospheres of *Taenia saginata*. Mol Biochem Parasitol 78:265–268

Bhattarai R, Budke CM, Carabin H, Proaño JV, Flores-Rivera J, Corona T, Ivanek R, Snowden KF, Flisser A (2012) Estimating the non-monetary burden of neurocysticercosis in Mexico. PLoS Negl Trop Dis 6:e1521

Bhattarai R, Carabin H, Budke C (2013) The burden of cysticercosis. In: Sibat HF (ed) Novel aspects on cysticercosis and neurocysticercosis. ISBN: 978-953-51-0956-3. InTech, Rijeka. https://doi.org/10.5772/51668. http://www.intechopen.com/books/novel-aspects-on-cysticercosis-andneurocysticercosis/the-burden-of-cysticercosis

Bhattarai R, Carabin H, Proaño JV, Flores-Rivera J, Corona T, Flisser A, León-Maldonado L, Budke CM (2019) The monetary burden of cysticercosis in Mexico. PLoS Negl Trop Dis 13: e0007501. https://doi.org/10.1371/journal.pntd.0007501

Brandt JR, Geerts S, De Deken R et al (1992) A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. Int J Parasitol 22:471–477

Bulaya C, Mwape KE, Michelo C, Sikasunge CS, Makungu C, Gabriel S, Dorny P, Phiri IK (2015) Preliminary evaluation of Community-Led Total Sanitation for the control of *Taenia solium* cysticercosis in Katete District of Zambia. Vet Parasitol 207:241–248. https://doi.org/10.1016/j.vetpar.2014.12.030

Carabin H, Krecek RC, Cowan LD, Michael L, Foyaca-Sibat H, Nash T, Willingham AL (2006) Estimation of the cost of *Taenia solium* cysticercosis in Eastern Cape Province, South Africa. Tropical Med Int Health 11:906–916

Carabin H, Ndimubanzi PC, Budke CM, Nguyen H, Qian Y et al (2011) Clinical manifestations associated with neurocysticercosis: a systematic review. PLoS Negl Trop Dis 5:e1152

Cárdenas G, Jung H, Ríos C, Fleury A, Soto-Hernández JL (2010) Severe cysticercal meningitis: clinical and imaging characteristics. Am J Trop Med Hyg 82:121–125

Carpio A (2002) Neurocysticercosis: an update. Lancet Infect Dis 2:751–762

Carpio A, Escobar A, Hauser WA (1998) Epilepsy and cysticercosis: a critical review. Epilepsia 39: 1025–1040

Carpio A, Kelvin E, Bagiella E, Leslie D, Leon P et al (2008) The effects of albendazole treatment on neurocysticercosis: a randomized controlled trial. J Neurol Neurosurg Psychiatry 79:1050–1055

Carpio A, Fleury A, Hauser WA (2013) Neurocysticercosis: five new things. Neurol Clin Pract 3: 118–125

Carpio A, Fleury A, Romo ML, Abraham R, Fandiño J, Durán JC, Cárdenas G, Moncayo J, Leite Rodrigues C, San-Juan D, Serrano-Dueñas M, Takayanagui O, Sander JW (2016) New diagnostic criteria for neurocysticercosis: reliability and validity. Ann Neurol 80:434–442. https://doi.org/10.1002/ana.24732

Carpio A, Chang M, Zhang H, Romo ML, Jaramillo A, Hauser WA, Kelvin EA (2019) Exploring the complex associations over time among albendazole treatment, cyst evolution, and seizure outcomes in neurocysticercosis. Epilepsia 60:1820–1828. https://doi.org/10.1111/epi.16302

Carrillo Mezo R, Lara García J, Arroyo M, Fleury A (2015) Relevance of 3D magnetic resonance imaging sequences in diagnosing basal subarachnoid neurocysticercosis. Acta Trop 152:60–65. https://doi.org/10.1016/j.actatropica.2015.08.017

CDC (1993) Recommendations of the International Task Force for Disease Eradication. MMWRRecomm Rep 42:1–38

Chembensofu M, Mwape KE, Van Damme I, Hobbs E, Phiri IK, Masuku M, Zulu G, Colston A, Willingham AL, Devleesschauwer B, Van Hul A, Chota A, Speybroeck N, Berkvens D, Dorny P, Gabriël S (2017) Re-visiting the detection of porcine cysticercosis based on full carcass dissections of naturally *Taenia solium* infected pigs. Parasit Vectors 10:572. https://doi.org/10.1186/s13071-017-2520-y

Copado BF, de Aluja AS, Mayagoitia L, Galindo F (2004) The behaviour of free ranging pigs in the Mexican tropics and its relationships with human faeces consumption. Appl Anim Behav Sci 88:243–252

Cortez Alcobedes MM, Boggio G, Guerra Mde L, de Gavidia MR, Rojas Reyes GC, Ferrer E, Lares M, Alviarez Y, Harrison LJ, Parkhouse RM (2010) Evidence that active transmission of porcine cysticercosis occurs in Venezuela. Trop Anim Health Prod 42:531–537

Croker C, Reporter R, Mascola L (2010) Use of statewide hospital discharge data to evaluate the economic burden of neurocysticercosis in Los Angeles County (1991–2008). Am J Trop Med Hyg 83:106–110

Croker C, Redelings M, Reporter R, Sorvillo F, Mascola L, Wilkins P (2012) The impact of neurocysticercosis in California: a review of hospitalized cases. PLoS Negl Trop Dis 6:e1480

Cruz M, Davis A, Dixon H, Pawlowski ZS, Proano J (1989) Operational studies on the control of *Taenia solium* taeniasis/cysticercosis in Ecuador. Bull World Health Organ 67:401–407

Del Brutto OH (2012) Neurocysticercosis in Western Europe: a re-emerging disease? Acta Neurol Belg 112:335–343

Del Brutto OH, García HH (2012) Neurocysticercosis in non endemic countries: time for a reappraisal. Neuroepidemiology 39:145–146

Del Brutto OH, Rajshekhar V, White AC Jr, Tsang VCW, Nash TE, Takayanagui OM et al (2001) Proposed diagnostic criteria for neurocysticercosis. Neurology 57:177–183

Diazgranados-Sánchez JA, Barrios-Arrázola G, Costa JL, Burbano-Pabon J, Pinzón-Bedoya J (2008) Ivermectin as a therapeutic alternative in neurocysticercosis that is resistant to conventional pharmacological treatment. Rev Neurol 46:671–674

Dixon HBF, Hargreaves WH (1944) Cysticercosis (*Taenia solium*) a further 10 years' clinical study covering 284 cases. QJM 13:107–122

Domke AV, Chartier C, Gjerde B, Höglund J, Leine N, Vatn S, Stuen S (2012) Prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep and goats in Norway. Parasitol Res 111:185–193

Escobar A (1983) The pathology of neurocysticercosis. In: Palacios E, Rodriguez-Carbajal J, Taveras JM (eds) Cysticercosis of central nervous system. Charles C Thomas, Springfield, pp 27–54

Eshitera EE, Githigia SM, Kitala P, Thomas LF, Fèvre EM, Harrison LJ, Mwihia EW, Otieno RO, Ojiambo F, Maingi N (2012) Prevalence of porcine cysticercosis and associated risk factors in Homa Bay District, Kenya. BMC Vet Res 8:234

Esquivel A, Diaz-Otero F, Gimenez-Roldan S (2005) Growing frequency of neurocysticercosis in Madrid (Spain). Neurologia 20:116–120

Estañol B, Corona T, Abad P (1986) A prognostic classification of cerebral cysticercosis: therapeutic implications. J Neurol Neurosurg Psychiatry 49:1131–1134

Flecker RH, Pray IW, Santivañez SJ, Ayvar V, Gamboa R, Muro C, Moyano LM, Benavides V, Garcia HH, O'Neal SE (2017) Assessing ultrasonography as a diagnostic tool for porcine cysticercosis. PLoS Negl Trop Dis 11:e0005282. https://doi.org/10.1371/journal.pntd.0005282

Fleury A, Dessein A, Preux PM, Dumas M, Tapia G, Larralde C, Sciutto E (2004) Symptomatic human neurocysticercosis – age, sex and exposure factors relating with disease heterogeneity. J Neurol 251:830–837

Fleury A, Hernández M, Avila M, Cárdenas G, Bobes RJ et al (2007) Detection of HP10 antigen in serum for diagnosis and follow-up of subarachnoidal and intraventricular human neurocysticercosis. J Neurol Neurosurg Psychiatry 78:970–974

Fleury A, Escobar A, Fragoso G, Sciutto E, Larralde C (2010) Clinical heterogeneity of human neurocysticercosis results from complex interactions among parasite, host and environmental factors. Trans R Soc Trop Med Hyg 104:243–250

Fleury A, Carrillo-Mezo R, Flisser A, Sciutto E, Corona T (2011) Subarachnoid basal neurocysticercosis: a focus on the most severe form of the disease. Expert Rev Anti-Infect Ther 9:123–133

Fleury A, Garcia E, Hernández M, Carrillo R, Govezensky T, Fragoso G, Sciutto E, Harrison LJ, Parkhouse RM (2013) Neurocysticercosis: HP10 antigen detection is useful for the follow-up of the severe patients. PLoS Negl Trop Dis 7:e2096

Ganaba R, Praet N, Carabin H, Millogo A, Tarnagda Z, Dorny P, Hounton S, Sow A, Nitiéma P, Cowan LD (2011) Factors associated with the prevalence of circulating antigens to porcine cysticercosis in three villages of Burkina faso. PLoS Negl Trop Dis 5:e927

Garcia H, Pretell E, Gilman R, Martinez et al (2004) A trial of antiparasitic treatment to reduce the rate of seizures due to cerebral cysticercosis. N Engl J Med 350:249–258

Garcia HH, Gonzalez AE, Gilman RH, Moulton LH, Verastegui M, Rodriguez S, Gavidia C, Tsang VC, Cysticercosis Working Group in Peru (2006) Combined human and porcine mass chemotherapy for the control of *T. solium*. Am J Trop Med Hyg 74:850–855

Garcia HH, Gonzalez AE, Rodriguez S, Gonzalvez G, Llanos-Zavalaga F, Tsang VC, Gilman RH (2010) Grupo de Trabajo en Cisticercosis en Perú. Epidemiology and control of cysticercosis in Peru. Rev Peru Med Exp Salud Publica 27:592–597

Garcia HH, Lescano AG, Gonzales I, Bustos JA, Pretell EJ, Horton J, Saavedra H, Gonzalez AE, Gilman RH, Cysticercosis Working Group in Peru (2016) Cysticidal efficacy of combined treatment with Praziquantel and Albendazole for parenchymal brain cysticercosis. Clin Infect Dis 62:1375–1379. https://doi.org/10.1093/cid/ciw134

Garcia-Dominguez C, Correa D, Rabiela MT, Flisser A (1991) Praziquantel treatment of muscle *Taenia solium* cysticercosis. 4. Reversible in vitro effect. Parasitol Res 77:691–696

Gonzalez AE, Cama V, Gilman RH, Tsang VC, Pilcher JB, Chavera A, Castro M, Montenegro T, Verastegui M, Miranda E et al (1990) Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. Am J TropMed Hyg 43:194–199

Gonzalez AE, Falcon N, Gavidia C, Garcia HH, Tsang VC, Bernal T, Romero M, Gilman RH (1997) Treatment of porcine cysticercosis with oxfendazole: a dose-response trial. Vet Rec 141: 420–422

Gonzalez AE, Bustos JA, Jimenez JA, Rodriguez ML, Ramirez MG, Gilman RH, Garcia HH, Cysticercosis Working Group in Peru (2012) Efficacy of diverse antiparasitic treatments for cysticercosis in the pig model. Am J Trop Med Hyg 87:292–296

Haby MM, Sosa Leon LA, Luciañez A, Nicholls RS, Reveiz L, Donadeu M (2020) Systematic review of the effectiveness of selected drugs for preventive chemotherapy for *Taenia solium* taeniasis. PLoS Negl Trop Dis 14:e0007873. https://doi.org/10.1371/journal.pntd.0007873

Hamamoto Filho PT, Singh G, Winkler AS, Carpio A, Fleury A (2020) Could differences in infection pressure be involved in cysticercosis heterogeneity? Trends Parasitol 36:826–834. https://doi.org/10.1016/j.pt.2020.07.003

Hamamoto Filho PT, Fragoso G, Sciutto E, Fleury A (2021) Inflammation in neurocysticercosis: clinical relevance and impact on treatment decisions. Expert Rev Anti-Infect Ther:1–16. https://doi.org/10.1080/14787210.2021.1912592

Hernández M, Astudillo OG, Diego G, de-la-Rosa-Arana JL, Meza-Lucas A, García-Rodea R, Romo ML, Toledo A, Parkhouse RM, Garate T, Sciutto E, Fleury A (2019) Immunodiagnosis of human neurocysticercosis: comparative performance of serum diagnostic tests in Mexico. Parasitol Res 118:2891–2899. https://doi.org/10.1007/s00436-019-06425-4

Herrera GC, de Aluja AS, Méndez AR (2007) El uso de la ultrasonografía para el diagnóstico de la cisticercosis porcina. Rev Vet Méx 38:125–133

Herrera-Araujo D, Mikecz O, Pica-Ciamarra U (2020) Placing a monetary value on the human health component of zoonotic diseases: a methodological note with an application to cysticercosis in Africa. Prev Vet Med 175:104862. https://doi.org/10.1016/j.prevetmed.2019.104862

Hotez PJ (2008) Forgotten people, forgotten diseases: the neglected tropical diseases and their impact on global health and development. ASM, Washington, p 218

Huerta M, de Aluja AS, Fragoso G, Toledo A, Villalobos N, Hernandez M, Gevorkian G, Acero G, Diaz A, Alvarez I, Avila R, Beltran C, Garcia G, Martinez JJ, Larralde C, Sciutto E (2001) Synthetic peptide vaccine against *Taenia solium* pig cysticercosis: successful vaccination in a controlled field trial in rural Mexico. Vaccine 20:262–266

Jayashi CM, Arroyo G, Lightowlers MW, García HH, Rodríguez S, Gonzalez AE (2012) Seroprevalence and risk factors for *Taenia solium* cysticercosis in rural pigs of northern Peru. PLoS Negl Trop Dis 6:e1733

Jayashi CM, Gonzalez AE, Castillo Neyra R, Rodríguez S, García HH, Lightowlers MW, Cysticercosis Working Group in Peru (2014) Validity of the Enzyme-linked Immunoelectrotransfer Blot (EITB) for naturally acquired porcine cysticercosis. Vet Parasitol 199:42–49. https://doi.org/10.1016/j.vetpar.2013.10.004

Kabululu ML, Johansen MV, Mlangwa JED, Mkupasi EM, Braae UC, Trevisan C, Colston A, Cordel C, Lightowlers MW, Ngowi HA (2020) Performance of Ag-ELISA in the diagnosis of *Taenia solium* cysticercosis in naturally infected pigs in Tanzania. Parasit Vectors 13:534. https://doi.org/10.1186/s13071-020-04416-4

Keilbach NM, de Aluja AS, Sarti GE (1989) Programme to control Taeniasis-cysticercosis (*T. solium*): experiences in a Mexican village. Acta Leiden 57:181–189

Kelvin EA, Carpio A, Bagiella E, Leslie D, Leon P, Andrews H et al (2009) The association of host age and gender with inflammation around neurocysticercosis cysts. Ann Trop Med Parasitol 103:487–499

Kelvin EA, Carpio A, Bagiella E, Leslie D, Leon P et al (2011) Seizure in people with newly diagnosed active or transitional neurocysticercosis. Seizure 20:119–125

Khaing TA, Bawm S, Wai SS, Htut Y, Htun LL (2015) Epidemiological survey on porcine cysticercosis in Nay Pyi Taw Area, Myanmar. J Vet Med 2015:340828. https://doi.org/10.1155/2015/340828. Epub 2015 Feb 26

Krecek RC, Mohammed H, Michael LM, Schantz PM, Ntanjana L, Morey L, WerreI SR, Willingham AL 3rd (2012) Risk factors of porcine cysticercosis in the Eastern Cape Province, South Africa. PLoS One 7:e37718

Lacey E (1990) Mode of action of benzimidazoles. Parasitol Today 6:112–115

Larralde C, de Aluja AS (2006) Cisticercosis guía para profesionales de la salud. FCE, Secretaría de Salud, Instituto Nacional de Salud Pública, Fundación Mexicana para la Salud, México

Lescano AG, Pray IW, Gonzalez AE, Gilman RH, VCW T, Gamboa R, Guezala MC, Aybar V, Rodriguez S, Moulton LH, Leontsini E, Gonzalvez G, O'Neal SE, Garcia HH, For The Cysticercosis Working Group In Peru (2019) Clustering of necropsy-confirmed porcine cysticercosis surrounding *Taenia solium* tapeworm carriers in Peru. Am J Trop Med Hyg 100:314–322. https://doi.org/10.4269/ajtmh.18-0296

Leuckart R (1879) The parasites of man and the diseases which proceed from them. A textbook for students and practitioners [trans by WE Hoyle]. Young J Pentland, Edinburgh, p 771

Lightowlers MW, Rolfe R, Gauci CG (1996) *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. Exp Parasitol 84:330–338

Lucato LT, Guedes MS, Sato JR et al (2007) The role of conventional MR imaging sequences in the evaluation of neurocysticercosis: impact on characterization of the scolex and lesion burden. Am J Neuroradiol 28:1501–1504

Machado LR (2010) The diagnosis of neurocysticercosis: a closed question? Arq Neuropsiquiatr 68:1–2

Maganira JD, Mwang'onde BJ, Kidima W, Mwita CJ, Höglund J (2019) Seroprevalence of circulating taeniid antigens in pigs and associated risk factors in Kongwa district, Tanzania. Parasite Epidemiol Control 7:e00123. https://doi.org/10.1016/j.parepi.2019.e00123

Marcin Sierra M, Arroyo M, Cadena Torres M, Ramírez Cruz N, García Hernández F, Taboada D, Galicia Martínez Á, Govezensky T, Sciutto E, Toledo A, Fleury A (2017) Extraparenchymal neurocysticercosis: demographic, clinicoradiological, and inflammatory features. PLoS Negl Trop Dis 11:e0005646. https://doi.org/10.1371/journal.pntd.0005646

Martins-Melo FR, Ramos AN Jr, Cavalcanti MG et al (2016) Neurocysticercosis-related mortality in Brazil, 2000–2011: epidemiology of a neglected neurologic cause of death. Acta Trop 153: 128–136

Mkupasi EM, Sikasunge CS, Ngowi HA, Johansen MV (2013) Efficacy and safety of anthelmintics tested against *Taenia solium* cysticercosis in pigs. PLoS Negl Trop Dis 7:e2200. https://doi.org/10.1371/journal.pntd.0002200

Molinari JL, Rodriguez D, Tato P, Soto R, Arechavaleta F, Solano S (1997) Field trial for reducing porcine *Taenia solium* cysticercosis in Mexico by systematic vaccination of pigs. Vet Parasitol 69:55–63

Monk EJM, Abba K, Ranganathan LN (2021) Anthelmintics for people with neurocysticercosis. Cochrane Database Syst Rev 6:CD000215. https://doi.org/10.1002/14651858.CD000215.pub5

Mont'Alverne Filho FE, Machado Ldos R, Lucato LT, Leite CC (2011) The role of 3D volumetric MR sequences in diagnosing intraventricular neurocysticercosis: preliminar results. Arq Neuropsiquiatr 69:74–78

Morales J, Martínez JJ, Garcia-Castella J, Peña N, Maza V, Villalobos N, Aluja AS, Fleury A, Fragoso G, Larralde C, Sciutto E (2006) *Taenia solium*: the complex interactions, of biological, social, geographical and commercial factors, involved in the transmission dynamics of pig cysticercosis in highly endemic areas. Ann Trop Med Parasitol 100:123–135

Morales J, Martínez JJ, Manoutcharian K, Hernández M, Fleury A, Gevorkian G, Acero G, Blancas A, Toledo A, Cervantes J, Maza V, Quet F, Bonnabau H, de Aluja AS, Fragoso G, Larralde C, Sciutto E (2008a) Inexpensive anti-cysticercosis vaccine: S3Pvac expressed in heat inactivated M13 filamentous phage proves effective against naturally acquired *Taenia solium* porcine cysticercosis. Vaccine 26:2899–2905

Morales J, Martínez JJ, Rosetti M, Fleury A, Maza V, Hernandez M, Villalobos N, Fragoso G, deAluja AS, Larralde C, Sciutto E (2008b) Spatial distribution of *Taenia solium* porcine cysticercosis within a rural area of Mexico. PLoS Negl Trop Dis 2:e284

Morales J, de Aluja AS, Martínez JJ, Hernández M, Rosas G et al (2011) Recombinant S3Pvacphage anticysticercosis vaccine: simultaneous protection against cysticercosis and hydatid disease in rural pigs. Vet Parasitol 176:53–58

Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD et al (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2197–2223. https://doi.org/10.1016/S0140-6736(12)61689-4

Murrieta I, Flores X, Osorio R, Kuschick Feher J, Carrillo-Mezo R, Fleury A (2021) Natural history of extraparenchymal neurocysticercosis. Trans R Soc Trop Med Hyg 15:traa174. https://doi.org/10.1093/trstmh/traa174

Nash TE, O'Connell EM, Hammoud DA, Wetzler L, Ware JM, Mahanty S (2020) Natural history of treated subarachnoid neurocysticercosis. Am J Trop Med Hyg 102:78–89. https://doi.org/10.4269/ajtmh.19-0436

Ng-Nguyen D, Stevenson MA, Dorny P, Gabriël S, Vo TV, Nguyen VT, Phan TV, Hii SF, Traub RJ (2017) Comparison of a new multiplex real-time PCR with the Kato Katz thick smear and copro-antigen ELISA for the detection and differentiation of *Taenia* Spp. in human stools. PLoS Negl Trop Dis 11:e0005743. https://doi.org/10.1371/journal.pntd.0005743

Ng-Nguyen D, Noh J, Breen K, Stevenson MA, Handali S, Traub RJ (2018) The epidemiology of porcine *Taenia solium* cysticercosis in communities of the Central Highlands in Vietnam. Parasit Vectors 11:360. https://doi.org/10.1186/s13071-018-2945-y

Ngowi HA, Carabin H, Kassuku AA, Mlozi MR, Mlangwa JE, Willingham AL 3rd (2008) A health-education intervention trial to reduce porcine cysticercosis in Mbulu District, Tanzania. Prev Vet Med 85:52–67

Ngowi HA, Kassuku AA, Carabin H, Mlangwa JE, Mlozi MR, Mbilinyi BP, Willingham AL 3rd (2010) Spatial clustering of porcine cysticercosis in Mbulu district, northern Tanzania. PLoS Negl Trop Dis 4:e652

Ngwing NA, Poné JW, Mbida M, Pagnah AZ, Njakoi H, Bilong CF (2012) A preliminary analysis of some epidemiological factors involved in porcine cysticercosis in Bafut and Santa subdivisions, North West Region of Cameroon. Asian Pac J Trop Med 5:814–817

Nsadha Z, Rutebarika C, Ayebazibwe C, Aloys B, Mwanja M, Poole EJ, Chesang E, Colston A, Donadeu M, Lightowlers MW (2021) Control trial of porcine cysticercosis in Uganda using a combination of the TSOL18 vaccination and oxfendazole. Infect Dis Poverty 10:34. https://doi.org/10.1186/s40249-021-00823-6

Nunes CM, Biondi GF, Heinemann MB, Richtzenhain LJ (2000) Comparative evaluation of an indirect ELISA test for diagnosis of swine cysticercosis employing antigen from *Taenia solium* and *Taenia crassiceps* metacestodes. Vet Parasitol 93:135–140

O'Neal S, Noh J, Wilkins P, Keene W, Lambert W, Anderson J, Compton Luman J, Townes J (2011) *Taenia solium* tapeworm infection, Oregon, 2006–2009. Emerg Infect Dis 17:1030–1036

O'Neal SE, Moyano LM, Ayvar V, Gonzalvez G, Diaz A, Rodriguez S, Wilkins PP, Tsang VC, Gilman RH, Garcia HH, Gonzalez AE, Cysticercosis Working Group in Peru (2012) Geographic correlation between tapeworm carriers and heavily infected cysticercotic pigs. PLoS Negl Trop Dis 6:e1953

O'Neal SE, Flecker RH (2015) Hospitalization frequency and charges for neurocysticercosis, United States, 2003–2012. Emerg Infect Dis 21:969–976. https://doi.org/10.3201/eid2106.141324

Osorio R, Carrillo-Mezo R, Romo ML, Toledo A, Matus C, González-Hernández I, Jung H, Fleury A (2019) Factors associated with cysticidal treatment response in extraparenchymal neurocysticercosis. J Clin Pharmacol 59:548–556. https://doi.org/10.1002/jcph.1346

Palomares-Alonso F, Toledo A, Palencia Hernández G, Jung-Cook H, Fleury A (2020) Effect of dexamethasone on albendazole cysticidal activity in experimental cysticercosis by *Taenia crassiceps* in BALB/c mice: in vitro and in vivo evaluation. Exp Parasitol 208:107801. https://doi.org/10.1016/j.exppara.2019.107801

Pearson RD, Guerrant RL (1983) Praziquantel: a major advance in anthelminthic therapy. Ann Intern Med 99:195–198

Pearson RD, Hewlett EL (1985) Niclosamide therapy for tapeworm infections. Ann Intern Med 102:550–551

Pérez-Torres S, Flores-Pérez FI, Orihuela A, Aguirre V, Bernal G, Nieto A, Vázquez R, Solano JJ (2012) Influence of tongue inspection during cysticercosis diagnosis on some behavioral and physiological stress measures in pigs (Sus scrofa domestica). Trop Anim Health Prod 44:383–384

Phiri IK, Dorny P, Gabriel S, Willingham AL 3rd, Sikasunge C, Siziya S, Vercruysse J (2006) Assessment of routine inspection methods for porcine cysticercosis in Zambian village pigs. J Helminthol 80:69–72. https://doi.org/10.1079/joh2005314

Pondja A, Neves L, Mlangwa J, Afonso S, Fafetine J, Willingham AL 3rd, Thamsborg SM, Johansen MV (2010) Prevalence and risk factors of porcine cysticercosis in Angónia District, Mozambique. PLoS Negl Trop Dis 4:e594

Porphyre V, Betson M, Rabezanahary H, Mboussou Y, Zafindraibe NJ, Rasamoelina-Andriamanivo H, Costard S, Pfeiffer DU, Michault A (2016) Taenia solium porcine cysticercosis in Madagascar: comparison of immuno-diagnostic techniques and estimation of the prevalence in pork carcasses traded in Antananarivo city. Vet Parasitol 219:77–83. https://doi.org/10.1016/j.vetpar.2015.08.027

Poudel I, Sah K, Subedi S, Kumar Singh D, Kushwaha P, Colston A, Gauci CG, Donadeu M, Lightowlers MW (2019) Implementation of a practical and effective pilot intervention against transmission of *Taenia solium* by pigs in the Banke district of Nepal. PLoS Negl Trop Dis 13:e0006838. https://doi.org/10.1371/journal.pntd.0006838

Praet N, Speybroeck N, Manzanedo R, Berkvens D, NsameNforninwe D, Zoli A, Quet F, Preux PM, Carabin H, Geerts S (2009) The disease burden of *Taenia solium* cysticercosis in Cameroon. PLoS Negl Trop Dis 3:e406

Praet N, Kanobana K, Kabwe C, Maketa V, Lukanu P, Lutumba P, Polman K, Matondo P, Speybroeck N, Dorny P, Sumbu J (2010) *Taenia solium* cysticercosis in the Democratic Republic of Congo: how does pork trade affect the transmission of the parasite? PLoS Negl Trop Dis 4:e817

Praet N, Verweij JJ, Mwape KE, Phiri IK, Muma JB, Zulu G, van Lieshout L, Rodriguez-Hidalgo R, Benitez-Ortiz W, Dorny P, Gabriël S (2013) Bayesian modelling to estimate the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis. Tropical Med Int Health 18:608–614. https://doi.org/10.1111/tmi.12089

Prakash A, Kumar GS, Rout M, Nagarajan K, Kumar R (2007) Neurocysticercosis in free roaming pigs–a slaughterhouse survey. Trop Anim Health Prod 39:391–394

Prasad KN, Chawla S, Prasad A, Tripathi M, Husain N, Gupta RK (2006) Clinical signs for identification of neurocysticercosis in swine naturally infected with *Taenia solium*. Parasitol Int 55:151–154

Proaño-Narvaez JV, Meza-Lucas A, Mata-Ruiz O, García-Jerónimo RC, Correa D (2002) Laboratory diagnosis of human neurocysticercosis: double-blind comparison of enzyme-linked immunosorbent assay and electroimmunotransfer blot assay. J Clin Microbiol 40:2115–2118

Rajkotia Y, Lescano AG, Gilman RH, Cornejo C, Garcia HH, Cysticercosis Working Group of Peru (2007) Economic burden of neurocysticercosis: results from Peru. Trans R Soc Trop Med Hyg 101:840–846

Ramiandrasoa NS, Ravoniarimbinina P, Solofoniaina AR, Andrianjafy Rakotomanga IP, Andrianarisoa SH, Molia S, Labouche AM, Fahrion AS, Donadeu M, Abela-Ridder B, Rajaonatahina D (2020) Impact of a 3-year mass drug administration pilot project for taeniasis control in Madagascar. PLoS Negl Trop Dis 14:e0008653. https://doi.org/10.1371/journal.pntd.0008653

Robles C, Chavarria M (1979) Presentación de un caso clínico de cisticercosis cerebral tratado medicamente con un nuevo farmaco: Praziquantel. Salud Publica Mex 21:603–618

Rodrigues CL, de Andrade DC, Livramento JA, Machado LR, Abraham R et al (2012) Spectrum of cognitive impairment in neurocysticercosis: differences according to disease phase. Neurology 78:861–866

Rodriguez-Hidalgo R, Benitez-Ortiz W, Praet N, Saa LR, Vercruysse J, Brandt J, Dorny P (2006) Taeniasis-cysticercosis in Southern Ecuador: assessment of infection status using multiple laboratory diagnostic tools. Mem Inst Oswaldo Cruz 101:779–782. https://doi.org/10.1590/s0074-02762006000700012

Royo Martínez R (1996) Hemograma de Cerdos Inoculados Experimentalmente con huevos de *Taenia solium*. Tesis de Licenciatura. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México

Ruiz S, García-Vázquez E, Picazo R, Hernández A, Herrero JA, Gómez J (2011) Neurocysticercosis in Murcia (Spain). Rev Clin Esp 211:133–138

Sáenz B, Ruíz-Garcia M, Jiménez E, Hernández-Aguilar J, Suastegui R et al (2006) Neurocysticercosis:clinical, radiologic, and inflammatory differences between children and adults. Pediatr Infect Dis J 25:801–803

Sánchez AL, Medina MT, Ljungström I (1998) Prevalence of taeniasis and cysticercosis in a population of urban residence in Honduras. Acta Trop 69:141–149

Santo AH (2007) Cysticercosis-related mortality in the State of Sao Paulo, Brazil, 1985–2004: a study using multiple causes of death. Cad Saude Publica 23:2917–2927

Sarti E, Flisser A, Schantz PM, Gleizer M, Loya M, Plancarte A, Avila G, Allan J, Craig P, Bronfman M, Wijeyaratne P (1997) Development and evaluation of a health education intervention against *Taenia solium* in a rural community in Mexico. Am J Trop Med Hyg 56:127–132

Sarti E, Schantz PM, Avila G, Ambrosio J, Medina-Santillán R, Flisser A (2000) Mass treatment against human taeniasis for the control of cysticercosis: a population-based intervention study. Trans R Soc Trop Med Hyg 94:85–89

Schantz PM, Moore AC, Muñoz JL, Hartman BJ, Schaefer JA, Aron AM, Persaud D, Sarti E, Wilson M, Flisser A (1992) Neurocysticercosis in an Orthodox Jewish community in New York City. N Engl J Med 327:692–695

Sciutto E, Martínez JJ, Villalobos NM, Hernández M, José MV, Beltrán C, Rodarte F, Flores I, Bobadilla JR, Fragoso G, Parkhouse ME, Harrison LJ, de Aluja AS (1998) Limitations of current diagnostic procedures for the diagnosis of *Taenia solium* cysticercosis in rural pigs. Vet Parasitol 79:299–313

Sciutto E, Fragoso G, Fleury A, Laclette JP, Sotelo J, Aluja A, Vargas L, Larralde C (2000) *Taenia solium* disease in humans and pigs: an ancient parasitosis disease rooted in developing countries and emerging as a major health problem of global dimensions. Microbes Infect 2:1875–1890

Serpa JA, Clinton White A Jr (2012) Neurocysticercosis in the United States. Pathog Glob Health 106:256–260

Shongwe NA, Byaruhanga C, Dorny P, Dermauw V, Qekwana DN (2020) Knowledge, practices and seroprevalence of Taenia species in smallholder farms in Gauteng, South Africa. PLoS One 15:e0244055. https://doi.org/10.1371/journal.pone.0244055

Shonyela SM, Mkupasi EM, Sikalizyo SC, Kabemba EM, Ngowi HA, Phiri I (2017) An epidemiological survey of porcine cysticercosis in Nyasa District, Ruvuma Region, Tanzania. Parasite Epidemiol Control 2:35–41. https://doi.org/10.1016/j.parepi.2017.09.002

Sikasunge CS, Phiri IK, Phiri AM, Siziya S, Dorny P, Willingham AL (2008) Prevalence of *Taenia solium* porcine cysticercosis in the Eastern, Southern and Western provinces of Zambia. Vet J 176:240–244

Singh G (1997) Neurocysticercosis in South-Central America and the Indian subcontinent. A comparative evaluation. Arq Neuropsiquiatr 55:349–356

Singh G, Rajshekhar V, Murthy JM, Prabhakar S, Modi M et al (2010) A diagnostic and therapeutic scheme for a solitary cysticercus granuloma. Neurology 75:2236–2245

Singh AK, Singh SK, Prasad KN, Singh A, Bajpai A, Rahman M, Rai RP, Gupta RK, Tripathi M, Husain N (2013) Evaluation of ELISA, neck muscle, tongue and eyelid examinations for the diagnosis of swine cysticercosis in a highly endemic area of north India. Exp Parasitol 134:313–317

Singh BB, Khatkar MS, Gill JP, Dhand NK (2016) Data and the associated R code used to estimate health and economic burden of neurocysticercosis in India. Data Brief 7:571–581. https://doi.org/10.1016/j.dib.2016.02.079

Somers R, Dorny P, Nguyen VK, Dang TC, Goddeeris B, Craig PS, Vercruysse J (2006) *Taenia solium* taeniasis and cysticercosis in three communities in north Vietnam. Tropical Med Int Health 11:65–72

Sorvillo FJ, Portigal L, DeGiorgio C, Smith L, Waterman SH, Berlin GW, Ash LR (2004) Cysticercosis-related deaths, California. Emerg Infect Dis 10:465–469

Sorvillo FJ, DeGiorgio C, Waterman SH (2007) Deaths from cysticercosis, United States. Emerg Infect Dis 13:230–235

Sreedevi C, Hafeez M, Kumar PA, Rayulu VC, Subramanyam KV, Sudhakar K (2012) PCR test for detecting *Taenia solium* cysticercosis in pig carcasses. Trop Anim Health Prod 44:95–99

Swastika K, Dharmawan NS, Suardita IK, Kepeng IN, Wandra T, Sako Y, Okamoto M, Yanagida T, Sasaki M, Giraudoux P, Nakao M, Yoshida T, Eka Diarthini LP, Sudarmaja IM, Purba IE, Budke CM, Ito A (2016) Swine cysticercosis in the Karangasem district of Bali, Indonesia: an evaluation of serological screening methods. Acta Trop 163:46–53. https://doi.org/10.1016/j.actatropica.2016.07.022

Thienpont D, Rochette F, Vanparijs OFJ (1986) Diagnosing helminthiasis by coprological examination, 2nd edn. Janssens Research Foundation, Beerse, p 205

Thys S, Mwape KE, Lefèvre P, Dorny P, Phiri AM, Marcotty T, Phiri IK, Gabriël S (2016) Why pigs are free-roaming: Communities' perceptions, knowledge and practices regarding pig management and taeniosis/cysticercosis in a *Taenia solium* endemic rural area in Eastern Zambia. Vet Parasitol 225:33–42. https://doi.org/10.1016/j.vetpar.2016.05.029

Toledo A, Osorio R, Matus C, Martinez Lopez Y, Ramirez Cruz N, Sciutto E, Fragoso G, Arauz A, Carrillo-Mezo R, Fleury A (2018) Human Extraparenchymal Neurocysticercosis: the control of inflammation favors the host. . .but also the parasite. Front Immunol 9:2652. https://doi.org/10.3389/fimmu.2018.02652

Torres-Corzo JG, Tapia-Perez JH, Vecchia RR et al (2010) Endoscopic management of hydrocephalus due to neurocysticercosis. Clin Neurol Neurosurg 112:11–16

Townes JM, Hoffmann CJ, Kohn MA (2004) Neurocysticercosis in Oregon, 1995–2000. Emerg Infect Dis 10:508–510

Trevisan C, Mkupasi EM, Ngowi HA, Forkman B, Johansen MV (2016) Severe seizures in pigs naturally infected with *Taenia solium* in Tanzania. Vet Parasitol 220:67–71. https://doi.org/10.1016/j.vetpar.2016.02.025

Trevisan C, Devleesschauwer B, Schmidt V, Winkler AS, Harrison W, Johansen MV (2017) The societal cost of *Taenia solium* cysticercosis in Tanzania. Acta Trop 165:141–154. https://doi.org/10.1016/j.actatropica.2015.12.021

Trevisan C, Devleesschauwer B, Praet N, Pondja A, Assane YA, Dorny P, Thamsborg SM, Magnussen P, Johansen MV (2018) Assessment of the societal cost of *Taenia solium* in Angónia district, Mozambique. BMC Infect Dis 18:127. https://doi.org/10.1186/s12879-018-3030-z

Tsang VC, Brand JA, Boyer AE (1989) An enzyme-linked immunoelectrotransfer blot assay and glycoproteins antigens for diagnosing human cysticercosis (*Taenia solium*). J Infect Dis 174: 1007–1009

Vega R, Pinero D, Ramanankandrasana B, Dumas M, Bouteille B et al (2003) Population genetic structure of *Taenia solium* from Madagascar and Mexico: implications for clinical profile diversity and immunological technology. Int J Parasitol 33:1479–1485

Waiswa C, Fèvre EM, Nsadha Z, Sikasunge CS, Willingham AL (2009) Porcine cysticercosis in Southeast Uganda: seroprevalence in Kamuli and Kaliro districts. J Parasitol Res 2009:375493. https://doi.org/10.1155/2009/375493

Wandra T, Swastika K, Dharmawan NS, Purba IE, Sudarmaja IM, Yoshida T, Sako Y, Okamoto M, Eka Diarthini NL, Sri Laksemi DA, Yanagida T, Nakao M, Ito A (2015) The present situation and towards the prevention and control of neurocysticercosis on the tropical island, Bali, Indonesia. Parasit Vectors 8:148. https://doi.org/10.1186/s13071-015-0755-z

Wertheim HFL, Horby P, Woodall JP (2012) Cysticercosis. In Atlas of human infectious diseases, 1st edn. Blackwell, Oxford, p 137

WHO (World Health Organization) (2007) Global plan to combat neglected tropical diseases 2008–2015

Willingham AL 3rd, Wu HW, Conlan J, Satrija F (2010) Combating *Taenia solium* cysticercosis in Southeast Asia an opportunity for improving human health and livestock production. Adv Parasitol 72:235–266

Winkler AS, Richter H (2015) Landscape analysis: management of neurocysticercosis with an emphasis on low- and middle-income countries. Commissioned by the World Health Organization, Geneva. http://apps.who.int/iris/bitstream/10665/152896/1/WHO_HTM_NTD_NZD_2015.05_eng.pdf

Wohlgemut J, Dewey C, Levy M, Mutua F (2010) Evaluating the efficacy of teaching methods regarding prevention of human epilepsy caused by *Taenia solium* neurocysticercosis in Western Kenya. Am J Trop Med Hyg 82:634–642

Wu W, Qian X, Huang Y, Hong Q (2012) A review of the control of clonorchiasis sinensis and *Taenia solium* taeniasis/cysticercosis in China. Parasitol Res 111:1879–1884

Xiao ZX, Zhao CY, Liu LP (1986) Albendazole treatment in cerebral cysticercosis. Chin J Int Med 25:100–102

Yap P, Fürst T, Müller I, Kriemler S, Utzinger J, Steinmann P (2012) Determining soil-transmitted helminth infection status and physical fitness of school-aged children. J Vis Exp 66:e3966

Toxoplasmosis: A Widespread Zoonosis Diversely Affecting Humans and Animals

16

Florence Robert-Gangneux, Dominique Aubert, and Isabelle Villena

Contents

F. Robert-Gangneux
Department of Parasitology-Mycology, University of Rennes 1 – UFR Medicine and CHU de Rennes, INSERM U1085, IRSET (Institut de Recherche en Santé Environnement Travail), Rennes Cedex, France
e-mail: florence.robert-gangneux@univ-rennes1.fr

D. Aubert · I. Villena (✉)
Department of Parasitology-Mycology, EA 7510, SFR Cap-Santé, UFR Medicine, University of Reims Champagne Ardenne and National Reference Centre on Toxoplasmosis, University Reims Hospital, Reims Cedex, France
e-mail: daubert@chu-reims.fr; ivillena@chu-reims.fr

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_14

Abstract

Infections by the protozoan parasite *Toxoplasma gondii* are widely prevalent in humans and animals worldwide. Since the discovery of the life cycle of the parasite (1969), several studies identified sources of contamination for humans through ingestion of viable tissue cysts in raw or undercooked meat or ingestion of food and water contaminated with sporulated oocysts after shedding in the feces of infected felids. Prevalence of toxoplasmosis varies between different countries and regions in the same country depending on age, social culture, eating habits, and environmental factors. In immunocompetent patients, toxoplasmosis is generally asymptomatic or benign, but severe infections are described in tropical areas due to atypical strains. In immunocompromised patients, *T. gondii* is an opportunistic parasite that may induce life-threatening disease; severe disease could occur in HIV-infected patients or in transplant patients. Finally, in case of congenital toxoplasmosis, infection can lead to abortion, cerebral damage, or ocular lesions. New genotyping tools were recently applied to field studies in different continents and revealed a complex population structure for *T. gondii* with a greater genetic diversity than expected, and a relation between genotype of *Toxoplasma* strain and severity of infection is described. According to different routes of transmission, hygienic measures can be recommended to avoid *Toxoplasma* contamination. These measures can be completed by a serological screening of patient at risk for toxoplasmosis as it is recommended in France. Although the cost of screening is expensive, the important preventive role of healthcare policies in the decrease of the burden of toxoplasmosis cannot be denied.

Keywords

Toxoplasma gondii · Oocysts · Cysts · Zoonosis · One health disease

16.1 Introduction

The protozoan parasite *Toxoplasma gondii* has a worldwide distribution and is one of the most frequent parasitic infections. This obligate intracellular parasite was first described in the common gundi *(Ctenodactylus gundi)*, a rodent from North Africa, by Nicolle and Manceaux in 1908, and was subsequently recognized as the agent of a widespread zoonosis involving humans as well as virtually all warm-blooded animals and birds. However, it took several decades until its entire life cycle was definitively understood in the late 1960s (Hutchison et al. 1969; Frenkel et al. 1970), with the

demonstration of the cat as definitive host responsible for oocysts shedding through feces and contamination of intermediate hosts. It is now well established that not only the cat, but all felids, can reproduce the sexual life cycle of the parasite and participate in the spread of the disease, which explains the wide distribution of toxoplasmosis.

Regarding human infection, the most remarkable events were the first reports on cases of congenital toxoplasmosis in 1939 (Schwartzman et al. 1948), the development of the first serologic test by Sabin and Feldman in 1948, and the recognition, in the middle 1970s, that past infection could reactivate in immunocompromised patients (Weiss and Dubey 2009). The high burden of congenital toxoplasmosis led to the progressive implementation of prevention policies in some European countries. During the last decade, the development of new genotyping tools and the multiplication of field studies have increased comprehension of the phylogenetic evolution of *T. gondii* in the world (Mercier et al. 2011), and advances have been achieved in the knowledge on the particular virulence associated with some genotypes (ElHajj et al. 2007; Behnke et al. 2015).

16.2 A Life Cycle Involving All Warm-Blooded Animals

16.2.1 Definitive Hosts and Contamination of the Environment: Not Only the Cat!

While only Felidae can act as definitive hosts and thus shed oocysts in their feces, almost all warm-blooded animals can serve as intermediate hosts. Many host species (birds, rodents, carnivorous, or herbivorous animals) from polar to tropical areas have been identified by serology or bioassay. *Toxoplasma gondii* undergoes sexual reproduction in the felid intestine, resulting in the production of millions of environmentally unsporulated oocysts. Oocysts take 1–5 days to sporulate in the environment and become infective and resistant in environment. Oocysts may survive for months in soil and water, thereby enhancing the probability of transmission to intermediate hosts such as birds, rodents, and humans. Cats become infected after consuming intermediate hosts harboring tissue cysts or directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Humans may acquire *T. gondii* infection via oral uptake of sporulated oocysts from the environment, consumption of raw or undercooked meat containing tissue cysts, or transplacental transmission of the parasite from the non-immune mother to the fetus (Fig. 1).

Oocysts are essential in the life cycle of *T. gondii*, and cats were everywhere, except the frozen artic. In general, the seropositivity increases with the age of the cat, indicating postnatal transmission of *T. gondii*. It is possible that in some young cats, the low antibody titers represented maternally transferred antibodies which generally disappear in the cat by 12 weeks of age (Dubey et al. 2020). At any given time, approximately 1% of cats are expected to shed oocysts (even if most cats only shed oocysts for about 1 week in their life), and this is supported by fecal survey. The

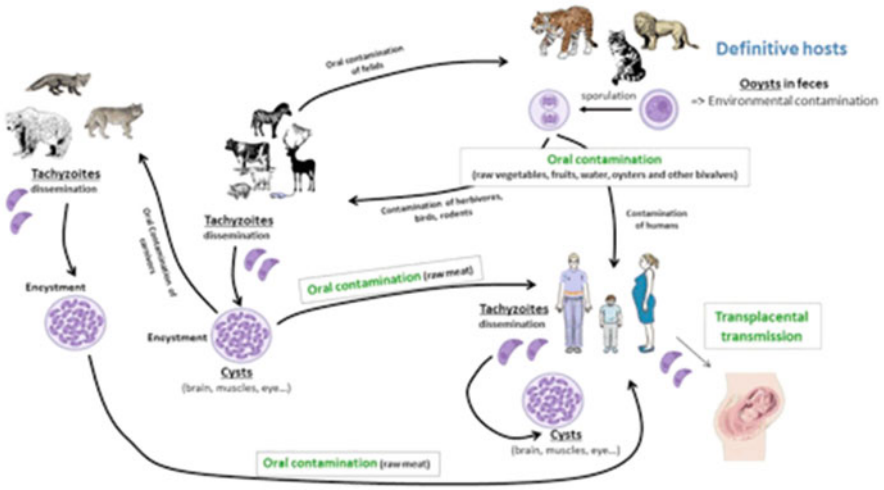

Fig. 1 Life cycle of *Toxoplasma gondii* (partially made using BioRender tools)

number of oocysts shed by naturally infected domestic cats is largely unknown, but probably several millions are disseminated into the environment according to experimental infection. Whether naturally infected cats shed oocysts more than once in their life is unknown. The number of oocysts shed during the secondary infection is usually lower than in primary infection; cats that had excreted oocysts did not re-excrete after challenge within 2–3 months after primary infection (Dubey and Frenkel 1974). Dubey recently reported that concurrent infections with certain feline pathogens can affect *T. gondii* infections in cats, but there is no evidence that they affect the seropositivity of *T. gondii* in cats (Dubey et al. 2020). In addition to domestic cats, wild felids can also shed oocysts. The role of wild felids in parasite transmission to humans may be important especially in areas where the domestic cat, *Felis catus*, is absent (e.g., tropical forest). Cats are essential for the maintenance of *T. gondii* in the environment, since infections are virtually absent from areas lacking cats. A single oocyst is able to infect pigs or mice, but pathogenicity depends on the strain, inoculum, and infection route (Dubey and Beatty 1988); oocysts are less infectious and pathogenic for cats than for intermediate hosts (Dubey and Frenkel 1974).

Based on serologic surveys, up to 74% of the adult cat population may be infected by *T. gondii (Tenter et al.* 2000*)*; but seroprevalence to *T. gondii* varied among countries, within different areas of a country, and within the same city (Dubey et al. 2020). In nature, cats are infected by eating small preys harboring tissue cysts or by ingesting oocysts from soil. Fatal toxoplasmosis is rare and occurs more often in immunocompromised cats and in kittens. Vertical transmission is uncommon and is probably not important for parasite propagation. Cats excrete millions of unsporulated oocysts in their feces after ingesting any of the three infectious stages; the prepatent period is 3–10 days after ingesting bradyzoites and more than or equal to 18 days after ingesting oocysts or tachyzoites. The patent period is only

1–3 weeks, but a re-excretion is possible, at least experimentally, after a second challenge with *T. gondii*, after corticoid treatment or superinfection by *Isospora felis*. Even within a shared habitat, exposure of wild and domestic felids to *T. gondii* may increase or decrease based upon access to prey and dietary preferences (VanWormer et al. 2013). Pet and feral domestic cats showed different prey consumption patterns, with higher predation levels observed in feral cat populations (Fitzgerald 2000). In regions where humans, livestock, and wildlife live in close contact, wild felid prey frequently include domestic livestock such as sheep, goats, and cattle as well as wildlife (Treves and Karanth 2003). In addition, inter-farm and seasonal variations in the risks of exposure to *T. gondii* oocysts for humans and livestock living on farms have been described (Simon et al. 2018).

16.2.2 Life Cycle of *T. gondii* in Domestic Animals and Wildlife

T. gondii is not an obligatory heteroxenous parasite and can propagate clonally (presumably indefinitely) by cycling among intermediate hosts. This can occur vertically through transplacental transmission from mother to offspring (Dubey et al. 1997). Tissue cysts are also orally infectious to carnivorous intermediate hosts, permitting *T. gondii* to bypass the sexual stage in the definitive host (Khan et al. 2007).

Oocysts are responsible for most of the *T. gondii* infections in noncarnivorous mammals and birds whereas other species are mainly infected by eating animals harboring tissue cysts. *T. gondii* infection in wildlife does not occur with the same probability in any species or place. Wild-living species first have variable levels of susceptibility and exposure. Exposure is largely determined by life history traits, especially feeding behavior. Herbivore intermediate hosts acquire infection by food or water contaminated with sporulated oocysts. Carnivores and omnivores may additionally become infected mainly by ingesting meat containing cysts. Hejlíček et al. (1997) suggested that carnivores have a higher prevalence of antibodies to *T. gondii* due to the cumulative ingestion of infected animals and that seroprevalences would be lower, by decreasing order, in omnivorous, herbivorous, and insectivorous animals.

16.2.3 A Wide Range of Opportunity for Human Infection

Human infection can occur from different transmission routes due to several stages of the parasite (cysts, oocysts, and tachyzoites). Even though the sources of *T. gondii* infection for humans are well known, their relative contribution is still unclear. The major routes of transmission vary between different human populations and depend on social culture, eating habits, and environmental factors (Tenter et al. 2000).

The most frequent transmission route appears due to cysts present in meat as a risk-factor analysis indicates that 30–63% of human infections can be attributed to the consumption of undercooked meat (Cook et al. 2000). All warm-blooded animal

species can be infected, but prevalence of infection in meat producing animals is variable among species and countries (Tenter et al. 2000; Dubey 2022). Sheep and pigs seem to be more infected while infection seems rarer in cattle. In Norway and France, consumption of undercooked lamb appears to be a stronger risk factor than consumption of pork (Kapperud et al. 1996; Baril et al. 1999), while in Poland consumption of undercooked pork was identified as the principal risk factor (Paul 1998). Moreover, there are few reported outbreaks due to ingestion of well-identified meat (Dubey 2021; Dubey et al. 2021), and absence of evidence of source of contamination is frequent due to lack of detection of cysts in meat.

Infection through cysts can be also occurring after organ transplantation when an organ from a *Toxoplasma*-seropositive donor is grafted to a *Toxoplasma*-seronegative recipient. Generally, heart, liver, and kidney transplantation is most frequent for this route of transmission (Speirs et al. 1988).

Infection through oocysts is another transmission route for humans. Sporulated oocysts are very resistant (especially to most of disinfectants) in environment, and they may survive during several years and disperse through water or soil movements and contaminate vegetables (Shapiro et al. 2019). They may be an important source of infection for humans, and several waterborne outbreaks of toxoplasmosis linked to oocyst contamination of drinking water are reported (Dubey 2022; Shapiro et al. 2019).

A recent meta-analysis of sporadic toxoplasma infections revealed the significance of transmission by environmental factors such as contact with soil and contact with animals, in particular cats, also the consumption of raw or undercooked meat and unwashed vegetables significantly increased the odds of acquiring an infection (Thebault et al. 2021). Moreover, a recent review (Dubey 2021) reports that there are no apparent differences in type or severity of symptoms in meat-versus oocyst-acquired infections.

The last transmission route of infection is through tachyzoites. Tachyzoites are delicate; they are destroyed by gastric secretions and unable to survive outside their host. So contamination by this route is very rare except in the case of congenital infection due to transplacental transmission from a mother to her fetus after maternal infection acquired during pregnancy. Tachyzoites can be rarely transmitted by transfusion (blood/bone marrow donor) or in case of laboratory accidents. Finally, toxoplasmosis in humans was occasionally reported after transmission by goat milk (Sacks et al. 1982; Skinner et al. 1990). However, few studies have attempted to detect *T. gondii* in milk of naturally infected animal species and dairy samples (Abadi et al. 2020).

16.3 Prevalence and Disease Burden in Humans

16.3.1 Pathophysiology of the Disease

The pathophysiology of toxoplasmosis results from the dissemination of tachyzoites. After transepithelial passage across the intestinal barrier, tachyzoites invade rapidly monocytes and gain access to the blood flow and from there virtually to all organs

(Robert-Gangneux and Darde 2012). In fact, tachyzoites can invade actively all nucleated cell types, which can explain the variety of clinical manifestations possibly observed, in particular in immunocompromised patients. After the onset of an efficient immune response, tachyzoites are not eradicated but convert into bradyzoites within cells and persist as cysts lifelong, mostly in the muscles, retina, and brain.

16.3.2 Toxoplasmosis in Immunocompetent Subjects: Seroprevalence in the World

It is widely admitted that toxoplasmosis is frequently asymptomatic in immunocompetent humans and is mostly responsible for mild nonspecific symptoms including fever, asthenia, and lymphadenopathy in about 20% of patients. Therefore, the diagnosis is often retrospective and is based on serology. Estimates indicate that approximately 25–30% of the world human population is infected by *Toxoplasma* (Montoya and Liesenfeld 2004); recent meta-analysis mention a global IgG seroprevalence 32.9% (95%CI: 29.4–36.4) in pregnant women with large variations among the countries (Bigna et al. 2020).

Seroprevalence varies among countries but also among regions within a same country. Low seroprevalence (10–30%) is observed in North America, in South East Asia, in Northern Europe, and in Sahelian countries of Africa. Moderate prevalence (30–50%) is found in countries from Central and Southern Europe and high prevalence in South America and in tropical African countries. Several factors can be put forward to explain this heterogeneity, e.g., (1) climatic factors affecting survival of oocysts in the environment (Dubey 1998), (2) the prevalence in meat-producing animals, (3) cooking or cultural habits, and (4) socioeconomic level and quality of water. Seroprevalence has declined over the last decades in most industrialized countries, probably as a result of combined factors (increased socioeconomic level and improvement of hygienic conditions, change of farming systems, consumption of frozen meat, and feeding of cats with sterilized food). For example, in France, the seroprevalence in pregnant women was about 80% in the early 1960s and declined to 54% and 44% in two national perinatal surveys in 1995 and 2003, respectively, while at the same time, the average age of first pregnancy increased (Villena et al. 2010). The seroprevalence observed in general population is increasing with age, and seroprevalence in men and women does not differ for the population aged 45 years and under (Bellali et al. 2013).

16.3.3 Congenital Toxoplasmosis: Determinants of Severity and Various Incidences in the World

Vertical transmission can occur when primary infection is acquired during pregnancy, by transplacental transfer of tachyzoites either during blood flow dissemination or at a later stage. The colonization of the placental tissue by the parasite is probably an

important factor in the process, since 50–72% of placentas from infected fetuses still harbor parasites at birth, which makes this biological sample a good tool for diagnosis (Robert-Gangneux et al. 2011). In France, the mean seroconversion rate during pregnancy was estimated to 6–7 per 1000 seronegative pregnant women (Berger et al. 2009). The frequency of vertical transmission and the severity of fetal damage depend on the stage of pregnancy when maternal infection occurs. Fetal infection occurs in less than 10% of cases during the first trimester but increases to 30% of cases in the second trimester and 60–70% in the third trimester and even more close to delivery (Dunn et al. 1999). The severity of fetal infection is inversely correlated, since neonates are usually asymptomatic in more than 80% of cases when infected during the third trimester of gestation (Desmonts and Couvreur 1974). Conversely, when transplacental transmission occurs during the first trimester of pregnancy, the consequences on fetal development are heavy, often leading to severe abnormalities involving the brain and eye tissues or to abortion. Major sequelae include mental retardation, seizures, microcephalus, hydrocephalus, microphthalmia, cataract, increased intraocular pressure, strabismus, uveitis, retinochoroiditis, and possibly blindness. During the second trimester, fetal infection may have variable consequences, including hepatosplenomegaly, intracranial calcifications, epilepsy, anemia, thrombocytopenia-induced petechiae, pneumonitis, or retinochoroiditis. Among the 272 cases collected in 2007 through the French surveillance for congenital toxoplasmosis network (Villena et al. 2010), 11 cases resulted in termination of pregnancy owing to cerebral lesions or fetal death, and 87% of live-born infants were asymptomatic. The remaining 13% of cases had intracranial calcifications (14 cases), hydrocephalus (3 cases), and/or retinochoroiditis of variable severity (12 cases). The tendencies are the same each year, among these 12 last years (data 2019, National Reference Center on Toxoplasmosis, France).

The incidence at birth was about 3 per 10,000 live births in 2007 in France (Villena et al. 2010), which is in the same range as incidence rates reported in other European countries, such as Denmark (2.1/10,000 live births) and Switzerland (4.3/10,000 live births), but is higher than that reported in Sweden (0.73/10,000) or in a pilot study in Massachusetts (1/10,000) (Guerina et al. 1994) and lower than that reported in Brazil (6/10,000) (Takahashi et al. 2019). The disease burden of congenital toxoplasmosis, as represented by disability-adjusted life years (DALY), is the highest among all food-borne pathogens (Havelaar et al. 2007). One study estimated 1.2 million disability-adjusted life years and an estimated 190,100 cases globally (Torgerson and Mastroiacovo 2013).

16.3.4 Toxoplasmosis in Immunocompromised Patients

Whereas toxoplasmosis is usually a mild or asymptomatic infection in immunocompetent subjects, it is a life-threatening infection in immunocompromised patients. Various factors severely impairing the cellular immune response, among which are HIV infection and immunosuppressive therapies, can lead to severe toxoplasmosis, due to either primary acquired infection or to reactivation of latent infection. Indeed, profound immunosuppression can favor cysts' rupture and tachyzoite multiplication and dissemination. Cyst reactivation is mostly localized to the brain and the retina

but can occur in other tissues, as *Toxoplasma* can invade all organs that can be subsequent potential sites for cyst reactivation (Patrat-Delon et al. 2010). This peculiarity puts transplant patients at risk for both reactivation and organ-transmitted infection. The risk for disseminated infection is closely related to the duration and degree of immunosuppression, with hematopoietic stem cell transplant (HSCT) patients being most at risk (Derouin and Pelloux 2008), whereas focal disease, such as cerebral toxoplasmosis or retinochoroiditis, is more commonly observed in HIV-infected patients. In HIV-positive patients, the incidence of toxoplasmosis is closely related to $CD4^+$ T cell counts, with an increasing risk when it falls below 100 cells/ μL. The incidence of TE has decreased and is now stabilized since the use of highly active antiretroviral therapy; it represents about 200 cases/year in France (Abgrall et al. 2001). In the absence of implementation of a recording system, there are no data available regarding the incidence of toxoplasmosis in transplant recipients.

16.3.5 New Insights into the Comprehension of Parasite Virulence: The Role of Genotype Strains

Although one single parasite species is responsible for toxoplasmosis in humans and animals, it has been shown from the 1990s that clinical isolates from Europe and USA could be divided into three major genotypes, types I, II, and III, equivalent to clonal lineages (Darde et al. 1992; Howe and Sibley 1995; Ajzenberg et al. 2002a).

However, more recently new genotyping tools such as multilocus sequence typing were applied to field studies in other continents and revealed a much more complex population structure with a greater genetic diversity, likely reflecting frequent exchanges of strains between hosts, as well as recombination of isolates during sexual life cycle within the definitive hosts (Ajzenberg et al. 2004). This led to the generation of recombinant isolates (I/II, I/III, or II/III) but also to new clonal haplogroups and, in some areas, particularly in South America, to atypical genotypes with many unique polymorphisms. Type II strains markedly predominate both in humans in Europe (Ajzenberg et al. 2002b; Aubert et al. 2010) and are isolated in more than 90% of congenital infections in France. Other clonal lineages are occasionally (type III) or exceptionally (type I) described in Europe. The exceptional isolation of atypical strains in France can be related to travels in South America or consumption of imported meat (Pomares et al. 2011). In North America, type II strains also predominate (Howe and Sibley 1995), but a higher prevalence of atypical strains is observed, and a clonal haplogroup (haplogroup 12) has been recently identified (Khan et al. 2011). By contrast, atypical genotypes largely predominate in South America, whereas type II isolates are rare (Pena et al. 2008). In Africa, clonal lineages known as *Africa 1-3* haplogroups coexist with type II and III lineages (Mercier et al. 2010) potentially leading to severe infection (Leroy et al. 2020). But, taken together with recent studies of *T. gondii* isolates from Africa, it is clear that the three clonal lineages (types I, II, and III) predominate not only in North America and Europe but also in Africa (Velmurugan et al. 2008). Until now, few data in humans are available from Asia, but some studies reveal a more limited genetic diversity than in South America, the presence of type III strains, and the widespread detection of a

clonal lineage in pigs in China (Zhou et al. 2010). Recently, clustering methods were used to organize the marked genetic diversity of 138 unique genotypes into 15 haplogroups that collectively define six major clades (Su et al. 2012).

It has long been known that clonal genotypes have variable virulence. Genotypes I and II have, respectively, a high and low virulence, whereas genotype III has an intermediate virulence. What emerges from recent epidemiologic studies using new genotyping data is that atypical strains are highly virulent and challenge the concepts of pathophysiology of the disease, at least in some parts of the world. First of all, the fact that infection is usually asymptomatic when it occurs in an immunocompetent subject is not questioned in Europe and North America, but recent experience from French Guiana shows that severe and even lethal toxoplasmosis can be observed in immunocompetent patients infected with atypical strains (Carme et al. 2002). Regarding congenital toxoplasmosis, a comparative prospective cohort study of infected children in Brazil and Europe showed that, independently of treatment, Brazilian children had a five times higher risk than European children for developing eye lesions and their lesions were larger, more multiple, more recurrent, and more likely to impair vision (Gilbert et al. 2008; Lago et al. 2021). More recently, phylogeography of *T. gondii* points to a South American origin which may explain geographic heterogeneities in disease burden (Bertranpetit et al. 2017).

Besides, recent observations have shown that (i) reinfection with an atypical strain of a previously immunized woman could lead to congenital transmission (Elbez-Rubinstein et al. 2009) and (ii) the rate of severe congenital infections was higher when women were infected with an atypical or recombinant strain than with a type II strain, whatever the stage of pregnancy at maternal infection was. Cumulative data (2006–2019) of the French surveillance for congenital toxoplasmosis network show that about 84% of congenital infections with type II strains are asymptomatic infections, whereas 83% of infections with atypical or recombinant genotypes are symptomatic. The large predominance of type II strains of low virulence in France (92% of cases), and more generally in Europe, explains the relatively low burden of the disease in those countries.

16.4 Prevalence and Disease Burden in Animals

Contact and interaction between wild fauna, domestic animals, and human beings may lead to an increased risk of transmission of zoonotic pathogens (Artois 1993). Wild mammals and birds are exposed to *T. gondii* through the ingestion of food or water contaminated with sporulated oocysts derived from felid feces (Dubey and Jones 2008). Additionally, wild carnivores and omnivores may also be infected by feeding birds and mammals with *T. gondii* cysts.

16.4.1 Wild Life

Serologic studies have assessed *T. gondii* infection in several species of wild animals from Europe (Jakubek et al. 2001; Sobrino et al. 2007; Richomme et al. 2009; Beral

et al. 2012) and other continents (Dubey et al. 1999). Mammals like cervids, wild boars, canids, viverrids and mustelids, as well as different kinds of birds, are among those wild animals found with antibodies to *T. gondii*, either at a group or on an individual level (Dubey et al. 2004). The modified agglutination test (MAT) has proved to be a very sensitive and specific assay for the serological diagnosis of *T. gondii* infection in many species of wild mammals and birds (Sobrino et al. 2007; Literák et al. 1992; Gauss et al. 2006; Gennari et al. 2016).

Exposure to *T. gondii* is highest in carnivorous species (Cabezon et al. 2011). High *T. gondii* seroprevalence is also reported in large predator species as lynx and the European wildcat (Sobrino et al. 2007) which is of epidemiological significance because infected felids shed oocysts in the wild environment.

In most species, *T. gondii* infection is generally unapparent, provoking only mild symptoms. However, a limited number of highly susceptible species have been discovered, in which *T. gondii* infection leads to frequent clinical disease and mortality. Marsupials and New World monkeys, which have evolved largely separately from cats, are among the most vulnerable species (Tenter et al. 2000). Fatal toxoplasmosis is also well-documented in hares (Jokelainen et al. 2011). *T. gondii* infection can be present at a high level in many wild birds without any clinical impact, but toxoplasmosis can be clinically severe in pigeons and canaries (Dubey 2002). The ingestion of infected birds is considered an important source of infection for cats (Khan et al. 2007; Dubey 2022).

16.4.2 Meat-Producing Animals

Tissue cysts of *T. gondii* contained in meat, meat-derived products may be important sources of infection for humans. However, for public health purposes, it is important to note that the tropism of *T. gondii* and the number of tissue cysts produced in a certain organ vary with the intermediate host species. In livestock, *T. gondii* tissue cysts are most frequently observed in various tissues of infected pigs, sheep, and goats and less frequently in infected poultry, rabbits, dogs, and horses. By contrast, tissue cysts are found only rarely in skeletal muscles of cattle or buffaloes (Tenter et al. 2000). Recently, a good correlation between seropositivity and the presence of tissues cysts in wild boar and roe deer was showed (Stollberg et al. 2021). Finally, prevalence in meat-producing animals varied among species and countries and is largely dependent on the methods of measure.

Prevalence of toxoplasmosis is highest in sheep and toxoplasmosis is implicated in 10–20% of sheep flocks with an abortion problem. The prevalence increases with age, reaching more than 90% in some studies (Tenter et al. 2000; Halos et al. 2010); the prevalence in ewes is more than twice than that in lamb. Viable *T. gondii* has been recovered from as many as 67% of sheep samples. Among the infected meat, lamb meat is supposed to be a major source of toxoplasmosis worldwide (Tenter et al. 2000). *T. gondii* has been recognized as one of the main causes of infective ovine abortion in New Zealand, Australia, the UK, Norway, and the USA (Dubey and Beatty 1988). Goats appear to be more susceptible to clinical toxoplasmosis, and even adult goats have died of acute toxoplasmosis.

Seroprevalence levels were lower in cattle, and very variable infection rates are found in pigs (1–60%) and poultry (0–30%), depending on their lifestyle (indoor or outdoor). In pigs, clinical cases of the infection are rare (leading to rare cases of myocarditis and encephalitis (Dubey and Jones 2008)), but the real problem of toxoplasmosis in pigs lies in the fact that tissues of infected animals may contain *T. gondii* tissue cysts. Infections with this parasite are common in pigs worldwide. The prevalence of *T. gondii* infection has decreased significantly with changes in pig production. It was suggested that infected pork products cause 50–75% of all cases of human toxoplasmosis in the USA, but the large study of Dubey et al. (2005) showed a seroprevalence of 0.57% and parasite isolation in only eight cases (0.38%). It is estimated that one pig is consumed by 200–400 individuals, and meat products are often made by combining the meat from different animals, thereby enhancing the risk of transfer of infection (Kijlstra and Jongert 2009). Moreover, an upsurge in consumer demand for "organically raised," "free-range" pork products has resulted in increasing numbers of hogs being raised in non-confinement systems. So, the prevalence of *T. gondii* in 33 market pigs raised under certified organic management conditions on two farms from Michigan, USA, was 90% (30/33) leading to the isolation of 17 strains (Dubey et al. 2012). This study indicated that organic pork meat may pose an increased risk of transmitting *T. gondii* to humans. Bayarri et al. (2012) have analyzed 50 samples of fresh pork meat and commercial cured ham collected in the city of Zaragoza (Spain), and *T. gondii* was detected in two samples of rib, reflecting a frequency of 8% positive fresh pork meat.

T. gondii has rarely been isolated from bovine tissue. It is unclear whether this is associated with fast elimination of cysts from cattle tissues or with inconsistent cyst formation following infection. In a study analyzing more than 2000 samples of beef, *T. gondii* was not isolated by bioassay in cat (Dubey et al. 2005). Moreover, a large French study reports a low prevalence in beef, but isolation of two strains (genotype II) was possible (Blaga et al. 2019). The prevalence of viable *T. gondii* in chickens produced in intensive farming is usually very low but may be high in free-range chickens. It is not known how many tissue cysts result in the infection of humans, but ingestion of one cyst is sufficient for a cat to become infected.

During the production of various meat products, meat of many animals is mixed, which also amplifies the risk in cases where only few animals would be infected (Aspinall et al. 2002).

Cases of acute toxoplasmosis and two recent outbreaks among hunters in Wisconsin and Illinois, USA, have already been linked to the consumption of insufficiently heated or raw game (Schumacher et al. 2021; Gaulin et al. 2020) underlining the risk of this kind of meat, as described a few years ago (Carme et al. 2002).

16.4.3 Genotype Distribution

In Europe, the majority of isolates from wildlife contain type II strains, with a few type III strains. From 26 *T. gondii* DNA extracts from red fox in Belgium submitted to a genotyping analysis with 15 microsatellite markers (Ajzenberg et al. 2010),

25 were type II, and only 1 type III (De Craeye et al. 2011). Similarly, using 6 loci microsatellite analysis, only type II strains were observed in 46 French isolates including 21 from wild boar (Richomme et al. 2009); 12 from roe deer; 9 from foxes; 1 from mouflon, red deer, and mallard (Aubert et al. 2010); and 1 from tawny owl (Aubert et al. 2008). Using the same molecular technique, Jokelainen et al. (2011) also identified the clonal type II in 15 DNA extracts from hare in Finland. In a recent study in Central and in Eastern Germany, Hermann et al. (2010) determined the complete genotype for 12 samples tissues from red foxes, using nine PCR-RFLP markers. Interestingly, this study showed evidence of a mixed infection, as well as infection with a *T. gondii* genotype that may represent a recombination of *T. gondii* types II and III. Su et al. (2006) developed a standardized restriction fragment length polymorphism (RFLP) typing scheme based on nine mostly unlinked nuclear genomic loci and one apicoplast marker. These markers enable one to distinguish the archetypal from atypical types. In addition, these markers can easily detect mixed strains in samples. Mixed infection of *T. gondii* strains in intermediary hosts has been previously reported (Ajzenberg et al. 2002a; Aspinall et al. 2002). In Svalbard, a Norwegian arctic archipelago, 55 artic foxes were found infected with *T. gondii*: 27 harbored clonal type II (17/27 were apico I and 10/27 apico II) and 4 had clonal type III (Prestrud et al. 2008). Strains from 22 foxes (40%) could not be fully genotyped, but 2 (3.6%) shared more than one allele at a given locus. Again, the most prevalent genotype in this study was clonal type II with a few types III genotypes. It is noteworthy that type II is also the dominant type in domestic mammals in Europe. For instance, Halos et al. (2010) analyzed 433 hearts of sheep by using PCR-restriction fragment length polymorphism and microsatellite markers on parasites isolated after bioassay in mice. All 46 genotypes belonged to type II, except for 1 strain from the Pyrenees mountains area, which belonged to genotype III, which is the first non-type II genotype found in sheep in Europe (Owen and Trees 1999) and Denmark (Jungersen et al. 2002). This similarity between strains found in wildlife and domestic species in Europe suggests that no clear separation exists between the two cycles.

In North America, strains of *T. gondii* are more diverse. A recent study analyzed 169 *T. gondii* isolates from various wildlife species and revealed the large dominance of the recently clonal type 12, followed by the type II and III lineages; these three major lineages accounted for 85% of strains from wildlife in North America (Dubey et al. 2011). The strains isolated from wildlife in North America are thus more diverse but may also be more different from strains found in the domestic environment than in Europe. Although type 12 has been identified from pigs and sheep in the USA, it may be more specifically found in wildlife (Su et al. 2012). The relative high diversity in *T. gondii* genotypes isolated from wildlife samples compared to those from domestic animals raised the question whether distinct gene pools exist for domestic and sylvatic hosts (Wendte et al. 2011).

This high genetic diversity in tropical wildlife in connection with a sylvatic life cycle has been firstly evoked in French Guiana where severe cases of human toxoplasmosis were detected after eating Amazonian undercooked game or drinking untreated river water (Darde et al. 1998; Carme et al. 2009). These cases were due to

highly atypical strains, all with a unique genotype, as determined by microsatellite analysis (Ajzenberg et al. 2004).

Finally, using multilocus genes sequencing (MLST) (in which the nucleotide sequences of several loci coding for housekeeping genes are analyzed) or whole genome sequencing, this diversity was shown to cluster into 16 haplogroups belonging to 6 ancestral groups (clades) and distributed throughout the world (Su et al. 2012; Lorenzi et al. 2016).

16.4.4 Most Frequent Sources for Human Infection: Case-Control Studies, Outbreaks

Different approaches have been used to estimate the relative importance of sources of contamination, using risk-factor analyses or estimation of the fraction of attributable risk, either in the general population (chronic infection) or in cases of seroconversion in pregnant women. These studies clearly identified the ingestion of undercooked meat as a risk factor (Cook et al. 2000; Kapperud et al. 1996; Baril et al. 1999; Berger et al. 2009). However, this result is probably partly due to this risk being easier to characterize than the risk due to oocysts. Several reports concerning toxoplasmosis outbreaks have been published in recent decades, mainly regarding outbreaks associated with the consumption of undercooked meat (Dubey 2022; AFSSA 2005). Moreover, it is difficult to link outbreaks due to *Toxoplasma* contamination, because most of infections are asymptomatic. In France, only few familial outbreaks were reported due to consumption of raw lamb (Ginsbourger et al. 2010).

However, outbreaks can be reported when infections are symptomatic due to virulent strains (from atypical genotype). In this way, previous studies demonstrated that game from the Amazonian forest was strongly associated with the risk of developing 10–20 days later severe toxoplasmosis (Carme et al. 2002). In French Guiana, atypical strains of *T. gondii* originating from a complex rainforest cycle involving wild felids have been linked to severe infections in humans, but these cases of Amazonian toxoplasmosis are sporadic, and outbreaks are rarely described. An outbreak of toxoplasmosis from December 2003 through mid-January 2004 involving 11 cases among the 38 inhabitants of a village in Suriname near the French Guiana border was described (Demar et al. 2007), and more recently another outbreak was reported linked to consumption of untreated water or meat or contact with soil (Blaizot et al. 2020). Severe toxoplasmosis after consumption of semi-raw game (Brazilian Tapir, locally known as Maipouri) was reported (Groh et al. 2012).

Recently, an emerging risk was observed in France with severe human infection transmitted by imported horse meat; the source of infection seems to be linked with consumption of raw horse meat from South America where atypical strains are circulating; these strains are virulent and responsible for severe congenital toxoplasmosis or death in immunocompetent humans (Pomares et al. 2011). This risk must be evaluated by survey of imported meat in large scale, and French authorities and EFSA are informed.

Moreover, severe toxoplasmosis was also observed after waterborne outbreaks due to contamination by oocysts. The first large outbreak was reported in British

Columbia, Canada, where drinking water taken from a reservoir was suspected to be contaminated by oocysts from cougar (Bowie et al. 1997). Unfortunately, oocysts were not detected after the outbreak in the reservoir. In contrast, *T. gondii* oocysts were isolated once in Brazil from samples taken from small reservoirs on roof tops. To assess this detection, the water was filtered through membranes, and the filters were fed to pigs and chickens, which developed toxoplasmic infection (de Moura et al. 2006). Although detection of oocysts in environmental samples is difficult (Dumetre and Darde 2007; Jones and Dubey 2010), methodological development is being made (Shapiro et al. 2019). Several methods to detect the presence of oocysts in filtered water is described using PCR to detect DNA (but not viable oocysts) from *T. gondii* after concentration (Kourenti and Karanis 2004; Villena et al. 2004). Oocysts can also contaminate vegetables and lead to human infection, but associated cases with identified vegetables are very rare. One case-control study of an outbreak of acute toxoplasmosis in Brazil was described with implication of escarole and green vegetables (Ekman et al. 2012).

While differentiating routes of *T. gondii* acquisition has been historically difficult, a recently recognized oocyst-specific antigen (Munoz-Zanzi et al. 2010) applied in a study of 76 mothers of congenitally infected infants in the USA demonstrated that 78% of these women had oocyst-acquired infections (Boyer et al. 2011).

Recently, a meta-analysis of the main identified and known risk factors for toxoplasmosis was published (Thebault et al. 2021). In this meta-analysis, the quality assessment stage was passed by 213 primary studies investigating risk factors for sporadic infection. The meta-analysis of toxoplasma sporadic infections revealed the significance of transmission by environmental factors such as drinking water, contact with soil, and contact with animals – in particular cats. The consumption of raw or undercooked meat and unwashed vegetables significantly increased the odds of acquiring the disease. Shellfish and raw milk were also identified as significant sources of toxoplasmosis. Almost all meat categories were identified as risk factors: pork, poultry, beef, processed meat, lamb, and game meat.

16.5 Impact on Public Health and Healthcare Decision-Makers

16.5.1 Implementation of Prevention Measures: Depending on Seroprevalence

16.5.1.1 Hygienic Measures

Hygienic measures can be recommended to seronegative patients (pregnant women or immunocompromised patients) to avoid *Toxoplasma* contamination. The prevention messages currently associate hygienic measures relative to the cat, to the consumption of well-cooked meat and thoroughly washed vegetables eaten raw, as well as to hands cleaning (Table 1). Drinking water has recently emerged as a new risk factor in some countries, depending on the source of the water supply network (surface or ground water) and on the sanitary level or the use of well water. Indeed, several outbreaks have been reported in Brazil, India, and Canada (Bahia-Oliveira et al. 2003; Bowie et al. 1997). Besides, ingestion of contaminated water from lakes or rivers during

Table 1 Hygienic measures according to prevention of *Toxoplasma* infection in seronegative population

Action or situation	Prevention measures
Cat contact	Wash hands carefully after stroking a cat Wear gloves when changing the cat litter Change the litter frequently and wash the tray with hot water (>60 °C) Avoid the litter in the kitchen
Meals	Cook the meat well-done or stew Avoid microwave cooking for meat Avoid raw vegetables at restaurants Avoid raw shellfish Avoid raw goat milk
Preparation of meals	Wash thoroughly vegetables, fruits, and herbs eaten raw, especially if they grow close to the ground Wash hands, knives, any containers, and table thoroughly after meat manipulation or cutting
Water	Prefer mineral water to tap water
Gardening or outdoor activities	Wash hands thoroughly and brush nails after any outdoor activities in contact with soil Wear gloves for gardening Avoid ingestion of water during recreation activities in lakes or rivers

recreational activities has been recently stressed out as a potential source for *Toxoplasma* infection (Jones and Dubey 2010), which could explain the large proportion of unexplained toxoplasmosis in pregnant women as shown in a study in Northern USA (Jones et al. 2009). The environmental contamination by wild felids is more difficult to master, but basic hygienic measures during or after external activities can help to overcome the risk. Finally, the trends of biologic food consumption should not lead to hazardous behavior, and it is worth being reminded that, even marginal, unpasteurized goat milk or raw shellfish can also be food at risk (Jones et al. 2009).

Recent knowledge on strain virulence should be taken into consideration, and these recommendations should now be applied to travelers who are visiting countries where virulent strains predominate, such as South America or Africa, even if they were previously immunized.

16.5.1.2 Serologic Screening

Serologic screening of pregnant women has been proposed to reduce the burden of congenital toxoplasmosis and has been implemented in France in 1987, with repeated monthly testing of seronegative women from 1992. The rationale of this approach relies on the possibility to perform a prenatal diagnosis and to treat the mother in case of seroconversion. Intuitively, this approach is pertinent when the seroprevalence is relatively high; thus the probability of infection during pregnancy is also high. Conversely, a low prevalence of the disease overweighs the cost of such public health policy. Therefore, the cost-benefit ratio should be carefully evaluated before the implementation of such screening measures. Other countries have a screening policy (Austria, Belgium, Italy, Lithuania, Slovenia) (Benard et al. 2008), but the frequency of serologic testing may vary from one- to three-monthly

testing. Some countries do not recommend this screening (the UK, Norway, Finland, and more recently Switzerland), arguing either for a too high cost or maternal stress in case of diagnosing a seroconversion or even fetal risk associated with amniocentesis. In France, a trend towards a regular decrease in seroprevalence has been observed since the 1970s and has been measured through three national perinatal surveys in 1995 (55%), 2003 (44%), and 2010 (37%), thus questioning the cost of maintaining a full screening of seronegative women (Robinson et al. 2021).

Postnatal screening of neonates at risk is another option, which has been implemented in some countries where prenatal screening was not considered to be a health priority, as is the case in Sweden, Denmark, Poland, or the USA (Massachusetts, New England). It allows the treatment of infected neonates in the aim to reduce the development of eye or neurologic sequelae. The cost of such screening has been also debated in Denmark, where it was estimated that the low burden of disease (1.6 per 10,000 live-born infants) did not justify continuing neonatal screening (Roser et al. 2010). In contrast, two cost-benefit analyses (CBA) found prenatal retesting for toxoplasmosis to be cost saving, one with the hypothesis of applying the French screening protocol in the USA (Stillwaggon et al. 2011) and the other examining the Austrian screening protocol (Prusa et al. 2017).

As for congenital toxoplasmosis, there is no consensus about serologic screening in immunodeficient patients, yet an annual serologic testing is usually recommended in HIV-infected patients previously seronegative for *Toxoplasma*. Prevention messages on how avoiding contamination should also be the rule, as for pregnant women. A chemoprophylaxis is recommended when CD4+ T cells is under 200/μL.

In transplant patients, the decision of *Toxoplasma* antibody screening varies highly among countries and is again mostly explained by the differences in the incidence rate of toxoplasmosis. A case-control study (Fernandez-Sabe et al. 2011) showed that primary infection observed in about solid organ transplant patients was due to a mismatch D+/R- in about half of patients, emphasizing the interest of primary prevention through both donor/recipient screening and hygienic measures. In France, serologic screening of the organ donor is mandatory and is strongly recommended (in practice, always done) in the recipient, whereas in the USA serologic screening of donors and recipients greatly depends on the transplant center. Serology of the donor is also routinely performed in 11 European countries (Derouin and Pelloux 2008). The knowledge of both recipient and donor serologic status allows starting primary chemoprophylaxis at the time of transplantation in case of a mismatch, particularly for heart transplant patients. More detailed information on the risk and prevention can be found in a recent review (Robert-Gangneux and Darde 2012).

16.5.2 Impact of Prevention and Screening on the Burden of Disease: Congenital Toxoplasmosis

A French retrospective study by Wallon et al. (2013) estimated the incidence of congenital transmission and the proportion of severely impaired infected neonates before and after onset of screening policy associated with prevention measures and maternal treatment, i.e., before and after 1992, respectively. They clearly found a

significant impact of monthly mandatory re-testing of seronegative women on the transmission rate of congenital infection, as well as an impact of treatment strategy adapted to prenatal diagnosis. The continuous treatment of women with positive prenatal diagnosis with pyrimethamine-sulfadiazine significantly reduced the severity of clinical signs in infected children.

In the USA, several longitudinal clinical studies showed that newborn postnatal screening and treatment were associated with better neurologic and developmental outcomes (Roizen et al. 1995; McLeod et al. 2006; Darde et al. 1992). By contrast, in ancient series, untreated infants, although asymptomatic at birth, developed high rates of ocular lesions or neurologic sequelae or suffered from recurrent episodes of retinochoroiditis (72%), despite spot treatment at time of diagnosis and at each recurrent lesion (Phan et al. 2008). More recently, Stillwaggon et al. (2011) evaluated the societal cost of congenital toxoplasmosis and concluded that implementation of a similar prevention program as that currently applied in France (maternal serologic screening, prenatal diagnosis and treatment) would be cost-saving at the scale of the USA, even if the seroprevalence is low, with an infection rate of 1 per 10,000 live births. However, the pattern of infection in North America could be more harmful than previously suspected, probably related to the genotypes of circulating strains. This study estimated the cost of mild vision loss and of severe toxoplasmosis at a rate of 500,000 US$ and 2.7 million $, respectively.

The role of the epidemiology of parasite strains in North America has recently been discussed because of the discovery of the circulation of type non-II strains, which could be more virulent than those responsible for toxoplasmosis in Europe (McLeod et al. 2012). It has been hypothesized that the disease burden in the USA could be due not only to the absence of prenatal management or postnatal treatment. However, the important preventive role of healthcare policies in the decrease of the burden of toxoplasmosis in France cannot be denied, and a recent cost effectiveness study modeling the monthly retesting programs vs. neonatal screening has demonstrated the benefit of the French prenatal screening program (Binquet et al. 2019).

16.6 Cross-References

▶ Cats – Revered and Reviled – and Associated Zoonoses
▶ Cryptosporidium and Cryptosporidiosis: Trickle or Treat?

References

Abadi MA, Rahimi E, Shakerian A (2020) Seasonal and age distribution of *Toxoplasma gondii* in milk of naturally infected animal species and dairy samples. Egypt J Vet Sci 51(2):171–180. https://doi.org/10.21608/ejvs.2020.20965.1143

Abgrall S, Rabaud C, Costagliola D (2001) Incidence and risk factors for toxoplasmic encephalitis in human immunodeficiency virus-infected patients before and during the highly active antiretroviral therapy era. Clin Infect Dis 33(10):1747–1755. CID001414 [pii]. https://doi.org/10.1086/322622

AFSSA (2005) Toxoplasmose: état des connaissances et évaluation du risqué lié à l'alimentation. Rapport du groupe de travail « *Toxoplasma gondii* » Maisons Alfort

Ajzenberg D, Banuls AL, Tibayrenc M, Darde ML (2002a) Microsatellite analysis of *Toxoplasma gondii* shows considerable polymorphism structured into two main clonal groups. Int J Parasitol 32(1):27–38. https://doi.org/10.1016/s0020-7519(01)00301-0

Ajzenberg D, Cogne N, Paris L, Bessieres MH, Thulliez P, Filisetti D, Pelloux H, Marty P, Darde ML (2002b) Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J Infect Dis 186(5):684–689. https://doi.org/10.1086/342663

Ajzenberg D, Banuls AL, Su C, Dumetre A, Demar M, Carme B, Darde ML (2004) Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. Int J Parasitol 34(10):1185–1196. https://doi.org/10.1016/j.ijpara.2004.06.007

Ajzenberg D, Collinet F, Mercier A, Vignoles P, Darde ML (2010) Genotyping of *Toxoplasma gondii* isolates with 15 microsatellite markers in a single multiplex PCR assay. J Clin Microbiol 48(12):4641–4645. https://doi.org/10.1128/JCM.01152-10

Artois M (1993) Epidemiology of contagious diseases, ecology of mammals, health, management and conservation biology: concluding remarks. Rev Sci Tech 12(1):203–234. https://doi.org/10.20506/rst.12.1.686

Aspinall TV, Marlee D, Hyde JE, Sims PF (2002) Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction – food for thought? Int J Parasitol 32(9):1193–1199. https://doi.org/10.1016/s0020-7519(02)00070-x

Aubert D, Terrier ME, Dumetre A, Barrat J, Villena I (2008) Prevalence of *Toxoplasma gondii* in raptors from France. J Wildl Dis 44(1):172–173. https://doi.org/10.7589/0090-3558-44.1.172

Aubert D, Ajzenberg D, Richomme C, Gilot-Fromont E, Terrier ME, de Gevigney C, Game Y, Maillard D, Gibert P, Darde ML, Villena I (2010) Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Vet Parasitol 171(3–4):346–349. https://doi.org/10.1016/j.vetpar.2010.03.033

Bahia-Oliveira LM, Jones JL, Azevedo-Silva J, Alves CC, Orefice F, Addiss DG (2003) Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. Emerg Infect Dis 9(1):55–62. https://doi.org/10.3201/eid0901.020160

Baril L, Ancelle T, Goulet V, Thulliez P, Tirard-Fleury V, Carme B (1999) Risk factors for Toxoplasma infection in pregnancy: a case-control study in France. Scand J Infect Dis 31(3):305–309. https://doi.org/10.1080/00365549950163626

Bayarri S, Gracia MJ, Perez-Arquillue C, Lazaro R, Herrera A (2012) *Toxoplasma gondii* in commercially available pork meat and cured ham: a contribution to risk assessment for consumers. J Food Prot 75(3):597–600. https://doi.org/10.4315/0362-028X.JFP-11-350

Behnke MS, Khan A, Lauron EJ, Jimah JR, Wang Q, Tolia NH, Sibley LD (2015) Rhoptry proteins ROP5 and ROP18 are major murine virulence factors in genetically divergent South American strains of *Toxoplasma gondii*. PLoS Genet 11(8):e1005434. https://doi.org/10.1371/journal.pgen.1005434

Bellali H, Pelloux H, Villena I, Fricker-Hidalgo H, Le Strat Y, Goulet V (2013) Prevalence of toxoplasmosis in France in 1998: is there a difference between men and women? At what age do children become infected? Rev Epidemiol Sante Publique 61(4):311–317. https://doi.org/10.1016/j.respe.2012.11.005

Benard A, Petersen E, Salamon R, Chene G, Gilbert R, Salmi LR, European Toxo Prevention Study G (2008) Survey of European programmes for the epidemiological surveillance of congenital toxoplasmosis. Euro Surveill 13(15). https://doi.org/10.2807/ese.13.15.18834-en

Beral M, Rossi S, Aubert D, Gasqui P, Terrier ME, Klein F, Villena I, Abrial D, Gilot-Fromont E, Richomme C, Hars J, Jourdain E (2012) Environmental factors associated with the seroprevalence of *Toxoplasma gondii* in Wild Boars (*Sus scrofa)*, France. Ecohealth 9(3):303–309. https://doi.org/10.1007/s10393-012-0786-2

Berger F, Goulet V, Le Strat Y, Desenclos JC (2009) Toxoplasmosis among pregnant women in France: risk factors and change of prevalence between 1995 and 2003. Rev Epidemiol Sante Publique 57(4):241–248. https://doi.org/10.1016/j.respe.2009.03.006

Bertranpetit E, Jombart T, Paradis E, Pena H, Dubey J, Su C, Mercier A, Devillard S, Ajzenberg D (2017) Phylogeography of *Toxoplasma gondii* points to a South American origin. Infect Genet Evol 48:150–155. https://doi.org/10.1016/j.meegid.2016.12.020

Bigna JJ, Tochie JN, Tounouga DN, Bekolo AO, Ymele NS, Youda EL, Sime PS, Nansseu JR (2020) Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. Sci Rep 10(1):12102. https://doi.org/10.1038/s41598-020-69078-9

Binquet C, Lejeune C, Seror V, Peyron F, Bertaux AC, Scemama O, Quantin C, Bejean S, Stillwaggon E, Wallon M (2019) The cost-effectiveness of neonatal versus prenatal screening for congenital toxoplasmosis. PLoS One 14(9):e0221709. https://doi.org/10.1371/journal.pone.0221709

Blaga R, Aubert D, Thebault A, Perret C, Geers R, Thomas M, Alliot A, Djokic V, Ortis N, Halos L, Durand B, Mercier A, Villena I, Boireau P (2019) *Toxoplasma gondii* in beef consumed in France: regional variation in seroprevalence and parasite isolation. Parasite 26:77. https://doi.org/10.1051/parasite/2019076

Blaizot R, Nabet C, Laghoe L, Faivre B, Escotte-Binet S, Djossou F, Mosnier E, Henaff F, Blanchet D, Mercier A, Darde ML, Villena I, Demar M (2020) Outbreak of Amazonian toxoplasmosis: a one health investigation in a remote Amerindian Community. Front Cell Infect Microbiol 10:401. https://doi.org/10.3389/fcimb.2020.00401

Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, Marion SA (1997) Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma Investigation Team. Lancet 350(9072):173–177. https://doi.org/10.1016/s0140-6736(96)11105-3

Boyer K, Hill D, Mui E, Wroblewski K, Karrison T, Dubey JP, Sautter M, Noble AG, Withers S, Swisher C, Heydemann P, Hosten T, Babiarz J, Lee D, Meier P, McLeod R, Toxoplasmosis Study G (2011) Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. Clin Infect Dis 53(11):1081–1089. https://doi.org/10.1093/cid/cir667

Cabezon O, Garcia-Bocanegra I, Molina-Lopez R, Marco I, Blanco JM, Hofle U, Margalida A, Bach-Raich E, Darwich L, Echeverria I, Obon E, Hernandez M, Lavin S, Dubey JP, Almeria S (2011) Seropositivity and risk factors associated with *Toxoplasma gondii* infection in wild birds from Spain. PLoS One 6(12):e29549. https://doi.org/10.1371/journal.pone.0029549

Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, Bichat S, Louvel D, Bourbigot AM, Peneau C, Neron P, Darde ML (2002) Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. J Clin Microbiol 40(11):4037–4044. https://doi.org/10.1128/JCM.40.11.4037-4044.2002

Carme B, Demar M, Ajzenberg D, Darde ML (2009) Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. Emerg Infect Dis 15(4):656–658. https://doi.org/10.3201/eid1504.081306

Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT (2000) Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ 321(7254):142–147. https://doi.org/10.1136/bmj.321.7254.142

Darde ML, Bouteille B, Pestre-Alexandre M (1992) Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. J Parasitol 78(5):786–794

Darde ML, Villena I, Pinon JM, Beguinot I (1998) Severe toxoplasmosis caused by a *Toxoplasma gondii* strain with a new isoenzyme type acquired in French Guyana. J Clin Microbiol 36(1):324. https://doi.org/10.1128/JCM.36.1.324-324.1998

De Craeye S, Speybroeck N, Ajzenberg D, Darde ML, Collinet F, Tavernier P, Van Gucht S, Dorny P, Dierick K (2011) *Toxoplasma gondii* and *Neospora caninum* in wildlife: common parasites in Belgian foxes and Cervidae? Vet Parasitol 178(1–2):64–69. https://doi.org/10.1016/j.vetpar.2010.12.016

de Moura L, Bahia-Oliveira LM, Wada MY, Jones JL, Tuboi SH, Carmo EH, Ramalho WM, Camargo NJ, Trevisan R, Graca RM, da Silva AJ, Moura I, Dubey JP, Garrett DO (2006) Waterborne toxoplasmosis, Brazil, from field to gene. Emerg Infect Dis 12(2):326–329. https://doi.org/10.3201/eid1202.041115

Demar M, Ajzenberg D, Maubon D, Djossou F, Panchoe D, Punwasi W, Valery N, Peneau C, Daigre JL, Aznar C, Cottrelle B, Terzan L, Darde ML, Carme B (2007) Fatal outbreak of human toxoplasmosis along the Maroni River: epidemiological, clinical, and parasitological aspects. Clin Infect Dis 45(7):e88–e95. https://doi.org/10.1086/521246

Derouin F, Pelloux H (2008) Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infect 14(12):1089–1101. CLM2091 [pii]. https://doi.org/10.1111/j.1469-0691.2008.02091.x

Desmonts G, Couvreur J (1974) Congenital toxoplasmosis. A prospective study of 378 pregnancies. N Engl J Med 290(20):1110–1116. https://doi.org/10.1056/NEJM197405162902003

Dubey JP (1998) *Toxoplasma gondii* oocyst survival under defined temperatures. J Parasitol 84(4): 862–865

Dubey JP (2002) A review of toxoplasmosis in wild birds. Vet Parasitol 106(2):121–153. https://doi.org/10.1016/s0304-4017(02)00034-1

Dubey JP (2021) Outbreaks of clinical toxoplasmosis in humans: five decades of personal experience, perspectives and lessons learned. Parasit Vectors 14(1):263. https://doi.org/10.1186/s13071-021-04769-4

Dubey JP (2022) Toxoplasmosis of animals and humans. CRC Press, Boca Raton

Dubey J, Beatty C (1988) Toxoplasmosis of animals and man. CRC Press, Boca Raton

Dubey JP, Frenkel JK (1974) Immunity to feline toxoplasmosis: modification by administration of corticosteroids. Vet Pathol 11(4):350–379. https://doi.org/10.1177/030098587401100407

Dubey JP, Jones JL (2008) *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol 38(11):1257–1278. https://doi.org/10.1016/j.ijpara.2008.03.007

Dubey JP, Shen SK, Kwok OC, Thulliez P (1997) Toxoplasmosis in rats (*Rattus norvegicus*): congenital transmission to first and second generation offspring and isolation of *Toxoplasma gondii* from seronegative rats. Parasitology 115(Pt 1):9–14. https://doi.org/10.1017/s0031182097008950

Dubey JP, Storandt ST, Kwok OC, Thulliez P, Kazacos KR (1999) *Toxoplasma gondi*i antibodies in naturally exposed wild coyotes, red foxes, and gray foxes and serologic diagnosis of Toxoplasmosis in red foxes fed T. *gondii* oocysts and tissue cysts. J Parasitol 85(2):240–243

Dubey JP, Graham DH, De Young RW, Dahl E, Eberhard ML, Nace EK, Won K, Bishop H, Punkosdy G, Sreekumar C, Vianna MC, Shen SK, Kwok OC, Sumners JA, Demarais S, Humphreys JG, Lehmann T (2004) Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. J Parasitol 90(1):67–71. https://doi.org/10.1645/GE-110R

Dubey JP, Hill DE, Jones JL, Hightower AW, Kirkland E, Roberts JM, Marcet PL, Lehmann T, Vianna MC, Miska K, Sreekumar C, Kwok OC, Shen SK, Gamble HR (2005) Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. J Parasitol 91(5):1082–1093. https://doi.org/10.1645/GE-683.1

Dubey JP, Velmurugan GV, Rajendran C, Yabsley MJ, Thomas NJ, Beckmen KB, Sinnett D, Ruid D, Hart J, Fair PA, McFee WE, Shearn-Bochsler V, Kwok OC, Ferreira LR, Choudhary S, Faria EB, Zhou H, Felix TA, Su C (2011) Genetic characterisation of *Toxoplasma gondii* in wildlife from North America revealed widespread and high prevalence of the fourth clonal type. Int J Parasitol 41(11):1139–1147. https://doi.org/10.1016/j.ijpara.2011.06.005

Dubey JP, Hill DE, Rozeboom DW, Rajendran C, Choudhary S, Ferreira LR, Kwok OC, Su C (2012) High prevalence and genotypes of *Toxoplasma gondii* isolated from organic pigs in northern USA. Vet Parasitol 188(1–2):14–18. https://doi.org/10.1016/j.vetpar.2012.03.008

Dubey JP, Cerqueira-Cezar CK, Murata FHA, Kwok OCH, Yang YR, Su C (2020) All about toxoplasmosis in cats: the last decade. Vet Parasitol 283:109145. https://doi.org/10.1016/j.vetpar.2020.109145

Dubey JP, Murata FHA, Cerqueira-Cezar CK, Kwok OCH (2021) Epidemiologic and public health significance of *Toxoplasma gondii* infections in venison: 2009-2020. J Parasitol 107(2): 309–319. https://doi.org/10.1645/20-162

Dumetre A, Darde ML (2007) Detection of *Toxoplasma gondii* in water by an immunomagnetic separation method targeting the sporocysts. Parasitol Res 101(4):989–996. https://doi.org/10.1007/s00436-007-0573-0

Dunn D, Wallon M, Peyron F, Petersen E, Peckham C, Gilbert R (1999) Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet 353(9167):1829–1833. S0140-6736(98)08220-8 [pii]. https://doi.org/10.1016/S0140-6736(98)08220-8

Ekman CC, Chiossi MF, Meireles LR, Andrade Junior HF, Figueiredo WM, Marciano MA, Luna EJ (2012) Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo 54(5):239–244. https://doi.org/10.1590/s0036-46652012000500001

Elbez-Rubinstein A, Ajzenberg D, Darde ML, Cohen R, Dumetre A, Yera H, Gondon E, Janaud JC, Thulliez P (2009) Congenital toxoplasmosis and reinfection during pregnancy: case report, strain characterization, experimental model of reinfection, and review. J Infect Dis 199(2): 280–285. https://doi.org/10.1086/595793

ElHajj H, Lebrun M, Arold ST, Vial H, Labesse G, Dubremetz JF (2007) ROP18 is a rhoptry kinase controlling the intracellular proliferation of *Toxoplasma gondii*. PLoS Pathog 3(2):e14. https://doi.org/10.1371/journal.ppat.0030014. 06-PLPA-RA-0306R3 [pii].

Fernandez-Sabe N, Cervera C, Farinas MC, Bodro M, Munoz P, Gurgui M, Torre-Cisneros J, Martin-Davila P, Noblejas A, Len O, Garcia-Reyne A, Del Pozo JL, Carratala J (2011) Risk factors, clinical features, and outcomes of toxoplasmosis in solid-organ transplant recipients: a matched case-control study. Clin Infect Dis. cir806 [pii]. https://doi.org/10.1093/cid/cir806

Fitzgerald BMTD (2000) Hunting behavior of domestic cats and their impact on prey populations. In: Turner DCBP (ed) The domestic cat: the biology of its behavior, 2nd edn. Cambridge University Press, Cambridge, pp 152–175

Frenkel JK, Dubey JP, Miller NL (1970) *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. Science 167(3919):893–896. https://doi.org/10.1126/science.167.3919.893

Gaulin C, Ramsay D, Thivierge K, Tataryn J, Courville A, Martin C, Cunningham P, Desilets J, Morin D, Dion R (2020) Acute toxoplasmosis among Canadian Deer Hunters associated with consumption of undercooked Deer Meat Hunted in the United States. Emerg Infect Dis 26(2): 199–205. https://doi.org/10.3201/eid2602.191218

Gauss CB, Dubey JP, Vidal D, Cabezon O, Ruiz-Fons F, Vicente J, Marco I, Lavin S, Gortazar C, Almeria S (2006) Prevalence of *Toxoplasma gondii* antibodies in red deer (Cervus elaphus) and other wild ruminants from Spain. Vet Parasitol 136(3–4):193–200. https://doi.org/10.1016/j.vetpar.2005.11.013

Gennari SM, Niemeyer C, Soares HS, Musso CM, Siqueira GC, Catao-Dias JL, Dias RA, Dubey JP (2016) Seroprevalence of *Toxoplasma gondii* in seabirds from Abrolhos Archipelago, Brazil. Vet Parasitol 226:50–52. https://doi.org/10.1016/j.vetpar.2016.06.016

Gilbert RE, Freeman K, Lago EG, Bahia-Oliveira LM, Tan HK, Wallon M, Buffolano W, Stanford MR, Petersen E (2008) Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PLoS Negl Trop Dis 2(8):e277. https://doi.org/10.1371/journal.pntd.0000277

Ginsbourger M, Guinard A, Villena I, King LA, El-Eid N, Schwoebel V (2010) Collective outbreak of food poisoning due to *Toxoplasma gondii* associated with the consumption of lamb meat, Aveyron (France), November 2010. Bull Epidemiol Hebd 16–17:195–197

Groh M, Faussart A, Villena I, Ajzenberg D, Carme B, Demar M, Joly V, Houze S, Simon S, Aubert D, Charlois-Ou C, Yeni P (2012) Acute lung, heart, liver, and pancreatic involvements with hyponatremia and retinochoroiditis in a 33-year-old French Guianan patient. PLoS Negl Trop Dis 6(10):e1802. https://doi.org/10.1371/journal.pntd.0001802

Guerina NG, Hsu HW, Meissner HC, Maguire JH, Lynfield R, Stechenberg B, Abroms I, Pasternack MS, Hoff R, Eaton RB et al (1994) Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. The New England Regional Toxoplasma Working Group. N Engl J Med 330(26):1858–1863. https://doi.org/10.1056/NEJM199406303302604

Halos L, Thebault A, Aubert D, Thomas M, Perret C, Geers R, Alliot A, Escotte-Binet S, Ajzenberg D, Darde ML, Durand B, Boireau P, Villena I (2010) An innovative survey underlining the significant level of contamination by *Toxoplasma gondii* of ovine meat consumed in France. Int J Parasitol 40(2):193–200. https://doi.org/10.1016/j.ijpara.2009.06.009

Havelaar AH, Kemmeren JM, Kortbeek LM (2007) Disease burden of congenital toxoplasmosis. Clin Infect Dis 44(11):1467–1474. https://doi.org/10.1086/517511

Hejlíček K, Literak I, Nezval J (1997) Toxoplasmosis in wild mammals from the Czech Republic. J Wildl Dis 33(3):480–485. https://doi.org/10.7589/0090-3558-33.3.480

Herrmann DC, Pantchev N, Vrhovec MG, Barutzki D, Wilking H, Frohlich A, Luder CG, Conraths FJ, Schares G (2010) Atypical *Toxoplasma gondii* genotypes identified in oocysts shed by cats in Germany. Int J Parasitol 40(3):285–292. https://doi.org/10.1016/j.ijpara.2009.08.001

Howe DK, Sibley LD (1995) *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis 172(6):1561–1566. https://doi.org/10.1093/infdis/172.6.1561

Hutchison WM, Dunachie JF, Siim JC, Work K (1969) Life cycle of *Toxoplasma gondii*. Br Med J 4(5686):806. https://doi.org/10.1136/bmj.4.5686.806-b

Jakubek EB, Brojer C, Regnersen C, Uggla A, Schares G, Bjorkman C (2001) Seroprevalences of *Toxoplasma gondii* and *Neospora caninum* in Swedish red foxes (*Vulpes vulpes*). Vet Parasitol 102(1–2):167–172. https://doi.org/10.1016/s0304-4017(01)00513-1

Jokelainen P, Isomursu M, Nareaho A, Oksanen A (2011) Natural *Toxoplasma gondii* infections in European brown hares and mountain hares in Finland: proportional mortality rate, antibody prevalence, and genetic characterization. J Wildl Dis 47(1):154–163. https://doi.org/10.7589/0090-3558-47.1.154

Jones JL, Dubey JP (2010) Waterborne toxoplasmosis – recent developments. Exp Parasitol 124(1):10–25. https://doi.org/10.1016/j.exppara.2009.03.013

Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG (2009) Risk factors for *Toxoplasma gondii* infection in the United States. Clin Infect Dis 49(6):878–884. https://doi.org/10.1086/605433

Jungersen G, Jensen L, Rask MR, Lind P (2002) Non-lethal infection parameters in mice separate sheep Type II *Toxoplasma gondii* isolates by virulence. Comp Immunol Microbiol Infect Dis 25(3):187–195. https://doi.org/10.1016/s0147-9571(01)00039-x

Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J (1996) Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. Am J Epidemiol 144(4):405–412. https://doi.org/10.1093/oxfordjournals.aje.a008942

Khan A, Fux B, Su C, Dubey JP, Darde ML, Ajioka JW, Rosenthal BM, Sibley LD (2007) Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. Proc Natl Acad Sci U S A 104(37):14872–14877. https://doi.org/10.1073/pnas.0702356104

Khan A, Dubey JP, Su C, Ajioka JW, Rosenthal BM, Sibley LD (2011) Genetic analyses of atypical *Toxoplasma gondii* strains reveal a fourth clonal lineage in North America. Int J Parasitol 41(6):645–655. S0020-7519(11)00046-4 [pii]. https://doi.org/10.1016/j.ijpara.2011.01.005

Kijlstra A, Jongert E (2009) *Toxoplasma*-safe meat: close to reality? Trends Parasitol 25(1):18–22. https://doi.org/10.1016/j.pt.2008.09.008

Kourenti C, Karanis P (2004) Development of a sensitive polymerase chain reaction method for the detection of *Toxoplasma gondii* in water. Water Sci Technol 50(1):287–291

Lago EG, Endres MM, Scheeren M, Fiori HH (2021) Ocular outcome of Brazilian patients with congenital toxoplasmosis. Pediatr Infect Dis J 40(1):e21–e27. https://doi.org/10.1097/INF.0000000000002931

Leroy J, Houze S, Darde ML, Yera H, Rossi B, Delhaes L, Gabriel F, Loubet P, Deleplancque AS, Senneville E, Ajana F, Sendid B, Malvy D (2020) Severe toxoplasmosis imported from tropical Africa in immunocompetent patients: a case series. Travel Med Infect Dis 35:101509. https://doi.org/10.1016/j.tmaid.2019.101509

Literák I, Hejlíček K, Nezval J, Folk Č (1992) Incidence of *Toxoplasma gondii* in populations of wild birds in the Czech Republic. Avian Pathol 21:659–665

Lorenzi H, Khan A, Behnke MS, Namasivayam S, Swapna LS, Hadjithomas M, Karamycheva S, Pinney D, Brunk BP, Ajioka JW, Ajzenberg D, Boothroyd JC, Boyle JP, Darde ML, Diaz-Miranda MA, Dubey JP, Fritz HM, Gennari SM, Gregory BD, Kim K, Saeij JP, Su C, White MW, Zhu XQ, Howe DK, Rosenthal BM, Grigg ME, Parkinson J, Liu L, Kissinger JC, Roos DS, Sibley LD (2016) Local admixture of amplified and diversified secreted pathogenesis determinants shapes mosaic *Toxoplasma gondii* genomes. Nat Commun 7:10147. https://doi.org/10.1038/ncomms10147

McLeod R, Boyer K, Karrison T, Kasza K, Swisher C, Roizen N, Jalbrzikowski J, Remington J, Heydemann P, Noble AG, Mets M, Holfels E, Withers S, Latkany P, Meier P, Toxoplasmosis Study G (2006) Outcome of treatment for congenital toxoplasmosis, 1981–2004: the National Collaborative Chicago-Based, Congenital Toxoplasmosis Study. Clin Infect Dis 42(10): 1383–1394. https://doi.org/10.1086/501360

McLeod R, Boyer KM, Lee D, Mui E, Wroblewski K, Karrison T, Noble AG, Withers S, Swisher CN, Heydemann PT, Sautter M, Babiarz J, Rabiah P, Meier P, Grigg ME, Toxoplasmosis Study G (2012) Prematurity and severity are associated with *Toxoplasma gondii* alleles (NCCCTS, 1981-2009). Clin Infect Dis 54(11):1595–1605. https://doi.org/10.1093/cid/cis258

Mercier A, Devillard S, Ngoubangoye B, Bonnabau H, Banuls AL, Durand P, Salle B, Ajzenberg D, Darde ML (2010) Additional haplogroups of *Toxoplasma gondii* out of Africa: population structure and mouse-virulence of strains from Gabon. PLoS Negl Trop Dis 4(11):e876. https://doi.org/10.1371/journal.pntd.0000876

Mercier A, Ajzenberg D, Devillard S, Demar MP, de Thoisy B, Bonnabau H, Collinet F, Boukhari R, Blanchet D, Simon S, Carme B, Darde ML (2011) Human impact on genetic diversity of *Toxoplasma gondii*: example of the anthropized environment from French Guiana. Infect Genet Evol. https://doi.org/10.1016/j.meegid.2011.05.003. S1567-1348(11)00142-0 [pii]

Montoya JG, Liesenfeld O (2004) Toxoplasmosis. Lancet 363(9425):1965–1976. https://doi.org/10.1016/S0140-6736(04)16412-X. S014067360416412X [pii]

Munoz-Zanzi CA, Fry P, Lesina B, Hill D (2010) *Toxoplasma gondii* oocyst-specific antibodies and source of infection. Emerg Infect Dis 16(10):1591–1593. https://doi.org/10.3201/eid1610.091674

Owen MR, Trees AJ (1999) Genotyping of *Toxoplasma gondii* associated with abortion in sheep. J Parasitol 85(2):382–384

Patrat-Delon S, Gangneux JP, Lavoue S, Lelong B, Guiguen C, le Tulzo Y, Robert-Gangneux F (2010) Correlation of parasite load determined by quantitative PCR to clinical outcome in a heart transplant patient with disseminated toxoplasmosis. J Clin Microbiol 48(7):2541–2545. JCM.00252-10 [pii]. https://doi.org/10.1128/JCM.00252-10

Paul M (1998) Potential risk factors for *Toxoplasma gondii* infection in cases with recently acquired toxoplasmosis. Przegl Epidemiol 52(4):447–454

Pena HF, Gennari SM, Dubey JP, Su C (2008) Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. Int J Parasitol 38(5):561–569. S0020-7519(07)00335-9 [pii]. https://doi.org/10.1016/j.ijpara.2007.09.004

Phan L, Kasza K, Jalbrzikowski J, Noble AG, Latkany P, Kuo A, Mieler W, Meyers S, Rabiah P, Boyer K, Swisher C, Mets M, Roizen N, Cezar S, Sautter M, Remington J, Meier P, Mc Leod R, Toxoplasmosis Study G (2008) Longitudinal study of new eye lesions in children with toxoplasmosis who were not treated during the first year of life. Am J Ophthalmol 146(3):375–384. https://doi.org/10.1016/j.ajo.2008.04.033

Pomares C, Ajzenberg D, Bornard L, Bernardin G, Hasseine L, Darde ML, Marty P (2011) Toxoplasmosis and horse meat, France. Emerg Infect Dis 17(7):1327–1328. https://doi.org/10.3201/eid1707.101642

Prestrud KW, Asbakk K, Mork T, Fuglei E, Tryland M, Su C (2008) Direct high-resolution genotyping of *Toxoplasma gondii* in arctic foxes *(Vulpes lagopus*) in the remote arctic Svalbard archipelago reveals widespread clonal Type II lineage. Vet Parasitol 158(1–2):121–128. https://doi.org/10.1016/j.vetpar.2008.08.020

Prusa AR, Kasper DC, Sawers L, Walter E, Hayde M, Stillwaggon E (2017) Congenital toxoplasmosis in Austria: prenatal screening for prevention is cost-saving. PLoS Negl Trop Dis 11(7): e0005648. https://doi.org/10.1371/journal.pntd.0005648

Richomme C, Aubert D, Gilot-Fromont E, Ajzenberg D, Mercier A, Ducrot C, Ferte H, Delorme D, Villena I (2009) Genetic characterization of *Toxoplasma gondii* from wild boar (*Sus scrofa*) in France. Vet Parasitol 164(2–4):296–300. https://doi.org/10.1016/j.vetpar.2009.06.014

Robert-Gangneux F, Darde ML (2012) Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev 25(2):264–296. https://doi.org/10.1128/CMR.05013-11. 25/2/264 [pii]

Robert-Gangneux F, Murat JB, Fricker-Hidalgo H, Brenier-Pinchart MP, Gangneux JP, Pelloux H (2011) The placenta: a main role in congenital toxoplasmosis? Trends Parasitol 27(12):530–536. https://doi.org/10.1016/j.pt.2011.09.005. S1471-4922(11)00169-3 [pii]

Robinson E, de Valk H, Villena I, Le Strat Y, Tourdjman M (2021) National perinatal survey demonstrates a decreasing seroprevalence of *Toxoplasma gondii* infection among pregnant women in France, 1995 to 2016: impact for screening policy. Euro Surveill 26(5). https://doi.org/10.2807/1560-7917.ES.2021.26.5.1900710

Roizen N, Swisher CN, Stein MA, Hopkins J, Boyer KM, Holfels E, Mets MB, Stein L, Patel D, Meier P et al (1995) Neurologic and developmental outcome in treated congenital toxoplasmosis. Pediatrics 95(1):11–20

Roser D, Nielsen HV, Petersen E, Saugmann-Jensen P, Norgaard-Pedersen B (2010) Congenital toxoplasmosis – a report on the Danish neonatal screening programme 1999–2007. J Inherit Metab Dis 33(Suppl 2):S241–S247. https://doi.org/10.1007/s10545-010-9124-4

Sacks JJ, Roberto RR, Brooks NF (1982) Toxoplasmosis infection associated with raw goat's milk. JAMA 248(14):1728–1732. https://doi.org/10.1001/jama.1982.03330140038029

Schumacher AC, Elbadawi LI, DeSalvo T, Straily A, Ajzenberg D, Letzer D, Moldenhauer E, Handly TL, Hill D, Darde ML, Pomares C, Passebosc-Faure K, Bisgard K, Gomez CA, Press C, Smiley S, Montoya JG, Kazmierczak JJ (2021) Toxoplasmosis outbreak associated with *Toxoplasma gondii*-contaminated venison-high attack rate, unusual clinical presentation, and atypical genotype. Clin Infect Dis 72(9):1557–1565. https://doi.org/10.1093/cid/ciaa285

Schwartzman J, Maffia A et al (1948) Congenital toxoplasmosis. J Pediatr 33(1):66–73. https://doi.org/10.1016/s0022-3476(48)80154-x

Shapiro K, Bahia-Oliveira L, Dixon B, Dumetre A, de Wit LA, VanWormer E, Villena I (2019) Environmental transmission of *Toxoplasma gondii*: oocysts in water, soil and food. Food Waterborne Parasitol 15:e00049. https://doi.org/10.1016/j.fawpar.2019.e00049

Simon JA, Pradel R, Aubert D, Geers R, Villena I, Poulle ML (2018) A multi-event capture-recapture analysis of *Toxoplasma gondii* seroconversion dynamics in farm cats. Parasit Vectors 11(1):339. https://doi.org/10.1186/s13071-018-2834-4

Skinner LJ, Timperley AC, Wightman D, Chatterton JM, Ho-Yen DO (1990) Simultaneous diagnosis of toxoplasmosis in goats and goatowner's family. Scand J Infect Dis 22(3): 359–361. https://doi.org/10.3109/00365549009027060

Sobrino R, Cabezon O, Millan J, Pabon M, Arnal MC, Luco DF, Gortazar C, Dubey JP, Almeria S (2007) Seroprevalence of *Toxoplasma gondii* antibodies in wild carnivores from Spain. Vet Parasitol 148(3–4):187–192. https://doi.org/10.1016/j.vetpar.2007.06.038

Speirs G, Hakim M, Calne R, Wreghitt T (1988) Relative risk of donor-transmitted *Toxoplasma gondii* infection in heart, liver and kidney transplant recipients. Clin Transpl 2:257–260

Stillwaggon E, Carrier CS, Sautter M, McLeod R (2011) Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. PLoS Negl Trop Dis 5(9): e1333. https://doi.org/10.1371/journal.pntd.0001333

Stollberg KC, Schares G, Mayer-Scholl A, Hrushetska I, Diescher S, Johne A, Richter MH, Bier NS (2021) Comparison of direct and indirect *Toxoplasma gondii* detection and genotyping in Game: relationship and challenges. Microorganisms 9(8). https://doi.org/10.3390/microorganisms9081663

Su C, Zhang X, Dubey JP (2006) Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. Int J Parasitol 36(7): 841–848. https://doi.org/10.1016/j.ijpara.2006.03.003

Su C, Khan A, Zhou P, Majumdar D, Ajzenberg D, Darde ML, Zhu XQ, Ajioka JW, Rosenthal BM, Dubey JP, Sibley LD (2012) Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proc Natl Acad Sci U S A 109(15):5844–5849. https://doi.org/10.1073/pnas.1203190109

Takahashi AFS, Bioni HO, Souza JM, Takizawa MGMH, Paiva JE (2019) Toxoplasmose congênita na cidade de Cascavel/PR no período de 2002–2016. Revista Thêma Sci 9:260–267

Tenter AM, Heckeroth AR, Weiss LM (2000) *Toxoplasma gondii*: from animals to humans. Int J Parasitol 30(12–13):1217–1258. https://doi.org/10.1016/s0020-7519(00)00124-7

Thebault A, Kooh P, Cadavez V, Gonzales-Barron U, Villena I (2021) Risk factors for sporadic toxoplasmosis : a systematic review and meta-analysis. Microb Risk Anal 17:100133

Torgerson PR, Mastroiacovo P (2013) The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ 91(7):501–508. https://doi.org/10.2471/BLT.12.111732

Treves A, Karanth K (2003) Human–carnivore conflict and perspectives on carnivore management worldwide. Conserv Biol 17:1491–1499

VanWormer E, Fritz H, Shapiro K, Mazet JA, Conrad PA (2013) Molecules to modeling: *Toxoplasma gondii* oocysts at the human-animal-environment interface. Comp Immunol Microbiol Infect Dis 36(3):217–231. https://doi.org/10.1016/j.cimid.2012.10.006

Velmurugan GV, Dubey JP, Su C (2008) Genotyping studies of *Toxoplasma gondii* isolates from Africa revealed that the archetypal clonal lineages predominate as in North America and Europe. Vet Parasitol 155(3–4, 314):–318. https://doi.org/10.1016/j.vetpar.2008.04.021

Villena I, Aubert D, Gomis P, Ferte H, Inglard JC, Denis-Bisiaux H, Dondon JM, Pisano E, Ortis N, Pinon JM (2004) Evaluation of a strategy for *Toxoplasma gondii* oocyst detection in water. Appl Environ Microbiol 70(7):4035–4039. https://doi.org/10.1128/AEM.70.7.4035-4039.2004

Villena I, Ancelle T, Delmas C, Garcia P, Brezin AP, Thulliez P, Wallon M, King L, Goulet V (2010) Congenital toxoplasmosis in France in 2007: first results from a national surveillance system. Euro Surveill 15(25):19600

Wallon M, Peyron F, Cornu C, Vinault S, Abrahamowicz M, Kopp CB, Binquet C (2013) Congenital toxoplasma infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. Clin Infect Dis 56(9):1223–1231. https://doi.org/10.1093/cid/cit032

Weiss LM, Dubey JP (2009) Toxoplasmosis: a history of clinical observations. Int J Parasitol 39(8):895–901. https://doi.org/10.1016/j.ijpara.2009.02.004

Wendte JM, Gibson AK, Grigg ME (2011) Population genetics of *Toxoplasma gondii*: new perspectives from parasite genotypes in wildlife. Vet Parasitol 182(1):96–111. https://doi.org/10.1016/j.vetpar.2011.07.018

Zhou P, Nie H, Zhang LX, Wang HY, Yin CC, Su C, Zhu XQ, Zhao JL (2010) Genetic characterization of *Toxoplasma gondii* isolates from pigs in China. J Parasitol 96(5):1027–1029. GE-2465 [pii]. https://doi.org/10.1645/GE-2465.1

Trichinella and Trichinellosis: From Wildlife to the Human Beings

17

Edoardo Pozio and María Ángeles Gomez Morales

Contents

Abstract

Trichinellosis is a cosmopolitan infection transmitted to humans through the consumption of raw or undercooked meat of domestic and wild pigs, horses, and carnivorous animals infected by nematode larvae of the genus *Trichinella*. These parasites are maintained in nature by a sylvatic cycle. Spillover from wild animals to domesticated animals can occur when there is improper management in segregating livestock and wildlife. The symptoms associated with trichinellosis vary with the number of infecting larvae ingested, the time after infection, and the *Trichinella* species. Progression of the disease follows the biological development of these

E. Pozio (✉) · M. Á. Gomez Morales
Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
e-mail: edoardo.pozio@iss.it; mariaangeles.gomezmorales@iss.it

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_58

nematodes. Most of the clinical features of trichinellosis are immunopathological in origin and are related to the capacity of these parasites to induce allergic responses.

Keywords

Trichinellosis · Trichinella · Meatborne disease · Swine · Game meat

17.1 Introduction

Trichinellosis is a foodborne disease caused by infection with nematode worms of the genus *Trichinella*. Infection results from the ingestion of meat of mammals, birds, and reptiles harboring the infective larvae of the parasite (Gottstein et al. 2009). The disease has long been known as trichinosis because the parasite genus was formerly called *Trichina* (Pozio 2021a). Today, the term trichinellosis has gained wide acceptance internationally. The parasite that causes trichinellosis was discovered in the United Kingdom in 1835. It was first observed in human muscles during a routine autopsy and was regarded as a mere zoological curiosity for a long time (Gaeta and Bruschi 2021). The worm was originally named as *Trichina spiralis* Owen, 1835. In 1895, the genus name was changed to *Trichinella* (Pozio 2021a). In 1860, *T. spiralis* was found in the muscle tissues of a patient who had died of a serious painful muscle disease, and this important case revealed both the pathogenicity of this zoonotic parasite and the link between the disease and the ingestion of undercooked pork. During the latter part of the nineteenth century, the essentials of the life cycle were worked out, and the pathogenicity of this roundworm was shown dramatically by several severe outbreaks in Germany (Gaeta and Bruschi 2021).

This group of zoonotic parasites shows a cosmopolitan distribution in all the continents except Antartica (Pozio and Zarlenga 2013). Today, 13 taxa separated into two clades are recognized in the genus (Zarlenga et al. 2020), one clade that encompasses species that encapsulate in host muscle tissues following muscle cell reprogramming and a second clade that does not encapsulate. The species and genotypes of the first clade parasitize only mammals. Among the three species that comprise the second clade, one infects mammals and birds (*Trichinella pseudospiralis*) and two parasitize mammals and reptiles (*Trichinella papuae* and *Trichinella zimbabwensis*) (Zarlenga et al. 2020).

These parasites are unusual among other nematodes in that the worm undergoes a complete developmental cycle, from larva to adult and then to larva, in the body of a single host, which strongly influences the epidemiology of these parasites and the human disease. When the cycle is complete, striated muscles of infected animals and humans contain a reservoir of larvae, capable of long-term survival. Animals and humans acquire the infection by ingesting muscle tissues infected by viable larvae.

17.2 Taxonomy

For more than 100 years, the genus *Trichinella* was considered monospecific, and *Trichinella spiralis* was considered the only species with a cosmopolitan distribution, infecting a very large number of mammals including humans. The domestic

cycle involving grazing domestic pigs and farm rats was considered the predominant mode of circulation for *T. spiralis*; surrounding wildlife were considered as an ancillary part of on-farm transmission. The expansion of helminthological investigations on wild animals starting from the 1950s highlighted the presence of nematodes of the genus *Trichinella* with biological characters different from those of *T. spiralis*, but without discernable morphological characters at any of the developmental stages that could be used to differentiate species (Pozio and Zarlenga 2021). The switch from a classical taxonomy to new taxonomic studies first based on crossbreeding experiments in laboratory animals, and then on biochemical (allozymes) and molecular characters, has allowed the identification of ten sibling species and three genotypes divided into two clades based on the presence or absence of a collagen capsule around the muscle larva. The clade in which the muscle larvae do not induce capsule formation infects mammals and birds (*T. pseudospiralis*) or mammals and reptiles (*T. papuae* and *T. zimbabwensis*). The clade characterized by the presence of a collagen capsule around the nurse-cell-larva complex infects only mammals. This clade is comprised of seven species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. patagoniensis*, and *T. chanchalensis*) and three genotypes (*Trichinella* T6, T8, and T9). Each taxon is characterized by a well-defined distribution area; however, there are two species which, for different reasons, have a cosmopolitan distribution: *T. spiralis* spread by the passive introduction of infected swine in most continents and *T. pseudospiralis* spread by birds worldwide. Carnivores are the main hosts of *Trichinella* species and genotypes, with the exception of *T. spiralis* which is also very well adapted to swine and consequently represents the main causative agent of human trichinellosis. This taxonomic scheme is supported by the analysis of more than ten thousand *Trichinella* isolates from different host species and continents. However, there remain some geographical areas from which no *Trichinella* parasites have been collected and identified at the species/genotype level such as Central America and most of South America and India. The phylogeny of the genus showed that the encapsulated and non-encapsulated clades diverged from their most recent common ancestor in Asia about 21 million years ago (mya) with taxon diversifications commencing about 10–7 mya. The currently described thirteen *Trichinella* taxa cannot be identified by their morphology at the species or genotype level. Each taxon can be reliably identified only by molecular methods (Pozio and La Rosa 2010; Sharma et al. 2020; Zarlenga et al. 2020).

17.3 Natural Cycle and Parasite Biology

Nematodes of the *Trichinella* genus develop two generations of worms in the same host. Gravid female worms (1.26–3.35 mm × 29–39 μm) embedded in the intestinal mucosa produce newborn larvae (NBL, 110 × 7 μm) which migrate into the lymphatic vessels and then enter the blood vessels to reach and penetrate striated muscle cells. In the muscle cell, NBL develop to the infective L1 (the muscle larva measure 0.65–1.1 mm × 25–40 μm) in about 15 days without making any molt unlike other nematodes (Despommier 1998). In muscle cells, larvae are coiled and

enclosed by a collagen capsule (encapsulated species) or appear to be free among the muscle fibers (nonencapsulated species). The size of the collagen capsule is about 350–450 × 180–300 μm. In this ecological niche, larvae can survive for many years (over 20 years in polar bears and up to 40 years in humans) waiting to be ingested by a new host. When a new host ingests infected muscle tissues, the larvae are released from the muscle cells in the stomach by digestion. In the duodenum, they penetrate the villi and within 2 days undergo four molts and rapidly develop to the adult stage. Males and females copulate, and within 6–7 days postinfection (p.i.), the females begin to produce NBL for at least 1–2 weeks or longer as influenced by the host's immune response at the gut level, which usually develops and results in the adult worm expulsion.

17.4 Epidemiology

17.4.1 In Animals

Trichinella spp. are prevalent in wildlife. The transmission cycles of the different *Trichinella* species and genotypes are closely related to their host species' biology and environmental characteristics. Predation probably accounts for the transmission of these pathogens in some instances, but the eating of carrion is of great epidemiological significance. Carnivores are the main reservoir hosts in the wild. Human behavior can strongly influence the sylvatic cycles both favoring and reducing the transmission of *Trichinella* spp. Carcasses of *Trichinella* sp.-infected animals left by hunters in the field after skinning, removing, and discarding the entrails, or road accidents represent a great biomass of these parasites readily available to the wild cycle. Epidemiological surveys carried out in Africa, Europe, and North America have shown that *Trichinella* spp. are more prevalent in wild animals living in natural or undisturbed areas such as parks and forests, protected areas, and mountainous regions, where human activity has not strongly modified the habitat (Pozio and Murrell 2006). Spillover from wild animals to domestic animals can occur when there is improper management in segregating livestock and wildlife (Pozio and Murrell 2006). There is no documented evidence of infection in pigs reared in herds kept under controlled management conditions (Pozio 2014). *Trichinella spiralis* is the species more adapted to swine, and, consequently, it is the species more frequently detected in domesticated pigs. The domestic and the sylvatic cycles can function either independently from each other or interactively. The term "domestic cycle" refers to the transmission pattern where the focus is on a swine herd being fed, e.g., uncooked pork scraps, carrion, uncooked garbage (i.e., garbage-fed pigs), or the pigs can feed on carcasses that are not promptly removed from the farm; transmission can also become domestic via synanthropic animals (e.g., rats and mustelids) living near swine herds (Pozio 2014). Horses fattened with pork scraps or with carcasses of carnivorous animals became infected with *Trichinella* spp. The use of *Trichinella*-infected meat of slaughtered crocodiles or farmed fur mammals (e.g., foxes) to feed other farmed crocodiles or foxes, respectively, has been reported

(Pozio and Murrell 2006; Pozio 2021b). Since nematodes of the genus *Trichinella* are mainly circulating among wildlife and backyard or free ranging pigs, these pathogens do not represent a great concern for the international meat trade. From the 1950s to today, according to the international literature, there are only 43 reports describing the importation of *Trichinella* sp.-infected animals or meat by the international trade to Europe. Most (60%) of these reports refer to live horses or their meat, 18.6% to pigs, 4.7% to wild boar, and 14.3% to bears. In contrast, the scientific literature is rich of reports on meat from pigs, wild boar, and bears, illegally introduced in personal baggage causing trichinellosis outbreaks in several European countries (Pozio 2015).

17.4.2 In Humans

The most common source of infection for humans is pork and pork-derived products from backyard and free-ranging pigs, wild boar, wild pigs, warthog, and bushpig; other sources responsible for outbreaks and single infections include meat and meat-derived products of horse, dog, bear, walrus, cougar, fox, badger, and jackal (Pozio 2014, 2021b; Rostami et al. 2017). Suspected meat sources were from soft-shelled turtle, monitor lizard, and squirrel. Among domestic animals, the meat of sheep and beef has been considered as the source of trichinellosis in China; however, *Trichinella* sp. larvae have never been detected in naturally infected sheep and cattle, suggesting that herbivorous animal meat may be mixed with infected meat from other sources and sold in restaurants and stores. With the exception of *T. zimbabwensis*, *Trichinella* T8, *T. patagoniensis*, and *T. chanchalensis*, all other species and genotypes of *Trichinella* have been reported from humans. In 2007, an average yearly incidence of ten thousand trichinellosis infections with a mortality rate of 0.2% was estimated, cumulating the highest number of infections detected worldwide in a year (Pozio 2007). Reliable estimates of the incidence of trichinellosis among humans and its effect on health were investigated between 1986 and 2009 in a systematic review of the international and grey literature (Murrell and Pozio 2011). During this period, 65,818 cases and 42 (0.06%) deaths were reported from 41 countries. The WHO European Region accounted for 87% of cases; 50% of those occurred in Romania, mainly during 1990–1999. The incidence in this country ranged from 1.1 to 8.5 cases per 100,000 inhabitants. Trichinellosis affected primarily adults (median age 33.1 years) and almost equally men (51%) and women. Out of 196 officially recognized countries in the world, *Trichinella* spp. infections in humans, acquired through the consumption of local animals, have been documented in 47 (23.9%) countries in the last 20 years (Pozio 2021b). *Trichinella* sp. infections in humans are more related to cultural food practices which include dishes based on raw or undercooked meat of different animal origins than to the presence of the parasite in the domestic and wild animals of the country. Muslim countries are not exempted from trichinellosis. The largest outbreak (about 500 infected people) occurred in Izmir (Turkey) for the consumption of meatballs fraudulently made with beef and pork from domestic pigs infected by *T. britovi* (Akkoc et al. 2009).

In France and in Italy, most of the trichinellosis cases are due to the consumption of raw horse meat, because this food habit is strongly related to the French culture imported also in Italy (Boireau et al. 2000; Pozio 2015). In Finland, where there is a high prevalence of infection in animals, no infection leading to disease has been documented in humans, due to the practice of eating only well cooked meat (Pozio 2007). In Romania, the highest prevalence of trichinellosis in humans occurs in the Transylvanian region, which was colonized by German people who have kept their food habits which are known to be risk factors for trichinellosis (Blaga et al. 2007). In the European Union (EU), the number of human cases and the notification rate (per 100,000 population) have been kept low from 2015 to 2019. The highest rate (0.03%) was reported in 2015 and 2017 and the lowest rate in 2018 (0.01%), which was the lowest rate ever reported since the beginning of *Trichinella* surveillance at the EU level in 2007. One death due to trichinellosis was reported in Portugal in 2019, resulting in an EU case fatality of 4.2% (EFSA 2021). In Israel, Lebanon, and Syria, human outbreaks of trichinellosis have been documented following consumption of pork from wild boars only among the Christian populations or immigrants from Thailand (Pozio 2007). In Algeria and Senegal, where the majority of the human population is Muslim, trichinellosis has only been documented in expatriates from France and very seldom in the local population. The migration of persons from eastern to western countries of Europe has resulted in several human outbreaks of trichinellosis in Denmark, Germany, Italy, Spain, and the United Kingdom (Pozio and Marucci 2003; Gallardo et al. 2007; Stensvold et al. 2007). The increasing number of international travelers has resulted in many reports of tourists who acquired *Trichinella* sp. infections for the consumption of warthog meat in Africa; of bear meat in Canada and Greenland; of pork in China, Egypt, Indonesia (Bali Island), Laos, and Malaysia; and wild boar meat in Turkey and Algeria (Pozio and Murrell 2006). In developed countries of North America and Europe in the last 20 years, there was a switch of the source of trichinellosis from pork and derived products to wild game meat (Rostami et al. 2017; Pozio et al. 2019).

17.5 Clinical Aspects

17.5.1 In Humans

The incubation period exhibits a variable length related to the number of larvae ingested, frequency of consumption of infected meat, type of meat consumed (raw, semi-raw), and the involved *Trichinella* species. The length of the incubation period ranges from 2 to 45 days (Table 1). It is generally thought that the shorter incubation period is correlated with a worse prognosis.

The clinical picture reflects the parasite cycle in the human body. In the early phase of the infection, i.e., during the intestinal phase, the most common sign and symptom are diarrhea and abdominal pain, which may be absent in individuals with a mild infection. The stools that can be loose up to 10–15 times a day are greenish brown in color and frequently with an admixture of mucus but containing no blood. Persistent diarrhea usually causes deterioration in the patient's general conditions,

Table 1 Clinical symptomatology, laboratory features, and convalescence in the course of trichinellosis

Clinical symptomatology	**Severity of the infection**	**Weeks p.i.**[a]
Enteral phase (diarrhea, nausea, vomiting)	Severe	0.5/5
	Moderate	1/3
	Mild	
Parenteral phase		
Myalgia	Severe	1.5/8
	Moderate	2/6
	Mild	2/5
Fever	Severe	1/6.5
	Moderate	2/5.5
	Mild	2/3.5
Periorbital edema, conjunctivitis	Severe	1/6
	Moderate	1.5/5.5
	Mild	1.5/4.5
Laboratory features		
Eosinophilia	Severe	1.5/10
	Moderate	2/8.5
	Mild	3/7
CPK	Severe	1.5/8
	Moderate	2.5/6
	Mild	3/5.5
Anti-*Trichinella* IgG	Severe	3–4/years
	Moderate	3–4/years
	Mild	4–6/15–20
Convalescence	Severe	6/16
	Moderate	5/12
	Mild	5/9

[a]Week of onset/disappearance

leading possibly to dyselectrolytemia and marked hypoproteinemia. Nausea and vomiting usually appear during the first days of the infection. This symptomatology usually precedes fever and myalgia by 3–4 days and then disappears in less than 1 week. The frequency of gastrointestinal disturbance is estimated at 6–60% of cases in various foci of trichinellosis. General weakness, chills, and headache are accompanied by fever (occasionally up to 40 °C), which may be continuous and persist up to 3 weeks during the acute phase in severe clinical forms. In mild cases, subfebrile body temperatures are frequently noted, which disappear after a few days of effective treatment. The frequency of fever varies from 41% to 100% of cases in various foci of the disease. Profuse sweating is frequently reported in trichinellosis (Kociecka 2000; Dupouy-Camet et al. 2002).

The parenteral or muscular phase (also known as systemic phase) corresponds to the acute phase of the infection, and it is associated with inflammatory and allergic responses caused by the larvae during migration and invasion of the skeletal muscle cells. Symmetrical eyelid and periocular edema frequently develop in 17–100% of

the patients, and the edema can involve the entire face. In severe cases, edema may even extend to the upper and lower extremities and usually vanishes in 5–6 days, particularly after glucocorticoid treatment. Eyelid edema is usually accompanied by conjunctival hyperemia, itching, lacrimation, and, occasionally, light intolerance. Eye pains upon ocular movements have been observed in around 77% of patients. Also, disturbed sight acuity and sometimes bilateral exophthalmia deserve attention. Nystagmus, reflecting involvement of ciliary muscles of patients, may occur. Approximately 25% of patients exhibit petechiae and subungual hemorrhages. Early symptoms develop at varying intervals and intensity. They also do not disappear in a defined constant sequence (Pozio et al. 2003).

Muscle pain involves various groups of muscles, and the intensity reflects the severity of the disease course. Pain develops in nuchal and trunk muscles, in the muscles of the upper and lower extremities, and less frequently in masseter muscles. It affects patients during execution of movements (particularly in lower extremities), while spontaneous pain is less frequently observed. Moreover, most persons with severe trichinellosis or phlebitis associated with trichinellosis also experience myalgia at rest. In some patients with severe course, adynamia dominates, which may persist for a long time, reflecting pronounced intensity of angiomyositis-type pathology or neuromuscular disturbances. Severe myalgia generally lasts 2–3 weeks (Pozio et al. 2003).

Itching and numbness or tingling sensation in various muscle groups frequently manifest together with muscle pain. Restricted motility due to muscle pain associated with movements may lead to contractions, particularly in knee and elbow joints, nuchal pseudorigidity, and difficulties in opening the mouth. The signs gradually subside during convalescence, to arrive to complete regression, especially with the aid of physiotherapy.

Fever, eyelid and periorbital edema, and muscle pains form the principal set of clinical signs/symptoms of trichinellosis. They are usually accompanied by hypereosinophilia and by high white blood cell counts. A correlation between the eosinophil levels and serum muscle enzymes such as lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) has been observed in these patients. According to a retrospective survey on trichinellosis outbreaks involving 5377 cases, carried out worldwide during 1986–2009, the typical clinical signs of trichinellosis were myalgia, diarrhea, fever, facial edema, and headaches that, after treatment, disappeared within 2–8 weeks. Their rapid recovery reflects improvements in diagnostic methods and drug treatment (Murrell and Pozio 2011).

17.5.1.1 Complications

Complications usually develop within the first 2 weeks in severe cases but also in moderate cases, for individuals improperly treated, or those for whom the treatment was started too late. A positive correlation has been observed between age and the frequency and severity of complications (Dupouy-Camet et al. 2002).

Cardiovascular disturbances can occur in moderate or severe cases, usually between the third and fourth week p.i. (Compton et al. 1993; Lazarević et al. 1999; Puljiz et al. 2005; Neghina et al. 2010; Messiaen et al. 2016). Myocarditis develops in 5–20% of symptomatic persons. The symptoms include pain in the heart region, tachycardia, and electrocardiogram abnormalities. (i.e., flattened T waves,

decreased ST, lowered QRS complex, acute Q waves, and disturbances in atrioventricular or interventricular conduction). Intensity of the signs/symptoms depends upon the time at which therapy was started and the health condition before the larval invasion began. Pain in the heart region (6.2%) and tachycardia (22.1%) occur, with a possible evolution to a fatal ventricle fibrillation (Pozio et al. 2003). Thromboembolic disease can also occur, specifically deep thrombophlebitis, intraventricular thrombi, and/or pulmonary embolism, which can all be fatal. Sudden death may result from embolism of the pulmonary artery (Kociecka 2000).

Neurological complications (neurotrichinellosis; Bruschi et al. 2013) include a variety of signs and symptoms (Ellrodt et al. 1987; Fourestie et al. 1993; Bruschi et al. 2013) and have been reported in 3–46% of patients, depending on the outbreaks. Persons with severe disease can show consciousness disorders or excessive excitement and frequently somnolence and apathy; some of the persons with these symptoms show signs of meningitis or encephalopathy. Dizziness, nausea, and tinnitus are transient. Anisocoria, facial nerve paresis, and Babinski reflex have also been observed in severe cases. Brain damage, which is usually observed within a few days after the onset of fever, can result in diffuse encephalopathy or focal signs such as disorientation, memory disturbances, frontal syndrome, behavioral disturbances, transient hemiparesis or hemiplegia, oculomotor dysfunction, aphasia, and cerebellar syndrome. Small hypodensities are seen with the computer tomography (CT) scan or magnetic resonance imaging (MRI) (Fourestie et al. 1993; Feydy et al. 1996). In most persons with neurological complications, there is an improvement of focal lesions within 2–4 weeks p.i. Most CT scan or MRI brain abnormalities disappear in 4–8 weeks p.i. Neurological complications could be less frequent if the infected person is treated early. Neurological complications and myocarditis, which are both life-threatening, are often simultaneously present (Fourestie et al. 1993).

Complications of the ocular lesions include edema and altered microcirculation at the level of the uvea, the retina, and, in some cases, in the optic nerve. Rarely, lesions of the retina may be induced by migrating *Trichinella* larvae, which penetrate ciliary arterioles and the central artery of the retina, leading to irreversible damage to eyesight. An intense invasion of the muscles of the ocular bulb provokes pain when moving the eyeballs, muscle paralysis, diplopia, or a disturbed accommodation (Pozio et al. 2003).

Dyspnea is relatively common and is caused primarily by parasite invasion and subsequent inflammation of respiratory muscles such as the diaphragm. Respiratory complications are uncommon. They can occur during both the early and late stages of trichinellosis. They consist of pneumonia, obstructive bronchitis, or Löffler-type infiltrates.

Digestive complications occur during the acute stage of infection, and they consist of massive proteinic exudation leading to hypoalbuminemia and localized edemas, acute intestinal necrosis, or prolonged diarrhea (Dupouy-Camet et al. 1998).

Death is rare. Of the more than 6500 infections reported in the EU from 1980 to 2005, only five fatalities were documented, all due to thromboembolic disease, in individuals over 65 years of age; deaths have been reported in two large outbreaks which involved more than 1000 cases (Ancelle et al. 1988). In a study period of 24 years (1986–2009), 42 deaths occurred worldwide (Murrell and Pozio 2011).

Most of the deaths were caused by *T. spiralis* due to its high pathogenicity to humans; less than 20 deaths were caused by other taxa (*T. nativa*, *T. murrelli*, *T. nelsoni*, and *T. papuae*) reported to have caused infections in humans.

The chronic stage begins when the adult females cease to release migrating larvae, and those already established have completed their development into the muscle cells. The transition to this stage is characterized by the progressive disappearance of the signs and symptoms of the disease and by the return of laboratory parameters to normal values. This stage usually begins between the sixth and the 8 week p.i., but infected persons might still have a severe asthenia for several weeks and chronic muscular pain for up to 6 months. Most persons will then become asymptomatic, although live larvae will persist in their muscles for years if they are not properly treated.

17.5.2 In Animals

Trichinellosis in animals has only been observed in the course of experimental infections, for both the acute and the chronic stages of the disease. In naturally infected pigs and horses, no clinical signs of trichinellosis can be observed. This makes control and prevention difficult, since farmers may mistakenly think that infected animals are healthy, as their appearance and growth remain within normal parameters. Significant differences in weight gain (10–15%) were observed in domesticated pigs experimentally infected with *T. spiralis* larvae from 40 to 100 days p.i. in comparison with uninfected animals (Ribicich et al. 2007). The impact of *T. spiralis* infection on weight gain was observed in pigs (Ribicich et al. 2007) but not in wild boar (Lacour et al. 2013). In experimentally infected horses, transient muscular disorders were observed, but none of the horses had fever (Soule et al. 1989).

17.6 Diagnosis

17.6.1 In Humans

The diagnosis of trichinellosis should be based on anamnesis (e.g., raw meat consumption), clinical signs and symptoms, and laboratory tests (immunodiagnosis or muscle biopsy). According to the European Centre for Disease Prevention and Control case definition, any person meeting the clinical criteria (i.e., at least three of the following six criteria: fever, muscle pain, diarrhea, facial edema, eosinophilia, and subconjunctival, subungual, and/or retinal hemorrhages) with an epidemiological link is considered as a probable case. Any person meeting the clinical criteria and the laboratory criteria (antibody response specific to the *Trichinella* spp. detected using indirect immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA), or Western blot (WB) is considered as a confirmed case (European Commission 2018). The International Commission on Trichinellosis (ICT) recommends ELISA for screening followed by WB to confirm ELISA-positive sera. Both tests should use excretory/secretory products as antigens, which are obtained from in vitro maintenance of *T. spiralis* muscle larvae. These antigens recognize anti-*Trichinella*

antibodies produced against the larvae of all species (Bruschi et al. 2019; Gómez-Morales et al. 2012). Seroconversion occurs between 12 and 60 days p.i. To recover the larvae for species identification or confirmation of infection, muscle biopsy (0.2–0.5 g of muscle tissue) should be collected preferentially from the deltoid muscle. *Trichinella* larvae can be detected in the biopsy by compressorium, HCl-pepsin digestion, or histological analysis. The collection of the muscle biopsy is seldom because it is an expensive, invasive, and a very painful method.

17.6.2 In Animals

According to the European Commission (2015) or the ISO 1870/2015, the artificial digestion of a muscle sample collected from preferential muscles or an equivalent method should be used to test the presence of *Trichinella* larvae in meat from all *Trichinella*-susceptible animals intended for human consumption, unless carcasses have undergone a freezing treatment. For species or genotype identification, a single multiplex PCR is recommended by the ICT (Pozio and Zarlenga 2021). Serological tests are recommended only for epidemiological studies, since animal hosts can harbor infective larvae before antibodies are detectable. On the other hand, for some species (*T. britovi* and *T. pseudospiralis*), the persistence of muscle larvae in the muscle is shorter than that of specific antibodies (Pozio et al. 2020). For these reasons, serological methods should not be used for the detection of *Trichinella* infection in individual food animal carcasses to assure food safety (Gajadhar et al. 2009; Gamble et al. 2004).

17.7 Therapy

The two drugs of choice are mebendazole (25 mg/kg 2–3 times day for 15 days) and albendazole (20 mg/kg 2–3 times a day for 15 days). Corticosteroids should be used for symptomatic treatment (e.g., prednisolone 30–60 mg/day) and always in combination with anthelmintic, but caution should be exercised due to the possibility of anaphylactic shock (Dupouy-Camet et al. 2002). Treatment with benzimidazoles is very effective during the intestinal phase, and as the development of the larvae progresses, the treatment becomes less and less effective (Pozio et al. 2001).

17.8 Prevention

Since parasites of the genus *Trichinella* circulate mainly among wildlife, the main preventative measures for farmed animals are to avoid the following: (1) access to wild animal carcasses, their scraps, and offal to domesticated animals; (2) the use of wild animal carcasses, their scraps, and offal for feeding domesticated animals; (3) access to pig carcasses, their scraps, and offal to domesticated animals; and (4) free range of domesticated pigs in the wild (Office International des Epizoties 2013; Pozio 2014). The best result to prevent *Trichinella* sp. transmission in the farm

and, at the same time, to prevent many other zoonotic and non-zoonotic infectious diseases at the farm level has been obtained from breeding pigs in high containment level farms, which must be periodically monitored by an independent body. An excellent example is represented by the European Commission (2015).

To reduce the spread of *Trichinella* sp. among wild animals, public health services must educate hunters not to leave the carcasses or parts thereof of hunted animals on the ground or in unfenced waste dumps. Carcasses of animals killed by cars should be quickly removed, and wild animals should be prevented from accessing food waste dumps.

17.8.1 Prevention at the Consumer Level

Consumer education about the cause and mode of *Trichinella* worm transmission gives the ability to avoid infection by eschewing meat that has not been frozen or cooked thoroughly. Ethnic or cultural practices that entail special risk may call for special educational measures. Cooking meat at an internal temperature of 70 °C kills the larvae in 1 minute, that is, when the meat color switches from pink to brown. Grilled meat or meat-derived products (e.g., sausages) can be at high risk, because larvae can survive in uncooked muscle portions in the core of the product or close to bones. Microwaves cannot be considered secure to kill *Trichinella* sp. larvae. Freezing meat is also an alternative way to kill the larvae, but the freezing time and freezing temperature should be monitored in the core of the meat products with a recording system. The use of a home freezer can represent a risk, because the temperature is not controlled, and frequent thermal shocks due to its frequent daily use may occur. Furthermore, muscle larvae of some *Trichinella* species can survive freezing for a long period of time; in fact, human infections caused by the consumption of previously frozen wild boar or bear meat have been documented (Gari-Toussaint et al. 2005; Houzé et al. 2009).

The survival time of *Trichinella* larvae in cured meat products is strictly related to the curing time and curing conditions. In industrialized products, fermentation or drying kills larvae in two weeks, that is, once the water activity value is reduced to 0.90, one could assume that *Trichinella* larvae are no longer infective (Porto-Fett et al. 2010). At high risk, there are homemade meat products from game and from free-ranging and backyard pigs, since the curing conditions and the curing times are not controlled and the prevalence of infection can be quite high in these animals. *Trichinella* larvae survive in meat for a long period of time under vacuum, lard, oil, or other substances that prevent dehydration.

17.9 Investigations in the Course of Trichinellosis Outbreaks

As previously reported, the clinical picture of trichinellosis is very complex and difficult for physicians to interpret, if they have no previous experience. Generally, *Trichinella* infection affects a group of individuals who have consumed the same

food based on raw or undercooked meats or their derivate products (e.g., sausages, salami). Single cases of trichinellosis are very rare and often appear as such only because a single individual has consumed a large amount of meat infected by a low number of larvae. Consequently, individuals who have consumed less of the same meat are asymptomatic or pauci-symptomatic. The intestinal phase hardly comes to the attention of physicians. It is the invasive phase which, due to the severity of the symptoms, induces patients to go to a hospital. When the physician suspects that it may be trichinellosis, he/she should immediately question the patient(s) about what kind of meat was consumed in the last 2–3 weeks, where this food was consumed, and with whom it was consumed. As soon as this initial information has been acquired, the physician must notify the public health service and the veterinary service to try to trace the infected meat that may have not yet been consumed and to identify the animal species and, if it is pig meat, the farm of origin. In most cases, investigations reveal that the source of the infection is to be traced back to a pig illegally bred or to an animal subject to hunting, often poached, and not subjected to veterinary controls. If the meat suspected to be the source of the infection can be traced in refrigerators, it must be immediately confiscated and tested by artificial digestion for the isolation of the larvae and their subsequent typing at the species level and the count of larvae per gram of muscle tissue. This information is very useful for physicians to evaluate the severity of the infection, the clinical course, and the antibody response and for veterinarians to know the risk of transmission of the pathogen to other animals at the farm or to wild animals.

17.10 Conclusions

Although the parasites of the genus *Trichinella* are among those most studied and controlled by the veterinary services, they continue to represent a public health problem that varies from country to country according to the conditions of pig farming and the consumption of game meat and, above all, based on food and cultural habits. Therefore, it is necessary that the public health services in conjunction with the veterinary services play a control and preventive role. Furthermore, this infection occurs sporadically in "leopard spots" in different countries for which most of the physicians do not know the disease and have diagnostic difficulties due to the lack of reliable tests.

References

Akkoc N, Kuruuzum Z, Akar S, Yuce A, Onen F, Yapar N, Ozgenc O, Turk M, Ozdemir D, Avci M, Guruz Y, Oral AM, Pozio E, Izmir Trichinellosis Outbreak Study Group (2009) A large-scale outbreak of trichinellosis caused by *Trichinella britovi* in Turkey. Zoonoses Public Health 56: 65–70

Ancelle T, Dupouy-Camet J, Bougnoux ME, Fourestie V, Petit H, Mougeot G, Nozais JP, Lapierre J (1988) Two outbreaks of trichinosis caused by horsemeat in France in 1985. Am J Epidemiol 127:1302–1311

Blaga R, Durand B, Antoniu S, Gherman C, Cretu CM, Cozma V, Boireau P (2007) A dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years: impact of political changes and regional food habits. Am J Trop Med Hyg 76:983–986

Boireau P, Vallee I, Roman T, Perret C, Mingyuan L, Gamble HR, Gajadhar A (2000) *Trichinella* in horses: a low frequency infection with high human risk. Vet Parasitol 93:309–320

Bruschi F, Brunetti E, Pozio E (2013) Neurotrichinellosis. Handb Clin Neurol 114:243–249

Bruschi F, Gómez-Morales MA, Hill DE (2019) International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and humans. Food Waterborne Parasitol 12:e00032

Compton SJ, Celum CL, Lee C, Thompson D, Sumi SM, Fritsche TR, Coombs RW (1993) Trichinosis with ventilatory failure and persistent myocarditis. Clin Infect Dis 16:500–504

Despommier DD (1998) How does *Trichinella spiralis* make itself at home? Parasitol Today 14: 318–323

Dupouy-Camet J, Ancelle T, Fourestie V, Boireau P, Soule C (1998) Trichinelloses. In: Maladies infectieuses Encycl Méd Chir (Ed.). Elsevier, Paris, p 8-517-A-10

Dupouy-Camet J, Kociecka W, Bruschi F, Bolas-Fernandez F, Pozio E (2002) Opinion on the diagnosis and treatment of human trichinellosis. Expert Opin Pharmacother 3:1117–1130

Ellrodt A, Halfon P, Le Bras P, Halimi P, Bourée P, Dési M, Caquet R (1987) Multifocal central nervous system lesions in three patients with trichinosis. Arch Neurol 44:432–434

European Commission (2015) Commission implementing regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat. Off J Eur Union L212:7–34

European Commission (2018) Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. Off J Eur Union L170:1–74

European Food Safety Agency (2021) The European Union One Health 2019 Zoonoses Report. EFSA J 19(2):6406

Feydy A, Touze E, Miaux Y, Bolgert F, Martin-Duverneuil N, Laplane D, Chiras J (1996) MRI in a case of neurotrichinosis. Neuroradiology 38(S1):S80–S82

Fourestie V, Douceron H, Brugieres P, Ancelle T, Lejonc JL, Gherardi RK (1993) Neurotrichinosis. A cerebrovascular disease associated with myocardial injury and hypereosinophilia. Brain 116: 603–616

Gaeta R, Bruschi F (2021) History of the parasite and disease. In: Bruschi F (ed) *Trichinella* and Trichinellosis. Academic Press. (in press)

Gajadhar AA, Pozio E, Gamble HR, Nöckler K, Maddox-Hyttel C, Forbes LB, Vallée I, Rossi P, Marinculić A, Boireau P (2009) *Trichinella* diagnostics and control: mandatory and best practices for ensuring food safety. Vet Parasitol 159:197–205

Gallardo MT, Mateos L, Artieda J, Wesslen L, Ruiz C, García MA, Galmés-Truyols A, Martin A, Hernández-Pezzi G, Andersson Y, Gárate T, Christensson D (2007) Outbreak of trichinellosis in Spain and Sweden due to consumption of wild boar meat contaminated with *Trichinella britovi*. Euro Surveill 12:E070315.1

Gamble HR, Pozio E, Bruschi F, Nöckler K, Kapel CM, Gajadhar AA (2004) International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and man. Parasite 11:3–13

Gari-Toussaint M, Tieulié N, Baldin J, Dupouy-Camet J, Delaunay P, Fuzibet JG, Le Fichoux Y, Pozio E, Marty P (2005) Human trichinellosis due to *Trichinella britovi* in southern France after consumption of frozen wild boar meat. Euro Surveill 10:117–118

Gómez-Morales MA, Ludovisi A, Amati M, Blaga R, Zivojinovic M, Ribicich M, Pozio E (2012) A distinctive Western blot pattern to recognize *Trichinella* infections in humans and pigs. Int J Parasitol 42:1017–1023

Gottstein B, Pozio E, Nöckler K (2009) Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin Microbiol Rev 22:127–145

Houzé S, Ancelle T, Matra R, Boceno C, Carlier Y, Gajadhar AA, Dupouy-Camet J (2009) Trichinellosis acquired in Nunavut, Canada in September 2009: meat from grizzly bear suspected. Euro Surveill 14:19383

Kociecka W (2000) Trichinellosis: human disease, diagnosis and treatment. Vet Parasitol 93:365–383
Lacour SA, Heckmann A, Macé P, Grasset-Chevillot A, Zanella G, Vallée I, Kapel CM, Boireau P (2013) Freeze-tolerance of *Trichinella* muscle larvae in experimentally infected wild boars. Vet Parasitol 194:175–178
Lazarević AM, Nesković AN, Goronja M, Golubovic S, Komić J, Bojić M, Popović AD (1999) Low incidence of cardiac abnormalities in treated trichinosis: a prospective study of 62 patients from a single-source outbreak. Am J Med 107:18–23
Messiaen P, Forier A, Vanderschueren S, Theunissen C, Nijs J, Van Esbroeck M, Bottieau E, De Schrijver K, Gyssens IC, Cartuyvels R, Dorny P, van der Hilst J, Blockmans D (2016) Outbreak of trichinellosis related to eating imported wild boar meat, Belgium, 2014. Euro Surveill 21: 30341
Murrell KD, Pozio E (2011) Worldwide occurrence and impact of human trichinellosis, 1986–2009. Emerg Infect Dis 17:2194–2202
Neghina R, Neghina AM, Marincu I, Iacobiciu I (2010) Cardiac involvement in patients with trichinosis hospitalized in western Romania. Foodborne Pathog Dis 7:1235–1238
Office International des Epizoties (2013) Chapter 8.14, Infection with *Trichinella* spp. In: Terrestrial animal health code, pp 1–4
Porto-Fett AC, Call JE, Shoyer BE, Hill DE, Pshebniski C, Cocoma GJ, Luchansky JB (2010) Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. Int J Food Microbiol 140:61–75
Pozio E (2007) World distribution of *Trichinella* spp. infections in animals and humans. Vet Parasitol 149:3–21
Pozio E (2014) Searching for *Trichinella*: not all pigs are created equal. Trends Parasitol 30:4–11
Pozio E (2015) *Trichinella* spp. imported with live animals and meat. Vet Parasitol 213:46–55
Pozio E (2021a) Scientific achievements of the last 60 years: from a single to a multispecies concept of the genus *Trichinella*. Vet Parasitol 297:109042
Pozio E (2021b) Epidemiology. In: Bruschi F (ed) *Trichinella* and Trichinellosis. Academic Press, pp 185–263
Pozio E, Gomez Morales MA, Dupouy-Camet J (2003) Clinical aspects, diagnosis and treatment of trichinellosis. Expert Rev Anti-Infect Ther 1:471–482
Pozio E, La Rosa G (2010) Trichinella. In: Liu D (ed) Molecular detection of foodborne pathogens. CRC Press, Boca Raton, pp 851–863
Pozio E, Ludovisi A, Pezzotti P, Bruschi F, Gómez-Morales MÁ (2019) Retrospective analysis of hospital discharge records for cases of trichinellosis does not allow evaluation of disease burden in Italy. Parasite 26:42
Pozio E, Marucci G (2003) *Trichinella*-infected pork products: a dangerous gift. Trends Parasitol 19:338
Pozio E, Merialdi G, Licata E, Della Casa G, Fabiani M, Amati M, Cherchi S, Ramini M, Faeti V, Interisano M, Ludovisi A, Rugna G, Marucci G, Tonanzi D, Gómez-Morales MA (2020) Differences in larval survival and IgG response patterns in long-lasting infections by *Trichinella spiralis*, *Trichinella britovi* and *Trichinella pseudospiralis* in pigs. Parasit Vectors 16:520
Pozio E, Murrell KD (2006) Systematics and epidemiology of *Trichinella*. Adv Parasitol 63:367–439
Pozio E, Sacchini D, Sacchi L, Tamburrini A, Alberici F (2001) Failure of mebendazole in the treatment of humans with *Trichinella spiralis* infection at the stage of encapsulating larvae. Clin Infect Dis 32:638–642
Pozio E, Zarlenga DS (2013) New pieces of the *Trichinella* puzzle. Int J Parasitol 43:983–997
Pozio E, Zarlenga DS (2021) Taxonomy of the *Trichinella* genus. In: Bruschi F (ed) *Trichinella* and Trichinellosis. Academic Press, pp 35–76
Puljiz I, Beus A, Kuzman I, Seiwerth S (2005) Electrocardiographic changes and myocarditis in trichinellosis: a retrospective study of 154 patients. Ann Trop Med Parasitol 99:403–411
Ribicich M, Gamble HR, Rosa A, Sommerfelt I, Marquez A, Mira G, Cardillo N, Cattaneo ML, Falzoni E, Franco A (2007) Clinical, haematological, biochemical and economic impacts of *Trichinella spiralis* infection in pigs. Vet Parasitol 147:265–270

Rostami A, Gamble HR, Dupouy-Camet J, Khazan H, Bruschi F (2017) Meat sources of infection for outbreaks of human trichinellosis. Food Microbiol 64:65–71

Sharma R, Thompson P, Hoberg EP, Scandrett WB, Konecsni K, Harms NJ, Kukka PM, Jung TS, Elkin B, Mulders R, Larter NC, Branigan M, Pongracz J, Wagner B, Kafle P, Lobanov VA, Rosentha BM, Jenkins EJ (2020) Hiding in plain sight: discovery and phylogeography of a cryptic species of *Trichinella* (Nematoda: Trichinellidae) in wolverine (*Gulo gulo*). Int J Parasitol 50:277–287

Soule C, Dupouy-Camet J, Georges P, Ancelle T, Gillet JP, Vaissaire J, Delvigne A, Plateau E (1989) Experimental trichinellosis in horses: biological and parasitological evaluation. Vet Parasitol 31:19–36

Stensvold CR, Nielsen HV, Mølbak K (2007) A case of trichinellosis in Denmark, imported from Poland, June 2007. Euro Surveill 12:E070809.3

Zarlenga D, Thompson P, Pozio E (2020) *Trichinella* species and genotypes. Res Vet Sci 133:289–296

Part III

Zoonoses in Food-Chain and Domestic Animals: Focus on Antimicrobial Resistance

Extended-Spectrum β-Lactamase and AmpC β-Lactamase-Producing Bacteria in Livestock Animals

18

Christa Ewers

ESBLs in livestock – How big is the piece in the complex puzzle on antimicrobial resistance?

Contents

Abstract

In the past decades, we faced a rapid increase in human infections caused by third-generation cephalosporin-resistant bacteria, e.g., *Escherichia* (*E.*) *coli*, *Klebsiella* (*K.*) *pneumoniae,* and *Salmonella*, mainly due to the plasmid-encoded production of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases. The primary reservoirs of ESBL/AmpC-producing bacteria are still contentious. However, it is believed that livestock animal- and food-related sources play a possible but yet not quantifiable role in the spread of such bacteria. The use of large quantities of antibiotics in this sector, but also in the medical field, undoubtedly contributes to

C. Ewers (✉)
Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Giessen, Germany
e-mail: christa.ewers@vetmed.uni-giessen.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_15

the global rise of antimicrobial resistant (AMR) bacteria. Entry of multidrug-resistant microorganisms early in the livestock production cycle, as well as their frequent occurrence in healthy animals and food products, together with the finding of common molecular characteristics suggests that a livestock animal-to-human transmission could occur. However, links between ESBL/AmpC-producing bacteria from these two sources have been drawn mainly from observational epidemiological data while studies providing unquestionable proofs for transfer directionality and quantifying the risk for human health are limited. In any case, food animal production should be regarded a major player in the expansion of the global resistome. Therefore, efforts need to be further directed toward reducing reliance on antimicrobials in this sector wherever possible. More than ever, good veterinary and farming practices, including responsible use of antibiotics and implementation of biosecurity, hygiene, and disease prevention, e.g., by vaccination or by improving the animals' gut health, are regarded essential in the containment of AMR. Many fields, including medicine, veterinary medicine, animal husbandry, environment, and trade, are involved in this complex issue. Thus, isolated, sectorial efforts in the food animal production field will not be that efficient unless concerted efforts from all those involved are applied on a global scale, following a One Health approach.

Keywords

ESBL · AmpC · Livestock · Food · Plasmid · HGT · Clonal spread · Antimicrobial usage

18.1 Resistance Mechanisms in Broad-Spectrum Cephalosporin-Resistant Gram-Negative Bacteria

Beta-lactams are the most widely used antibiotics in clinical practice over the world and strong selective pressures upon them have resulted in a continuous increase of AMR bacteria (Bush and Bradford 2016; WHO 2018a,b). The β-lactams interfere with the metabolism of the bacterial cell wall by mimicking and thereby inactivating one of the building blocks, i.e., penicillin-binding proteins, used by enzymes to construct peptidoglycan (Bush and Bradford 2016). Resistance to β-lactams can be due to mutations in the penicillin-binding proteins, a reduced permeability of the cell wall, and the production of β-lactamase enzymes able to hydrolyze and inactivate the β-lactam-ring, which is by far the most common mechanism in *Enterobacterales*. Penicillin was one of the first β-lactams developed for clinical use in humans, and as early as 1940, β-lactamase activity was described as a penicillin-inactivating mechanism that threatened the use of this critical class of β-lactam antibiotics (Bush and Fisher 2011). With the introduction of new β-lactams (e.g., the penicillin derivatives methicillin and oxacillin, first to fourth generation) and most recently fifth-generation cephalosporins, monobactams, and carbapenems with improved activity on specific bacteria and increasing stability to hydrolysis, new β-lactamases emerged

and are continuously evolving with the ability to hydrolyze the β-lactam bond in almost all β-lactam-containing molecules (Bush and Fisher 2011; Bush and Bradford 2020). To date almost 3000 unique, naturally occurring small-, broad-, and extended-spectrum β-lactamases grouped into four Ambler classes A–D based upon amino acid sequence homology have been described. Class A and C β-lactamases are the most commonly found in *Enterobacterales* and are mainly represented by extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases (in this chapter referred to as "AmpCs"), respectively (Smet et al. 2010; Bush and Fisher 2011; Bush and Bradford 2020).

Enterobacteriaceae producing ESBLs are resistant to third- and fourth- generation cephalosporins and to aztreonam, while they are usually inhibited by β-lactamase-inhibitors, such as clavulanate, sulbactam, tazobactam, and avibactam. They are also not hydrolyzed by cephamycins and carbapenems. While early ESBLs mainly evolved from natural narrow-spectrum TEM-1/TEM-2 (named after a Greek patient "Temoneira") and SHV-1 ("sulfhydryl reagent variable") β-lactamases, CTX-M (cefotaximase Munich)-type enzymes, all of which reveal an ESBL phenotype, succeeded over the last decades and currently represent the most common ESBLs (Livermore et al. 2007; Liakopoulos et al. 2016; Bevan et al. 2017; Bush and Bradford 2020). Other ESBL groups, such as OXA, GES, PER, and VEB β-lactamases, have been described more recently and are found only sporadically and often in nonfermenting gram-negative bacteria, including *Pseudomonas* and *Acinetobacter* spp. (Potron et al. 2015). The genes that encode CTX-M-type ESBLs have once been mobilized from the chromosomes of environmental *Klyuvera* spp. and nowadays are predominantly encoded on transferable plasmids in both clinical relevant and commensal bacteria (Livermore et al. 2007). Currently, members of the *Enterobacterales* order are the most often encountered bacteria possessing ESBL/AmpC enzymes. After their initial observation in humans in the 1980s, ESBL/AmpC-producing *Enterobacteriales* emerged in companion animals more than a decade later and shortly after that in food-producing animals as well as in wildlife and various environmental sources (Guenther et al. 2011; Ewers et al. 2012; Lee et al. 2020; Palmeira et al. 2021). The rapid and extensive dissemination of CTX-M-type ESBLs in medical and veterinary settings, among commensal bacteria of humans and animals and in the environment, is regarded as one of the most successful histories of AMR observed in the antibiotic era. It is probably driven by one or a combination of the following factors: (i) efficient capture and spread of $bla_{\text{CTX-M}}$ genes by mobile genetic elements; (ii) association of these mobile elements with highly successful bacterial genotypes; and (iii) extensive use of extended-spectrum cephalosporins and other antimicrobials which can co-select ESBL-producing strains resulting in high selective pressure (D'Andrea et al. 2013).

AmpC- β-lactamases historically were chromosomal cephalosporinases *in Pseudomonas aeruginosa* and many *Enterobacterales* (Jacoby 2009; Bush and Bradford 2020). Since the 1980s, a growing number of genes for AmpC enzymes have escaped from the chromosome of different gram-negative bacteria onto transmissible plasmids. These "acquired" or "plasmidic" AmpCs consequently can now appear in bacteria

usually lacking or poorly expressing chromosomal bla_{ampC} genes, such as *E. coli* or *K. pneumoniae*. AmpC-β-lactamase mechanisms can be divided into the following categories: (a) inducible resistance by chromosomally encoded *ampC* genes (e.g., *Enterobacter cloacae*, *Citrobacter freundii*, and *P. aeruginosa*); (b) resistance due to mutations in promoter and/or attenuator sequences (e.g., *E. coli* and *Acinetobacter baumannii*); or (c) plasmid-mediated resistance (e.g., *K. pneumoniae*, *E. coli*, *Salmonella*, etc.) (Tamma et al. 2019). Although many gram-negative bacteria produce a chromosomal β-lactamase (cAmpC), it is the transferable plasmidic AmpC that is responsible for most of the multidrug resistance observed in gram-negative isolates (Bush and Fisher 2011; Tamma et al. 2019). AmpC-β-lactamases mediate resistance to most penicillins, first- to third-generation cephalosporins, cephamycins, and inhibitor β-lactam combinations (e.g., amoxicillin/clavulanate), but usually not to fourth-generation cephalosporins and carbapenems (Jacoby 2009). According to their original chromosomal producers they once developed from, AmpCs can be divided into at least five phylogenetic groups: the *Citrobacter freundii* group (CMY-2-like, LAT, and CFE), the *Enterobacter* group (MIR and ACT), the *Morganella morganii* group (DHA), the *Hafnia alvei* group (ACC), and the *Aeromonas* group (CMY-1-like, MOX, and FOX) with the most prevalent and widely disseminated being CMY-2-like enzymes (Meini et al. 2019). Although to date resistance due to pAmpCs is less common than ESBL production in most parts of the world, it may be (i) more difficult to detect in the clinical laboratory, probably underestimating its occurrence, and (ii) broader in substrate spectrum, probably resulting in significant therapeutic failure (Jacoby 2009; Meini et al. 2019).

Broad-spectrum cephalosporins have long been the treatment of choice for serious infections with enterobacterial and other gram-negative pathogens. Since ESBL/AmpC-producing bacteria rapidly increased over the past two decades, other critically important antimicrobials had to be used alternatively (Bush and Bradford 2016). However, mobile genetic elements, such as plasmids and transposons, frequently encode not only ESBL/AmpC genes but in addition genes coding for resistance, e.g., to aminoglycosides, quinolones, sulfonamides, and tetracyclines. Thus, the way was paved for the spread of bacteria with broad-spectrum cephalosporin resistance in both hospital settings and the community. As a result, clinicians were increasingly forced to use carbapenems for the treatment of severe infections caused by multidrug-resistant bacteria. This in turn has led to the selection of acquired carbapenemases among *Enterobacterales*, e.g., KPC (*K. pneumoniae* carbapenemase), OXA (oxacillinase)-48-like, NDM (New Delhi metallo-β-lactamase), VIM (Verona integron metallo β-lactamase), and other gram-negative bacteria, such as *A. baumannii* (e.g., OXA-23-like and OXA-58-like). Resistance to carbapenems is currently one of the most pressing public health threats relating to antibiotic resistance (Nordmann 2013; Dropa and Daoud 2022). Again, it was first described in humans and later, and still relatively rare, in companion and livestock animals (Fischer et al. 2012; Poirel et al. 2012; Nordmann 2013; Stolle et al. 2013; Ewers et al. 2016; Koeck et al. 2018).

18.2 Livestock Animals and Food as a Possible Source of ESBL/AmpC-Producing Bacteria

The primary reservoirs of ESBL/AmpC-producing *Enterobacteriaceae* are still controversial. Early reports described the occurrence of these bacteria in healthy humans (Valverde et al. 2004; Pitout et al. 2009; Overdevest et al. 2011), companion animals (Ewers et al. 2010, 2011; Dierikx et al. 2012; Pomba et al. 2014), wild animals (Literak et al. 2010; Fischer et al. 2014), and the environment (Mesa et al. 2006; da Costa et al. 2013). Since the late 1990s, ESBLs/AmpCs have been detected in poultry (Hasman et al. 2005; Kojima et al. 2005; Girlich et al. 2007; Fischer et al. 2017; Saliu et al. 2017), pigs (Riano et al. 2006; Djeffal et al. 2017; Vidovic and Vidovic 2020; De Koster et al. 2021), cattle (Frye et al. 2008; Wieler et al. 2011; Madec et al. 2012; Reist et al. 2013; Hering et al. 2014; Duse et al. 2015; Bergspica et al. 2020; De Koster et al. 2021), and retail meat (Jensen et al. 2006; Jouini et al. 2007; Bergenholtz et al. 2009; Doi et al. 2010; Cohen Stuart et al. 2012; Geser et al. 2012a,b; Kola et al. 2012; Vidovic and Vidovic 2020) almost globally. Hence, several studies raised questions in particular about the possible role of livestock animal- and food-related reservoirs in the spread of ESBL/AmpC-producing bacteria (Canton et al. 2008; Carattoli 2008; Hunter et al. 2010; Smet et al. 2010; Ewers et al. 2012; Seiffert et al. 2013; Vidovic and Vidovic 2020). Notably, healthy animals might not only carry multidrug-resistant commensal, i.e., nonpathogenic microorganisms, but may particularly be a source for primary or secondary pathogens leading to various diseases (e.g., gastroenteritis, urinary tract infection, wound infection, bacteremia, and septicemia) in immunocompromised patients or even in healthy individuals (Canton et al. 2008; Smet et al. 2010). One of the earliest descriptions of poultry as carriers of ESBLs and AmpCs was by Brinas et al. (2003), who recovered CTX-M-14, SHV-12, and CMY-2 β-lactamases in *E. coli* from the feces of healthy chickens in Spain in 2000 (Brinas et al. 2003). After that, studies from numerous countries followed and reported about the frequent presence of different types of ESBLs and AmpCs in *E. coli* and less frequently in *Salmonella* isolates from poultry (Girlich et al. 2007; Smet et al. 2008, 2010; Randall et al. 2011; Ewers et al. 2012; Saliu et al. 2017; Lee et al. 2020). First indications for the presence of ESBLs/AmpCs in healthy pigs came from Spain, where an SHV-12-producing *Salmonella* isolate was identified in 1999 (Riano et al. 2006), and from the USA, where researchers detected CMY-2-producing *E. coli* isolates during the years 1998 and 1999 (Winokur et al. 2000). Nearly within the same time frame, initial reports were published from the first findings of AmpC-producing *Salmonella* and ESBL-producing *E. coli* isolates in healthy cattle in the USA (Gupta et al. 2003) and Japan (Duse et al. 2015), respectively. Likewise, ESBL/AmpC-producing bacteria were recovered more than a decade ago from sick animals, animal manure, and the farm environment, indicating additional sources (Mesa et al. 2006; Carattoli 2008; Smet et al. 2010; Ewers et al. 2012; Friese et al. 2013).

18.3 Occurrence of ESBL/AmpC-Producing Bacteria in Livestock Animals

Total prevalences of ESBL/AmpC-producing bacteria in healthy animals of the major livestock species, i.e., poultry, pigs, and cattle, vary largely with study type. Nevertheless, poultry production was considered as the major contributor to the selection and spread of third-generation cephalosporin-resistant bacteria. Here, percentages of ESBLs (mainly targeted in the cited studies) and/or AmpCs range from 0.1–26.6% for *Salmonella* spp. (Smet et al. 2008, 2010; Randall et al. 2011; Ewers et al. 2012; Bai et al. 2015; Djeffal et al. 2017; Sabry et al. 2020) and from 1.7–96.4% in *E. coli* isolates (Kojima et al. 2005; Riano et al. 2006; Girlich et al. 2007; Smet et al. 2008, 2010; Ewers et al. 2012, 2021; Geser et al. 2012a,b; Dierikx et al. 2013; Laube et al. 2013; Hering et al. 2014; Huijbers et al. 2015; Nguyen et al. 2019; Gazal et al. 2020; Kuhnke et al. 2020; Kakooza et al. 2021) mainly originating from broilers, but also from turkeys in different European, Asian, and African countries and in North and South America. Also *Klebsiella pneumoniae*, which are of critical importance in human medicine, have been identified as ESBL producers in broiler carcasses (Wu et al. 2016; Projahn et al. 2019; Chenouf et al. 2021).

Rates of *Salmonella* and *E. coli* isolates producing ESBLs/AmpCs described for pigs are between 0.2% and 8% and between 1% and 84% (Riano et al. 2006; Wu et al. 2008; Smet et al. 2010; Ewers et al. 2012, 2021; Hammerum et al. 2014; Hansen et al. 2014; Randall et al. 2014; Stefani et al. 2014; von Salviati et al. 2014; Dohmen et al. 2017; Kraemer et al. 2017; Kaesbohrer et al. 2019; Bergspica et al. 2020; Kuhnke et al. 2020; Lay et al. 2021), and those for cattle these range from 0.6–2.4% and from 0.7–91.0%, respectively, in the cited studies (Gupta et al. 2003; Frye et al. 2008; Hunter et al. 2010; Smet et al. 2010; Wieler et al. 2011; Ewers et al. 2012, 2021; Hordijk et al. 2013; Reist et al. 2013; Duse et al. 2015; Hille et al. 2017; Palmeira and Ferreira 2020; Weber et al. 2021; Gelalcha and Kerro Dego 2022). The overall heterogeneity of these global data may not simply refer to differences between animal species or national/local variations in pathogen prevalence and susceptibility patterns or to global differences in antimicrobial use. It could be also due to different study designs, i.e., with respect to inclusion criteria (e.g., animal age, production system, and previous antimicrobial treatment) and microbiological procedures (e.g., usage of selective or nonselective cultivation media; sample enrichment). However, it was commonly agreed that livestock should be regarded as major player in the maintenance and/or expansion of the global bacterial resistome with a noticeable trend toward a more frequent colonization with ESBL/AmpC-producing bacteria of poultry compared to pigs and cattle (in rank).

With the increased pressure of AMR in the veterinary field, several European countries, including Germany (GERMAP), Finland (FINRES), Sweden (SVARM), Denmark (DANMAP), the Netherlands (MARAN), and Norway (NORM-VET), established active surveillance and monitoring programs to provide an estimation of whether the level of resistance has increased or decreased over time and to measure the impact of specific interventions or programs implemented at the national level (Mader et al. 2022). Such programs have also been initiated by non-European

countries, such as Canada (CIPARS 2002–2021), the USA (NARMS 2003–2018), and Japan (JVARM 2000–2017). Since 2004, Member States of the European Union are required to monitor and report AMR data on *Salmonella* and *Campylobacter* on mandatory basis and on indicator bacteria, i.e., commensal *E. coli* and enterococci on voluntary basis from food-producing animals (at farm or slaughterhouse level) and food thereof. In accordance with Commission Implementing Decision 2013/652/EU, the monitoring in indicator *E. coli* isolates has also become mandatory. Under the framework of Directive 2003/99/EC, the European Food Safety Authority (EFSA) publishes these data in a yearly joint summary report with the European Center for Disease Prevention and Control (ECDC) (EFSA/ECDC 2022). In addition, non-governmental initiatives, such as the AMR monitoring undertaken by the Centre Européen d'Etudes pour la Santé Animale (CEESA), initiated pan-European programs to create insight into the AMR development to provide an alert for the pharmaceutical companies to increase resources in research and development of novel antibiotics (Schrijver et al. 2018). Among the four different programs conducted by CEESA, the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) examines the AMR of zoonotic and commensal bacteria in healthy food animals, and VetPath surveys AMR of bacterial pathogens from diseased animals. CEESA programs are financed by the veterinary pharmaceutical industry, but the results are presented to peer-reviewed journals to ensure independent reporting (de Jong et al. 2013; Schrijver et al. 2018; Ewers et al. 2021). It should be noted that EFSA and EASSA make use of different readouts. EFSA categorizes all isolates with a MIC value above the epidemiological cutoff (ECOFF) as "resistant," while EASSA categorizes strains into "decreased susceptible" and "clinically resistant" as they apply both ECOFFs and clinical breakpoints (Moyaert et al. 2014).

In the years 2019–2020, presumptive ESBL producers (i.e., isolates with minimal inhibitory concentartions [MICs] >1 mg/L for cefotaxime and/or ceftazidime and a synergy test positive for any of these antimicrobials together with susceptibility to meropenem) and presumptive AmpC producers (i.e., isolates with MICs >1 mg/L for cefotaxime and/or ceftazidime and cefoxitin MIC >8 mg/L together with susceptibility to meropenem) determined by routine monitoring (i.e., by using non-selective culture medium) in *Salmonella* isolates from broilers, fattening turkeys, and laying hens in EU Member States were observed at very low levels, varying between 0% and 1.9% (EFSA/ECDC 2022). For indicator *E. coli* isolates from broilers, fattening turkeys, fattening pigs, and bovines >1 year, the levels ranged from 0.1–1.5%. To ensure comparability of official monitoring data across the European Union, the EFSA provided technical specifications on the harmonized monitoring (e.g., usage of selective primary isolation media) and reporting (e.g., usage of epidemiological cutoffs instead of clinical breakpoints) of AMR (EFSA 2012). Data from the specific monitoring of ESBL and AmpC producers (using selective culture medium containing cefotaxime at 1 mg/L, as recommended by EUCAST) differ considerably from routine monitoring data. Percentages for presumptive ESBL producers ranged between 29.7% (broilers) and 34.1% (pigs), and those for AmpC producers between 3.6% (bovines, <1 year) and 11.3% (broilers). Marked variations in the prevalence of presumptive *E. coli* ESBL/AmpC producers

were reported between the EU Member States. It ranged from 2.1% (Cyprus) to 99.2% (Italy) in fattening pigs, from 1.1% (Italy) to 70.8% (Germany) in bovine animals >1 year of age, from 0.3% (Finland) to 98.6% (Slovakia) in broilers, and from 0% (Sweden) to 70.4% (Spain) in fattening turkeys (EFSA/ECDC 2022). The median occurrence of presumptive ESBL/AmpC producers was quite similar to the figures obtained in 2018. It appears that *E. coli* is the major source of third-generation cephalosporin resistance, which is less frequently found in Salmonella isolates. Of note, a statistically significant decrease in the prevalence of ESBL/AmpC producing *E. coli* has been observed in both broilers and turkeys over the period 2016–2020. A previous study from the Netherlands demonstraed a decreasing prevalence of contamination with ESBL *E. coli* in retail chicken already in the period from 2013–2015 (Huizinga et al. 2019). Another study from France reported that the nationwide drastic reduction of ceftiofur use and all other antibiotics in chicken production late 2011 had no impact yet on the ESBL/AmpC prevalence in retail chicken meat in that country (Casella et al. 2017).

The percentages of livestock animals colonized with third-generation cephalosporin resistant and/or ESBL/AmpC-producing *E. coli* mostly exceed what has been determined for human healthy carriers so far. While initially human fecal carriage of predominantly ESBL-producing isolates has been mainly reported along with nosocomial outbreaks, these isolates passed into the community almost in the mid-2000s (Canton et al. 2008). Proportions of humans colonized with ESBL-producing bacteria strongly vary by the study population and only exceptionally amount up to 80%, if, for example, travel-related fecal colonization, i.e., colonization after people had traveled to ESBL/AmpC "high-risk countries," is also considered (Ewers et al. 2012; Geser et al. 2012a,b; Nicolas-Chanoine et al. 2013; Seiffert et al. 2013; Kuenzli et al. 2014; Lubbert et al. 2015; Arcilla et al. 2017). This emphasizes the importance of the community reservoir in the evolution and dynamics of ESBL/AmpC-producing pathogens.

The contribution of food sources to the burden of antimicrobial resistance in humans is another controversial issue. Contamination of meat products with AMR bacteria may not only contribute to a theoretical spread of these organisms within the human population but also to a rapid transfer of AMR genes from foodborne commensals to human pathogens (da Costa et al. 2013). People could ingest ESBL/AmpC-producing bacteria by consuming the contaminated food directly or through cross-contamination on noncooked foods. Indeed, the presence of ESBL/AmpC-producing bacteria on retail meat has been documented repeatedly since many years (Hasman et al. 2005; Bergenholtz et al. 2009; Doi et al. 2010; Egea et al. 2012; Geser et al. 2012a,b; Casella et al. 2017; Kaesbohrer et al. 2019). Mainly following selective enrichment procedures, rates of ESBLs/AmpCs and/or presumptive ESBLs/AmpCs detected in beef and pork meat ranged from 0–21.7% (Jensen et al. 2006; Jouini et al. 2007; Lavilla et al. 2008; Ewers et al. 2012; Geser et al. 2012a,b; Kaesbohrer et al. 2019; Vidovic and Vidovic 2020). Much higher proportions, almost ranging between 30% and 100% have been reported in several studies performed on poultry meat in the recent couple of years worldwide (Hasman et al. 2005; Jouini et al. 2007; Bergenholtz et al. 2009; Doi et al. 2010;

Leverstein-van Hall et al. 2011; Overdevest et al. 2011; Randall et al. 2011; Cohen Stuart et al. 2012; Egea et al. 2012; Ewers et al. 2012; Kola et al. 2012; Kluytmans et al. 2013; Casella et al. 2017).

According to the latest report from EFSA/ECDC, in 2019–2020, presumptive ESBL/AmpC producers were observed in *Salmonella* isolates from broiler, pig, and bovine meat at very low levels during routine monitoring, varying between 0% and 0.2% (EFSA/ECDC 2022). Specific monitoring revealed presumptive *E. coli* ESBL/AmpC producers in pig, bovine, and broiler meat varying between 0.7% and 9.3% (AmpC) and between 4.3% and 23.4% (ESBL). These data again varied considerably between countries. The prevalence of presumptive *E. coli* ESBL/AmpC producers ranged from 0.3% (Finland) to 100% (Malta) in broiler meat, while it was less variable in meat from pigs, ranging from 0% (Finland and the Netherlands) to 24.4% (Portugal) and from bovine animals, where it ranged from 0.3% (UK) to 24.0% (Bulgaria).

Notably, reports about differences in ESBL/AmpC contamination of conventional versus organic (restricted antimicrobial use in animal rearing) chicken or retail chicken products are controversial. According to studies from Germany (43.9% vs. 36%) (Kola et al. 2012) and from the Netherlands (100% vs. 84%) (Cohen Stuart et al. 2012), there was a difference but it was not significant. It was suggested that ESBL-colonized 1-day-old chickens were introduced into organic farms or that cross-contamination between conventional and organic flocks during rearing or slaughtering or through an ESBL-contaminated environment accounted for the colonization of animals. Colonization of 1-day-old chickens and farm environmental contamination with ESBL-producing bacteria has also been shown in other studies (Bortolaia et al. 2010; Laube 2013). Also in a study from the USA, substantial variations in bacteria resistant to critically important antimicrobials were not found among organic and conventional retail chicken products (Mollenkopf et al. 2014). In contrast, significant differences were observed in studies from Italy (27.9% vs. 12.9% cloacal isolates; 31.3% vs. 1.4% skin isolates) (Musa et al. 2020) and Turkey (46% vs. 22%) (Uyanik et al. 2021). Regarding pigs, a recent study demonstrated that the percentage of ESBL-positive pens was significantly higher on conventional (55.2%) than on organic farms (44.8%) (Meissner et al. 2022). However, the authors determined similar proportions of ESBL-positive pens on conventional farms (54.3–61.9%) and a wide variation (7.7–84.2%) on organic farms. They found that the original farms, from which weaner pigs were purchased, had a major influence on the ESBL status.

Another important criterion in the complex issue of AMR is age-related and production cycle–dependent colonization of animals. The industrial production of broiler meat is the final or bottom level of a four-step pyramid, below the parent, grandparent, and primary breeder steps, and few reports suggest that ESBL/AmpC-producing bacteria are not uncommon in the top of some production pyramids. For instance, in Sweden, transmission of such bacteria from imported breeding chickens was documented by findings of *E. coli* carrying respective resistance genes in environmental samples from hatcheries rearing production animals or breeding stock (parent animals) (SVARM 2003–2021). Obviously, ESBL/AmpC-producing

E. coli have also been introduced in the Dutch poultry production chain through imported day-old grandparent chickens and the occurrence of these bacteria in the different levels of layers are likely attributed to vertical transmission (MARAN 2003–2022). In a longitudinal study from Denmark, a significant decrease in the carriage of identical ESBL-producing *E. coli* was detected from piglets to weaners and finishers (AbuOun et al. 2021). A reverse relationship between the prevalence of AMR bacteria in the intestinal microbiota and animal age has been demonstrated at dairy farms as well, which has been attributed either to a higher fitness of the resistant strains in young calves or selection pressure due to the feeding of waste milk that may contain antimicrobial residues (Geser et al. 2012a,b; Hordijk et al. 2013; Weber et al. 2021). A scoping review published by Gaire et al. (2020) also revealed an age-dependent AMR phenomenom in cattle and swine that was irrespective of geographic location and specific production practices (Gaire et al. 2020). Such findings emphasize that knowledge about the epidemiology of ESBLs/AmpC at the farm level should be improved as it may be of great value for the proper design of surveillance and intervention studies.

18.4 ESBL/AmpC Types

Irrespective of the origin, i.e., associated with fecal carriage or food contamination, CTX-M-1 has long been by far the most frequent ESBL type identified in all major groups of livestock animals in Europe and in other parts of the world, followed by CTX-M-14, TEM-52, and SHV-12. Other ESBL types frequently isolated include variants belonging to the CTX-M (e.g., CTX-M-2, -3, -8, -9, -15, 20, -27, -32, and -55), SHV (e.g., SHV-2 and -5), TEM (e.g., TEM-20, -24, -71, -106, and -126), and OXA (e.g., OXA-10) families (Ewers et al. 2012; Seiffert et al. 2013; Saliu et al. 2017; Palmeira and Ferreira 2020; Widodo et al. 2020). The type of AmpC β-lactamase detected was almost always the CMY-2 variant, followed by DHA-1, while other types are detected less often. CMY-2 is also one of the major enzyme isolated from poultry in Europe, while it is less common in bacteria from pigs and cattle (Smet et al. 2008; Doi et al. 2010; Kola et al. 2012; Fischer et al. 2017; Saliu et al. 2017; MARAN 2022).

The most frequent ESBL types in humans worldwide are CTX-M-15 and CTX-M-14, while CTX-M-1, which was initially rarely present in humans, slightly increased in recent years (Bevan et al. 2017). The $bla_{\text{CTX-M-15}}$ gene has spread in a pandemic fashion, mainly driven by plasmids of incompatibility group IncF and by a certain *E. coli* clonal lineage, namely multilocus sequence-type ST131 (Livermore et al. 2007; Pitout and DeVinney 2017). CTX-M-14 is also prevalent in poultry and cattle in Asia and to a moderate extent in livestock animals in non-Asian countries, suggesting possible transmission scenarios in the respective area. It is rather interesting to note that the most common ESBL type in humans, namely CTX-M-15, has invaded animal production compartments only in the past years, after a long period of low impact in livestock animals compared with a common occurrence in companion animals (Ewers et al. 2012). This enzyme type is no longer rare in bacteria

from livestock sources and its presence was increasingly detected in bacteria from cattle, poultry, and pig sources (Smet et al. 2010; Randall et al. 2011; Wieler et al. 2011; Hammerum et al. 2012; Madec et al. 2012; Fischer et al. 2014; Diab et al. 2017; Djeffal et al. 2017; Saliu et al. 2017; Fournier et al. 2020; Palmeira and Ferreira 2020; Ramos et al. 2020). Also, the shiga toxin producing *E. coli* O104:H4 strain, that was responsible for a severe outbreak of enterohaemorrhagic *E. coli* (EHEC) infection via contaminated food in Germany in 2011, carried ESBL-type CTX-M-15 (Ewers et al. 2011). For a detailed spatial and temporal distribution of ESBL/AmpC type in livestock animals, the author would like to refer to scientific reviews (Ewers et al. 2012; Saliu et al. 2017; Palmeira and Ferreira 2020; Ramos et al. 2020) and the annual EFSA/ECDC and national EU Member State reports.

The frequent carriage of ESBL/AmpC-producing bacteria by healthy cattle, pigs, or poultry and high prevalence of these organisms in food products indicates that food-producing animals may be the origin of at least part of the human infections. The following chapter will show that knowledge on the genetic makeup and epidemiology of plasmid and bacterial host is the minimum necessary for further assessing the foodborne risk and may also be valuable for source attribution. It is generally accepted that thorough cooking destroys bacteria in food, while cross-contamination to uncooked food may occur in case of inadequate hygiene measures, and these are important lessons from foodborne outbreaks due to *Salmonella*, *Campylobacter*, and enterohemorrhagic *E. coli* (EHEC) bacteria. As we currently cannot determine the magnitude of the mode of transmission of ESBL/AmpC-producing bacteria via the food chain, this theoretical hazard to human health requires further assessment.

18.5 Antimicrobial Drug Usage and Its Supposed Impact on the Emergence of ESBL/AmpC-Producing Bacteria in Livestock Animals

Antimicrobials are essential for both human and animal health. Many antimicrobial drugs administered to food animals are the same classes as those utilized in human medicine. Any antimicrobial usage (AMU), whether in the human or animal sector, can select for AMR and promotes the dissemination of AMR bacteria, regardless they are commensals, pathogens, or environmental strains. This greatly influences the population structure of microbial communities resulting in unpredictable consequences for public health (EFSA/ECDC 2013). Even though this is widely accepted, the evidence linking antibiotic use in food-producing animals with AMR bacteria emerging in humans is a long and controversially discussed issue. With the increasing emergence of ESBLs/AmpCs and more recently of carbapenemases in bacteria implicated in human infections, this debate reached a novel peak. Antimicrobial resistance genes and AMR bacteria have mainly evolved as a result of selective pressure, which is amplified by misuse (e.g., unnecessary use, subinhibitory dosage, or inappropriate duration of treatment) and overuse, particularly of broad-spectrum antimicrobials (Chantziaras et al. 2014; Guardabassi et al. 2018). In addition, we

must be aware that AMR may also be of ancient origin, implying that we all basically live in a surrounding filled with AMR genes and their precursors, the so-called resistome (D'Costa et al. 2011). The resistome refers to the collection of all AMR genes associated with microbiota in a given environment and is greatly influenced by the AMU in the different medical sectors. Based on metagenomic sequencing, it has been shown that there are abundant AMR genes in the fecal microbiota of food-producing animals which were not always always directly related to AMU, but were undoubtedly influenced by the use of injectable antimicrobials or their administration through feed or water (Ma et al. 2021). Understanding the complex events in the microevolution and dissemination of AMR in different compartments is therefore essential to estimate the impact of food animal production on this global health crisis.

Antimicrobial usage can differ in humans and food-producing animals in terms of methods of administration and quantities administered. There are also important variations between and within food-producing animal species, as well as between countries (Guardabassi and Kruse 2008; EFSA/ECDC 2013). In humans, antibiotics are administered individually to patients for therapeutic purposes, i.e., to treat infections, whereas prophylactic use of drugs to prevent the emergence of infections is the exception. This is quite comparable to what we encounter in small-animal and equine veterinary medicine. Antimicrobial prophylaxis refers to the administration of an antimicrobial substance to a healthy animal in the presence of a specific risk factor or stressors, such as weaning for piglets, intra- or interfarm transportation of calves, or drying off for dairy cows (Guardabassi et al. 2018; EMA 2019; Jerab et al. 2022). According to the WHO (2018a,b) and the European Medicines Agency (2019), unnecessary prophylactic AMU should be avoided, particulary if it is used to compensate poor health management and biosecurity. It should be restricted to individual animals or a group of animals at high risk of developing infections that have a significant impact on animal health and herd productivity (Guardabassi et al. 2018, WHO 2018a,b, EMA 2019).

In industrial farming, antimicrobials are commonly given to a whole group of animals (herd or group medication) to treat both diseased and clinically healthy in-contact animals that are presumably infected. Metaphylaxis shall prevent healthy animals from developing clinical signs and to prevent further spread of the disease (Guardabassi et al. 2018). The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report from 2021 revealed that 86.9% of the antimicrobial products sold for veterinary care in Europe were products suitable for group medication (i.e., oral powders, oral solutions, and premixes) (EMA 2021; Jerab et al. 2022). Metaphylaxis in commercially reared cattle and pig holdings was most often implemented after weaning, transportation, and regrouping as these stressors often result in disease (Kasabova et al. 2021; Jerab et al. 2022). The new VMP Regulation (EU) 2019/6, which came into force in January 2022, states: "Antimicrobial medicinal products shall be used for metaphylaxis only when the risk of spread of an infection or of an infectious disease in the group of animals is high and when no other appropriate alternatives are available" (EC 2019; Jerab et al. 2022). It also provided for a wide range of additional, concrete measures to fight AMR and to promote a prudent and responsible AMU in animals. These include the obligation for

Member States to collect data on the sales and use of antimicrobials in animals per species; the possibility to reserve certain antimicrobials for human use only; a ban on the preventive use of antibiotics in groups of animals; and a reinforced ban on the use of antimicrobials for promoting growth and increasing yield (EC 2019). Notably, several member states have already implemented programs to collect antimicrobial sales data for animals, but often not per animal species. In Germany, for example, obligatory monitoring was adopted by the 16th Amendment of the German Pharmaceuticals Act ("Arzneimittelgesetz," AMG) in 2014, which regulates the official monitoring of the use of antibiotic drugs for weaners and fattening pigs, beef cattle, and poultry (Schaekel et al. 2017). Indeed, veterinary professional associations strongly advocate the principles of antimicrobial stewardship and responsible use. However, there is some concern that a ban of metaphylactic group treatment could likely result in high morbidity and mortality, for example, due to infections with *E. coli* in all major livestock species and with *Streptococcus suis* in pigs (Jerab et al. 2022). A recent study performed over a period of 11 months demonstrated a significant decline of multidrug-resistant *E. coli* and a significant increase in the proportion of isolates that were fully susceptible to the tested antimicrobials after withdrawal of group treatment of pigs on a farm (De Lucia et al. 2021). The authors suggested that their findings might support policymakers in the implementation of measures to control AMR and reduce antimicrobial use.

Another practice still common in livestock production in some countries is the use of antimicrobials to spur animal growth and to improve feed efficiency (Gilbert 2012). Antimicrobial use for growth promotion refers to the exposure of healthy animals to subtherapeutic concentrations in feed to improve growth rate, efficiency of feed utilization, and reproductive performance (Guardabassi et al. 2018). Since 1997, the WHO, together with the Food and Agriculture Organization (FAO) and the World Animal Health Organization (OIE), has consistently recommended restrictions on nontherapeutic uses of antimicrobials in food animals, which resulted in several countries adapting their legislation over time (Anonymous 2007). The European Union banned most antimicrobial growth promoters (AGPs) (e.g., avoparcin, tylosin phophate, bacitracin zinc, virginiamycin, and spiramycin) at the end of the 1990s because their in-feed application has been associated with the selection of resistance to clinically important antimicrobials in human medicine (Aarestrup et al. 2001). For example, in the Netherlands, 14% of people living near turkey farms where the growth promoter avoparcin was used were found to carry vancomycin-resistant enterococci (VRE), which are frequent causes of nosocomial infections (Bergspica et al. 2020). Soon after the remaining growth promoters have been phased out in Europe from January 2006, some types of antimicrobial resistances were indeed substantially reduced and the supposed benefits of growth promoters could obviously be achieved by other measures aiming at disease control, such as good health management conditions, biosecurity, and vaccination (Guardabassi and Kruse 2008). In recent years, many countries worlwide prohibited or restricted the addition of AGPs to feed. For example, the USA prohibited the use of medically important antimicrobials for growth promotion in 2017, while they still allow the use of nonmedically important antimicrobials, such as the ionophores

(Food and Drug Administration Center for Veterinary Medicine 2017). Recently, the Chinese government launched a regulation to withdraw medicated feed additives in accordance with the National Action Plan to Combat AMR from Animal Resources (2017–2020). Vietnam announced the ban of AGPs in 2020, and Bangladesh, Bhutan, Indonesia, Myanmar, Nepal, Sri Lanka, and Thailand have announced some AGP restrictions (Ma et al. 2021).

Nowadays a wide range of antimicrobial agents are authorized for use in the food animal production and these are frequently the same, or belong to the same classes, as those used in the human medicine (EFSA/ECDC 2013). Due to public health concerns, a much greater scrutiny is focused on therapeutic use of antimicrobials in food-producing animals, particularly for substances that have analogues in human medicine (Meini et al. 2019), in particular if these are ranked as "critically important." To be classified in this category, an antimicrobial agent must serve the following criteria: the antimicrobial class is (i) the sole, or one of limited available therapies, to treat serious bacterial infections in people alternatives to treat human diseases, and (ii) used to treat diseases caused by (1) bacteria that may be transmitted from nonhuman sources, or (2) bacteria that may acquire resistance genes from nonhuman sources (WHO 2018a,b). Some antibiotic classes, including third- and fourth-generation cephalosporins, quinolones, and aminoglycosides, are defined as "critically important" for both human and animal health, by WHO and OIE, respectively (Pomba et al. 2018). This raises a particular concern in the prioritized use of these antimicrobials in the veterinary area to assure an appropriate balance between animal health needs and public health considerations (Anonymous 2007). Regarding the emergence of ESBL/AmpC-producing bacteria, the possible selective pressure by β-lactam antibiotics is of special interest. Systematic reviews and guidelines about β-lactams currently licensed for use in food-producing animals have been published previously (Burch et al. 2008; Constable et al. 2008; Smet et al. 2010; Liebana et al. 2013). Due to a very broad spectrum, short or zero withdrawal times for milk and the availability of "long acting" formulations for certain indications cefquinom and ceftiofur are commonly used in veterinary medicines where they are authorized for the treatment of various diseases caused by defined pathogens in cattle and pigs (Burch et al. 2008; Constable et al. 2008; Liebana et al. 2013). The former authorization of ceftiofur for injection of day-old chicken for prevention of septicemia in some Member States of the EU has been phased out and currently no cephalosporin-containing products are licensed for poultry species in the EU and in many other countries.

According to a joint report by the ECDC, EFSA, and EMA, an analysis of AMU and ESBL/AmpC prevalence data from food-producing animals based on the time periods 2015–2016, 2016–2017, and 2017–2018 showed a statistically significant association between the prevelance of ESBL/AmpC-producing *E. coli* and consumption of third- and forth-generation cephalosporins in livestock production (ECDC (European Centre for Disease Prevention and Control) 2021). In addition, several experimental and on-farm studies have been published supporting the hypothesis that the veterinary use of β-lactams, and also undetermined factors not related to antimicrobial use, may select for ESBL/AmpC-producing *Enterobacteriaceae* in

animals. There is, for example, evidence that parental therapy with ceftiofur significantly increases the likelihood of dairy cows to be colonized with third-generation cephalosporin-resistant *E. coli* (Tragesser et al. 2006; Volkova et al. 2012; MARAN 2022). In Denmark, an increased frequency of ESBL-producing *E. coli* was identified on farms with high versus no consumption of third- and fourth-generation cephalosporins (70% ESBL versus 20% ESBL) (Hammerum et al. 2014). In pigs inoculated intragastrically with an *E. coli* expressing CTX-M-1 an increase in the number of CTX-M-1-producing *E. coli* was highest after administration of cephalosporins and interestingly this was mainly due to the proliferation of indigenous isolates that probably acquired the ESBL plasmids via conjugation (Cavaco et al. 2008). The authors pointed out that pigs treated with cephalosporins and sent to slaughterhouses shortly after the end of the withdrawal time may still shed high numbers of ESBLs (up to 10^6 CFU/g feces), favoring contamination of food products and the environment. In Canada, a strong correlation between a reduction in ceftiofur-resistant *Salmonella* Heidelberg and *E. coli* (both producing AmpC) from human infections and retail poultry and withdrawal of ceftiofur use for disease prophylaxis in hatcheries has been reported (Dutil et al. 2010). Usage of ceftiofur and cefquinom and probably also environmental contamination due to the excretion of their metabolites mainly with the urine may have influenced the emergence of acquired AmpCs and ESBLs in both gram-negative pathogens and commensals in livestock animals (Aarestrup 2005; Tragesser et al. 2006; Subbiah et al. 2012). A study from Japan revealed a decreased resistance to broad-spectrum cephalosporins in *E. coli* from healthy broilers after voluntary withdrawal of ceftiofur (Hiki et al. 2015). Finally, an association between the prophylactic ("off-label") use of ceftiofur in 1-day-old piglets for disease prevention and the occurrence of CTX-M-1-producing *E. coli*, which could not be detected on control farms without a recent history of ceftiofur usage, was recently demonstrated (Apostolakos et al. 2020). Conversely, the transfer of a CMY-2 plasmid to *Salmonella* spp. and commensal *E. coli* in cattle was not attributed to ceftiofur treatment (Poirel et al. 2018). Similarly, CTX-M-producing *E. coli* persisted on a dairy farm in the absence of the use of any β-lactam for longer than 6 months (Liebana et al. 2006).

Most ESBL/AmpC-producing bacteria carry further resistances to commonly used veterinary drugs, e.g., amoxicillin, sulfonamides, trimethoprim, fluoroquinolones, and aminoglycosides, such that dropping the use of cephalosporins may be only one out of several necessary steps in reducing AMR resistance (EFSA/ECDC 2013). Co-selection of third-generation cephalosporin resistance, as evidenced by the isolation of ceftiofur-resistant CMY-2-producing *E. coli* upon administration of florfenicol, has, for example, been reported recently for French cattle (Meunier et al. 2010). In a study from Belgium, the administration of amoxicillin in poultry was significantly associated with the emergence of third-generation cephalosporin-resistant *E. coli* (Persoons et al. 2011). Likewise, an experimental chicken model clearly illustrated how, in *E. coli*, "old" antimicrobials, e.g., amoxicillin, may co-select antimicrobial resistance to third-generation cephalosporins by favoring resistance plasmid exchange (Dheilly et al. 2012). However, beyond antimicrobials the authors identified poor hygienic condition, lack of acidification of drinking water, repeated

feed changes during the production cycle, and hatchery of origin as additional risk factors possibly promoting the spread of resistant bacteria in poultry.

Since clear evidence has been provided for a linkage between AMU and selection of third-generation cephalosporin-resistant organisms in livestock animals, a significant reduction and rationale therapeutic use of antimicrobial agents is warranted. With respect to this, various national and international veterinary organizations have developed general ethical guidelines to encourage a prudent use of antibiotics in line with a "good veterinary practice" (Guardabassi and Kruse 2008). This would for instance include: (i) the use of antimicrobials by veterinary prescription and oversight only (which is a standard prerequisite for the use of veterinary medical products containing antimicrobials for food-producing animals in the European Union already); (ii) decision on therapeutic options guided by susceptibility testing of the identified pathogen; and (iii) prioritization of antimicrobial use according to the critical importance of the respective substance for humans, and several other issues aiming to reduce AMR in livestock animals while ensuring animal welfare at the same time. The decision on exceptional off-label use of antimicrobials (i.e., use in a different species, for a different disease, or at a dosage different to that on the label) to avoid causing unacceptable suffering should strictly follow a specific cascade (Directive 2011/82/EC). This is also to ensure a restrictive use of substances of critical importance for human health. In several countries, off-label use of cephalosporins in food-producing animals is no longer allowed, after it has long been used in the USA and Europe to prevent early mortality due to septicemia in poultry hatcheries or to treat diarrhea or prevent systemic infection in piglets (EFSA/ECDC 2013; Seiffert et al. 2013). Several national guidelines from EU Member States, including the German guideline for prudent use of antimicrobials in veterinary medicine, recommend a restrictive use of antimicrobials ranked as critically important for human health in sick individual animals and only in case microbiological identification and susceptibility testing of the target pathogen have been performed (BTK-AGTAM 2015). The US Food and Drug Administration has proposed a ban on the use of ceftiofur in livestock which was heavily criticized by representatives from the veterinary field as an intrusion on veterinary practice, whereas people from food safety agencies considered it at best a minor step (Kluytmans et al. 2013). As mentioned before, pig and poultry producers in some countries have introduced voluntary bans on the use of broad-spectrum cephalosporins; in other countries, e.g., in Germany, third- and fourth-generation cephalosporins are not authorized for poultry (DANMAP 2003–2021; MARAN 2003–2022).

In Denmark, the occurrence of ESBL *E. coli* in pigs at slaughter fell from 11.8% in 2010 to 3.6% in 2011, that of pigs tested on farms from 11% in 2010 to 0% in 2011 after the voluntary ban was introduced in July 2010 (Agerso and Aarestrup 2013). Also in the Netherlands, some benefits regarding a drop in AMR are seen after the reduction in the use of antimicrobials (>50%) by the veterinarians over the last couple of years. Compared with 2009, a steady decrease in resistance in several animal species, including pigs, to antimicrobials of critical importance for human health has been reported (MARAN 2003–2022). Finally, two systematic reviews, that analyzed a large body of literature, showed that interventions designed to reduce

use of antibiotics in food-producing animals have a positive effect on reducing the prevalence of AMR in both animals and humans, particulary those humans that are in direct contact with food-producing animals (Tang et al. 2017; Scott et al. 2018). Although these data are very encouraging, long-term implications for animal health cannot entirely be foreseen yet.

The overall quantity of antibiotics used in the food-producing animal industry is difficult to assess due to a number of confounding factors in the provided data (e.g., use of different technical units, lack of precise information on specific purpose for antibiotic use, and clear separation of antibiotic use for food-producing animal species and companion animals) (Seiffert et al. 2013). The majority of high-income countries have national monitoring programs that capture antibiotic prescriptions in animals. In some countries, the date are stratified by animal species, age, and disease indication (Sanders et al. 2020). In contrast, due to a lack of capacitiy or resources, only 6% of low-income countries monitor AMU in animals, most probably due to lack of capacities (WHO 2021).

In 2009, the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) was launched by the European Medicine Agency (EMA) to develop a harmonized approach for the collection and reporting of data on veterinary antimicrobials based on national sales figures, and combined with estimations of consumption in at least the major groups of species, including poultry, pigs, and veal valves (EMA 2021). The ESVAC strategy for 2016–2020 aimed to enable the analysis of European-level trends in AMU per animal species using data that are standardized between countries. Werner et al. (2018) provided an excellent, comprehensive summary about relevant methods and applications in national, EU-wide, and global programs to monitor the usage of antimicrobial drugs in animals. They further discuss key figures and variables that are essential to describe AMU in animals and give an overview of monitoring systems in the European Union and in non-European countries.

18.6 Transmission of ESBL/AmpC-Producing Bacteria or Resistance Genes Between Livestock Animals and Humans and Zoonotic Aspects

A number of ESBL/AmpC-producing bacteria, such as *E. coli*, *K. pneumoniae*, *Salmonella*, and other gram-negative bacteria, are not only of clinical and economic impact in human medicine, but can also cause infections in animals and are potent colonizers of the gut of healthy animals at the same time (Canton et al. 2008; Smet et al. 2010; Ramos et al. 2020). This makes a transmission of these microorganisms, whether they are AMR or not, between animals and humans a possible scenario. Transmission of ESBL/AmpC-producing bacteria can occur through direct contact or indirectly via the food chain and the environment (Carattoli 2008). The transfer and spread of ESBLs/AmpCs among different habitats (i.e., animals, humans, and the environment) is mainly driven by mobile genetic elements, such as plasmids, insertion sequences, integrons, and transposons. Due to an often occurring physical

linkage of multiple genetic determinants on the same plasmid, self-conjugative properties, and the capability to acquire additional mobile genetic elements (e.g., insertion sequences and transposons), plasmids have been signified as major vehicles of ESBLs/AmpCs in gram-negative bacteria over the past decades (Bush and Fisher 2011; Carattoli 2013). Different ESBL/AmpC types are linked with distinct plasmid incompatibility (Inc) groups (based on replicon types) and these plasmid scaffolds are important factors in understanding the spread of AMR bacteria across the different habitats. For instance, the $bla_{\text{CTX-M-1}}$ gene is frequently carried by IncN, IncFII, IncFIB, and IncI1 plasmids and those plasmids have been observed in human samples as well (Carattoli 2013; EFSA/ECDC 2013). Several *bla* genes are frequently, although not exclusively, associated with plasmids of distinct Inc. groups, such as $bla_{\text{CTX-M-1}}$ with IncN, IncI1 and IncF, $bla_{\text{CTX-M-14}}$ with IncK and IncF, $bla_{\text{CTX-M-15}}$ with IncF and IncI1, $bla_{\text{CMY-2}}$ with IncI1 and IncA/C, and SHV-12 with IncF, IncI1, and IncX3 (Carattoli 2009, 2013; Seiffert et al. 2013; Hammerum et al. 2014; Liakopoulos et al. 2016). Apart from plasmid dissemination by horizontal gene transfer, clonal expansion of distinct bacterial genotypes largely contributed to the public health burden of AMR. This is probably best exemplified by the pandemic dissemination extraintestinal pathogenic *E. coli* O25b:H4-ST131 subclades C1/H30R (associated with fluoroquinolone resistance) and C2/H30-Rx (associated with extended-spectrum cephalosporin resistance conferred by the ESBL CTX-M-15) that cause millions of AMR infections annually (Pitout and DeVinney 2017; Duprilot et al. 2020).

Accordingly, investigating outbreaks due to ESBL/AmpC-producing bacteria, tracing the spread of AMR determinants in epidemiologically linked strains, and comparison of strains from different sources on a global scale substantially relies on a combinational methodological approach, considering both bacterial host and resistance genes carrying plasmid. An integral part of plasmid epidemiological surveillance is plasmid replicon typing to determine incompatibility groups. Plasmid multilocus sequence typing (pMLST), by which plasmids are assigned to sequence types (STs) (http://pubmlst.org/plasmid/), allow for an even finer resolution, e.g., of same plasmid families revealing identical replicon types. The latter is in analogy to the MLST for bacterial genomes (e.g., for *E. coli* [http://mlst.ucc.ie/mlst/dbs/Ecoli/]) which also aims at identifying groups of clonally related strains (Carattoli 2011; Seiffert et al. 2013). With the increasing affordability of whole genome and plasmid sequencing, higher-resolution methods became available that will have a great impact on the assessment of possible transmission events of ESBL/AmpC-producing bacteria and their plasmids between different ecological compartments.

Although the majority of cases of colonization with ESBL/AmpC-producing bacteria among humans cannot be clearly linked to livestock and food-producing animals (on a sound scientific basis), several studies suggested that direct contact with livestock animals can be a risk factor for human colonization. In an experimental model mimicking the human gut microbiota, an *E. coli* strain of poultry origin established itself very well and easily transferred its plasmid (IncI) and *bla* gene (TEM-52) to commensal human *E. coli* even in the absence of antimicrobial selective pressure (Smet et al. 2011). This may not simply demonstrate a theoretical

scenario as supported by previous findings that the prevalence of ESBL-producing *E. coli* among people working in meat-processing companies or among farmers was higher than usually recorded for the general population at the given time and region (Lavilla et al. 2008; Geser et al. 2012a,b; Dierikx et al. 2013). Moreover, in some studies, patients suffering from gastrointestinal disease due to infections with ESBL-producing *Salmonella* had more contact with food-producing animals than subjects with susceptible isolates (Gupta et al. 2003; Fey et al. 2000). In a study from the Netherlands, human and pig isolates within the same farm harbored similar ESBL gene types and had identical sequence and plasmid types on two farms, suggesting clonal transmission. The ESBL carriage in humans (6%) was associated with the average number of hours working on the farm per weak and with the presence of ESBLs in pigs (Dohmen et al. 2015). Genetically related IncI1 plasmids (i.e., with the same plasmid sequence type) carrying the $bla_{\text{CTX-M-1}}$ gene were determined in *E. coli* and *Salmonella* isolates from colonized and diseased humans, food-producing animals, and from meat samples in various European countries (Carattoli 2011; Leverstein-van Hall et al. 2011). Similar reports refer to $bla_{\text{CTX-M-1}}$ gene carrying IncN plasmids that are extensively distributed among different livestock animal species, but were also identified in *Enterobacteriaceae* from humans and retail meat (Cavaco et al. 2008; Moodley and Guardabassi 2009; Bortolaia et al. 2010; Carattoli 2011; Randall et al. 2011; Ewers et al. 2021). In a study from Germany, an ESBL *E. coli* isolate from a farm worker and a cattle fecal sample from the same farm shared an identical sequence type (ST3891) and CTX-M allele, indicating a zoonotic transfer. Two other pairs of human-pig and human-cattle *E. coli* isolates encoded the same ESBL genes but did not share the same ST, which may indicate resistance plasmid transfer (Dahms et al. 2015). In 6 of 18 farmers (33%), Dierikx et al. (2013) identified isolates producing ESBL/AmpC types (CTX-M-1, SHV-12, and CMY-2), which were also present in the samples from their animals. Five farmers even showed identical plasmid families (IncI1, IncK, and IncN) and in isolates from two farmers the genes were carried on identical plasmid subtypes (IncI1-ST12 and IncN-ST1) indicating plasmid transfer between animals and farmers in the Netherlands (Dierikx et al. 2013). A Danish study demonstrated the presence of *E. coli* with identical CTX-M enzymes, macrorestriction profile, and MLST type in both pigs and farmers at 4 of 20 investigated pig farms (Hammerum et al. 2014). According to their findings, the authors considered it likely that the CTX-M-1-, CTX-M-14-, and CTX-M-27-producing *E. coli* frequently detected in patients in Denmark could be of animal origin. However, they suggested that further studies would be neccessary to quantify a possible zoonotic link between ESBL-producing *E. coli* and human infections. Using whole genome sequence analysis, van Hoek et al. (2020) could only confirm six of eight previously suggested (based on *E. coli* STs, plasmid families, and ESBL/AmpC genes) transmission events between broilers, farmers, and their family members on the same farm (van Hoek et al. 2020), underlining the added value of high-resolution methods. Likewise, de Been et al. (2014) could demonstrate that the majority of ESBL/AmpC-producing *E. coli* isolates from broilers, meat, and human infections, that were supposed to be identical based on lower-resolution methods, were indeed rather dissimilar based on

WGS analysis (de Been et al. 2014). Plasmid reconstructions revealed three distinct plasmid lineages of the IncI1 and IncK type that carried ESBL/AmpC genes. As the plasmid backbones within each lineage were virtually identical and were shared by genetically unrelated human and animal isolates, the authors suggested that ESBL/AmpC genes are mainly disseminated in animals and humans via distinct plasmids rather than by clonal transmission of strains.

There are also studies that found no transmission link between zoonotic and/or AMR pathogens of animal and human origins. Ludden et al. (2019) compared more than 430 *E. coli* isolates, including 155 ESBL-producing isolates from livestock and retail meat with the genomes of 1517 *E. coli* isolates associated with blood stream infections from the UK. They found that these two groups of *E. coli* were genetically distinct populations that in addition revealed only a limited overlap in AMR-gene-carrying mobile elements (Ludden et al. 2019). Investigating genomic and plasmid backbone, Kluytmans et al. (2013) found just one perfect match between geographically and temporally matched *E. coli* from humans and chicken meat (Kluytmans et al. 2013), implicating chicken meat as a source of human colonization with ESBL-producing *E. coli* isolates. Also other authors found that ESBL-producing *E. coli* from humans were generally different to that from animals, i.e., chickens, cattle, turkey, and pigs in the same region (only 1.2% [3/258] related strains), as judged from antimicrobial and virulence gene profiles in combination with clonal complexes, suggesting a widespread human-to-human transmission as a strong possibility (Wu et al. 2013). Likewise, in a recent Swedish study there was no indication of the spread of *E. coli* carrying $bla_{\text{CMY-2}}$ from broilers to human clinical settings (Borjesson et al. 2013).

We need to be clear that the majority of studies performed around the topic of AMR transfer so far did not particularly focus on confirming precise transmission paths, but aimed to explore whether bacteria similar at the genetic level occurred in different animal species and humans, indicating possible epidemiological links (Wu et al. 2013). If such links, mainly created on the basis of observational findings, are indeed consistent with transmission through the food chain, remains inconclusive, unless infections with ESBL/AmpC-producing bacteria cannot be clearly traced back to a foodborne source. This in turn would implicitly assume that (i) *bla*-gene carrying plasmid and bacteria are transmitted jointly through a single event, and (ii) the strain transmitted is capable of causing disease in humans in a given period of time, which for instance has been the case in one of the first foodborne nosocomial outbreaks due to an ESBL-producing (SHV-1, CTX-M-15) *K. pneumoniae* isolate in Spain (Calbo et al. 2011). A very good example showing that even with an apparently sufficient molecular dataset the direct contribution of livestock animals to the transmission of AMR microorganisms might be overstated has been published previously. By using comparative whole genome analyses, Mather et al. (2013) could demonstrate that, contrary to current belief, the epidemic multidrug-resistant *Salmonella Typhimurium* DT104, responsible for human gastrointestinal infections worldwide, was largely maintained within animal and human populations separately (Jerab et al. 2022). Based on WGS, they could show that the isolates and their resistance genes were mainly kept within their host orgns with

limited transmission, pointing toward alternative sources for these MDR strains. This study emphasizes the critical importance of integrated genotypic datasets, including whole genome analysis, in understanding the ecology of bacterial zoonosis and AMR.

Interestingly, and this is best known for *E. coli*, bacterial isolates supposed to be nonpathogenic for otherwise healthy individuals seem to be more prone to acquire ESBL/AmpC β-lactamase genes than their pathogenic counterparts are. For example, extraintestinal pathogenic *E. coli* (ExPEC), i.e., highly virulent *E. coli* strains that are basically decipherable by their virulence gene profile and assignment to phylogenetic group and/or multilocus sequence type, are not among the majority of ESBL/AmplC-producing strains identified from infected and colonized humans and animals (Ewers et al. 2012). The sudden worldwide increase of *E. coli* clone O25:H4-ST131-CTX-M-15 in hospital- and particularly in community-onset infections is the most compelling exception from that (i.e., with respect to the extent of its spread) (Nicolas-Chanoine et al. 2014; Pitout and DeVinney 2017; Duprilot et al. 2020). ST131 is part of the highly virulent phylogenetic group B2 and since its first recognition in 2008 it developed to the most dominant ExPEC genotype worldwide (Pitout and DeVinney 2017). *E. coli* strains of this clonal group are commonly associated with bacteremia, urinary tract infections, and urosepsis, and due to their multidrug resistance, discordant antimicrobial therapy and increased morbidity and mortality are increasingly observed (Nicolas-Chanoine et al. 2014). The number of reported livestock animal and food-associated ST131 isolates, whether they harbor ESBLs or not, is negligible compared to its incredible frequency in humans. Nevertheless, there are findings of ESBL-producing ST131 isolates in livestock animals, for example, in healthy poultry from Spain (CTX-M-9) (Cortes et al. 2010; Mora et al. 2010), in diarrheic poultry from Tunisia (CTX-M-15) (Jouini et al. 2021), in broiler liver in Algeria (Chenouf et al. 2021), or in diarrheal pig in China (CTX-M-9 & CTX-M-14) (Liu et al. 2018). Several other researchers failed to detect this genotype among ESBL producers, e.g., from poultry and retail poultry meat (Overdevest et al. 2011; Egea et al. 2012) and from cattle (Madec et al. 2012; Diab et al. 2017). In contrast, a much wider distribution of CTX-M-type-producing ST131 *E. coli* strains has been observed in companion animals, only a few years after its first emergence in humans (Ewers et al. 2010; Dierikx et al. 2012; Pomba et al. 2014). Thus, this clonal group basically circulates among humans, but crossed the species barrier to dogs and cats in particular, suggesting a spillover from humans and a subsequent dissemination among companion animals. In any case, it is clear that livestock animals are currently not a threatening source of this highly virulent and multidrug resistant clonal group, while the reasons for low carriage rates with such strains are yet unsolved (Ewers et al. 2012).

Nevertheless, there is evidence for the existence of shared clones of ESBL/AmpC-producing *E. coli* in food-producing animals and humans, indicating that some common genotypes, plasmid, and β-lactamase types could indeed be circulating between them (Lavilla et al. 2008). Though less prevalent among highly virulent B2 ExPEC strains, ESBL/AmpC-producing *E. coli* are generally dispersed over the entire population of this bacterial species (Ewers et al. 2012). There is an apparent accumulation of AMR isolates in certain non-B2 genotypes and strains of various

clonal groups, including ST10, ST23, ST38, ST117, ST167, ST405, ST410, ST617, and ST648. These lineages are circulating widely across species and sources, i.e., humans, animals, and food products (Oteo et al. 2009; Cortes et al. 2010; Bortolaia et al. 2011; Leverstein-van Hall et al. 2011; Overdevest et al. 2011; Ewers et al. 2012, 2014; Liu et al. 2018), which does not automatically indicate clonal transmission between these compartments.

In summary, robust studies providing unquestionable proofs for the livestock animal-to-human transmission and quantifying the true burden for public health are still rare. Overlaps in bacterial strain and plasmid characteristics among humans and food animals almost often refer to one of these criteria only. Accordingly, several studies consistently emphasized the key role of plasmid versus clonal dissemination in the spread of ESBL/AmpC genes between animals and humans and vice versa. The notion that people who have direct contact with infected animals or contaminated meat, such as farm workers, slaughterers, or people working in the food-producing industry, face a particular risk to acquire AMR bacteria requires further consideration. With the increasing availability of molecular epidemiological data, particularly of combined WGS and plasmid sequence data, knowledge about initial microevolutionary events leading to the introduction and further enrichment of ESBL/AmpC-producing bacteria in food-producing animals will hopefully increase in the near future.

18.7 Conclusions

The frequent occurrence of ESBL/AmpC-producing bacteria in the food production chain, i.e., in healthy livestock animals and food thereof, and its unpredictable impact on food safety and environmental pollution is a major concern in the global debate on AMR. Problems related to AMR are inherently associated with the use of antibiotics in any environment, i.e., related to veterinary and human medicine. Accordingly, its use in the livestock production cycle may be one among other important factors in promoting the development of MDR bacteria and genetic resistance determinants of zoonotic relevance.

Even if the impact of food-producing animals to AMR in humans cannot be quantified yet, antimicrobial consumption in these animals undisputedly contributes its part in the maintenance or even expansion of the global bacterial resistome. Therefore, AMU in this sector needs to be kept at levels as low as possible without losing sight of any aspects of animal welfare. Generally, the judicious and rational use of antimicrobials, particularly those that are “critically important” for human health, should be regarded as a naturally ethical issue in the veterinary profession. However, this would be only one step toward preserving the benefits of antimicrobials for people. Beyond antimicrobial consumption, animal husbandry, extensive trade, farm hygiene, and biosecurity as well as intensive farming are considered the most compelling risk factors contributing to the global emergence of ESBL/AmpC-producing *Enterobacteriaceae* in livestock. In all major food animal production systems, massive movement of animals from reproduction to fattening farms

occurs and also food is traded globally, facilitating a virtually boundless transfer of MDR microorganisms between farms and countries, which might weaken regional or national mitigation measures considerably. This makes joining transnational forces to contain the risks of spreading AMR one essential part in an overall holistic approach. In this context, risk assessment, i.e., identification of stages within the food production chain (e.g., slaughter of animals, distribution, handling, and consumption of foods) that may pose an increased risk of human infection with AMR bacteria, should be generally included as a mature part of this global effort.

Resistance will probably never return to pre-antibiotic use levels; weak market encouragements and increasing difficulty and cost to develop new effective antimicrobial substances have greatly discouraged investment in this area. Not only for these reasons are alternatives to antimicrobials urgently required in veterinary medicine. Implementation and optimization of biosecurity on farms and overall good hygiene practices at all stages of the food chain, i.e., pre- and postharvest, have already shown excellent results in reducing the impact of multidrug-resistant bacteria in livestock animals. Several other options that have been proposed to lower the burden of AMR, such as pre- and probiotics, antimicrobial peptides, phytocompounds, competitive exclusion products, and vaccines, bacteriophages have shown variable effects so far, but represent promising approaches that need to be intensified, as there is no more time to lose. Finally, more recent concepts, such as modified CRISPR-Cas approaches, that target AMR genes in bacteria and reverse the selective pressure of resistance, or nanoparticles, that could help in blockage of enzyme pathways and alteration of cell wall might be essential pieces in the global effort to combat antimicrobial resistance.

References

Aarestrup FM (2005) Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin Pharmacol Toxicol 96(4):271–281

Aarestrup FM et al (2001) Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother 45(7):2054–2059

AbuOun M et al (2021) A genomic epidemiological study shows that prevalence of antimicrobial resistance in *Enterobacterales* is associated with the livestock host, as well as antimicrobial usage. Microb Genom 7(10):000630

Agerso Y, Aarestrup FM (2013) Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing *Escherichia coli* in slaughter pigs. J Antimicrob Chemother 68(3):569–572

Anonymous (2007) Joint FAO/WHO/OIE expert meeting on critically important antimicrobials. Report of the FAO/WHO/OIE expert meeting, 26–30 Nov 2007. FAO, Rome

Apostolakos I et al (2020) High-resolution characterisation of ESBL/pAmpC-producing *Escherichia coli* isolated from the broiler production pyramid. Sci Rep 10(1):11123

Arcilla MS et al (2017) Import and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis 17(1):78–85

Bai L et al (2015) Prevalence of *Salmonella* isolates from chicken and pig slaughterhouses and emergence of ciprofloxacin and cefotaxime co-resistant *S. enterica* Serovar Indiana in Henan, China. PLoS One 10(12):e0144532

Bergenholtz RD et al (2009) Characterization of genetic determinants of extended-spectrum cephalosporinases (ESCs) in *Escherichia coli* isolates from Danish and imported poultry meat. J Antimicrob Chemother 64(1):207–209

Bergspica I et al (2020) Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* in pigs and pork meat in the European Union. Antibiotics (Basel) 9(10):678

Bevan ER et al (2017) Global epidemiology of CTX-M beta-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72(8):2145–2155

Borjesson S et al (2013) Characterization of plasmid-mediated AmpC-producing *E. coli* from Swedish broilers and association with human clinical isolates. Clin Microbiol Infect 19(7): E309–E311

Bortolaia V et al (2010) *Escherichia coli* producing CTX-M-1, -2, and -9 group beta-lactamases in organic chicken egg production. Antimicrob Agents Chemother 54(8):3527–3528

Bortolaia V et al (2011) Potential pathogenicity and host range of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from healthy poultry. Appl Environ Microbiol 77(16):5830–5833

Brinas L et al (2003) Beta-lactamase characterization in *Escherichia coli* isolates with diminished susceptibility or resistance to extended-spectrum cephalosporins recovered from sick animals in Spain. Microb Drug Resist 9(2):201–209

BTK-AGTAM (2015) Leitlinien für den sorgfältigen Umgang mit antibakteriell wirksamen Tierarzneitmitteln. https://www.bundestieraerztekammer.de/tieraerzte/leitlinien/. Accessed 03 Aug 2022

Burch DGS et al (2008) Guidelines for antimicrobial use in swine. In: Guardabassi L, Jensen LB, Kruse H (eds) Guide to antimicrobial use in animals. Blackwell Publishing, Ames, pp 102–125

Bush K, Bradford PA (2016) Beta-lactams and beta-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med 6(8):a025247

Bush K, Bradford PA (2020) Epidemiology of beta-lactamase-producing pathogens. Clin Microbiol Rev 33(2):e00047–e00019

Bush K, Fisher JF (2011) Epidemiological expansion, structural studies, and clinical challenges of new beta-lactamases from gram-negative bacteria. Annu Rev Microbiol 65:455–478

Calbo E et al (2011) Foodborne nosocomial outbreak of SHV-1 and CTX-M-15-producing *Klebsiella pneumoniae*: epidemiology and control. Clin Infect Dis 52(6):743–749

Canton R et al (2008) Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. Clin Microbiol Infect 14(Suppl 1):144–153

Carattoli A (2008) Animal reservoirs for extended spectrum beta lactamase producers. Clin Microbiol Infect 14:117–123

Carattoli A (2009) Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother 53(6):2227–2238

Carattoli A (2011) Plasmids in gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol 301(8):654–658

Carattoli A (2013) Plasmids and the spread of resistance. Int J Med Microbiol 303(6–7):298–304

Casella T et al (2017) High prevalence of ESBLs in retail chicken meat despite reduced use of antimicrobials in chicken production, France. Int J Food Microbiol 257:271–275

Cavaco LM et al (2008) Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrob Agents Chemother 52(10):3612–3616

Chantziaras I et al (2014) Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. J Antimicrob Chemother 69(3):827–834

Chenouf NS et al (2021) Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from broiler liver in the center of Algeria, with detection of CTX-M-55 and B2/ST131-CTX-M-15 in *Escherichia coli*. Microb Drug Resist 27(2):268–276

CIPARS (2002–2021) Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) reports. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars.html. Accessed 03 Aug 2022

Cohen Stuart J et al (2012) Comparison of ESBL contamination in organic and conventional retail chicken meat. Int J Food Microbiol 154(3):212–214

Constable PD et al (2008) Guidelines for antimicrobial use in cattle. In: Guardabassi L, Jensen LB, Kruse H (eds) Guide to antimicrobial use in animals. Blackwell Publishing, Ames, pp 143–160

Cortes P et al (2010) Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl Environ Microbiol 76(9): 2799–2805

D'Andrea MM et al (2013) CTX-M-type beta-lactamases: a successful story of antibiotic resistance. Int J Med Microbiol 303(6–7):305–317

D'Costa VM et al (2011) Antibiotic resistance is ancient. Nature 477(7365):457–461

da Costa PM et al (2013) Transfer of multidrug-resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment. Int J Environ Res Public Health 10(1):278–294

Dahms C et al (2015) Occurrence of ESBL-producing *Escherichia coli i*n livestock and farm workers in Mecklenburg-Western Pomerania, Germany. PLoS One 10(11):e0143326

DANMAP (2003–2021) Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) Reports. https://www.danmap.org. Accessed 03 Aug 2022

de Been M et al (2014) Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet 10(12): e1004776

de Jong A et al (2013) Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals. Int J Antimicrob Agents 41(5):403–409

De Koster S et al (2021) ESBL-producing, carbapenem- and ciprofloxacin-resistant *Escherichia coli* in Belgian and Dutch broiler and pig farms: a cross-sectional and cross-border study. Antibiotics (Basel) 10(8):945

De Lucia A et al (2021) Reduction in antimicrobial resistance prevalence in *Escherichia coli* from a pig farm following withdrawal of group antimicrobial treatment. Vet Microbiol 258:109125

Dheilly A et al (2012) Resistance gene transfer during treatments for experimental avian colibacillosis. Antimicrob Agents Chemother 56(1):189–196

Diab M et al (2017) High prevalence of non-ST131 CTX-M-15-producing *Escherichia coli* in healthy cattle in Lebanon. Microb Drug Resist 23(2):261–266

Dierikx CM et al (2012) Occurrence and characteristics of extended-spectrum-beta-lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. J Antimicrob Chemother 67(6):1368–1374

Dierikx C et al (2013) Extended-spectrum beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother 68(1):60–67

Djeffal S et al (2017) Prevalence and clonal relationship of ESBL-producing *Salmonella* strains from humans and poultry in northeastern Algeria. BMC Vet Res 13(1):132

Dohmen W et al (2015) Carriage of extended-spectrum beta-lactamases in pig farmers is associated with occurrence in pigs. Clin Microbiol Infect 21(10):917–923

Dohmen W et al (2017) Risk factors for ESBL-producing *Escherichia coli* on pig farms: a longitudinal study in the context of reduced use of antimicrobials. PLoS One 12(3):e0174094

Doi Y et al (2010) Extended-spectrum and CMY-type beta-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. Clin Microbiol Infect 16(1):33–38

Dropa M, Daoud Z (2022) Editorial: the global threat of carbapenem-resistant gram-negative bacteria. Front Cell Infect Microbiol 12:974268

Duprilot M et al (2020) Success of *Escherichia coli* O25b:H4 sequence type 131 clade C associated with a decrease in virulence. Infect Immun 88(12):e00576–e00520

Duse A et al (2015) Risk factors for antimicrobial resistance in fecal *Escherichia coli* from preweaned dairy calves. J Dairy Sci 98(1):500–516

Dutil L et al (2010) Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis 16(1):48–54

EC (2019) Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on Veterinary Medicinal Products and Repealing Directive 2001/82/EC

Egea P et al (2012) Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing *Escherichia coli* in the south of Spain. Int J Food Microbiol 159(2):69–73

European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Medicines Agency (EMA) (2021) Third joint inter-agency report on integrated analysis of consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the EU/EEA, JIACRA III. 2016–2018. ESCD/EFSA/EMA, Stockholm/Parma/Amsterdam, 2021. https://doi.org/10.2903/j.efsa.2021.6712

European Food Safety Authority (EFSA) (2012) Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. EFSA J 10(6): 2742

European Food Safety Authority (EFSA)/European Centre for Disease Prevention and Control (ECDC) (2013) EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food 2011. EFSA J 11(5):3196

European Food Safety Authority (EFSA)/European Centre for Disease Prevention and Control (ECDC) (2022) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA J 20(3:7209):197

European Medicines Agency (EMA) (2019) Reflection paper on the prophylactic use of antimicrobials in animals in the context of Article 107(3) of Regulation (EU) 2019/6

European Medicines Agency (EMA), European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) (2021) Sales of Veterinary Antimicrobial Agents in 31 European Countries in 2019 and 2020; (EMA/58183/2021)

Ewers C et al (2010) Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum beta-lactamase-producing *Escherichia coli* among companion animals. J Antimicrob Chemother 65:651–660

Ewers C et al (2011) Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted! Berl Munch Tierarztl Wochenschr 124(3–4): 94–101

Ewers C et al (2012) Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18(7):646–655

Ewers C et al (2014) CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? J Antimicrob Chemother 69(5):1224–1230

Ewers C et al (2016) Genome sequence of OXA-23 producing *Acinetobacter baumannii* IHIT7853, a carbapenem-resistant strain from a cat belonging to international clone IC1. Gut Pathog 8:37

Ewers C et al (2021) Genomic diversity and virulence potential of ESBL- and AmpC-beta-lactamase-producing *Escherichia coli* strains from healthy food animals across Europe. Front Microbiol 12:626774

Fey PD et al (2000) Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N Engl J Med 342(17):1242–1249

Fischer J et al (2012) *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. J Antimicrob Chemother 67(7):1793–1795

Fischer J et al (2014) *bla*CTX-M-15-carrying *Escherichia coli* and *Salmonella* isolates from livestock and food in Germany. J Antimicrob Chemother 69(11):2951–2958

Fischer J et al (2017) Simultaneous occurrence of MRSA and ESBL-producing *Enterobacteriaceae* on pig farms and in nasal and stool samples from farmers. Vet Microbiol 200:107–113

Food and Drug Administration Center for Veterinary Medicine FaDACfV (2017) FDA announces implementation of GFI#213, outlines continuing efforts to address antimicrobial resistance CVM update 3 January 2017. https://www.aasv.org/news/story.php?id=9531. Accessed 03 Aug 2022

Fournier C et al (2020) Occurrence of CTX-M-15- and MCR-1-producing *Enterobacterales* in pigs in Portugal: evidence of direct links with antibiotic selective pressure. Int J Antimicrob Agents 55(2):105802

Friese A et al (2013) Faecal occurrence and emissions of livestock-associated methicillin-resistant *Staphylococcus aureus* (laMRSA) and ESBL/AmpC-producing *E. coli* from animal farms in Germany. Berl Munch Tierarztl Wochenschr 126(3–4):175–180

Frye JG et al (2008) Analysis of *Salmonella enterica* with reduced susceptibility to the third-generation cephalosporin ceftriaxone isolated from U.S. cattle during 2000–2004. Microb Drug Resist 14(4):251–258

Gaire TN et al (2020) Age dependence of antimicrobial resistance among fecal bacteria in animals: a scoping review. Front Vet Sci 7:622495

Gazal LES et al (2020) Detection of ESBL/AmpC-producing and fosfomycin-resistant *Escherichia coli* from different sources in poultry production in Southern Brazil. Front Microbiol 11:604544

Gelalcha BD, Kerro Dego O (2022) Extended-spectrum beta-lactamases producing *Enterobacteriaceae* in the USA dairy cattle farms and implications for public health. Antibiotics (Basel) 11 (10):1313

Geser N et al (2012a) Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. BMC Vet Res 8:21

Geser N et al (2012b) Molecular identification of extended-spectrum-beta-lactamase genes from *Enterobacteriaceae* isolated from healthy human carriers in Switzerland. Antimicrob Agents Chemother 56(3):1609–1612

Gilbert N (2012) Rules tighten on use of antibiotics on farms. Nature 481(7380):125

Girlich D et al (2007) Extended-spectrum beta-lactamase CTX-M-1 in *Escherichia coli* isolates from healthy poultry in France. Appl Environ Microbiol 73(14):4681–4685

Guardabassi L, Kruse H (2008) Principles of prudent and rational use of antimicrobials in animals. In: Guardabassi L, Jensen LB, Kruse H (eds) Guide to antimicrobial use in animals. Blackwell Publishing, Ames, pp 1–12

Guardabassi L et al (2018) Optimization of antimicrobial treatment to minimize resistance selection. Microbiol Spectr 6(3):ARBA-0018-2017

Guenther S et al (2011) Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Front Microbiol 2:246

Gupta A et al (2003) Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. J Infect Dis 188 (11):1707–1716

Hammerum AM et al (2012) Characterization of CTX-M-14- and CTX-M-15-producing *Escherichia coli* of porcine origin. J Antimicrob Chemother 67(8):2047–2049

Hammerum AM et al (2014) Characterization of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. J Antimicrob Chemother 69(10):2650–2657

Hansen KH et al (2014) Strain diversity of CTX-M-producing *Enterobacteriaceae* in individual pigs: insights into the dynamics of shedding during the production cycle. Appl Environ Microbiol 80(21):6620–6626

Hasman H et al (2005) Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother 56(1):115–121

Hering J et al (2014) Prevalence and potential risk factors for the occurrence of cefotaxime resistant *Escherichia coli* in German fattening pig farms--a cross-sectional study. Prev Vet Med 116(1–2): 129–137

Hiki M et al (2015) Decreased resistance to broad-spectrum cephalosporin in *Escherichia coli* from healthy broilers at farms in Japan after voluntary withdrawal of ceftiofur. Foodborne Pathog Dis 12(7):639–643
Hille K et al (2017) Cefotaxime-resistant *E. coli* in dairy and beef cattle farms-joint analyses of two cross-sectional investigations in Germany. Prev Vet Med 142:39–45
Hordijk J et al (2013) Within-farm dynamics of ESBL/AmpC-producing *Escherichia coli* in veal calves: a longitudinal approach. J Antimicrob Chemother 68(11):2468–2476
Huijbers PM et al (2015) Methicillin-resistant *Staphylococcus aureus* and extended-spectrum and AmpC beta-lactamase-producing *Escherichia coli* in broilers and in people living and/or working on organic broiler farms. Vet Microbiol 176(1–2):120–125
Huizinga P et al (2019) Decreasing prevalence of contamination with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) in retail chicken meat in The Netherlands. PLoS One 14(12):e0226828
Hunter PA et al (2010) Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. J Antimicrob Chemother 65:i3–i17
Jacoby GA (2009) AmpC beta-lactamases. Clin Microbiol Rev 22(1):161–182
Jensen LB et al (2006) First description of an oxyimino-cephalosporin-resistant, ESBL-carrying *Escherichia coli* isolated from meat sold in Denmark. J Antimicrob Chemother 57(4):793–794
Jerab J et al (2022) Real-world data on antibiotic group treatment in European livestock: drivers, conditions, and alternatives. Antibiotics (Basel) 11(8):1046
Jouini A et al (2007) Characterization of CTX-M and SHV extended-spectrum beta-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. J Antimicrob Chemother 60(5):1137–1141
Jouini A et al (2021) First detection of human ST131-CTX-M-15-O25-B2 clone and high-risk clonal lineages of ESBL/pAmpC-producing *E. coli* isolates from diarrheic poultry in Tunisia. Antibiotics (Basel) 10(6):670
JVARM (2000–2017) Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) reports. https://www.maff.go.jp/nval/english/AMR/Monitoring/index.html. Accessed 03 Aug 2022
Kaesbohrer A et al (2019) Diversity in prevalence and characteristics of ESBL/pAmpC producing *E. coli* in food in Germany. Vet Microbiol 233:52–60
Kakooza S et al (2021) Epidemiological dynamics of extended-spectrum beta-lactamase- or AmpC beta-lactamase-producing *Escherichia coli* screened in apparently healthy chickens in Uganda. Scientifica (Cairo) 2021:3258059
Kasabova S et al (2021) Antibiotic usage pattern in broiler chicken flocks in Germany. Front Vet Sci 8:673809
Kluytmans JA et al (2013) Extended-spectrum beta-lactamase-producing *Escherichia coli* from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clin Infect Dis 56(4):478–487
Koeck R et al (2018) Carbapenem-resistant *Enterobacteriaceae* in wildlife, food-producing, and companion animals: a systematic review. Clin Microbiol Infect 24(12):1241–1250
Kojima A et al (2005) Extended-spectrum-beta-lactamase-producing *Escherichia coli* strains isolated from farm animals from 1999 to 2002: report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. Antimicrob Agents Chemother 49(8):3533–3537
Kola A et al (2012) High prevalence of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in organic and conventional retail chicken meat, Germany. J Antimicrob Chemother 67(11):2631–2634
Kraemer JG et al (2017) Prevalence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and Methicillin-resistant *Staphylococcus aureus* in pig farms in Switzerland. Sci Total Environ 603-604:401–405
Kuenzli E et al (2014) High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Swiss travellers to South Asia- a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. BMC Infect Dis 14:528

Kuhnke D et al (2020) Occurrence of ESBL-producing *Escherichia coli* in healthy, living food-producing animals in Europe: a systematic review. CAB Rev 15:1–13
Laube H et al (2013) Longitudinal monitoring of extended-spectrum beta-lactamase/AmpC-producing *Escherichia coli* at German broiler chicken fattening farms. Appl Environ Microbiol 79 (16):4815–4820
Lavilla S et al (2008) Dissemination of extended-spectrum beta-lactamase-producing bacteria: the food-borne outbreak lesson. J Antimicrob Chemother 61(6):1244–1251
Lay KK et al (2021) Colistin resistance and ESBL production in *Salmonella* and *Escherichia coli* from pigs and pork in the Thailand, Cambodia, Lao PDR, and Myanmar Border Area. Antibiotics (Basel) 10(6):657
Lee S et al (2020) Prevalence of extended-spectrum beta-lactamases in the local farm environment and livestock: challenges to mitigate antimicrobial resistance. Crit Rev Microbiol 46(1):1–14
Leverstein-van Hall MA et al (2011) Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17(6):873–880
Liakopoulos A et al (2016) A review of SHV extended-spectrum beta-lactamases: neglected yet ubiquitous. Front Microbiol 7:1374
Liebana E et al (2006) Longitudinal farm study of extended-spectrum beta-lactamase-mediated resistance. J Clin Microbiol 44(5):1630–1634
Liebana E et al (2013) Public health risks of enterobacterial isolates producing extended-spectrum beta-lactamases or AmpC beta-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. Clin Infect Dis 56(7): 1030–1037
Literak I et al (2010) Antimicrobial-resistant faecal *Escherichia coli* in wild mammals in Central Europe: multiresistant *Escherichia coli* producing extended-spectrum beta-lactamases in wild boars. J Appl Microbiol 108(5):1702–1711
Liu X et al (2018) Molecular characterization of extended-spectrum beta-lactamase-producing multidrug resistant *Escherichia coli* from swine in Northwest China. Front Microbiol 9:1756
Livermore DM et al (2007) CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 59(2):165–174
Lubbert C et al (2015) Colonization with extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacteriaceae* in international travelers returning to Germany. Int J Med Microbiol 305(1):148–156
Ludden C et al (2019) One health genomic surveillance of *Escherichia coli* demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. mBio 10(1): 02693–02618
Ma T et al (2021) A review of the resistome within the digestive tract of livestock. J Anim Sci Biotechnol 12(1):121
Madec JY et al (2012) Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*(CTX-M-15)-carrying plasmids. J Antimicrob Chemother 67(3):578–581
Mader R et al (2022) Review and analysis of national monitoring systems for antimicrobial resistance in animal bacterial pathogens in Europe: a basis for the development of the European Antimicrobial Resistance Surveillance Network in Veterinary Medicine (EARS-Vet). Front Microbiol 13:838490
MARAN (2003–2022) Monitoring of Antimicrobial Resistance and antibiotic usage in Animals in the Netherlands (MARAN) reports. https://www.wur.nl/en/Research-Results/Research-Institutes/Bioveterinary-Research/In-the-spotlight/Antibiotic-resistance/MARAN-reports.htm. Accessed 03 Aug 2022
MARAN (2022) Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2021. https://www.wur.nl/en/research-results/research-institutes/bioveterinary-research/show-bvr/less-antibiotic-resistance-in-animal-husbandry.htm. Accessed 03 Aug 2022
Mather AE et al (2013) Distinguishable epidemics of multidrug-resistant Salmonella Typhimurium DT104 in different hosts. Science 341(6153):1514–1517
Meini S et al (2019) AmpC beta-lactamase-producing *Enterobacterales*: what a clinician should know. Infection 47(3):363–375

Meissner K et al (2022) Extended-spectrum beta-lactamase-producing *Escherichia coli* in conventional and organic pig fattening farms. Microorganisms 10(3):603
Mesa RJ et al (2006) Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). J Antimicrob Chemother 58(1):211–215
Meunier D et al (2010) Plasmid-borne florfenicol and ceftiofur resistance encoded by the floR and bla_{CMY-2} genes in *Escherichia coli* isolates from diseased cattle in France. J Med Microbiol 59 (4):467–471
Mollenkopf DF et al (2014) Organic or antibiotic-free labeling does not impact the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken. Foodborne Pathog Dis 11(12):920–929
Moodley A, Guardabassi L (2009) Transmission of IncN plasmids carrying $bla_{CTX-M-1}$ between commensal *Escherichia coli* in pigs and farm workers. Antimicrob Agents Chemother 53(4): 1709–1711
Mora A et al (2010) Recent emergence of clonal group O25b:K1:H4-B2-ST131 *ibeA* strains among *Escherichia coli* poultry isolates, including CTX-M-9-producing strains, and comparison with clinical human isolates. Appl Environ Microbiol 76(21):6991–6997
Moyaert H et al (2014) Antimicrobial resistance monitoring projects for zoonotic and indicator bacteria of animal origin: common aspects and differences between EASSA and EFSA. Vet Microbiol 171(3–4):279–283
Musa L et al (2020) Antimicrobial susceptibility of *Escherichia coli* and ESBL-producing *Escherichia coli* diffusion in conventional, organic and antibiotic-free meat chickens at slaughter. Animals (Basel) 10(7):1215
NARMS (2003–2018) National Antimicrobial Resistance Monitoring System (NARMS) Integrated Reports. https://www.cdc.gov/narms/reports/index.html. Accessed 03 Aug 2022
Nguyen VT et al (2019) Limited contribution of non-intensive chicken farming to ESBL-producing *Escherichia coli* colonization in humans in Vietnam: an epidemiological and genomic analysis. J Antimicrob Chemother 74(3):561–570
Nicolas-Chanoine MH et al (2013) 10-fold increase (2006–11) in the rate of healthy subjects with extended-spectrum beta-lactamase-producing *Escherichia coli* faecal carriage in a Parisian check-up centre. J Antimicrob Chemother 68(3):562–568
Nicolas-Chanoine MH et al (2014) *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev 27(3):543–574
Nordmann P (2013) Carbapenemase-producing *Enterobacteriaceae*: overview of a major public health challenge. Med Mal Infect 44(2):51–56
Oteo J et al (2009) Extended-spectrum beta-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. Int J Antimicrob Agents 34(2):173–176
Overdevest I et al (2011) Extended-spectrum beta-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. Emerg Infect Dis 17(7):1216–1222
Palmeira JD, Ferreira HMN (2020) Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* in cattle production - a threat around the world. Heliyon 6(1):e03206
Palmeira JD et al (2021) Emergence and spread of cephalosporinases in wildlife: a review. Animals (Basel) 11(6):1765
Persoons D et al (2011) Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiol Infect 139(5):765–771
Pitout JD, DeVinney R (2017) *Escherichia coli* ST131: a multidrug-resistant clone primed for global domination. F1000Res 6
Pitout JD et al (2009) Molecular characteristics of travel-related extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates from the Calgary Health Region. Antimicrob Agents Chemother 53(6):2539–2543
Poirel L et al (2012) Carbapenemase-producing *Acinetobacter* spp. in Cattle, France. Emerg Infect Dis 18(3):523–525
Poirel L et al (2018) Antimicrobial resistance in *Escherichia coli*. Microbiol Spectr 6(4):ARBA-0026-2017

Pomba C et al (2014) Within-lineage variability of ST131 *Escherichia coli* isolates from humans and companion animals in the south of Europe. J Antimicrob Chemother 69(1):271–273
Pomba C et al (2018) Licensing and approval of antimicrobial agents for use in animals. Microbiol Spectr 6(4):ARBA-0016-2017
Potron A et al (2015) Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii:* mechanisms and epidemiology. Int J Antimicrob Agents 45(6): 568–585
Projahn M et al (2019) Contamination of chicken meat with extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* during scalding and defeathering of broiler carcasses. Food Microbiol 77:185–191
Ramos S et al (2020) *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: health implications of extended spectrum beta-lactamase (ESBL) production. Animals (Basel) 10(12):2239
Randall LP et al (2011) Prevalence of *Escherichia coli* carrying extended-spectrum beta-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J Antimicrob Chemother 66(1):86–95
Randall L et al (2014) Detection of antibiotic residues and association of cefquinome residues with the occurrence of extended-spectrum beta-lactamase (ESBL)-producing bacteria in waste milk samples from dairy farms in England and Wales in 2011. Res Vet Sci 96(1):15–24
Reist M et al (2013) ESBL-producing *Enterobacteriaceae*: occurrence, risk factors for fecal carriage and strain traits in the Swiss slaughter cattle population younger than 2 years sampled at abattoir level. PLoS One 8(8):e71725
Riano I et al (2006) Detection and characterization of extended-spectrum beta-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. J Antimicrob Chemother 58(4): 844–847
Sabry MA et al (2020) Extended-spectrum beta-lactamase-producing *Salmonella* serovars among healthy and diseased chickens and their public health implication. J Glob Antimicrob Resist 22: 742–748
Saliu EM et al (2017) Types and prevalence of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in poultry. Anim Health Res Rev 18(1):46–57
Sanders P et al (2020) Monitoring of farm-level antimicrobial use to guide stewardship: overview of existing systems and analysis of key components and processes. Front Vet Sci 7:540
Schaekel F et al (2017) Antibiotic drug usage in pigs in Germany-are the class profiles changing? PLoS One 12(8):e0182661
Schrijver R et al (2018) Review of antimicrobial resistance surveillance programmes in livestock and meat in EU with focus on humans. Clin Microbiol Infect 24(6):577–590
Scott AM et al (2018) Is antimicrobial administration to food animals a direct threat to human health? A rapid systematic review. Int J Antimicrob Agents 52(3):316–323
Seiffert SN et al (2013) Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: an emerging problem for human health? Drug Resist Updat 16(1–2):22–45
Smet A et al (2008) Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. Antimicrob Agents Chemother 52(4):1238–1243
Smet A et al (2010) Broad-spectrum beta-lactamases among *Enterobacteriaceae* of animal origin: molecular aspects, mobility and impact on public health. FEMS Microbiol Rev 34(3):295–316
Smet A et al (2011) *In situ* ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration. J Appl Microbiol 110(2):541–549
Stefani S et al (2014) Prevalence and characterization of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in food-producing animals in Northern Italy. New Microbiol 37(4): 551–555
Stolle I et al (2013) Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. J Antimicrob Chemother 68(12):2802–2808
Subbiah M et al (2012) Urine from treated cattle drives selection for cephalosporin resistant *Escherichia coli* in soil. PLoS One 7(11):e48919

SVARM (2003–2021) Swedish Veterinary Antibiotic Resistance Monitoring (SVARM) Reports. https://www.sva.se/en/our-topics/antibiotics/svarm-resistance-monitoring/swedres-svarm-reports/. Accessed 03 Aug 2022

Tamma PD et al (2019) A primer on AmpC beta-lactamases: necessary knowledge for an increasingly multidrug-resistant world. Clin Infect Dis 69(8):1446–1455

Tang KL et al (2017) Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet Health 1(8):e316–e327

Tragesser LA et al (2006) Association between ceftiofur use and isolation of *Escherichia coli* with reduced susceptibility to ceftriaxone from fecal samples of dairy cows. Am J Vet Res 67(10): 1696–1700

Uyanik T et al (2021) Characterization of extended-spectrum beta-lactamase-producing *Enterobacterales* from organic and conventional chicken meats. Lett Appl Microbiol 72(6): 783–790

Valverde A et al (2004) Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* during nonoutbreak situations in Spain. J Clin Microbiol 42(10):4769–4775

van Hoek A et al (2020) Transmission of ESBL-producing *Escherichia coli* between broilers and humans on broiler farms. J Antimicrob Chemother 75(3):543–549

Vidovic N, Vidovic S (2020) Antimicrobial resistance and food animals: influence of livestock environment on the emergence and dissemination of antimicrobial resistance. Antibiotics (Basel) 9(2):52

Volkova VV et al (2012) Mathematical model of plasmid-mediated resistance to ceftiofur in commensal enteric *Escherichia coli* of cattle. PLoS One 7(5):e36738

von Salviati C et al (2014) Extended-spectrum beta-lactamases (ESBL)/AmpC beta-lactamases-producing *Escherichia coli* in German fattening pig farms: a longitudinal study. Berl Munch Tierarztl Wochenschr 127(9–10):412–419

Weber LP et al (2021) Prevalence and risk factors for ESBL/AmpC-*E. coli* in pre-weaned dairy calves on dairy farms in Germany. Microorganisms 9(10):2135

Werner N et al (2018) Monitoring antimicrobial drug usage in animals: methods and applications. Microbiol Spectr 6(4). https://doi.org/10.1128/microbiolspec.ARBA-0015-2017

Widodo A et al (2020) Extended-spectrum beta-lactamase (ESBL)-producing *Eschericia coli* from livestock. Syst Rev Pharm 11:382–392

Wieler LH et al (2011) No evidence of the Shiga toxin-producing *E. coli* O104:H4 outbreak strain or enteroaggregative *E. coli* (EAEC) found in cattle faeces in northern Germany, the hotspot of the 2011 HUS outbreak area. Gut Pathog 3(1):17

Winokur PL et al (2000) Animal and human multidrug-resistant, cephalosporin-resistant salmonella isolates expressing a plasmid-mediated CMY-2 AmpC beta-lactamase. Antimicrob Agents Chemother 44(10):2777–2783

World Health Organization (WHO) (2018a) WHO Critically Important Antimicrobials for Human Medicine 6th revision. https://apps.who.int/iris/bitstream/handle/10665/325036/WHO-NMH-FOS-FZD-19.1-eng.pdf?ua=1. Accessed 03 Aug 2022

World Health Organization (WHO) (2018b) WHO report on surveillance of antibiotic consumption: 2016–2018 early implementation. World Health Organization, Geneva. https://www.who.int/publications/i/item/who-report-on-surveillance-of-antibiotic-consumption. Accessed 03 Aug 2022

World Health Organization (WHO) (2021) Global Antimicrobial Restistance and Use Surveillance Systems (GLASS). https://www.who.int/initiatives/glass. Accessed 03 Aug 2022

Wu S et al (2008) Detection of a single isolate of CTX-M-1-producing *Escherichia coli* from healthy pigs in Denmark. J Antimicrob Chemother 61(3):747–749

Wu G et al (2013) Comparative analysis of ESBL-positive *Escherichia coli* isolates from animals and humans from the UK, The Netherlands and Germany. PLoS One 8(9):e75392

Wu H et al (2016) Characterization of antimicrobial resistance in *Klebsiella* species isolated from chicken broilers. Int J Food Microbiol 232:95–102

Zoonotic Transmission of Antimicrobial-Resistant Enterococci: A Threat to Public Health or an Overemphasized Risk?

19

Valeria Bortolaia and Luca Guardabassi

Contents

Abstract

Enterococci are intrinsically resistant to various antimicrobial classes and able to acquire resistance to clinically relevant drugs via chromosomal mutations and horizontal gene transfer. Consequently, therapeutic options for treatment of enterococcal infections are limited. Zoonotic transfer of antimicrobial resistance in enterococci has been studied for many years. The first studies hypothesizing possible animal-to-human transmission of resistant strains and mobile genetic

V. Bortolaia (✉)
Department of Bacteria, Parasites and Fungi, Statens Serum Institute, Copenhagen, Denmark
e-mail: vabo@ssi.dk

L. Guardabassi
Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_16

elements are dated 1993. Since then, a considerable amount of papers has been published on this subject, providing the groundwork for important decisions limiting antimicrobial use in animal husbandry. In this chapter, the relative contribution by animal enterococci to antimicrobial resistance in infections in humans was reviewed taking into consideration the potential impact associated with different enterococcal species, animal hosts, epidemiological routes, and mechanisms of transfer. The authors conclude that potential zoonotic risks mainly concern horizontal transfer of resistance genes and clonal transmission of multidrug-resistant *Enterococcus faecalis* lineages such as ST16 from a variety of animal species. The risk of clonal transmission appears to be negligible for *Enterococcus faecium*, which is markedly more host-specific than *E. faecalis*, and mainly limited to companion animals, which are a potential reservoir of ampicillin-resistant, hospital-associated lineages such as ST78 and ST192. Of note, such conclusions are largely based on studies from developed countries that never used or banned the use of antimicrobial growth promoters in livestock for nearly two decades (at the time of writing this review), which has significantly reduced the occurrence of resistance to clinically relevant antimicrobials in enterococci in animals. As for horizontal transfer of mobile genetic elements, although it has been demonstrated experimentally that antimicrobial-resistant enterococci of animal origin can transiently colonize the human digestive tract and transfer their resistance genes to the indigenous microbiota, the magnitude and clinical implications of this phenomenon, which currently appear to be limited, have not been fully elucidated. Further research is warranted to explore the ecology and epidemiology of enterococcal mobile genetic elements carrying resistance genes of clinical relevance, especially aminoglycoside and linezolid resistance in *E. faecalis*.

Keywords

Enterococcus faecium · *Enterococcus faecalis* · *Enterococcus* · Antimicrobial resistance · AMR · Food · Meat · Livestock · Companion animals · VRE · AREF · Plasmid · Tn*1546* · Foodborne · Zoonosis · Horizontal gene transfer · Clonal transmission · Growth promoters · WGS · MLST

19.1 Introduction

Enterococci are commensal bacteria in the intestinal microbiota of humans and animals, but are also opportunistic pathogens that can cause a variety of infections (Gilmore et al. 2013). In humans, *E. faecalis* and *E. faecium* are well-known causes of hospital-acquired infections, including endocarditis, bacteremia, meningitis, wound and urinary tract infections, and peritonitis (Arias and Murray 2012). Together, these two species are ranked as the second and fourth most frequently reported cause of nosocomial infections in the USA and Europe, respectively (Weiner-Lastinger et al. 2020; Suetens et al. 2018), with *E. faecalis* being the species most frequently isolated, followed by *E. faecium* (Weiner-Lastinger et al. 2020).

Therapeutic options to treat *E. faecalis* and *E. faecium* infection are limited because enterococci are intrinsically resistant to important antimicrobial classes in clinical practice as they tolerate low concentrations of β-lactams, quinolones, aminoglycosides, and lincosamides and are able to metabolize preformed folic acid, thereby bypassing inhibition of folate synthesis by trimethoprim and sulfonamides (Murray 1990; Hollenbeck and Rice 2012). Treatment of severe enterococcal infections usually consists of a penicillin (ampicillin or penicillin) either as monotherapy or in combination with a cephalosporin (ceftriaxone) or an aminoglycoside (gentamicin or streptomycin) (Rosselli del Turco et al. 2021). Glycopeptides such as vancomycin are the best alternative if the causative strain is resistant to one or more first-line drugs or if the patient has β-lactam allergy or renal impairment (Rosselli del Turco et al. 2021). Daptomycin and linezolid are also good options if the patient has renal impairment and if the local prevalence of vancomycin resistance is high. Other antimicrobials which may be used for treatment of enterococcal infections include quinupristin-dalfopristin (for *E. faecium* only), tigecycline, and fifth-generation cephalosporins. Older antibiotics such as chloramphenicol, doxycycline, minocycline, and nitrofurantoin may be used for specific indications (Arias and Murray 2008). Unfortunately, enterococci have a particular ability to acquire exogenous resistance genes via conjugative transposons and plasmids, which has resulted in the emergence of resistance to virtually all available therapeutic options (Werner et al. 2013). Globally, *E. faecalis* and *E. faecium* were responsible for some 112,000 and 200,000 deaths associated with antimicrobial resistance, including 30,000 and 51,000 deaths attributable to antimicrobial resistance, respectively, in 2019 (Antimicrobial Resistance Collaborators 2022).

Several authors have hypothesized zoonotic transmission of antimicrobial-resistant enterococci since the early 1990s. At that time, vancomycin-resistant enterococci (VRE) were emerging as nosocomial pathogens worldwide (Leclercq et al. 1988; Uttley et al. 1988; Sundsfjord et al. 2001). Although initially it was believed that nosocomial use of vancomycin was the only factor selecting for VRE, this assumption was partly revisited after a considerable reservoir of VRE was reported in the community and in production animals in Europe (Bates et al. 1993; Klare et al. 1993; Torres et al. 1994; Goossens 1998; Martone 1998). In 1995, two independent studies established a correlation between usage of the vancomycin-analogue avoparcin as a growth promoter in livestock and occurrence of VRE in chickens and pigs (Klare et al. 1995; Aarestrup 1995). Subsequent studies confirmed that (i) there is cross-resistance between avoparcin and vancomycin (van den Bogaard et al. 1997a, b), (ii) avoparcin use was associated with occurrence of VRE in animal feces and meat products (Bager et al. 1997; Aarestrup et al. 2000a), and (iii) occurrence of VRE in food animals and meat products was correlated to occurrence of VRE in fecal samples of community-dwelling humans (Pantosti et al. 1999; Klare et al. 1999; van den Bogaard et al. 2000). The risk that animal VRE could be transmitted through the food chain was regarded as high since enterococci are particularly resistant to heat, disinfectants, and other decontamination procedures used at slaughterhouses (Giraffa 2002). All these evidences provided the basis for establishing and maintaining the ban on avoparcin use enforced in the

EU since 1997 in accordance with the precautionary principle (Anonymous 1997). Additional links between antimicrobial-resistant enterococci in production animals and antimicrobial-resistant enterococci in healthy humans were hypothesized on the basis of similarities in the patterns of resistance to quinupristin/dalfopristin, erythromycin, tetracyclines, gentamicin, and chloramphenicol, as well as in the distribution of genes conferring resistance to these antimicrobials (Welton et al. 1998; Werner et al. 1998; Aarestrup et al. 2000a, b; Del Campo et al. 2003; Klare et al. 2003; Kieke et al. 2006). These findings contributed to support the ban on use of all antimicrobials as growth promoters in the EU, which was ratified through different EU Regulations enforced in 1999 and in 2006 (Anonymous 1998a, b, 2003). Although the public health benefits of the ban on the use of antimicrobials for growth promotion have been debated (Wallinga and Burch 2013), and such use is still practiced in about 26% of countries in the world (World Organization for Animal Health 2021), there is compelling evidence showing that the diminished use of avoparcin achieved with the ban has been effective in reducing the presence of VRE in animal populations (Nobrega et al. 2021). On the basis of a systematic review and meta-analysis on this subject, it was concluded that the ban on avoparcin use was also significantly associated with a diminished presence of VRE in human samples (Nobrega et al. 2021). However, these findings most probably referred to samples from healthy individuals in the community since, in hospital settings, the population-weighted mean percentage of vancomycin resistance in *E. faecium* increased significantly in the EU/EEA countries in the period 2016–2020, according to data retrieved from the Surveillance Atlas of the European Centre for Disease Prevention and Control (ECDC) (https://www.ecdc.europa.eu/en).

This chapter is a narrative review of the literature regarding the possible contribution by enterococci of animal origin to antimicrobial resistance problems in human medicine. The topic is reviewed taking into consideration the roles played by different enterococcal species (*E. faecium* vs. *E. faecalis*), animal hosts (food vs. companion animals), epidemiological routes (foodborne transmission vs. transmission by contact with animals), and mechanisms of transfer (clonal transmission vs. horizontal gene transfer). Aquatic animals and derived seafood were not included in the review, as enterococci isolated from these sources often derive from anthropogenic contamination. The chapter is organized into four sections addressing occurrence of antimicrobial resistance in human, animal, and food isolates (section "Occurrence of Antimicrobial Resistance"), evidence of transmission between animals and humans (section "Transmission of Antimicrobial Resistance Between Animals and Humans"), genetic links between clinical and animal strains (section "Genetic Links Between Clinical and Animal Strains"), and conclusions by the authors (section "Conclusions").

19.2 Occurrence of Antimicrobial Resistance

Occurrence of antimicrobial resistance varies significantly depending on host species and geographical regions. Local data on occurrence of antimicrobial resistance often reflect the specific patterns of antimicrobial usage within each host species, and

marked differences between hosts may provide useful epidemiological indications on potential reservoirs of antimicrobial resistance within defined geographical areas. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) founded in 1995 was the first initiative for systematic collection of country-wide data on occurrence of antimicrobial resistance in *E. faecium* and *E. faecalis* in human patients, healthy production animals, and retail meat, with the purpose of identifying possible zoonotic links (www.danmap.org). Since then, several national and international antimicrobial resistance surveillance programs have been established with similar purposes worldwide. The two following paragraphs summarize the most recent publicly available information on the occurrence of antimicrobial resistance in *E. faecium* and *E. faecalis* from human patients, production animals, and meat obtained from national surveillance programs monitoring these bacteria within the last 10 years at the time of writing this review, and reflect the content of Tables 1 and 2, respectively. Typically, isolates included in these surveillance programs derived from invasive infections in humans, from cecal content or feces of healthy production animals, and from meat at retail. Occurrence of antimicrobial resistance, defined as the percentage of resistant isolates as a proportion of the isolates tested, is defined rare (<0.1%), very low (0.1–1.0%), low (>1–10.0%), moderate (>10.0–20.0%), high (>20.0–50.0%), very high (>50.0–70.0%), and extremely high (>70.0%), following the categorization used by the European Food Safety Authority (EFSA) (EFSA and ECDC 2021).

19.2.1 Patterns of Antimicrobial Resistance in Animal and Human *E. faecium*

Clinically relevant antimicrobial resistance phenotypes in *E. faecium* include resistance to ampicillin, daptomycin, gentamicin, linezolid, quinupristin-dalfopristin, teicoplanin, tigecycline, and vancomycin. Clear host-specific patterns in occurrence of resistance to ampicillin and gentamicin were observed in the reports examined (Table 1). Ampicillin resistance occurred at extremely high level in human clinical isolates, whereas it ranged from rare to moderate in isolates from animals and meat irrespective of the geographical origin (Table 1). Similarly, occurrence of gentamicin resistance ranged between moderate to high in human clinical isolates and from rare to low in isolates from animals and meat in any country (Table 1). Based on these observations, and on the fact that occurrence of ampicillin and gentamicin resistance has been stable or decreased in several animal and meat sources from most countries based on 5- and 10-year trends (Table 1), it seems unlikely that animal sources constitute an important reservoir of ampicillin- and gentamicin-resistant *E. faecium* causing severe infections in humans. A possible exception could be represented by ampicillin-resistant *E. faecium* (AREFm) in companion animals (see section "Transmission via Direct Contact with Companion Animals"), which are not covered by national surveillance programs.

Geographical patterns of vancomycin resistance are useful to discern potential epidemiological links between animals and humans. Occurrence of vancomycin resistance ranged from rare to low in human clinical isolates in different European

Table 1 Prevalence of antimicrobial resistance in *Enterococcus faecium* isolated from humans (invasive isolates) and animals (indicator isolates) obtained from publicly available national surveillance program reports

	Source[a]	Country										
		Belgium				Denmark			Japan			The Netherlands
		No.[a]	R[b]	Trends[c]		No.[a]	R[b]	Trends[c]	No.[a]	R[d]	Trends[c]	No.[a]
Antimicrobial		(year)	(%)	5y[e]	10y	(year)	(%)	10y	(year)	(%)	5y[f]	(year)
Ampicillin	Broilers	169 (2019)	29	↓[g]		258 (2020)	0	↓	22 (2017)	0	↓	
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	8.1	↓[g]					4 (2017)	0	—	92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	5.1	↓[g]		100 (2019)	12	↑	11 (2017)	0	—	
	Pork											
	Turkeys	37 (2019)	16.2	↓[g]								
	Turkey meat											17 (2016)
	Humans	341 (2019)	85	—	↓	768 (2020)	92.4	—	47,046 (2017)	87.9	↑	474 (2016)
Daptomycin	Broilers	169 (2019)	23.7			258 (2020)	4					
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	12.1									92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	9			100 (2019)	0					
	Pork											
	Turkeys	37 (2019)	18.9									
	Turkey meat											17 (2016)
	Humans											
Erythromycin	Broilers	169 (2019)	77.5	↓[g]		258 (2020)	9	↓	22 (2017)	27.3	↓	
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	33.3	↓[g]					4 (2017)	0	↓	92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	11.8	↓[g]		100 (2019)	20	—	11 (2017)	45.5	↑	
	Pork											
	Turkeys	37 (2019)	51.4	↓[g]								
	Turkey meat											17 (2016)
	Humans								42,259 (2017)	83.1	↓	
Gentamicin (HLR)[h]	Broilers	169 (2019)	2.4	—[g]		258 (2020)	0		22 (2017)	9.1	↑	
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	2.3	—[g]					4 (2017)	0	—	92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	0	—[g]		100 (2019)	1	—	11 (2017)	0	↓	
	Pork											
	Turkeys	37 (2019)	0	—[g]								
	Turkey meat											17 (2016)
	Humans	262 (2019)	23.7	↓	↓	298 (2020)	30					
Linezolid	Broilers	169 (2019)	0	—[g]		258 (2020)	0					
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	1.7	—[g]								92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	1.1	—[g]		100 (2019)	0					
	Pork											
	Turkeys	37 (2019)	0	—[g]								
	Turkey meat											17 (2016)
	Humans					621 (2020)	0.5		39,584 (2017)	<0.05	—	

			Norway				Sweden				Switzerland				USA			
R[b]	Trends[c]		No.[a]	R[b]	Trends[c]		No.[a]	R[b]	Trends[c]		No.[a]	R[b]	Trends[c]		No.[a]	R[d]	Trends[c]	
(%)	5y	10y	(year)	(%)	2y	6y	(year)	(%)	5y	10y	(year)	(%)	4y	10y	(year)	(%)	5y	10y
			237 (2020)	0.8	↑	↑					247 (2016)	4	—	↓	28 (2018)	0	—	
7.1	↓	—													110 (2018)	0.9	↓	↓
0	↓	↓	20 (2019)	0							129 (2017)	0.8	—	—	143 (2018)	0	—	
0	↓	↓													114 (2018)	0	↓	↓
			106 (2019)	6.6											34 (2018)	0	—	
															84 (2018)	0	↓	—
			115 (2020)	7.8	↑		70 (2016)	7							21 (2018)	0	—	
17.6	↓														60 (2018)	15	↓	↓
86			144 (2020)	71.5	↓	↓	554 (2016)	84.1	↓	↑	387 (2017)	82.4	—	—	808 (2012)	87	—	↑
			237 (2020)	0	↓	↓					247 (2016)	6.5			28 (2018)	0	—	
14.3															110 (2018)	5.5	—	↑
0			20 (2019)	0							129 (2017)	1.6			143 (2018)	0	↓	
6.5															114 (2018)	0.9	↑	↓
			106 (2019)	0											34 (2018)	0	—	
															84 (2018)	1.2	↑	↓
			115 (2020)	0	↓										21 (2018)	4.8	↓	
0															60 (2018)	3.3	↓	↓
			237 (2020)	11	↑	↑					247 (2016)	21.5	↑	—	28 (2018)	21.4	↑	
57.1	↓	↑													110 (2018)	20.9	↓	↓
30.4	↓	↓	20 (2019)	0							129 (2017)	20.2	↑	—	143 (2018)	7	↑	
10.9	—	↑													114 (2018)	11.4	↑	↓
			106 (2019)	3.8											34 (2018)	2.9	↓	
															84 (2018)	11.9	↑	↓
			115 (2020)	16.5	↓		70 (2016)	33							21 (2018)	19	↑	
58.8	↑														60 (2018)	31.7	↓	↓
			237 (2020)	0	—	—					247 (2016)	0			28 (2018)	7.1	↑	
3.6	↑	↑													110 (2018)	1.8	↓	↓
2.2	↓	↑	20 (2019)	0							129 (2017)	0			143 (2018)	0	—	
0	—	—													114 (2018)	0	—	—
			106 (2019)	0											34 (2018)	0	—	
															84 (2018)	0	—	—
			115 (2020)	0	—		70 (2016)	0							21 (2018)	4.8	↑	
0	—														60 (2018)	6.7	↓	↓
			144 (2020)	43.8	↑	↑	385 (2016)	20.5	↓	↑	248 (2017)	33.9	—	—	679 (2012)	13	↓	↓
			237 (2020)	0.8	↑	↑					247 (2016)	0			28 (2018)	0	—	
0	—	↓													110 (2018)	0	—	—
5.4	↑	↑	20 (2019)	0							129 (2017)	0			143 (2018)	0	—	
0	—	—													114 (2018)	0	—	—
			106 (2019)	0											34 (2018)	0	—	
															84 (2018)	0	—	—
			115 (2020)	0.9	↑		70 (2016)	0							21 (2018)	0	—	
0	—														60 (2018)	0	—	—
			144 (2020)	0	—	—					292 (2017)	0	—	—				

(continued)

Table 1 (continued)

	Source[a]	Country										
		Belgium				Denmark			Japan			The Netherlands
		No.[a]	R[b]	Trends[c]		No.[a]	R[b]	Trends[c]	No.[a]	R[d]	Trends[c]	No.[a]
Antimicrobial		(year)	(%)	5y[e]	10y	(year)	(%)	10y	(year)	(%)	5y[f]	(year)
Quinupristin-dalfopristin	Broilers	169 (2019)	82.8	↓[g]		258 (2020)	44	—				
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	85.1	↓[g]								92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	86	↓[g]		100 (2019)	6	↑				
	Pork											
	Turkeys											
	Turkey meat	37 (2019)	62.2	↓[g]								17 (2016)
	Humans											
Teicoplanin	Broilers	169 (2019)	0			258 (2020)	<1					
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	0									92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	0			100 (2019)	0					
	Pork											
	Turkeys	37 (2019)	0									
	Turkey meat											17 (2016)
	Humans					253 (2020)	3.2		47,321 (2017)	0.4	—	
Tetracycline	Broilers	169 (2019)	71.6	—[g]		258 (2020)	12	↑	22 (2017)	31.8	↓	
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	41.4	—[g]					4 (2017)	0	↓	92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	51.1	—[g]		100 (2019)	54	—	11 (2017)	54.5	↑	
	Pork											
	Turkeys	37 (2019)	67.6	—[g]								
	Turkey meat											17 (2016)
	Humans								52,494 (2017)	36.2	↑	
Tigecycline	Broilers	169 (2019)	0			258 (2020)	0					
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	0									92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	0			100 (2019)	0					
	Pork											
	Turkeys	37 (2019)	0									
	Turkey meat											17 (2016)
	Humans					120 (2020)	2.5					
Vancomycin	Broilers	169 (2019)	0	↓[g]		258 (2020)	<1		22 (2017)	0	—	
	Broiler meat											28 (2016)
	Beef cattle	174 (2019)	0	↓[g]					4 (2017)	0	—	92 (2015)
	Beef											46 (2015)
	Pigs	178 (2019)	0	↓[g]		100 (2019)	0		11 (2017)	0	—	
	Pork											
	Turkeys	37 (2019)	0	↓[g]								
	Turkey meat											17 (2016)
	Humans	343 (2019)	0.6	↓	↓	790 (2020)	9.4	↑	52,127 (2017)	0.8	↑	474 (2016)

			Norway				Sweden				Switzerland				USA			
R[b]	Trends[c]		No.[a]	R[b]	Trends[c]		No.[a]	R[b]	Trends[c]		No.[a]	R[b]	Trends[c]		No.[a]	R[d]	Trends[c]	
(%)	5y	10y	(year)	(%)	2y	6y	(year)	(%)	5y	10y	(year)	(%)	4y	10y	(year)	(%)	5y	10y
			237 (2020)	0.4	↓	↓					247 (2016)	57.1	↓	—	28 (2018)	53.6	↑	
42.9	↓	↓													110 (2018)	64.5	↑	↑
72.8	↑	↑	20 (2019)	0							129 (2017)	96.1	↑	↑	143 (2018)	60.8	↑	
54.3															114 (2018)	77.2	↑	↑
			106 (2019)	0											34 (2018)	58.8	↑	
															84 (2018)	79.8	↑	↑
			115 (2020)	0	—										21 (2018)	61.9	↑	
58.8	↓														60 (2018)	61.7	↑	↓
			237 (2020)	0	—	—					247 (2016)	0						
0																		
0			20 (2019)	0							129 (2017)	0						
0																		
			106 (2019)	0														
			115 (2020)	0	—													
0																		
			237 (2020)	4.2	↓	↓					247 (2016)	22.7	—	↓	28 (2018)	42.9	↓	
25	↓	↓													110 (2018)	43.6	↓	↓
41.3	↓	↓	20 (2019)	20							129 (2017)	4.7	↓	↓	143 (2018)	25.9	↓	
10.9	—	—													114 (2018)	14.9	↓	↓
			106 (2019)	22.6											34 (2018)	44.1	↑	
															84 (2018)	39.3	↓	↓
			115 (2020)	20	↑		70 (2016)	29							21 (2018)	66.7	↑	
76.5	↓														60 (2018)	58.3	↓	↓
											48 (2017)	18.8	—	↑				
			237 (2020)	0	—	—					247 (2016)	1.2			28 (2018)	0	—	
0															110 (2018)	0	—	—
0			20 (2019)	0							129 (2017)	3.1			143 (2018)	0	—	
0															114 (2018)	0.9	↑	↑
			106 (2019)	0											34 (2018)	0	—	
															84 (2018)	1.2	↑	↑
			115 (2020)	0	—										21 (2018)	0	—	
0															60 (2018)	0	—	—
			144 (2020)	12.5	↑	↑												
			237 (2020)	0	—	—					247 (2016)	0	—	—	28 (2018)	0	—	
0	—	↓													110 (2018)	0	—	—
0	—	—	20 (2019)	0							129 (2017)	0	—	—	143 (2018)	0	—	
0	—	—													114 (2018)	0	—	—
			106 (2019)	0											34 (2018)	0	—	
															84 (2018)	0	—	—
			115 (2020)	0	—		70 (2016)	0							21 (2018)	0	—	
0	—														60 (2018)	0	—	—
1	↓		144 (2020)	0	↓	↓	546 (2016)	0.4	—	—	416 (2017)	2.2	—	—	1110 (2016)	68	↓	—

countries and in Japan and was nearly absent in isolates from animal sources from the same countries (Table 1). In the USA, vancomycin resistance was also virtually absent in animal and meat *E. faecium* isolates, whereas it occurred at very high level (68%) in human clinical isolates (Table 1). This strongly indicates that occurrence of vancomycin resistance among human clinical isolates is primarily driven by hospital use of glycopeptides.

Different considerations can be made with regard to the occurrence of quinupristin/dalfopristin resistance, which ranged almost invariably from moderate to extremely high among isolates from animals and meat in Europe and in the USA. The frequent recovery and increasing occurrence of quinupristin/dalfopristin-resistant strains in animals according to 5- and 10-year trends is surprising since the quinupristin/dalfopristin analogue virginiamycin, which is likely selecting for quinupristin/dalfopristin resistance, has not been used since 1999, at least in European countries. Genetic linkage of quinupristin/dalfopristin resistance genes with genes conferring resistance to other antimicrobials used in animal production (e.g., macrolides) may explain the persistence of these genes in food animals in Europe (Hammerum et al. 2001). Data on occurrence of quinupristin/dalfopristin resistance in human isolates were not readily available. Occurrence of this resistance phenotype was lower in human isolates compared to animal isolates, suggesting the existence of a potential animal reservoir for quinupristin/dalfopristin resistance (Donabedian et al. 2006; Kieke et al. 2006; Hammerum et al. 2009) which, however, does not seem to pose a major resistance problem in human infections at present. A recent study investigating risks for selection for quinupristin/dalfopristin-resistant *E. faecium* in humans derived from virginiamycin use in food animals in China concluded that such risks are close to zero (Cox et al. 2020). However, the study investigated risks for selection for quinupristin/dalfopristin resistance in vancomycin-resistant *E. faecium* (VREFm) only and was based on the unproven assumption that resistance cannot be transferred from animal-origin strains to strains causing infections in humans.

Occurrence of daptomycin resistance was rare or low in isolates from animal and meat sources from all countries, except Belgium (Table 1). In this country, occurrence of daptomycin resistance ranged from moderate in *E. faecium* from calves and pigs to high in *E. faecium* from broilers (Table 1). No national surveillance data on occurrence of daptomycin resistance in *E. faecium* from humans were readily available for comparison. A study investigating patients with daptomycin-resistant

Data sources: Belgium, Garcia-Graells et al. 2019 and ECDC Surveillance Atlas: Antimicrobial Resistance 2019; Denmark, DANMAP 2019, 2020; Japan, NAOR 2019; the Netherlands, Nethmap-MARAN 2016, 2017; Norway, NORM/NORM-VET 2019, 2020; Sweden, Swedres-Svarm 2016 and ECDC Surveillance Atlas: Antimicrobial Resistance 2016; Switzerland, Swiss Antibiotic Resistance Report 2018; USA, NARMS Now and ResistanceMap: Antibiotic resistance 2022. Blank cells indicate that no information was available

[a]Animal sources represent healthy meat-producing animals, meat sources represent meat sold at retail, and human sources represent diseased patients with invasive infections. From the USA, bovine isolates are from steers and heifers (and not from calves). No., number of isolates tested

[b]Resistance (R) defined according to EUCAST ECOFFs for animal and meat isolates and EUCAST clinical breakpoints for human isolates (www.eucast.org). Of note, quinupristin-dalfopristin resistance in animal isolates is defined using EFSA cutoff values as no EUCAST ECOFF is available (EFSA et al. 2021)

[c]Trends indicate increased, decreased, or stable percentage of resistance in the time period indicated (y, years)

[d]Resistance (R) defined according to CLSI breakpoints. Limited to Japan, tetracycline refers to minocycline in humans and oxytetracycline in animals

[e]6y trend for animal isolates

[f]2y trend for vancomycin resistance in isolates of animal origin

[g]Data on trends of antimicrobial resistance for *E. faecium* isolates of animal origin are available only for all animal categories merged

[h]*HLR* high-level resistance

E. faecium infections found a possible association between acquisition of daptomycin resistance and residential proximity to animal or crop operations (Kelesidis and Chow 2013). However, the number of patients was too limited for drawing definitive conclusions (Kelesidis and Chow 2013).

Occurrence of resistance to linezolid, teicoplanin, and tigecycline was rare in human, animal, and meat isolates, with few exceptions, which however are not indicative of any large animal or human reservoir (Table 1).

It is important to note that occurrence of resistance to macrolides (e.g., erythromycin) and tetracyclines was frequently observed among animal and meat isolates, likely as a consequence of veterinary use of these drugs (Table 1). Genetic linkage between macrolide and tetracycline resistance genes and genes conferring resistance to clinically relevant antimicrobials may explain the occurrence of linezolid, quinupristin/dalfopristin, and glycopeptide resistance in animals, as a result of co-selection by veterinary use of macrolides and tetracyclines (Hammerum et al. 2001; Novais et al. 2005; Tyson et al. 2018; Rushton-Green et al. 2019). Unfortunately, data on resistance to macrolides and tetracyclines, which represent useful epidemiological markers indicating flow of antimicrobial resistance genes from livestock to humans, are generally not generated for human clinical isolates in surveillance programs from most countries, with the exception of Japan and Switzerland (Table 1). In these countries, erythromycin and tetracycline resistance occurred at similarly high and even very high level in *E. faecium* from most animal and human sources (Table 1), which indicates a possible animal reservoir, mainly linked to broilers and pigs, of enterococci resistant to these antimicrobials.

19.2.2 Patterns of Antimicrobial Resistance in Animal and Human *E. faecalis*

From a contemporary clinical perspective, the only antimicrobial resistance phenotype in *E. faecalis* that could be possibly linked to animal reservoirs is gentamicin resistance. Resistance to ampicillin, daptomycin, linezolid, teicoplanin, tigecycline, and vancomycin was rare or even not detected in animal isolates, with stable 5- and 10-year trends, based on national antimicrobial surveillance programs from several countries worldwide (Table 2).

Occurrence of gentamicin resistance was similar in *E. faecalis* from most animal and human sources (Table 2). These data indicate possible existence of an animal reservoir of gentamicin-resistant *E. faecalis*, though linked to different animal sources in different geographical areas. For example, in European countries and in Japan, the proportion of *E. faecalis* displaying gentamicin resistance ranged from moderate to high in cattle and pigs, and from rare to low in poultry, with the exception of turkeys in Belgium (Table 2). In the USA, occurrence of gentamicin resistance was rare in isolates from cattle, whereas it ranged from low to high in isolates from pigs and poultry (Table 2).

Remarkably, the occurrence of gentamicin resistance was higher in animals than meat thereof, with the biggest differences observed between cattle and beef and between pigs and pork (Table 2). Although comparison of occurrence of gentamicin

Table 2 Prevalence of antimicrobial resistance in *Enterococcus faecalis* isolated from humans (invasive isolates) and animals (indicator isolates) obtained from publicly available national surveillance program reports

Antimicrobial	Source[a]	Country											
		Belgium				Denmark				Japan			The Netherlands
		No.	R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[d]	Trends[c]	No.
		(year)	(%)	5y[e]	10y	(year)	(%)	4y	10y	(year)	(%)	5y[f]	(year)
Ampicillin	Broilers	173 (2019)	0.6	↓[g]		21 (2020)	0	—	—	85 (2017)	0	—	
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	0.9	↓[g]						10 (2017)	0	—	17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	0	↓[g]		91 (2019)	0	—	—	13 (2017)	0	—	
	Pork												
	Turkeys	67 (2019)	0	↓[g]									
	Turkey meat												63 (2016)
	Humans	496 (2019)	0.4	—	↓	650 (2020)	0			116,493 (2017)	0.2	↓	
Daptomycin	Broilers	173 (2019)	0			21 (2020)	0						
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	0										17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	3.6			91 (2019)	3						
	Pork												
	Turkeys	67 (2019)	0										
	Turkey meat												63 (2016)
	Humans												
Erythromycin	Broilers	173 (2019)	83.8	↓[g]		21 (2020)	38	↑	↑	85 (2017)	58.8	↓	
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	84.3	↓[g]						10 (2017)	10	↑	17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	41.1	↓[g]		91 (2019)	63	↑	↑	13 (2017)	61.5	↓	
	Pork												
	Turkeys	67 (2019)	74.6	↓[g]									
	Turkey meat												63 (2016)
	Humans									101,379 (2017)	53.8	↓	
Gentamicin (HLR)[h]	Broilers	173 (2019)	2.9	↑[g]		21 (2020)	0			85 (2017)	3.5	↓	
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	11.3	↑[g]						10 (2017)	0	↓	17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	10.7	↑[g]		91 (2019)	11	—	—	13 (2017)	7.7	↓	
	Pork												
	Turkeys	67 (2019)	10.4	↑[g]									
	Turkey meat												63 (2016)
	Humans	363 (2019)	16.8	↓	↓	349 (2020)	7.4		↓				
Linezolid	Broilers	173 (2019)	0	↓[g]		21 (2020)	0						
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	3.5	↓[g]									17 (2015)

			Norway				Sweden				Switzerland				USA			
R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[d]	Trends[c]	
(%)	5y	10y	(year)	(%)	2y	6y	(year)	(%)	5y	10y	(year)	(%)	4y	10y	(year)	(%)	5y	10y
			87 (2020)	0	—	—					31 (2016)	0	—	—	238 (2018)	0	—	
0	↓	↓													152 (2018)	0	—	—
0	—	—	12 (2019)	0							46 (2017)	0	—	—	91 (2018)	0	—	
0	—	—													375 (2018)	0	—	—
			46 (2019)	0											252 (2018)	0	—	
															392 (2018)	0	—	↓
			24 (2020)	0	—		41 (2016)	0							168 (2018)	0	—	
0	—														389 (2018)	0	—	—
			482 (2020)	0	—	—	1014 (2016)	0.5	—	—	515 (2017)	0.2	—	↓	1537 (2012)	1	—	—
			87 (2020)	1.1	↑	—					31 (2016)	0			238 (2018)	0	—	
0															152 (2018)	0	—	—
0			12 (2019)	0							46 (2017)	0			91 (2018)	0	—	
0															375 (2018)	0	—	—
			46 (2019)	0											252 (2018)	0	—	
															392 (2018)	0.3	↑	↑
			24 (2020)	0	↓										168 (2018)	0	—	
0															389 (2018)	0	—	↓
			87 (2020)	11.5	↓	↓					31 (2016)	35.5	↑	—	238 (2018)	39.1	↑	
55.4	↓	↑													152 (2018)	22.4	↓	↓
41.2	—	↓	12 (2019)	0							46 (2017)	37	↓	↓	91 (2018)	6.6	↓	
2.2	—	↓													375 (2018)	0.8	↓	↓
			46 (2019)	0											252 (2018)	66.7	↑	
															392 (2018)	7.7	—	—
			24 (2020)	7	↓		41 (2016)	49							168 (2018)	34.5	↓	
65.1	↑														389 (2018)	27.2	↓	↓
			87 (2020)	0	—	—					31 (2016)	0	—	—	238 (2018)	9.7	↓	
0	↓	↓													152 (2018)	8.6	↓	↓
5.9	↑	↓	12 (2019)	0							46 (2017)	23.9	↑	↑	91 (2018)	0	↓	
0	↓	—													375 (2018)	0	↓	↓
			46 (2019)	0											252 (2018)	14.3	↑	
															392 (2018)	1	—	↑
			24 (2020)	0	—		41 (2016)	0							168 (2018)	22	↓	
1.6	↓														389 (2018)	17.7	↓	↓
			482 (2020)	12	↓	↓	722 (2016)	13.4	↓	↓	337 (2017)	9.2	—	↓	1155 (2012)	34	↑	↓
			87 (2020)	0	—	—					31 (2016)	0			238 (2018)	0	—	
0	—	—													152 (2018)	0	—	—
0	↓	—	12 (2019)	0							46 (2017)	0			91 (2018)	0	—	

(continued)

Table 2 (continued)

	Source[a]	Country											
		Belgium				Denmark				Japan			The Netherlands
		No.	R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[d]	Trends[c]	No.
Antimicrobial		(year)	(%)	5y[e]	10y	(year)	(%)	4y	10y	(year)	(%)	5y[f]	(year)
	Beef												137 (2015)
	Pigs	56 (2019)	3.5	↓[g]		91 (2019)	0						
	Pork												
	Turkeys	67 (2019)	0	↓[g]									
	Turkey meat												63 (2016)
	Humans					507 (2020)	1.2						
Teicoplanin	Broilers	173 (2019)	0			21 (2020)	0			85 (2017)			
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	0							10 (2017)			17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	0			91 (2019)	0			13 (2017)			
	Pork												
	Turkeys	67 (2019)	0										
	Turkey meat												63 (2016)
	Humans					224 (2020)	0			113,501 (2017)	<0.05	—	
Tetracycline	Broilers	173 (2019)	82.1	↓[g]		21 (2020)	62	↑	↑	85 (2017)	65.9	↓	
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	91.3	↓[g]						10 (2017)	10	↓	17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	60.7	↓[g]		91 (2019)	91	↑	↑	13 (2017)	84.6	↑	
	Pork												
	Turkeys	67 (2019)	95.5	↓[g]									
	Turkey meat												63 (2016)
	Humans									125,728 (2017)	50.3	↑	
Tigecycline	Broilers	173 (2019)	0			21 (2020)	0						
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	0										17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	0			91 (2019)	0						
	Pork												
	Turkeys	67 (2019)	0										
	Turkey meat												63 (2016)
	Humans					112 (2020)	0.9						
Vancomycin	Broilers	173 (2019)	0	↓[g]		21 (2020)	0			85 (2017)	0	—	
	Broiler meat												56 (2016)
	Beef cattle	115 (2019)	0	↓[g]						10 (2017)	0	—	17 (2015)
	Beef												137 (2015)
	Pigs	56 (2019)	1.8	↓[g]		91 (2019)	0			13 (2017)	0	—	
	Pork												
	Turkeys	67 (2019)	0	↓[g]									
	Turkey meat												63 (2016)
	Humans	495 (2019)	1	↑	↑	622 (2020)	0	—	—	126,510 (2017)	<0.05	—	507 (2016)

			Norway				Sweden				Switzerland				USA			
R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[b]	Trends[c]		No.	R[d]	Trends[c]	
(%)	5y	10y	(year)	(%)	2y	6y	(year)	(%)	5y	10y	(year)	(%)	4y	10y	(year)	(%)	5y	10y
0	—	—													375 (2018)	0	—	—
			46 (2019)	0											252 (2018)	0	—	
															392 (2018)	0	—	—
			24 (2020)	0	—		41 (2016)	0							168 (2018)	0	—	
0	—														389 (2018)	0	—	—
			482 (2020)	0	—	—					460 (2017)	0.4	—	↓				
			87 (2020)	0	—	—					31 (2016)	0						
0																		
0			12 (2019)	0							46 (2017)	0						
0																		
			46 (2019)	0														
			24 (2020)	0	—													
0																		
			87 (2020)	66.7	↑	↑					31 (2016)	64.5	↑	↓	238 (2018)	70.2	↑	
66.1	↓	↓													152 (2018)	65.1	↑	↓
52.9	—	↓	12 (2019)	33.3							46 (2017)	67.4	↓	—	91 (2018)	16.5	↓	
14.6	↓	↓													375 (2018)	25.6	↑	↓
			46 (2019)	63											252 (2018)	86.5	↑	
															392 (2018)	75.5	↓	↓
			24 (2020)	7	↓		41 (2016)	80							168 (2018)	89.9	↓	
88.9	↑														389 (2018)	82	↓	↓
											116 (2017)	47.4	—	↑				
			87 (2020)	0	—	—					31 (2016)	0			238 (2018)	0	—	
0															152 (2018)	0	—	—
0			12 (2019)	0							46 (2017)	2.2			91 (2018)	0	—	
0.7															375 (2018)	0	—	—
			46 (2019)	0											252 (2018)	0	—	
															392 (2018)	0	—	—
			24 (2020)	0	—										168 (2018)	0	—	
0															389 (2018)	0.3	↑	↑
			482 (2020)	3.9	↑	↑												
			87 (2020)	0	—	—					31 (2016)	3.2	—	—	238 (2018)	0	—	
0	↓	↓													152 (2018)	0	—	—
0	↓	—	12 (2019)	0							46 (2017)	0	—	—	91 (2018)	0	—	
0	↓	—													375 (2018)	0	—	—
			46 (2019)	0											252 (2018)	0	—	
															392 (2018)	0	—	—
			24 (2020)	0	—		41 (2016)	0							168 (2018)	0	—	
0	—														389 (2018)	0	—	—
0	↓		482 (2020)	0	—	—	956 (2016)	0	—	—	622 (2017)	0.3	—	—	3278 (2012)	5	—	—

resistance in *E. faecalis* obtained from animals and meat should be done with caution due to the overall low number of animal isolates included and to the fact that meat may also be of imported origin, these data indicate low risk of carcass contamination in cattle and pig slaughtering and consequent low human exposure to gentamicin-resistant *E. faecalis* through consumption of beef and pork. As observed for *E. faecium*, resistance to macrolides and tetracycline is widespread among *E. faecalis* isolates from animals and meat, possibly as a consequence of the widespread use of these antibiotics in livestock production (Table 2). Macrolide and tetracycline resistance data for human *E. faecalis* isolates were generated in the national surveillance programs of Japan and Switzerland only (Table 2). In these countries, erythromycin and tetracycline resistance occurred at similarly high and even very high level in *E. faecalis* from most animal and human sources (Table 2), which indicates a possible animal reservoir of enterococci resistant to erythromycin and tetracycline. This potential animal reservoir may represent a risk to human health in case of genetic linkage between erythromycin and tetracycline resistance genes and genes conferring resistance to clinically relevant antimicrobials, but unfortunately this type of genetic information is not routinely generated in national surveillance programs.

19.3 Transmission of Antimicrobial Resistance Between Animals and Humans

Transmission of antimicrobial resistance between animal and human enterococci may happen through different epidemiological routes and mechanisms. Humans can acquire animal enterococci by direct exposure to animals and animal-contaminated environments or indirectly, through consumption of contaminated food of animal origin and vegetables from crops treated with animal manure.

19.3.1 Foodborne Transmission

Foodborne transmission may result from consumption of contaminated animal food products and cross-contamination in the kitchen (Wegener et al. 1997). *E. faecium* and *E. faecalis* generally contaminate raw meat and cheese at concentrations of

Data sources: Belgium, Garcia-Graells et al. 2019 and ECDC Surveillance Atlas: Antimicrobial Resistance 2019; Denmark, DANMAP 2019, 2020; Japan, NAOR 2019; the Netherlands, Nethmap-MARAN 2016, 2017; Norway, NORM/NORM-VET 2019, 2020; Sweden, Swedres-Svarm 2016 and ECDC Surveillance Atlas: Antimicrobial Resistance 2016; Switzerland, Swiss Antibiotic Resistance Report 2018; USA, NARMS Now and ResistanceMap: Antibiotic resistance 2022. Blank cells indicate that no information was available

[a]Animal sources represent healthy meat-producing animals, meat sources represent meat sold at retail, and human sources represent diseased patients with invasive infections. From the USA, bovine isolates are from steers and heifers (and not from calves). No., number of isolates tested

[b]Resistance (R) defined according to CLSI breakpoints. Limited to Japan, tetracycline refers to minocycline in humans and oxytetracycline in animals

[c]Trends indicate increased, decreased, or stable percentage of resistance in the time period indicated (y, years)

[d]Resistance (R) defined according to EUCAST ECOFFs for animal and meat isolates and EUCAST clinical breakpoints for human isolates (www.eucast.org). Of note, quinupristin-dalfopristin resistance in animal isolates is defined using EFSA cutoff values as no EUCAST ECOFF is available (EFSA et al. 2021)

[e]6y trend for animal isolates

[f]2y trend for vancomycin resistance in isolates of animal origin

[g]Data on trends of antimicrobial resistance for *E. faecalis* isolates of animal origin are available only for all animal categories merged

[h]*HLR* high-level resistance

10^2–10^4 and 10^5–10^7 colony forming units (CFU) per gram, respectively (Giraffa 2002). Experiments conducted on healthy human volunteers have shown that animal enterococcal strains occurring in ingested food are shed with feces for a limited time period of about 1–3 weeks. In an experiment performed on himself, Berchieri established that a minimum concentration of 10^7 CFU of VREFm of poultry and pig origin was necessary to be able to isolate the same strain from feces for a period of 20 days (Berchieri 1999). Sørensen et al. demonstrated that VREFm from poultry meat and quinupristin/dalfopristin-resistant *E. faecium* from pork ingested at 10^7 CFU could be detected in the feces of 8 out of 12 volunteers 6 days after ingestions, at different concentrations (Sørensen et al. 2001). One out of 12 volunteers excreted the strain also 14 days after ingestion (Sørensen et al. 2001). In a similar experiment, Lester et al. demonstrated that animal VREFm transiently colonizing the human gut could transfer the *vanA* vancomycin resistance operon to resident commensal *E. faecium* strains in three out of six volunteers, indicating that occurrence of VREFm in food may result in the transfer of vancomycin resistance to the microbiota of consumers (Lester et al. 2006). Furthermore, Al-Ahmad et al. showed that foodborne *E. faecalis* could integrate into dental oral biofilm in five out of six volunteers for at least 5 days, indicating a potential risk for endodontic infections that may evolve into bacteremia (Al-Ahmad et al. 2010).

According to a study in Switzerland (Collineau et al. 2018), poultry meat poses the highest risk of transmission of antimicrobial-resistant enterococci for the consumers, especially transfer of tetracycline and macrolide resistance. The authors attributed the highest risk mainly to cross-contamination during the slaughter process in poultry production and to a lesser extent to high consumption of poultry meat. An American study assessing exposure to antimicrobial-resistant bacteria in ground beef consumers estimated that the probabilities of exposure to tetracycline-resistant enterococci are on average 6.2% per serving, which are double than the probabilities of being exposed to tetracycline-resistant *E. coli* (3.1% per serving) (Zhang et al. 2021). To our knowledge, no data are available on foodborne exposure to enterococci that are resistant to clinically important antimicrobials but this is likely to be low due to infrequent occurrence of resistant isolates in meat products (Tables 1 and 2).

The hypothesis that antimicrobial-resistant enterococci of animal origin could be transferred to the intestine of healthy humans via food is indirectly supported also by studies describing clonally related strains in meat products and in the feces of healthy meat consumers. Donabedian et al. described closely related gentamicin-resistant *E. faecalis* strains in multiple pork samples and one human sample, and indistinguishable strains in a chicken meat sample and a human sample in the USA (Donabedian et al. 2003). Agerso et al. demonstrated clonal relatedness between five vancomycin-resistant *E. faecalis* (VREFs) from turkey meat and from the intestine of healthy humans in Denmark (Agerso et al. 2008). Similarly, in an additional study from Denmark, Hammerum et al. reported the occurrence of highly related VREFm isolates in Danish pig samples and in the intestine of a healthy human who reported no contact with pigs but had eaten pork (Hammerum et al. 2004).

In conclusion, based on the current knowledge, ingested antimicrobial-resistant enterococci of animal origin can be shed with feces for a variable time, likely depending on the numbers of enterococci ingested as well as on host factors, but it

remains unknown whether animal strains are able to permanently colonize the human intestine and to what extent their transit through the intestinal lumen allows for a significant transfer of antimicrobial resistance genes to the indigenous microbiota.

19.3.2 Transmission via Direct Contact with Food Animals

Farm and slaughterhouse workers and veterinarians are the main categories at risk for this transmission route since they are daily exposed to high numbers of animals. High density of animals and animal excreta implies a high load of fecal bacteria in farm environments. Various studies showed that genetically related antimicrobial-resistant enterococci can be isolated from animal feces, insects, dust, and air inside and in proximity of farms, which indicates the existence of multiple sources of human exposure to animal enterococci, as extensively reviewed by the European Food Safety Authority (EFSA BIOHAZ Panel 2021). Different studies reported occurrence of genetically related enterococci strains displaying specific resistance phenotypes in the feces of animals and healthy farm workers. VREFm clones shared by turkey, turkey farmers, and turkey slaughterers and by broiler and broiler farmers were detected in the Netherlands and in Norway (van den Bogaard et al. 1997a, b; Simonsen et al. 1998; Stobberingh et al. 1999; Jensen et al. 2003). Clonally related quinupristin-/dalfopristin-resistant *E. faecium* were isolated from a poultry farmer and his animals in the Netherlands (Jensen et al. 1998). Closely related plasmids and indistinguishable Tn*1546* variants harboring *vanA* have been reported in genetically unrelated VREFm isolated from poultry and workers within farms (Stobberingh et al. 1999; van den Bogaard et al. 2002; Sletvold et al. 2007), suggesting that *vanA* of animal origin may be horizontally transferred to the intestinal microbiota of farm workers. Evidence of human infections caused by antimicrobial-resistant enterococci transmitted by direct contact with production animals is limited to a single study. Das et al. reported a VREF-infected wound in a worker who was injured while working at a factory packaging chickens (Das et al. 1997). The strain isolated from the wound had the same resistance profile of isolates from the factory and the patient had no risk factors for a VREF infection, strongly supporting animal origin of the infection (Das et al. 1997).

19.3.3 Transmission via Direct Contact with Companion Animals

The role of companion animals as reservoirs of antimicrobial-resistant enterococci was first hypothesized in 1996, when van Belkum et al. discovered that (i) 17% of dogs and cats examined harbored VREFm while the incidence among people living in the same area was 2–3% and (ii) VREFm isolates from a dog, a cat, and a human carrier were indistinguishable by pulsed-field gel electrophoresis (PFGE) (van Belkum et al. 1996). The authors concluded their article by raising the question, “which dog poses a greater risk to the postman: the one that barks or the one that wags its tail?” (van Belkum et al. 1996). Companion animals represent potential

sources of antimicrobial-resistant bacteria since they live in close contact with their owners and are often administered antimicrobials belonging to the same classes used for treatment in humans (Guardabassi et al. 2004; Jackson et al. 2009). Antimicrobial-resistant enterococci can be isolated from different animal body sites and from feces, which may represent a source of contamination of domestic and urban environment (Jackson et al. 2009; Ghosh et al. 2011). AREFm were detected in a considerable proportion of dogs and cats in different European countries, being reported in 23% (of 183), 30% (of 79), and 76% (of 25) of dogs in the UK, the Netherlands, and Denmark, respectively, and in 13% (of 85) of cats in the Netherlands (Damborg et al. 2009; de Regt et al. 2012). This is in contrast with food-producing animals, where AREFm are rare or not detected by national surveillance programs (Table 1), and may reflect the frequent usage of aminopenicillins and other β-lactam antibiotics in small animal veterinary medicine. In addition, VREFm were detected in 1.4% (out of 71) and 13% (out of 87) of dog feces samples examined in Portugal and Spain, respectively (Herrero et al. 2004; Poeta et al. 2005). Occurrence of VRE in dogs was reported also outside Europe. The first VREFm reported in a dog in the USA was shown to harbor a mutated form of Tn*1546* described in human patients (Simjee et al. 2002). A VREF from a dog with mastitis displayed a PFGE profile prevalent among human isolates in New Zealand (Manson et al. 2003). A recent meta-analysis and systematic review indicated a significant heterogeneity of the data on the prevalence of VRE in companion animals with a pooled prevalence in dogs and cats of 18.2% and 12.3%, respectively (Wada et al. 2021). These studies suggest that VREFm and *vanA* may be exchanged between humans and dogs but the importance and prevalent direction of this transmission route is impossible to assess.

19.4 Genetic Links Between Clinical and Animal Strains

Even if it appears plausible that antimicrobial-resistant enterococci of animal origin transiently colonize the digestive tract of humans and/or transfer resistance genes to human-adapted strains, the magnitude and the clinical consequences of this biological phenomenon are controversial. This section reviews the knowledge of the genetic similarities between human clinical and animal strain populations of *E. faecium* and *E. faecalis*. This information, complemented with epidemiological data, is of paramount importance to assess the risk of zoonotic transmission of antimicrobial resistance by clonal propagation and horizontal gene transfer.

19.4.1 Genetic Links Between Clinical and Animal *E. faecium*

E. faecium can be divided into two genomically distinct groups, A and B, which include hospital-associated and community-associated isolates, respectively (Lebreton et al. 2013). Within group A, an additional separation into clade A1, including the vast majority of hospital-associated isolates, and clade A2, mainly associated with animals but also including clinical isolates, has been proposed and validated with different strain collections (Lebreton et al. 2013; Raven et al. 2016a;

Manson et al. 2019; van Hal et al. 2022). Clade A1 comprises the epidemic lineages associated with nosocomial infections worldwide, which are clonal complexes 17 (including, among others, sequence types ST16 and ST17), 18 (ST18), and 78 (ST78 and ST192), as identified using multilocus sequence typing (MLST) (Willems et al. 2012). These hospital-associated lineages are generally characterized by ampicillin resistance and are particularly enriched in genes encoding colonization and adhesion factors, which likely play a role in virulence (Somarajan and Murray 2013). Animal strains rarely overlap with the hospital-associated lineages, with the notable exception of canine *E. faecium* strains. Indeed, AREFm belonging to the hospital-associated ST78 and ST192 have been frequently detected in dogs but they generally lack genes encoding putative virulence factors (Damborg et al. 2009). Thus, it appears that animal and clinical *E. faecium* strains constitute two distinct subpopulations in relation to ampicillin resistance and occurrence of putative virulence factors. Similar conclusions have been drawn for VREFm. The population structures of VREFm isolated from human patients and animals are generally diverse and overlap only sporadically (Woodford et al. 1998; Jung et al. 2006; Biavasco et al. 2007; Donabedian et al. 2010; Freitas et al. 2011; Hammerum 2012; Tzavaras et al. 2012; Willems et al. 2012; Getachew et al. 2013). Among lineages of clinical relevance, there are single reports of *vanA*-positive VREFm ST132 (related to ST18) in swine in Portugal, *vanA*-positive VREFm ST78 in rabbit meat, and *vanB*-positive VREFm ST17 in chicken meat and veal in Spain (Lopez et al. 2009). VREFm lineages grouped in clonal complex CC5, which are common among porcine strains of diverse geographical origin, have been sporadically reported as a cause of urinary tract infections in hospitalized patients (Freitas et al. 2011). Altogether, these findings suggest that animal VREFm strains have a limited zoonotic potential.

A series of evolutionary studies based on comparative genome analyses of large collections of *E. faecium* with different antimicrobial resistance profiles and isolated from various geographical regions has conclusively substantiated the limited genetic links between animal and clinical *E. faecium*. These studies have shown that animal and clinical strains, although evolutionary linked, constitute different subpopulations or clades that diversified mainly through recombination and acquisition or loss of mobile genetic elements (MGEs) and eventually adapted to different ecological niches (van Schaik et al. 2010; de Regt et al. 2012; Galloway-Pena et al. 2012; Willems et al. 2012; de Been et al. 2013; Lebreton et al. 2013; Gouliouris et al. 2018; O'Dea et al. 2019; Arredondo-Alonso et al. 2020; Lee et al. 2021). Recent taxonomy studies support that the strains of *E. faecium* clade B should be classified as *Enterococcus lactis* (Belloso Daza et al. 2021).

19.4.2 Genetic Links Between Clinical and Animal *E. faecalis*

Also in *E. faecalis*, few genetic lineages such as CC2, CC9, CC87, and ST16 are particularly enriched among nosocomial isolates and associated with multidrug resistance, as shown by molecular epidemiological studies using MLST and further confirmed by studies based on whole genome sequence (WGS) data analyses

(McBride et al. 2007; Freitas et al. 2009; Willems et al. 2011; Palmer et al. 2012; Tedim et al. 2015; Raven et al. 2016b). However, differently from *E. faecium*, the *E. faecalis* hospital-associated clones are phylogenetically closely related to human commensal and animal strains, indicating the absence of a clear boundary between clinical and non-clinical strains (Palmer et al. 2012; Kim and Marco 2014; Guzman-Prieto et al. 2016; Pöntinen et al. 2021). For some of the lineages enriched among hospitalized patients, like CC2 and CC87, the genetic link between clinical and animal strains seems to be weak as descriptions in animal sources have been only sporadic and included, for example, findings in a black rat in Spain; in dogs, pigs, and natural gilthead seabream in Portugal; and in crows in the USA (McBride et al. 2007; Freitas et al. 2009; Freitas et al. 2011; Barros et al. 2012; Kuch et al. 2012; Oravcova et al. 2014; Lozano et al. 2015; Marques et al. 2018). On the contrary, ST16 is well represented among isolates in hospitalized patients as well as healthy humans and animals (Ruiz-Garbajosa et al. 2006; Willems et al. 2011; Kuch et al. 2012; Tedim et al. 2015; León-Sampedro et al. 2019). In a study examining 386 contemporary human *E. faecalis* from hospital and community sources in 6 European countries, ST16 represented 11% and 15% of the total hospital- and community-associated strains, respectively (Kuch et al. 2012). Half of the ST16 strains from each source displayed gentamicin resistance, suggesting that up to 6% of nosocomial infections by gentamicin-resistant strains may be acquired in the community (Kuch et al. 2012). Notably, ST16 was found highly predominant among gentamicin-resistant *E. faecalis* isolated from pigs and pork in Denmark in 2001–2002 and represented 9% of 22 *E. faecalis* isolates from endocarditis patients in the same country in 1996–2002 (Larsen et al. 2010). These porcine and human strains were shown to be closely related genetically using pulsed-field gel electrophoresis (PFGE), which suggested a link between gentamicin-resistant *E. faecalis* ST16 in pigs and human patients in Denmark (Larsen et al. 2010). When sequencing 32 *E. faecalis* from retail chicken meat in the USA and comparing them with available sequences of 149 clinical, commensal, and animal isolates, Manson et al. identified a cluster of gentamicin-resistant ST16 *E. faecalis* including 2 chicken meat strains, 9 porcine strains, and 1 clinical strain. The strains in this cluster differed for 56–92 single nucleotide polymorphisms (SNPs) based on single-copy core genome phylogeny, strongly indicating high genetic similarity between clinical and animal strains (Manson et al. 2019). Gentamicin-resistant ST16 strains have also been associated with nosocomial infections in Cuba, Japan, and Saudi Arabia, with urinary tract infection patients and their chickens in Vietnam, with urinary tract infections in cats, and with swine and poultry sources in Portugal (Freitas et al. 2009; Quinones et al. 2009; Watanabe et al. 2009; Poulsen et al. 2012; Novais et al. 2013; Marques et al. 2018; Farman et al. 2019). Multidrug-resistant ST16 strains displaying additional resistance to linezolid have been reported in patients in Belgium, China, Colombia, Cuba, Denmark, Germany, Greece, Japan, Korea, Malaysia, Thailand and the UK (Quinones et al. 2009; Spiliopoulou et al. 2011; Diaz et al. 2012; Weng et al. 2013; Kudo et al. 2014; Vorobieva et al. 2017; Bender et al. 2018; Angeles Argudín et al. 2019; Tsilipounidaki et al. 2019; Saavedra et al. 2020; Park et al. 2020; Ma et al. 2021; McHugh et al. 2022); in healthy humans in Portugal and the USA (Freitas et al. 2009; Freitas et al. 2020); in pigs in Colombia, Italy, Korea,

and Portugal (Novais et al. 2013; Tamang et al. 2017; Fioriti et al. 2020; Freitas et al. 2020); and in goats in China (Yang et al. 2022). Vancomycin-resistant ST16 strains have been isolated from poultry meat in Portugal (Freitas et al. 2009), American crows in the USA (Oravcova et al. 2014), and migrating birds in Tunisia (Ben Yahia et al. 2018). The widespread distribution of *E. faecalis* ST16 resistant to clinically important antimicrobials across several host species and countries provides ample opportunities for spillover from a reservoir to another, which may ultimately result in disease in humans. Also other *E. faecalis* lineages are shared between human patients and animals. For example, ST108 is predominant among VREFs isolated from humans and poultry in New Zealand and also shows bacitracin resistance, which is consistent with a possible transfer to humans after resistance to this veterinary antibiotic is acquired in poultry production (Rushton-Green et al. 2019). Linezolid-resistant ST480 of bovine, porcine, and human clinical origin in Belgium has been shown to differ only by 10–25 SNPs out of 1945 loci used to build the phylogeny, and such close phylogenetic relationship along with the common geographical origin suggests an epidemiological link between these strains (Timmermans et al. 2021).

In summary, a zoonotic transmission of antimicrobial-resistant *E. faecalis* clones appears to be possible based on the available epidemiological studies, but there have not been enough studies to date to prove the robustness of this information.

19.4.3 Genetic Links Between Mobile Genetic Elements in Clinical and Animal Enterococci

The transfer of MGEs carrying antimicrobial resistance genes from animal enterococci to human pathogenic strains may be considered a zoonosis. Transferability of MGEs from animal to human enterococci strains has been demonstrated in vitro and/or in animal models, including transferability of gentamicin resistance in *E. faecalis* and of ampicillin, gentamicin, and vancomycin resistance in *E. faecium* (Lester and Hammerum 2010; Ghosh et al. 2011; Sparo et al. 2012; Novais et al. 2013). However, these studies were not designed to determine if the strains with the newly acquired, animal-origin antimicrobial resistance caused infections in humans. Early studies based on traditional typing methods indicated the occurrence of host-specific mutations in transposons and resistance genes and inferred possible animal-to-human transmission based on the occurrence of animal-specific mutations in strains of human origin. For example, a point mutation (G to T) in *vanX* in Tn*1546* has been consistently associated with *E. faecium* isolated from poultry (G) and pigs (T), while both types occur among human clinical isolates (Jensen 1998; Hammerum 2012). Similarly, different *erm*(B) alleles occurred at different frequencies among macrolide-resistant *E. faecium* isolates from pigs and poultry, and all variants were present among isolates from healthy and diseased humans (De Leener et al. 2005). These studies, as well as evidence of indistinguishable MGEs in healthy animal and clinical human strains (Hegstad et al. 2010; Werner et al. 2013), suggested exchange of MGEs carrying antimicrobial resistance genes between animal and clinical enterococci. However, studies using WGS have

minimized this risk. A study investigated a large ($n = 1644$) collection of *E. faecium* isolates from hospitals, healthy individuals, and animals using short- and long-read sequencing in combination with a machine learning classifier. The study revealed that evolution of plasmids in *E. faecium* is driven by host and ecological factors that hinder transmission between different reservoirs (Arredondo-Alonso et al. 2020). Another large-scale study conducted on *E. faecium* isolates from livestock, retail meat, wastewater, and bloodstream infections in the UK provided further evidence for niche adaptation and limited exchange of antimicrobial resistance genes between these reservoirs on a national scale (Gouiliouris et al. 2018). The picture seems to be rather different for *E. faecalis*. WGS analysis of a unique collection of 2207 *E. faecalis* strains isolated from different sources over a period of 82 years (1936–2018) showed that the accessory genome of this species is shared between various host types, including animals and human patients (Pöntinen et al. 2021). The authors concluded that *E. faecalis* is an ecological generalist microbe that carries genes or adaptive variants that enable its survival in different ecological niches, as opposed to *E. faecium*, which appears to be highly host-specific and comprises lineages that are clearly adapted to specific hosts.

19.5 Conclusions

Enterococci are among the leading causes of life-threatening infections in humans, such as bacteremia and endocarditis. These infections are generally treated empirically and the consequences of treatment failure may be fatal to the patient if infection is caused by a strain resistant to first-line agents. By this chapter, the authors made an attempt to evaluate to what extent resistance problems in enterococcal infections in humans are attributable to strains and MGEs of animal origin. Aquatic animals and derived seafood were not included in the review, as enterococci isolated from these sources often derive from anthropogenic contamination. The authors' conclusion is that, in general, clinical *E. faecium* strains are not genetically linked to animal sources, whereas the boundary between animal and clinical *E. faecalis* strains is not well defined. Although there is a potential risk of clonal transmission of AREFm from companion animals, which are frequent carriers of hospital-associated lineages such as ST78 and ST192, the magnitude of such risk appears to be limited since strains isolated from these animals usually lack the putative virulence factors present in hospital strains. On the contrary, clear overlap is evident for multidrug-resistant *E. faecalis* ST16, which has been associated with both human patients and various animal species. Detailed population genetic analysis of *E. faecalis* indicates that this species is an ecological generalist able to adapt to different hosts, thereby providing opportunities for transfer of antimicrobial resistance between farms and hospitals, as opposed to *E. faecium* that appears to be an ecological specialist.

Even though resistance genes of clinical relevance have been reported in enterococci isolated from animals, comparison of data on occurrence of antimicrobial resistance in animal, meat, and human clinical isolates indicates that antimicrobial-resistant enterococci of animal origin pose an overall limited zoonotic risk, with differences linked to geographical region, specific types of resistance, and animal

sources. The major risk seems to be associated to horizontal transfer of gentamicin resistance genes through consumption of poultry meat, especially in the USA, where resistance to this first-line agent is relatively frequent in isolates from broiler and turkey meat. Farm-to-fork transmission of gentamicin resistance genes is plausible since aminoglycosides are used in livestock production but hardly ever used for systemic antimicrobial therapy in the primary health-care sector because of parenteral administration and high toxicity. However, lack of prevalence and genomic data on carriage of gentamicin resistance strains in the community hampers quantification of this zoonotic risk. Similarly, zoonotic implications of linezolid-resistant strains from bovines and pigs have been described in Belgium but based on circumstantial evidence only. VRE strains causing human infections are not obviously linked to animals as indicated by the fact that they are prevalent in countries where avoparcin has never been used in livestock. Indigenous anaerobes in the patient's digestive tract seem to be a more important source of *vanB* operons than farm animals. As for *vanA* operons, the risk of zoonotic transfer was significantly reduced by the ban on avoparcin and by the consequent decrease of VRE in livestock.

The risk of foodborne transmission of antimicrobial-resistant enterococci is significantly higher for poultry meat than for other food products of animal origin, mainly due to higher risk of carcass contamination in poultry slaughtering and higher consumption of poultry meat. Although horizontal transfer of resistance genes from animal to human enterococci has been demonstrated to occur in the human digestive tract under in vivo conditions, the clinical significance of this phenomenon appears to be negligible, or at least limited, for *E. faecium* but not for *E. faecalis*. Thus, future research should focus on the ecology and epidemiology of MGEs carrying resistance genes of clinical relevance to assess the animal contribution to antimicrobial resistance in *E. faecalis* infections in humans.

It should be emphasized that these conclusions are largely based on studies from developed countries that either never used or banned the use of antimicrobial growth promoters in livestock for nearly two decades (at the time of writing this review), which has significantly reduced the occurrence of clinically relevant antimicrobial-resistant enterococci in the animal reservoir. Further research is needed to assess the risk of antimicrobial resistance transmission in countries where growth promoters continue to be used, which include developing and least developed countries that lack policies governing antimicrobial use and apply less efficient biosecurity measures to prevent zoonotic transmission.

References

Aarestrup FM (1995) Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb Drug Resist 1:255–257

Aarestrup FM, Kruse H, Tast E et al (2000a) Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. Microb Drug Resist 6:63–70

Aarestrup FM, Agerso Y, Gerner-Smidt P et al (2000b) Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. Diagn Microbiol Infect Dis 37:127–137

Agerso Y, Lester CH, Porsbo LJ et al (2008) Vancomycin-resistant *Enterococcus faecalis* isolates from a Danish patient and two healthy human volunteers are possibly related to isolates from imported Turkey meat. J Antimicrob Chemother 62:844–845

Al-Ahmad A, Maier J, Follo M et al (2010) Food-borne enterococci integrate into oral biofilm: an in vivo study. J Endod 36:1812–1819

Angeles Argudín M, Youzaga S, Dodémont M et al (2019) Detection of *optrA*-positive enterococci clinical isolates in Belgium. Eur J Clin Microbiol Infect Dis 38:985–987

Anonymous (1997) Commission directive 97/6/EC. Off J Eur Commun Legis L35:11–13

Anonymous (1998a) Council regulation 2821/98. Off J Eur Commun Legis L351:4–8

Anonymous (1998b) Commission regulation 2788/98. Off J Eur Commun Legis L347:31–32

Anonymous (2003) Commission regulation (EC) No 1831/2003. Off J Eur Union L268:29–43

Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399:629–655

Arias CA, Murray BE (2008) Emergence and management of drug-resistant enterococcal infections. Expert Rev Anti-Infect Ther 6:637–655

Arias CA, Murray BE (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. Nat Rev Microbiol 10:266–278

Arredondo-Alonso S, Top J, McNally A et al (2020) Plasmids shaped the recent emergence of the major nosocomial pathogen *Enterococcus faecium*. MBio 11:e03284–e03219

Bager F, Madsen M, Christensen J et al (1997) Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Prev Vet Med 31:95–112

Barros J, Andrade M, Radhouani H et al (2012) Detection of *vanA*-containing *Enterococcus* species in faecal microbiota of gilthead seabream (*Sparus aurata*). Microbes Environ 27:509–511

Bates J, Jordens Z, Selkon JB (1993) Evidence for an animal origin of vancomycin-resistant enterococci. Lancet 342:490–491

Belloso Daza MV, Cortimiglia C, Bassi et al (2021) Genome-based studies indicate that the Enterococcus faecium clade B strains belong to Enterococcus lactis species and lack of the hospital infection associated markers. Int J Syst Evol Microbiol 71. https://doi.org/10.1099/ijsem.0.004948

Ben Yahia H, Chairat S, Hamdi N et al (2018) Antimicrobial resistance and genetic lineages of faecal enterococci of wild birds: emergence of *vanA* and *vanB2* harbouring *Enterococcus faecalis*. Int J Antimicrob Agents 52:936–941

Bender JK, Fleige C, Lange D et al (2018) Rapid emergence of highly variable and transferable oxazolidinone and phenicol resistance gene *optrA* in German *Enterococcus* spp. clinical isolates. Int J Antimicrob Agents 52:819–827

Berchieri A (1999) Intestinal colonization of a human subject by vancomycin-resistant *Enterococcus faecium*. Clin Microbiol Infect 5:97–100

Biavasco F, Foglia G, Paoletti C et al (2007) VanA-type enterococci from humans, animals, and food: species distribution, population structure, Tn*1546* typing and location, and virulence determinants. Appl Environ Microbiol 73:3307–3319

Collineau L, Carmo LP, Endimiani A et al (2018) Risk ranking of antimicrobial-resistant hazards found in meat in Switzerland. Risk Anal 38:1070–1084

Cox LA Jr, Popken DA, Sun J et al (2020) Quantifying human health risks from virginiamycin use in food animals in China. Risk Anal 40:1244–1257

Damborg P, Top J, Hendrickx AP et al (2009) Dogs are a reservoir of ampicillin-resistant *Enterococcus faecium* lineages associated with human infections. Appl Environ Microbiol 75: 2360–2365

DANMAP (2019) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032. Available at www.danmap.org

DANMAP (2020) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032. Available at www.danmap.org

Das I, Fraise A, Wise R (1997) Are glycopeptide-resistant enterococci in animals a threat to human beings? Lancet 349:997–998

de Been M, van Schaik W, Cheng L et al (2013) Recent recombination events in the core genome are associated with adaptive evolution in *Enterococcus faecium*. Genome Biol Evol 5: 1524–1535

De Leener E, Martel A, De Graef EM et al (2005) Molecular analysis of human, porcine, and poultry *Enterococcus faecium* isolates and their *erm*(B) genes. Appl Environ Microbiol 71: 2766–2770

de Regt MJ, van Schaik W, van Luit-Asbroek M et al (2012) Hospital and community ampicillin-resistant *Enterococcus faecium* are evolutionarily closely linked but have diversified through niche adaptation. PLoS One 7:e30319

Del Campo R, Ruiz-Garbajosa P, Sanchez-Moreno MP et al (2003) Antimicrobial resistance in recent fecal enterococci from healthy volunteers and food handlers in Spain: genes and phenotypes. Microb Drug Resist 9:47–60

Diaz L, Kiratisin P, Mendes RE et al (2012) Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. Antimicrob Agents Chemother 56:3917–3922

Donabedian SM, Thal LA, Hershberger E et al (2003) Molecular characterization of gentamicin-resistant Enterococci in the United States: evidence of spread from animals to humans through food. J Clin Microbiol 41:1109–1113

Donabedian SM, Perri MB, Vager D et al (2006) Quinupristin-dalfopristin resistance in *Enterococcus faecium* isolates from humans, farm animals, and grocery store meat in the United States. J Clin Microbiol 44:3361–3365

Donabedian SM, Perri MB, Abdujamilova N et al (2010) Characterization of vancomycin-resistant *enterococcus faecium* isolated from swine in three Michigan counties. J Clin Microbiol 48: 4156–4160

ECDC Surveillance Atlas: Antimicrobial resistance. Available at: https://www.ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc. Date accessed: Feb 18, 2022

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (2021) The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA J 19:6490. https://doi.org/10.2903/j.efsa.2021.6490

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A et al (2021) Scientific opinion on the role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. EFSA J 19:6651, 188 pp

Farman M, Yasir M, Al-Hindi RR et al (2019) Genomic analysis of multidrug-resistant clinical *enterococcus faecalis* isolates for antimicrobial resistance genes and virulence factors from the western region of Saudi Arabia. Antimicrob Resist Infect Control 8:55. https://doi.org/10.1186/s13756-019-0508-4

Fioriti S, Morroni G, Coccitto SN et al (2020) Detection of oxazolidinone resistance genes and characterization of genetic environments in enterococci of swine origin, Italy. Microorganisms 8(12):2021. https://doi.org/10.3390/microorganisms8122021

Freitas AR, Novais C, Ruiz-Garbajosa P et al (2009) Clonal expansion within clonal complex 2 and spread of vancomycin-resistant plasmids among different genetic lineages of *Enterococcus faecalis* from Portugal. J Antimicrob Chemother 63:1104–1111

Freitas AR, Coque TM, Novais C et al (2011) Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn *1546* on indistinguishable plasmids. J Clin Microbiol 49:925–931

Freitas AR, Tedim AP, Novais C et al (2020) Comparative genomics of global *optrA*-carrying *Enterococcus faecalis* uncovers a common chromosomal hotspot for *optrA* acquisition within a diversity of core and accessory genomes. Microb Genom 6:e000350. https://doi.org/10.1099/mgen.0.000350

Galloway-Pena J, Roh JH, Latorre M et al (2012) Genomic and SNP analyses demonstrate a distant separation of the hospital and community-associated clades of *Enterococcus faecium*. PLoS One 7:e30187

Garcia-Graells C, Ghenne D, Boland C et al (2019) La résistance antimicrobienne chez les *E. coli* ESBL et commensales, *Campylobacter* spp., *Salmonella* spp., *Staphylococcus aureus* résistants

à la méthicilline (MRSA) et *Enterococcus faecalis* et *faecium* isolés des populations d'animaux producteurs d'aliments (production primaire) et des denrées alimentaires en 2019. Available at: Microsoft Word - Rapport AMR_2019_FR_final.docx (favv-afsca.be)

Getachew Y, Hassan L, Zakaria Z et al (2013) Genetic variability of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans, chickens, and pigs in Malaysia. Appl Environ Microbiol 79:4528–4533

Ghosh A, Dowd SE, Zurek L (2011) Dogs leaving the ICU carry a very large multi-drug resistant enterococcal population with capacity for biofilm formation and horizontal gene transfer. PLoS One 6:e22451

Gilmore MS, Lebreton F, van Schaik W (2013) Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. Curr Opin Microbiol 16:10–16

Giraffa G (2002) Enterococci from foods. FEMS Microbiol Rev 26:163–171

Goossens H (1998) Spread of vancomycin-resistant enterococci: differences between the United States and Europe. Infect Control Hosp Epidemiol 19:546–551

Gouliouris T, Raven KE, Ludden C et al (2018) Genomic surveillance of *Enterococcus faecium* reveals limited sharing of strains and resistance genes between livestock and humans in the United Kingdom. mBio 9:e01780-18

Guardabassi L, Schwarz S, Lloyd DH (2004) Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother 54:321–332

Guzman Prieto AM, van Schaik W, Rogers MRC et al (2016) Global emergence and dissemination of Enterococci as nosocomial pathogens: attack of the clones? Front Microbiol 7:788. https://doi.org/10.3389/fmicb.2016.00788

Hammerum AM (2012) Enterococci of animal origin and their significance for public health. Clin Microbiol Infect 18:619–625

Hammerum AM, Flannagan SE, Clewell DB et al (2001) Indication of transposition of a mobile DNA element containing the *vat*(D) and *erm*(B) genes in *Enterococcus faecium*. Antimicrob Agents Chemother 45:3223–3225

Hammerum AM, Lester CH, Neimann J et al (2004) A vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected 7 years after the ban of avoparcin, is possibly related to pig isolates. J Antimicrob Chemother 53:547–549

Hammerum AM, Agerso Y, Garcia-Migura L et al (2009) Evaluation of the quinupristin/dalfopristin breakpoints for *Enterococcus faecium*. Int J Antimicrob Agents 34:288–290

Hegstad K, Mikalsen T, Coque TM et al (2010) Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. Clin Microbiol Infect 16:541–554

Herrero IA, Fernandez-Garayzabal JF, Moreno MA et al (2004) Dogs should be included in surveillance programs for vancomycin-resistant enterococci. J Clin Microbiol 42:1384–1385

Hollenbeck BL, Rice LB (2012) Intrinsic and acquired resistance mechanisms in *Enterococcus*. Virulence 3:421–433

Jackson CR, Fedorka-Cray PJ, Davis JA et al (2009) Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. J Appl Microbiol 107:1269–1278

Jensen LB (1998) Differences in the occurrence of two base pair variants of Tn*1546* from vancomycin-resistant enterococci from humans, pigs, and poultry. Antimicrob Agents Chemother 42:2463–2464

Jensen LB, Hammerum AM, Aerestrup FM et al (1998) Occurrence of *satA* and *vgb* genes in streptogramin-resistant *Enterococcus faecium* isolates of animal and human origins in the Netherlands. Antimicrob Agents Chemother 42:3330–3331

Jensen LB, Willems RJ, van den Bogaard AE (2003) Genetic characterization of glycopeptide-resistant enterococci of human and animal origin from mixed pig and poultry farms. APMIS 111:669–672

Jung WK, Hong SK, Lim JY et al (2006) Phenotypic and genetic characterization of vancomycin-resistant enterococci from hospitalized humans and from poultry in Korea. FEMS Microbiol Lett 260:193–200

Kelesidis T, Chow AL (2013) Proximity to animal or crop operations may be associated with de novo daptomycin-non-susceptible *Enterococcus* infection. Epidemiol Infect 15:1–4

Kieke AL, Borchardt MA, Kieke BA et al (2006) Use of streptogramin growth promoters in poultry and isolation of streptogramin-resistant *Enterococcus faecium* from humans. J Infect Dis 194: 1200–1208

Kim EB, Marco ML (2014) Nonclinical and clinical *Enterococcus faecium* strains, but not *Enterococcus faecalis* strains, have distinct structural and functional genomic features. Appl Environ Microbiol 80:154–165

Klare I, Heier H, Claus H et al (1993) Environmental strains of *Enterococcus faecium* with inducible high-level resistance to glycopeptides. FEMS Microbiol Lett 106:23–29

Klare I, Heier H, Claus H et al (1995) *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol Lett 125:165–171

Klare I, Badstubner D, Konstabel C et al (1999) Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Resist 5:45–52

Klare I, Konstabel C, Badstubner D et al (2003) Occurrence and spread of antibiotic resistances in *Enterococcus faecium*. Int J Food Microbiol 88:269–290

Kuch A, Willems RJ, Werner G et al (2012) Insight into antimicrobial susceptibility and population structure of contemporary human *Enterococcus faecalis* isolates from Europe. J Antimicrob Chemother 67:551–558

Kudo M, Nomura T, Yomoda S et al (2014) Nosocomial infection caused by vancomycin-susceptible multidrug-resistant *Enterococcus faecalis* over a long period in a university hospital in Japan. Microbiol Immunol 58:607–614

Larsen J, Schonheyder HC, Lester C et al (2010) Porcine-origin gentamicin-resistant *Enterococcus faecalis* in humans, Denmark. Emerg Infect Dis 16:682–684

Lebreton F, van Schaik W, Manson MA et al (2013) Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains. MBio 4:e00534–e00513

Leclercq R, Derlot E, Duval J et al (1988) Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N Engl J Med 319:157–161

Lee T, Jordan D, Sahibzada S, Abraham R et al (2021) Antimicrobial resistance in porcine enterococci in Australia and the ramifications for human health. Appl Environ Microbiol 87: e03037–e03020

León-Sampedro R, Del Campo R, Rodriguez-Baños M et al (2019) Phylogenomics of *Enterococcus faecalis* from wild birds: new insights into host-associated differences in core and accessory genomes of the species. Environ Microbiol 21:3046–3062

Lester CH, Hammerum AM (2010) Transfer of *vanA* from an *Enterococcus faecium* isolate of chicken origin to a CC17 *E. faecium* isolate in the intestine of cephalosporin-treated mice. J Antimicrob Chemother 65:1534–1536

Lester CH, Frimodt-Moller N, Sørensen TL et al (2006) *In vivo* transfer of the *vanA* resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother 50:596–599

Lopez M, Saenz Y, Rojo-Bezares B et al (2009) Detection of *vanA* and *vanB2*-containing enterococci from food samples in Spain, including *Enterococcus faecium* strains of CC17 and the new singleton ST425. Int J Food Microbiol 133:172–178

Lozano C, González-Barrio D, García JT et al (2015) Detection of vancomycin-resistant *Enterococcus faecalis* ST6-vanB2 and *E. faecium* ST915-*vanA* in faecal samples of wild *Rattus rattus* in Spain. Vet Microbiol 177:168–174

Ma X, Zhang F, Bai B et al (2021) Linezolid resistance in *Enterococcus faecalis* associated with urinary tract infections of patients in a tertiary hospitals in China: resistance mechanisms, virulence, and risk factors. Front Public Health 9:570650. https://doi.org/10.3389/fpubh.2021.570650

Manson JM, Keis S, Smith JM et al (2003) Characterization of a vancomycin-resistant *Enterococcus faecalis* (VREF) isolate from a dog with mastitis: further evidence of a clonal lineage of VREF in New Zealand. J Clin Microbiol 41:3331–3333

Manson AL, Van Tyne D, Straub TJ et al (2019) Chicken meat-associated enterococci: influence of agricultural antibiotic use and connection to the clinic. Appl Environ Microbiol 85: e01559–e01519

Marques C, Belas A, Franco A et al (2018) Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. J Antimicrob Chemother 73:377–384

Martone WJ (1998) Spread of vancomycin-resistant enterococci: why did it happen in the United States? Infect Control Hosp Epidemiol 19:539–545

McBride SM, Fischetti VA, Leblanc DJ et al (2007) Genetic diversity among *Enterococcus faecalis*. PLoS One 2:e582

McHugh MP, Parcell BJ, Pettigrew KA et al (2022) Presence of *optrA*-mediated linezolid resistance in multiple lineages and plasmids of *Enterococcus faecalis* revealed by long read sequencing. Microbiology (Reading) 168(2). https://doi.org/10.1099/mic.0.001137

Murray BE (1990) The life and times of the *enterococcus*. Clin Microbiol Rev 3:46–65

NAOR (Nippon AMR One Health Report) (2019) The AMR One Health Surveillance Committee. Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, Ministry of Health, Labour and Welfare, Tokyo

NARMS (National Antimicrobial Resistance Monitoring System) Now. Available at: NARMS Now: Integrated Data | FDA. Date accessed 18 Feb 2022

NethMap-MARAN (2016) Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2015; Nethmap: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. Available at MARAN reports - WUR

NethMap-MARAN (2017) Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2016; Nethmap: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. Available at MARAN reports - WUR

Nobrega DB, Tang KL, Caffrey NP et al (2021) Prevalence of antimicrobial resistance genes and its association with restricted antimicrobial use in food-producing animals: a systematic review and meta-analysis. J Antimicrob Chemother 76:561–575

NORM/NORM-VET (2019) Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø / Oslo 2020. ISSN:1502-2307 (print) / 1890-9965 (electronic). Available at: NORM-VET reports (vetinst.no)

NORM/NORM-VET (2020) Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø / Oslo 2021. ISSN:1502-2307 (print) / 1890–9965 (electronic). Available at: NORM and NORM-VET: Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway - NIPH (fhi.no)

Novais C, Coque TM, Costa MJ et al (2005) High occurrence and persistence of antibiotic-resistant enterococci in poultry food samples in Portugal. J Antimicrob Chemother 56:1139–1143

Novais C, Freitas AR, Silveira E et al (2013) Spread of multidrug-resistant *Enterococcus* to animals and humans: an underestimated role for the pig farm environment. J Antimicrob Chemother 68: 2746. https://doi.org/10.1093/jac/dkt289

O'Dea M, Sahibzada S, Jordan D et al (2019) Genomic, antimicrobial resistance, and public health insights into *Enterococcus* spp. from Australian chickens. J Clin Microbiol 57:e00319–e00319

Oravcova V, Zurek L, Townsend A et al (2014) American crows as carriers of vancomycin-resistant enterococci with *vanA* gene. Environ Microbiol 16:939–949

Palmer KL, Godfrey P, Griggs A et al (2012) Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. MBio 3:e00318–e00311

Pantosti A, Del Grosso M, Tagliabue S et al (1999) Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. Lancet 354:741–742

Park K, Jeong YS, Chang J et al (2020) Emergence of *optrA*-mediated linezolid-nonsusceptible *Enterococcus faecalis* in a tertiary care hospital. Ann Lab Med 40:321–325

Poeta P, Costa D, Rodrigues J et al (2005) Study of faecal colonization by *vanA*-containing *Enterococcus* strains in healthy humans, pets, poultry and wild animals in Portugal. J Antimicrob Chemother 55:278–280

Pöntinen AK, Top J, Arredondo-Alonso S et al (2021) Apparent nosocomial adaptation of *Enterococcus faecalis* predates the modern hospital era. Nat Commun 12:1523. https://doi.org/10.1038/s41467-021-21749-5

Poulsen LL, Bisgaard M, Son NT et al (2012) *Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam. Emerg Infect Dis 18:1096–1100

Quinones D, Kobayashi N, Nagashima S (2009) Molecular epidemiologic analysis of *Enterococcus faecalis* isolates in Cuba by multilocus sequence typing. Microb Drug Resist 15:287–293

Raven KE, Reuter S, Reynolds R et al (2016a) A decade of genomic history for healthcare-associated *Enterococcus faecium* in the United Kingdom and Ireland. Genome Res 26: 1388–1396

Raven KE, Reuter S, Gouliouris T et al (2016b) Genome-based characterization of hospital-adapted *Enterococcus faecalis* lineages. Nat Microbiol 1:15033. https://doi.org/10.1038/nmicrobiol.2015.33

ResistanceMap: Antibiotic resistance (2022) The Center for Disease, Dynamics Economics & Policy. ResistanceMap: Antibiotic resistance. Available at: https://resistancemap.cddep.org/CountryPage.php?country=United States. Date accessed 18 Feb 2022

Rosselli Del Turco E, Bartoletti M, Dahl A et al (2021) How do I manage a patient with enterococcal bacteraemia? Clin Microbiol Infect 27:364–371

Ruiz-Garbajosa P, Bonten MJ, Robinson DA et al (2006) Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of recombination. J Clin Microbiol 44:2220–2228

Rushton-Green R, Darnell RL, Taiaroa G et al (2019) Agricultural origins of a highly persistent lineage of vancomycin-resistant *Enterococcus faecalis* in New Zealand. Appl Environ Microbiol 85:e00137–e00119

Saavedra SY, Bernal JF, Montilla-Escudero E et al (2020) Vigilancia nacional de aislamientos clínicos de *Enterococcus faecalis* resistentes al linezolid portadores del gen *optrA* en Colombia, 2014-2019 [National surveillance of clinical isolates of Enterococcus faecalis resistant to linezolid carrying the optrA gene in Colombia, 2014-2019]. Rev Panam Salud Publica 44: e104. Spanish. https://doi.org/10.26633/RPSP.2020.104

Simjee S, White DG, McDermott PF et al (2002) Characterization of Tn*1546* in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. J Clin Microbiol 40:4659–4665

Simonsen GS, Haaheim H, Dahl KH et al (1998) Transmission of VanA-type vancomycin-resistant enterococci and vanA resistance elements between chicken and humans at avoparcin-exposed farms. Microb Drug Resist 4:313–318

Sletvold H, Johnsen PJ, Simonsen GS et al (2007) Comparative DNA analysis of two *vanA* plasmids from *Enterococcus faecium* strains isolated from poultry and a poultry farmer in Norway. Antimicrob Agents Chemother 51:736–739

Somarajan SR, Murray BE (2013) Could a phosphotransferase system provide the means to control outbreaks of *Enterococcus faecium* infection? J Infect Dis 207:1633–1636

Sørensen TL, Blom M, Monnet DL et al (2001) Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. N Engl J Med 345:1161–1166

Sparo M, Urbizu L, Solana MV et al (2012) High-level resistance to gentamicin: genetic transfer between *Enterococcus faecalis* isolated from food of animal origin and human microbiota. Lett Appl Microbiol 54:119–125

Spiliopoulou I, Damani A, Chini V et al (2011) Linezolid-resistant enterococci in Greece: epidemiological characteristics. Chemotherapy 57:181–185

Stobberingh E, van den Bogaard AE, London N et al (1999) Enterococci with glycopeptide resistance in turkeys, Turkey farmers, Turkey slaughterers, and (sub)urban residents in the south of the Netherlands: evidence for transmission of vancomycin resistance from animals to humans? Antimicrob Agents Chemother 43:2215–2221

Suetens C, Latour K, Kärki T et al (2018) Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term

care facilities: results from two European point prevalence surveys, 2016 to 2017. Euro Surveill 23:1800516. https://doi.org/10.2807/1560-7917.ES.2018.23.46.1800516

Sundsfjord A, Simonsen GS, Courvalin P (2001) Human infections caused by glycopeptide-resistant *Enterococcus* spp: are they a zoonosis? Clin Microbiol Infect 7(Suppl4):16–33

Swedres-Svarm (2016) Consumption of antibiotics and occurrence of resistance in Sweden. Solna/Uppsala ISSN1650-6332. Available at: Swedres-Svarm reports - SVA

Swiss Antibiotic Resistance Report 2018 (2018) Federal Office of Public Health and Federal Food Safety and Veterinary Office. Usage of Antibiotics and Occurrence of Antibiotic Resistance in Bacteria from Humans and Animals in Switzerland. FOPH publication number: 2018-OEG-87. Available at: www.bag.admin.ch

Tamang MD, Moon DC, Kim SR et al (2017) Detection of novel oxazolidinone and phenicol resistance gene *optrA* in enterococcal isolates from food animals and animal carcasses. Vet Microbiol 201:252–256

Tedim AP, Ruiz-Garbajosa P, Corander J et al (2015) Population biology of intestinal *Enterococcus* isolates from hospitalized and non-hospitalized individuals in different age groups. Appl Environ Microbiol 81:1820–1831

Timmermans M, Bogaerts B, Vanneste K et al (2021) Large diversity of linezolid-resistant isolates discovered in food-producing animals through linezolid selective monitoring in Belgium in 2019. J Antimicrob Chemother 77:49–57

Torres C, Reguera JA, Sanmartin MJ et al (1994) *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. J Antimicrob Chemother 33:553–561

Tsilipounidaki K, Gerontopoulos A, Papagiannitsis C et al (2019) First detection of an *optrA*-positive, linezolid-resistant ST16 *Enterococcus faecalis* from human in Greece. New Microbes New Infect 29:100515. https://doi.org/10.1016/j.nmni.2019.01.010

Tyson GH, Sabo JL, Hoffmann M et al (2018) Novel linezolid resistance plasmids in *Enterococcus* from food animals in the USA. J Antimicrob Chemother 73:3254–3258

Tzavaras I, Siarkou VI, Zdragas A et al (2012) Diversity of *vanA*-type vancomycin-resistant *Enterococcus faecium* isolated from broilers, poultry slaughterers and hospitalized humans in Greece. J Antimicrob Chemother 67:1811–1818

Uttley AH, Collins CH, Naidoo J et al (1988) Vancomycin-resistant enterococci. Lancet 1:57–58

van Belkum A, van den Braak N, Thomassen R et al (1996) Vancomycin-resistant enterococci in cats and dogs. Lancet 348:1038–1039

van den Bogaard AE, Mertens P, London NH et al (1997a) High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in the Netherlands: is the addition of antibiotics to animal feeds to blame? J Antimicrob Chemother 40:454–456

van den Bogaard AE, Jensen LB, Stobberingh EE (1997b) Vancomycin-resistant enterococci in turkeys and farmers. N Engl J Med 337:1558–1559

van den Bogaard AE, Bruinsma N, Stobberingh EE (2000) The effect of banning avoparcin on VRE carriage in the Netherlands. J Antimicrob Chemother 46:146–148

van den Bogaard AE, Willems RJ, London N et al (2002) Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 49:497–505

van Hal SJ, Willems RJL, Gouliouris T et al (2022) The interplay between community and hospital *Enterococcus faecium* clones within health-care settings: a genomic analysis. Lancet Microbe 3:e133–e141

van Schaik W, Top J, Riley DR et al (2010) Pyrosequencing-based comparative genome analysis of the nosocomial pathogen *Enterococcus faecium* and identification of a large transferable pathogenicity island. BMC Genomics 11:239

Vorobieva V, Roer L, Justesen US et al (2017) Detection of the *optrA* gene in a clinical ST16 *Enterococcus faecalis* isolate in Denmark. J Glob Antimicrob Resist 10:12–13

Wada Y, Irekeola AA, EAR ENS et al (2021) Prevalence of vancomycin-resistant *Enterococcus* (VRE) in companion animals: the first meta-analysis and systematic review. Antibiotics (Basel) 10:138

Wallinga D, Burch DG (2013) Does adding routine antibiotics to animal feed pose a serious risk to human health? BMJ 347:f4214

Watanabe S, Kobayashi N, Quiñones D et al (2009) Genetic diversity of enterococci harboring the high-level gentamicin resistance gene *aac(6′)-Ie-aph(2″)-Ia* or *aph(2″)-Ie* in a Japanese hospital. Microb Drug Resist 15:185–194

Wegener HC, Madsen M, Nielsen N et al (1997) Isolation of vancomycin resistant *Enterococcus faecium* from food. Int J Food Microbiol 35:57–66

Weiner-Lastinger LM, Abner S, Edwards JR et al (2020) Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015-2017. Infect Control Hosp Epidemiol 41:1–18

Welton LA, Thal LA, Perri MB et al (1998) Antimicrobial resistance in enterococci isolated from Turkey flocks fed virginiamycin. Antimicrob Agents Chemother 42:705–708

Weng PL, Ramli R, Shamsudin MN et al (2013) High genetic diversity of *Enterococcus faecium* and *Enterococcus faecalis* clinical isolates by pulsed-field gel electrophoresis and multilocus sequence typing from a hospital in Malaysia. Biomed Res Int 2013:938937. https://doi.org/10.1155/2013/938937

Werner G, Klare I, Witte W (1998) Association between quinupristin/dalfopristin resistance in glycopeptide-resistant *Enterococcus faecium* and the use of additives in animal feed. Eur J Clin Microbiol Infect Dis 17:401–402

Werner G, Coque TM, Franz CM et al (2013) Antibiotic resistant enterococci-Tales of a drug resistance gene trafficker. Int J Med Microbiol 303:360–379

Willems RJ, Hanage WP, Bessen DE et al (2011) Population biology of gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS Microbiol Rev 35:872–900

Willems RJ, Top J, van Schaik W (2012) Restricted gene flow among hospital subpopulations of *Enterococcus faecium*. MBio 3:e00151–e00112

Woodford N, Adebiyi AM, Palepou MF et al (1998) Diversity of VanA glycopeptide resistance elements in enterococci from humans and nonhuman sources. Antimicrob Agents Chemother 42:502–508

World Organization for Animal Health (OIE) (2021) Fifth OIE annual report on antimicrobial agents intended for use in animals. Available at: www.oie.int

Yang W, Xu Q, Yang Q et al (2022) Characterization of a novel RepA_N-family plasmid harbouring the phenicol-oxazolidinone resistance gene *optrA* in *Enterococcus faecalis* ST16 high-risk clone of goat origin. Vet Microbiol 266:109340. https://doi.org/10.1016/j.vetmic.2022.109340

Zhang Y, Schmidt JW, Arthur TM et al (2021) A comparative quantitative assessment of human exposure to various antimicrobial-resistant bacteria among U.S. ground beef consumers. J Food Prot 84:736–759

Methicillin-Resistant *Staphylococcus aureus* in Food Animals

20

Host-Adaptive Evolution, Epidemiology, and Public Health Threat

Anders Rhod Larsen, J. Ross Fitzgerald, and Jesper Larsen

Contents

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic human pathogen primarily associated with skin and soft tissue infections (SSTIs), but it is also an important cause of invasive and life-threatening infections. The emergence of livestock-associated MRSA (LA-MRSA) clones over the last two decades is worrisome, as they pose an additional threat to human health. LA-MRSA is most prevalent in industrial pig production systems in Europe and Asia but has also been increasingly recognized in other food animals and geographical regions. *S. aureus* has a remarkable ability to adapt to different host species, which is mediated by fixation of beneficial mutations and acquisition of mobile genetic elements encoding antimicrobial resistance determinants as well as colonization and virulence factors. LA-MRSA is a major cause of SSTIs among livestock workers, who are thought to serve as a source of transmission

A. R. Larsen · J. Larsen (✉)
Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark
e-mail: jrl@ssi.dk

J. R. Fitzgerald
The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_51

to their household members and into the local rural community, from where the bacterium can spread into healthcare settings. Importantly, these populations include a higher proportion of elderly and immunocompromised people with an elevated risk of developing invasive staphylococcal illnesses. In contrast, the risk of foodborne transmission appears to be low, even though LA-MRSA is a frequent contaminant of retail foods. Thus, there is an urgent need to identify and implement effective control measures to prevent spillover of LA-MRSA from livestock workers into the general population.

Keywords

Staphylococcus aureus · Methicillin-resistant *Staphylococcus aureus* · MRSA; Antibiotic resistance; Antimicrobial resistance · Methicillin resistance · Humans · Animals · Food animals · Livestock; Zoonosis · Adaptation · Evolution · Epidemiology · Colonization · Transmission · Infection · Infectious diseases · Disease burden · Public health · One Health

20.1 Introduction

Staphylococcus aureus is an opportunistic pathogen that colonizes the nares, pharynx, perineum, and other mucosal surfaces of about 50% of the human population worldwide, of whom about 20% are persistent carriers (Lowy 1998; Wertheim et al. 2005; van Belkum et al. 2009). *S. aureus* usually spreads through direct exposure (e.g., via hands) to colonized persons, and people colonized with *S. aureus* are at increased risk for developing infections, especially when the skin or mucosal barriers are breached or if the immune system is suppressed (Lowy 1998; von Eiff et al. 2001; Wertheim et al. 2005). *S. aureus* is primarily associated with skin and soft tissue infections (SSTIs) but is also a prominent cause of invasive, life-threatening infections, including bacteremia, endocarditis, osteomyelitis, and arthritis (Lowy 1998; Wertheim et al. 2005).

Methicillin-resistant *S.* aureus (MRSA) continues to be one of the most common antibiotic-resistant bacteria causing invasive disease in humans. It is estimated that in Europe alone MRSA causes approximately 171,000 invasive infections each year (ECDC and EMA 2009). MRSA is resistant to nearly all β-lactam antibiotics, which has serious implications for the treatment of severe infections. For example, it has been estimated that MRSA was responsible for more than 100,000 deaths and 3.5 million disability-adjusted life-years (DALYs) attributable to methicillin resistance based on an alternative scenario in which all MRSA infections were replaced by methicillin-susceptible *S. aureus* (MSSA) infections (Antimicrobial Resistance Collaborators 2022). As a consequence, the World Health Organization now considers MRSA to be one of the greatest threats to human health (WHO 2017). MRSA was first described in patients in 1961 shortly after the introduction of the β-lactam antibiotic, methicillin, as a treatment option against emerging penicillin-resistant *S. aureus* (Jevons 1961). For a long while, hospitals and other healthcare institutions

were the only reservoir for MRSA, but this changed dramatically in the 1990s with the emergence of numerous MRSA clones in the community (Chambers and DeLeo 2009). These geographically and evolutionary distinct community-associated MRSA (CA-MRSA) clones, such as USA300 in North America, continue to pose a major threat to public health due to their rapid spread in the general population and their ability to cause infections in young and otherwise healthy people.

Methicillin resistance in *S. aureus* is mediated by the *mecA* or *mecC* gene encoding enzymes called penicillin-binding protein 2a (PBP2a) and penicillin-binding protein 2c (PBP2c), respectively, which confer resistance to virtually all β-lactam antibiotics, including penicillinase-labile penicillins (e.g., penicillin G), penicillinase-stabile penicillins (e.g., methicillin), and cephalosporins (e.g., cefoxitin). The *mecA* and *mecC* genes are located on chromosomally integrated mobile genetic elements (MGEs) known as staphylococcus cassette chromosome *mec* (SCC*mec*), which can be classified into types defined by the combination of the type of *ccr* gene complex, which encodes unique site-specific cassette chromosome recombinases, and the class of the *mec* gene complex, which is composed of *mecA* or *mecC* and their regulatory genes (IWG-SCC 2009). MRSA clones are commonly defined by their multilocus sequence type (e.g., ST8) or clonal complex (e.g., CC8; a CC is a group of STs sharing at least five of seven identical alleles with at least one other ST in the group) and the SCC*mec* type (e.g., SCC*mec* IV).

The first reports of MRSA in pigs and pig farmers originated from France and the Netherlands and were published in 2005 (Armand-Lefevre et al. 2005; Voss et al. 2005), which marked the beginning of a new wave of methicillin resistance. Livestock-associated MRSA (LA-MRSA) is most prevalent in industrial pig production systems but has also been reported from other food animals worldwide, although the specific clones that have emerged vary with geographical location. For example, CC398 is the predominant LA-MRSA clone in Europe, whereas most LA-MRSA isolates from Asia belong to CC9. There is a wealth of literature concerning the presence, characterization, and geographic distribution of LA-MRSA clones in food animals, including the latest edition of this book and recent literature dedicated entirely to this subject (Fitzgerald 2012; Guardabassi et al. 2013; Chuang and Huang 2015; Butaye et al. 2016; Fitzgerald and Holden 2016; Aires-de-Sousa 2017; Haag et al. 2019). Our goal here is to discuss the origin, evolution, and host adaptation of *S. aureus* in animals and the epidemiology and disease burden of LA-MRSA in the human population, with special emphasis on CC398.

20.2 Origin and Evolution of MRSA in Animals

S. aureus is a model multihost pathogen responsible for an array of important infections in both humans and food animals. The evolutionary origin of *S. aureus* in different animal hosts has been an area of significant research interest over the last 10 years. In particular, the remarkable ability of *S. aureus* to adapt to different host species has underpinned its expansion into new host niches and its corresponding

economic and veterinary health impact. An understanding of the mechanisms facilitating *S. aureus* host adaptation can reveal novel targets for the development of therapeutics for controlling infections, some of which might be relevant to multiple host species, including humans.

The availability of large numbers of whole genome sequences for *S. aureus* isolates from different clinical, geographic, temporal, and host sources has led to significant improvements in our ability to investigate the evolution of *S. aureus* (Fitzgerald and Holden 2016). Although studies of animal *S. aureus* clones have traditionally lagged behind research of human *S. aureus* clones, large numbers of whole genome sequences have recently become available for animal isolates, thus enabling us to trace the events that have promoted the evolution of animal-specific clones (Richardson et al. 2018; Hoekstra et al. 2020; O'Dea et al. 2020; Ekesi et al. 2021; Shittu et al. 2021). These population genomic studies also provide a framework to investigate the mechanisms of pathogenesis that underpin adaptation to different host species.

Several studies have employed a population genomic approach to explore the evolutionary history of animal *S. aureus* isolates and their relatedness to human *S. aureus* isolates. Richardson et al. provided the most comprehensive analysis to date, involving over 800 *S. aureus* isolates from 43 different host species across 50 countries (Richardson et al. 2018). The analysis demonstrated that much of the diversity within *S. aureus* is represented by human isolates, while animal isolates often represent animal-specific subtypes that are interspersed among human clones in the *S. aureus* species phylogeny. Evolutionary analysis revealed that animal clones have evolved through host switch events with humans being a major hub, although cows also represent a reservoir for new pathogenic clones emerging in human populations. The earliest of these host switch events occurred several thousand years ago and have continued up to recent decades, correlating with the Neolithic era and the subsequent expansion, industrialization, and globalization of agriculture.

After a host switch event, *S. aureus* has been shown to be able to overcome narrow bottlenecks and high levels of genetic drift that reduce fixation of beneficial mutations in order to adapt to the new host species. In an experimental model of host switching, adaptive mutations were rapidly selected for and swept through the infecting population (Bacigalupe et al. 2019). In fact, single mutations can have a powerful impact on the capacity for bacterial host adaptation. For example, Viana et al. demonstrated that clinical *S. aureus* isolates recovered from natural infections of rabbits contain mutations in the *dltB* gene that are essential and sufficient for pathogenicity (Viana et al. 2015). In addition to mutations, acquisition of genes that are beneficial in the new host niche is critical for survival in the early stages after a host switch. MGEs such as prophages, staphylococcal pathogenicity islands (SaPIs), and plasmids have been identified that encode effectors facilitating innate immune evasion in different host species. For example, human *S. aureus* clones harbor the so-called immune evasion cluster 1 (IEC1) on a ΦSa3int prophage integrated into the *hlb* gene on the bacterial chromosome (Bae et al. 2006; Richardson et al. 2018). The IEC1 element encodes one or more determinants, including staphylococcal complement inhibitor (SCIN), chemotaxis inhibitory protein of staphylococci

(CHIPS), staphylokinase (SAK), staphylococcal enterotoxin A (SEA), and staphylococcal enterotoxin P (SEP), each of which interacts specifically with components of the human innate immune system (Thammavongsa et al. 2015). In addition, a family of phages encodes leukocidin toxins, which have a host-dependent tropism for neutrophils (Spaan et al. 2017). SaPIs commonly encode superantigens that contribute to immune evasion by stimulating the activation of host-specific subsets of T cells, leading to dysregulation of the T cell immune response (Deringer et al. 1997; Wilson et al. 2018). SaPIs can also encode von Willebrand factor binding proteins (vWbps) that stimulate coagulation of plasma promoting abscess formation (McAdow et al. 2012). While all *S. aureus* isolates contain a chromosomal copy of the *vwb* gene, the encoded vWbp does not coagulate plasma from ruminants but the SaPI-encoded variant does, thereby conferring the capacity for abscess formation in cows, sheep, and horses (Viana et al. 2010). In parallel to gene acquisition, gene loss is a common feature of host adaptation as demonstrated particularly for ruminant *S. aureus* (Guinane et al. 2010; Richardson et al. 2018). A particularly striking example is the evolution of a subtype of *S. aureus* subsp. *anaerobius,* a species that is a highly host-specialized pathogen associated with a specific pathology of superficial lymph nodes in goats and sheep known as Morel's disease. During host restriction, large chromosomal rearrangements have occurred along with the accumulation of over 200 pseudogenes, resulting in a highly fastidious metabolism (Yebra et al. 2021). Moreover, the number of insertion sequences (IS) has expanded, particularly in intergenic regions where they provide distinct mechanisms for the control of expression of flanking genes (Yebra et al. 2021). In addition to gene acquisition and loss, over a longer period of time in the new host, mutation and recombination contribute to further refinement to differences in nutrient availability that exist in the new host. For example, bovine *S. aureus* exhibits enhanced utilization of lactose, the major source of carbohydrate in milk, which might be the result of mutations affecting expression of sugar transport systems (Richardson et al. 2018).

The evolutionary history of important *S. aureus* clones in food animals has been explored previously. For example, the first report of a host switch event leading to the emergence of a poultry-adapted *S. aureus* CC5 clone provided evidence for its origin in humans followed by acquisition of avian-specific MGEs that promote protection against killing by avian heterophils (Lowder et al. 2009; Ekesi et al. 2021). The *S. aureus* CC5 clone was subsequently disseminated around the world via the globalized broiler poultry industry.

Subsequently, Price et al. used a comparative genomic approach to dissect the evolutionary history of *S. aureus* CC398, which comprises the predominant LA-MRSA clone in Europe (Price et al. 2012). The analysis showed that LA-MRSA CC398 evolved from a human variant of *S. aureus* CC398, and that the host switch was accompanied by simultaneous loss of the IEC1-harboring ΦSa3int prophage and acquisition of a transposon containing the tetracycline resistance gene *tet*(M). This was later followed by acquisition of different SCC*mec* elements and other determinants conferring resistance to some of the most frequently used veterinary antimicrobials (Price et al. 2012; Sieber et al. 2018). LA-MRSA CC398 has become particularly widespread in large-scale confined pig holdings (EFSA 2009, 2010) but

is also found in other animals such as veal calves, horses, mink, and poultry, with some of the isolates showing signs of further host adaptation through gene acquisition (Abdelbary et al. 2014; Larsen et al. 2016; Hansen et al. 2020; EFSA and ECDC 2021). The rapid spread of LA-MRSA CC398 is primarily facilitated by animal movements, although spread via human carriers and contaminated fomites has also been documented (Grøntvedt et al. 2016; Sieber et al. 2018; Smith et al. 2018).

The other major LA-MRSA clone, CC9, is common in pigs in some parts of the world particularly in Asia (Ji et al. 2021; Jiang et al. 2021; Yu et al. 2021). Yu et al. showed that LA-MRSA CC9 evolved from a human variant of *S. aureus* CC9 through a stepwise trajectory resembling that of LA-MRSA CC398, including initial loss of the IEC1-harboring ΦSa3int prophage and subsequent acquisition of different SCC*mec* elements and other determinants, such as the tetracycline resistance gene *tet*(L) and the SaPI-encoded vWbp protein associated with abscess formation in cows, sheep, and horses (Yu et al. 2021).

20.3 Epidemiology and Disease Burden of LA-MRSA CC398 in Humans

Much of our knowledge about the epidemiology and disease burden of LA-MRSA in humans comes from studies on the CC398 clone in Europe.

20.3.1 Epidemiology

The mechanisms leading to *S. aureus* colonization, transmission, and infection are multifactorial and include both host and bacterial determinants (Wertheim et al. 2005). For example, *S. aureus* has to overcome several obstacles in order to colonize the nares. First, *S. aureus* needs to come in contact with the nose. Second, *S. aureus* must adhere to receptors in the nose. Third, *S. aureus* must be able to resist the host defenses. Finally, *S. aureus* needs to be able to propagate in the nose. Hands are considered to be the most important vector for *S. aureus* transmission to the nasal niche. Most *S. aureus* infections are caused by the patient's own *S. aureus* inhabiting the skin or mucosal membranes prior to disease onset, and persistent carriers therefore have a higher risk for developing an *S. aureus* infection (von Eiff et al. 2001). On the other hand, the mortality rate from *S. aureus* bacteremia is higher in non-carriers than in carriers, suggesting that partial immunity might play an important role (Wertheim et al. 2004).

LA-MRSA CC398 is frequently transmitted to persons who have direct exposure to food animals, leading to carriage rates of up to 87% (Cuny et al. 2009; Garcia-Graells et al. 2013). The frequency and duration of carriage seems to depend on the intensity of livestock exposure. Livestock workers might carry LA-MRSA CC398 outside the farm environment for long periods up to several weeks. For example, Köck et al. showed that the prevalence among pig farm workers decreased from 77% to 46% during summer vacation (Köck et al. 2012), while Graveland et al. found that

the prevalence among veal calf farmers was 26% to 11% in periods with and without animal exposure, respectively (Graveland et al. 2011). In contrast, persons with short-term exposure to positive animals are less likely to establish and maintain LA-MRSA CC398 carriage for more than a few days (van Cleef et al. 2011a; Angen et al. 2017). LA-MRSA CC398 is also commonly found in household contacts of colonized or infected livestock workers and veterinarians, albeit usually at relatively low frequencies of up to around 5% (Cuny et al. 2009; Garcia-Graells et al. 2013; van Cleef et al. 2014; Verkade et al. 2014). LA-MRSA CC398 is primarily associated with SSTIs among young and otherwise healthy livestock workers but is also capable of causing serious illness and even death in the general population, which includes a higher proportion of elderly and immunocompromised people with an elevated risk of developing invasive staphylococcal illnesses (Lekkerkerk et al. 2012, 2015; van Rijen et al. 2014; Deiters et al. 2015; Larsen et al. 2015, 2017; Kinross et al. 2017; Sieber et al. 2020). This is illustrated by surveillance data from Denmark, which showed that approximately 30% of all LA-MRSA CC398 SSTIs and 60% of all LA-MRSA CC398 bloodstream infections (BSIs) occur in people without livestock exposure (Larsen et al. 2017).

There is a clear spatiotemporal relationship between LA-MRSA CC398 infections in people with and without livestock exposure, suggesting that persons with direct livestock exposure might serve as a source of transmission to their household members (indirect exposure) and into the local rural community, from where the bacterium can spread into healthcare settings (Larsen et al. 2015). It should be noted, however, that the transmissibility of LA-MRSA CC398 in community and healthcare settings is significantly lower than that of other MRSA clones (Bootsma et al. 2011; Wassenberg et al. 2011; Hetem et al. 2013; Verkade et al. 2014), which might explain why LA-MRSA CC398 only causes sporadic illness in persons living in urban areas and minor outbreaks in healthcare institutions. LA-MRSA CC398 has undergone several genetic changes that in theory could have reduced the bacterium's ability to survive and cause disease in the human population. For example, LA-MRSA CC398 lacks many of the colonization and virulence factors found in human *S. aureus* clones, including extracellular matrix-binding protein-binding protein (Empbp), elastin-binding protein (EbpS), Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin 1 (TSST1), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C (SEC), staphylococcal enterotoxin K (SEK), staphylococcal enterotoxin L (SEL), staphylococcal enterotoxin Q (SEQ), and exfoliatin A (Hallin et al. 2011). LA-MRSA CC398 also lacks the IEC1 element and the associated ability to evade our innate immune system due to loss of the ΦSa3int prophage during the human-to-animal host switch event (Price et al. 2012). Finally, LA-MRSA CC398 has acquired several MGEs encoding resistance to a wide range of the most frequently used antimicrobial drugs in pigs, which are likely to exert a considerable fitness cost outside the livestock reservoir (Andersson and Hughes 2010; Sieber et al. 2018).

LA-MRSA CC398 is a frequent contaminant of retail foods in Europe, including pork, beef, veal, chicken, turkey, lamb, and mutton, and it therefore seems reasonable to assume that people are exposed to this bacterium with some frequency

through consumption or handling of contaminated food products (Kluytmans 2010). Indeed, it has been shown that contaminated poultry meat can be a source of LA-MRSA CC398 infection (Larsen et al. 2016). However, the majority of LA-MRSA CC398 infections occurs in persons living in rural areas where food animals are raised, as mentioned above, suggesting that the spread of LA-MRSA CC398 into the general population is mainly due to local transmission from nearby animal farms. LA-MRSA CC398 has also been detected in low concentrations in air and soil samples collected around pig farms, but it is currently unknown whether, and to what extent, these environments play a role in LA-MRSA CC398 transmission to humans (Schulz et al. 2012; Angen et al. 2021).

20.3.2 Disease Burden

MRSA continues to pose a significant public health threat worldwide. For example, MRSA accounted for approximately 22% of infections caused by antibiotic-resistant bacteria of public health concern within the European Union and European Economic Area (EU/EEA) during 2015, 21% of attributable deaths, and 19% of DALYs (Cassini et al. 2019). In a more recent study, MRSA was found to be the leading pathogen-drug combination worldwide during 2019, when it caused more than 100,000 deaths and 3.5 million DALYs (Antimicrobial Resistance Collaborators 2022). In contrast, the relative disease burden of LA-MRSA CC398 is geographically heterogeneous. Kinross et al. estimated the burden of human infections caused by MRSA CC398 in seven EU/EEA countries during 2013, measured as the proportion of LA-MRSA CC398 cases among the total number of MRSA cases (Kinross et al. 2017). Overall, LA-MRSA CC398 accounted for 9.0% of the total number of MRSA cases. Denmark, the Netherlands, and Spain had a substantially higher estimated disease burden of LA-MRSA CC398 than France, Germany, Norway, and Poland (16.7%, 21.5%, and 9.7% vs. 1.7%, 2.6%, 1.5%, and 1.8%, respectively). In comparison, the burden of LA-MRSA CC398 in Denmark and the Netherlands was much lower in a similar study conducted by van Cleef et al. in 2007 (1.6% and 11.9%, respectively) (van Cleef et al. 2011b). This temporal trend was paralleled by a shift in the proportion of MRSA bloodstream infections (BSIs) caused by LA-MRSA CC398, which increased from 0.6% in 2007 to 4.6% in 2013 (van Cleef et al. 2011b; Kinross et al. 2017). Surveillance data from Denmark and the Netherlands suggest that LA-MRSA CC398 still accounts for a substantial proportion of the total number of MRSA cases (DANMAP 2021; NethMap 2021).

There are two main reasons that explain the high relative burden of LA-MRSA CC398 in Denmark and the Netherlands. First, both countries are relatively small with a population of 5.8 and 17.3 million, respectively (https://data.worldbank.org/), but have large industrial pig production systems (https://www.fao.org/faostat/) with a high prevalence of LA-MRSA CC398 (EFSA and ECDC 2021). Second, Denmark and the Netherlands have very low levels of other MRSA clones compared to other European countries. This is illustrated by the fact that MRSA accounted for only 2.2% and 1.6% of all episodes of *S. aureus* BSI in Denmark and the Netherlands in

2019, respectively, when the population-weighted EU/EEA mean was 15.5% (ECDC 2020).

Surveillance data from Denmark illustrate the connectivity between the prevalence of LA-MRSA CC398 in pigs and the disease burden. Larsen et al. showed that the prevalence of LA-MRSA CC398 in Danish pig farms increased from 16% in 2010 to more than 60% in 2014, which was paralleled by an increasing number of LA-MRSA CC398 SSTIs and BSIs (Larsen et al. 2017). In 2014, LA-MRSA CC398 accounted for 16% and 21% of all MRSA BSIs and SSTIs, corresponding to 1.2 and 37.4 cases of BSI and SSTI per 1,000,000 person-years, respectively. Approximately 30% of all patients with LA-MRSA CC398 SSTI and 60% of all patients with LA-MRSA CC398 BSI reported neither direct nor indirect livestock exposure, reflecting the fact that the general population comprises a higher proportion of people at elevated risk of developing invasive infections, compared to people with livestock exposure. The 30-day case-fatality rates among patients with LA-MRSA CC398 BSI and other types of MRSA BSI were not significantly different from each other (35% vs. 21%), which suggests that LA-MRSA CC398 is as capable as other MRSA clones of causing death once it has entered the bloodstream.

20.3.3 Emerging Public Health Threats?

The last 10 years of population genomics have improved our understanding of the evolution of LA-MRSA. However, it is evident that new clones are emerging and existing clones continue to evolve. For example, two recent studies from Australia identified a hitherto unrecognized LA-MRSA clone, CC93 (Sahibzada et al. 2017, 2020). Analyses of the isolates showed that they are closely related to isolates belonging to the predominant CA-MRSA clone in Australia, CC93, suggesting that they might have evolved through a recent human-to-animal host switch event. In contrast to LA-MRSA CC398, a substantial proportion of the LA-MRSA CC93 isolates carry the same IEC1 element and PVL-encoding *lukS-PV* and *lukF-PV* genes as their presumed CA-MRSA CC93 progenitor. The presence of PVL in LA-MRSA CC93 has raised public health concerns because of the association between PVL and development of severe staphylococcal diseases, including sepsis, in young and otherwise healthy people (Tong et al. 2010).

Other studies have shown that LA-MRSA CC398 might be capable of adapting to the human host through acquisition the phage-encoded immune modulators. For example, a Danish study showed that 6% of human LA-MRSA CC398 isolates collected during 2004–2011 harbored the IEC1 element, while a German study found a low, albeit slightly increasing, prevalence of IEC1 among LA-MRSA CC398 isolates from hospital patients during 2000–2015, ranging from 1.1% during 2000–2006 to 3.9% during 2007–2015 (Larsen et al. 2015; van Alen et al. 2018). In another study, Gerlach et al. showed that 40% of LA-MRSA CC398 isolates from pigs in Denmark produce another phage-encoded immune modulator enzyme known as TarP, which enables *S. aureus* to evade antibody-mediated immune recognition by altering the dominant cell surface epitope known as wall teichoic acids (Gerlach

et al. 2018). In a later study, Sieber et al. were able to demonstrate that reacquisition of the IEC1-harboring ΦSa3int prophage by LA-MRSA CC398 facilitates household transmission and further spread into the community and healthcare settings (Sieber et al. 2020). The additional disease burden attributable to IEC1-positive LA-MRSA CC398 isolates was nevertheless relatively low, and the authors found no evidence to suggest that they have become self-sustainable in the general population. In contrast to IEC1, TarP did not influence household transmission of LA-MRSA CC398.

Antimicrobials are widely used for treatment of animals with clinical infectious disease, disease prevention, and growth promotion in food animals. In EU/EEA, antimicrobial use in food animals is estimated to account for approximately 50% of the annual antimicrobial consumption, some of which are important for human medicine, including the treatment of *S. aureus* infections (EMA 2019; ECDC et al. 2021). The widespread use of antimicrobials in food animals is thought to be a major driver of antimicrobial resistance and, in the worst case, might lead to the emergence of multidrug-resistant bacteria that are difficult to treat in humans. In accord with this view, most LA-MRSA isolates are resistant to multiple classes of veterinary antimicrobials such as β-lactams (mediated by the *mecA* or *mecC* gene), aminoglycosides, lincosamides, macrolides, pleuromutilins, quinolones, streptogramins, and tetracyclines. However, there are still several alternatives available for the treatment of LA-MRSA infections, including rifampin, fosfomycin, vancomycin, daptomycin, linezolid, fusidic acid, and tigecycline, although it should be noted that resistance to rifampin and linezolid has been described previously in LA-MRSA CC398 and LA-MRSA CC9 (Kehrenberg et al. 2009; Li et al. 2016).

20.4 Research Needs

The dynamic nature of *S. aureus* genome evolution and its striking host adaptability underscores the need for continued surveillance at the human-animal interface to detect genetic as well as epidemiologic changes that affect public health. Most LA-MRSA clones and isolates lack the IEC1 element, and many studies have used PCR-based detection of the *scn* gene encoding SCIN (a marker of IEC1) to differentiate between LA and human variants. However, many variables can influence the performance of this test in different settings, including the genetic background of the local *S. aureus* populations (e.g., a relatively high proportion of LA-MRSA CC93 harbors the IEC1 element) and local evolutionary events (e.g., reacquisition of the IEC1-harboring ΦSa3int prophage by LA-MRSA CC398). Whole genome sequencing (WGS) is increasingly used for *S. aureus* outbreak investigation and surveillance and, as summarized in the current chapter, has been used to investigate the epidemiology and population structure of LA-MRSA clones (Price et al. 2012; Yu et al. 2021). These reference population structures enable rapid and accurate typing of isolates based on their phylogenetic relationships with the reference isolates as well as identification of genetic changes that might affect the epidemiology. Furthermore,

machine learning approaches could be applied to identify high-risk clones with potential to spread and cause disease in human populations.

The impact of LA-MRSA on human morbidity and mortality will likely increase in the near future if LA-MRSA CC398 and other LA-MRSA clones are allowed to spread into the general population, and it is therefore important to identify and implement effective control measures to reduce the rate or level of LA-MRSA carriage among livestock workers when they leave the farm (e.g., handwashing and disinfection and change of clothes and footwear) in order to minimize the risk of transmission outside the farm environment. There is also a need for studies that evaluate the role of foodborne transmission and environmental contamination in the expansion of LA-MRSA into the general population.

Food animals also carry MSSA isolates (Hasman et al. 2010). Yet, their impact on public health has not been well studied. Preliminary surveillance data from Denmark show that two-thirds of all LA *S. aureus* CC398 BSI isolates are methicillin-susceptible, of which one-fourth harbor the IEC1 element (A. Larsen & J. Larsen, unpublished data). Future studies should aim to determine the relative disease burden of LA-MRSA and LA-MSSA in other parts of the world.

20.5 Conclusions

In summary, *S. aureus* has a remarkable capacity to adapt to different host species and acquire resistance to antimicrobials. These traits demand close and continued scrutiny via surveillance employing high-throughput WGS and phylodynamic and machine learning approaches in order to identify high-risk clones and implement measures to limit spread into and within human populations.

References

Abdelbary MM, Wittenberg A, Cuny C, Layer F, Kurt K, Wieler LH, Walther B, Skov R, Larsen J, Hasman H, Fitzgerald JR, Smith TC, Wagenaar JA, Pantosti A, Hallin M, Struelens MJ, Edwards G, Böse R, Nübel U, Witte W (2014) Phylogenetic analysis of *Staphylococcus aureus* CC398 reveals a sub-lineage epidemiologically associated with infections in horses. PLoS One 9:e88083

Aires-de-Sousa M (2017) Methicillin-resistant *Staphylococcus aureus* among animals: current overview. Clin Microbiol Infect 23:373–380

Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8:260–271

Angen Ø, Feld L, Larsen J, Rostgaard K, Skov R, Madsen AM, Larsen AR (2017) Transmission of methicillin-resistant *Staphylococcus aureus* to human volunteers visiting a swine farm. Appl Environ Microbiol 83:e01489–e01417

Angen Ø, Nielsen MW, Løfstrøm P, Larsen AR, Hendriksen NB (2021) Airborne spread of methicillin resistant *Staphylococcus aureus* from a swine farm. Front Vet Sci 8:644729

Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399:629–655

Armand-Lefevre L, Ruimy R, Andremont A (2005) Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11:711–714

Bacigalupe R, Tormo-Mas MÁ, Penadés JR, Fitzgerald JR (2019) A multihost bacterial pathogen overcomes continuous population bottlenecks to adapt to new host species. Sci Adv 5:eaax0063

Bae T, Baba T, Hiramatsu K, Schneewind O (2006) Prophages of *Staphylococcus aureus* Newman and their contribution to virulence. Mol Microbiol 62:1035–1047

Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ (2011) The nosocomial transmission rate of animal-associated ST398 meticillin-resistant *Staphylococcus aureus*. J R Soc Interface 8:578–584

Butaye P, Argudín MA, Smith TC (2016) Livestock-associated MRSA and its current evolution. Curr Clin Microbiol Rep 3:19–31

Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, Colomb-Cotinat M, Kretzschmar ME, Devleesschauwer B, Cecchini M, Ouakrim DA, Oliveira TC, Struelens MJ, Suetens C, Monnet DL, Burden of AMR Collaborative Group (2019) Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis 19:56–66

Chambers HF, DeLeo FR (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7:629–641

Chuang YY, Huang YC (2015) Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: an emerging issue? Int J Antimicrob Agents 45:334–340

Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W (2009) Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. PLoS One 4:e6800

Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (2021) DANMAP 2020. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. https://www.danmap.org/-/media/sites/danmap/downloads/reports/2020/danmap_2020_18102021_version-4_low.pdf?la=da&hash=6BCD0F486746BE9DBE7653C1B04F1681C99E3074

Deiters C, Günnewig V, Friedrich AW, Mellmann A, Köck R (2015) Are cases of methicillin-resistant Staphylococcus aureus clonal complex (CC) 398 among humans still livestock-associated? Int J Med Microbiol 305:110–113

Deringer JR, Ely RJ, Monday SR, Stauffacher CV, Bohach GA (1997) Vbeta-dependent stimulation of bovine and human T cells by host-specific staphylococcal enterotoxins. Infect Immun 65:4048–4054

Ekesi NS, Dolka B, Alrubaye AAK, Rhoads DD (2021) Analysis of genomes of bacterial isolates from lameness outbreaks in broilers. Poult Sci 100:101148

European Centre for Disease Prevention and Control (ECDC) (2020) Antimicrobial resistance in the EU/EEA (EARS-Net). Annual epidemiological report for 2019. https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2019.pdf

European Centre for Disease Prevention and Control (ECDC) & European Medicines Agencies (EMA) (2009) The bacterial challenge: time to react. A call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents. https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) & European Medicines Agencies (EMA) (2021) Third joint inter-agency report on integrated analysis of consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the EU/EEA. EFSA J 19:6712

European Food Safety Authority (EFSA) (2009) Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008. Part A: MRSA prevalence estimates. EFSA J 7:1376

European Food Safety Authority (EFSA) (2010) Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008. Part B: factors associated with MRSA contamination of holdings. EFSA J 8:1597

European Food Safety Authority (EFSA) & European Centre for Disease Prevention and Control (ECDC) (2021) The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA J 19:6490

European Medicines Agencies (EMA) (2019) Categorisation of antibiotics used in animals promotes responsible use to protect public and animal health. https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf

Fitzgerald JR (2012) Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends Microbiol 20:192–198

Fitzgerald JR, Holden MTG (2016) Genomics of natural populations of *Staphylococcus aureus*. Annu Rev Microbiol 70:459–478

Garcia-Graells C, van Cleef BA, Larsen J, Denis O, Skov R, Voss A (2013) Dynamic of livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 in pig farm households: a pilot study. PLoS One 8:e65512

Gerlach D, Guo Y, De Castro C, Kim SH, Schlatterer K, Xu FF, Pereira C, Seeberger PH, Ali S, Codée J, Sirisarn W, Schulte B, Wolz C, Larsen J, Molinaro A, Lee BL, Xia G, Stehle T, Peschel A (2018) Methicillin-resistant *Staphylococcus aureus* alters cell wall glycosylation to evade immunity. Nature 563:705–709

Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D (2011) Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS One 6: e16830

Grøntvedt CA, Elstrøm P, Stegger M, Skov RL, Skytt Andersen P, Larssen KW, Urdahl AM, Angen Ø, Larsen J, Åmdal S, Løtvedt SM, Sunde M, Bjørnholt JV (2016) Methicillin-resistant *Staphylococcus aureus* CC398 in humans and pigs: a "One Health" perspective on introduction and transmission. Clin Infect Dis 63:1431–1438

Guardabassi L, Larsen J, Weese JS, Butaye P, Battisti A, Kluytmans J, Lloyd DH, Skov RL (2013) Public health impact and antimicrobial selection of meticillin-resistant staphylococci in animals. J Glob Antimicrob Resist 1:55–62

Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, Lowder BV, Cartwright RA, Smyth DS, Smyth CJ, Lindsay JA, Gould KA, Witney A, Hinds J, Bollback JP, Rambaut A, Penadés JR, Fitzgerald JR (2010) Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol Evol 2:454–466

Haag AF, Fitzgerald JR, Penadés JR (2019) *Staphylococcus aureus* in animals. Microbiol Spectr 7: GPP3-0060-2019

Hallin M, De Mendonça R, Denis O, Lefort A, El Garch F, Butaye P, Hermans K, Struelens MJ (2011) Diversity of accessory genome of human and livestock-associated ST398 methicillin resistant *Staphylococcus aureus* strains. Infect Genet Evol 11:290–299

Hansen JE, Stegger M, Pedersen K, Sieber RN, Larsen J, Larsen G, Lilje B, Chriél M, Andersen PS, Larsen AR (2020) Spread of LA-MRSA CC398 in Danish mink (*Neovison vison*) and mink farm workers. Vet Microbiol 245:108705

Hasman H, Moodley A, Guardabassi L, Stegger M, Skov RL, Aarestrup FM (2010) *spa* type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. Vet Microbiol 141:326–331

Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ (2013) Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 19:1797–1802

Hoekstra J, Zomer AL, Rutten VPMG, Benedictus L, Stegeman A, Spaninks MP, Bennedsgaard TW, Biggs A, De Vliegher S, Mateo DH, Huber-Schlenstedt R, Katholm J, Kovács P, Krömker V, Lequeux G, Moroni P, Pinho L, Smulski S, Supré K, Swinkels JM, Holmes MA, Lam TJGM, Koop G (2020) Genomic analysis of European bovine *Staphylococcus aureus* from clinical versus subclinical mastitis. Sci Rep 10:18172

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (2009) Classification of Staphylococcal Cassette Chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. Antimicrob Agents Chemother 53:4961–4967

Jevons MP (1961) "Celbenin"-resistant staphylococci. Br Med J 1:124–125
Ji X, Krüger H, Tao J, Wang Y, Feßler AT, Bai R, Wang S, Dong Y, Shen J, Wang Y, Schwarz S, Wu C (2021) Comparative analysis of genomic characteristics, fitness and virulence of MRSA ST398 and ST9 isolated from China and Germany. Emerg Microbes Infect 10:1481–1494
Jiang N, Wyres KL, Li J, Feßler AT, Krüger H, Wang Y, Holt KE, Schwarz S, Wu C (2021) Evolution and genomic insight into methicillin-resistant *Staphylococcus aureus* ST9 in China. J Antimicrob Chemother 76:1703–1711
Kehrenberg C, Cuny C, Strommenger B, Schwarz S, Witte W (2009) Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr*. Antimicrob Agents Chemother 53:779–781
Kinross P, Petersen A, Skov R, Van Hauwermeiren E, Pantosti A, Laurent F, Voss A, Kluytmans J, Struelens MJ, Heuer O, Monnet DL, The European Human LA-MRSA Study Group (2017) Livestock-associated meticillin-resistant *Staphylococcus aureus* (MRSA) among human MRSA isolates, European Union/European Economic Area countries, 2013. *Euro Surveill* 22:pii=16-00696
Kluytmans JA (2010) Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? Clin Microbiol Infect 16:11–15
Köck R, Loth B, Köksal M, Schulte-Wülwer J, Harlizius J, Friedrich AW (2012) Persistence of nasal colonization with livestock-associated methicillin-resistant *Staphylococcus aureus* in pig farmers after holidays from pig exposure. Appl Environ Microbiol 78:4046–4047
Larsen J, Petersen A, Sørum M, Stegger M, van Alphen L, Valentiner-Branth P, Knudsen LK, Larsen LS, Feingold B, Price LB, Andersen PS, Larsen AR, Skov RL (2015) Meticillin-resistant *Staphylococcus aureus* CC398 is an increasing cause of disease in people with no livestock contact in Denmark, 1999 to 2011. Euro Surveill 20:pii=30021
Larsen J, Stegger M, Andersen PS, Petersen A, Larsen AR, Westh H, Agersø Y, Fetsch A, Kraushaar B, Käsbohrer A, Feβler AT, Schwarz S, Cuny C, Witte W, Butaye P, Denis O, Haenni M, Madec JY, Jouy E, Laurent F, Battisti A, Franco A, Alba P, Mammina C, Pantosti A, Monaco M, Wagenaar JA, de Boer E, van Duijkeren E, Heck M, Domínguez L, Torres C, Zarazaga M, Price LB, Skov RL (2016) Evidence for human adaptation and foodborne transmission of livestock-associated methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 63:1349–1352
Larsen J, Petersen A, Larsen AR, Sieber RN, Stegger M, Koch A, Aarestrup FM, Price LB, Skov RL, Danish MRSA Study Group (2017) Emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* bloodstream infections in Denmark. Clin Infect Dis 65:1072–1076
Lekkerkerk WS, van de Sande-Bruinsma N, van der Sande MA, Tjon-A-Tsien A, Groenheide A, Haenen A, Timen A, van den Broek PJ, van Wamel WJ, de Neeling AJ, Richardus JH, Verbrugh HA, Vos MC (2012) Emergence of MRSA of unknown origin in the Netherlands. Clin Microbiol Infect 18:656–661
Lekkerkerk WS, van Wamel WJ, Snijders SV, Willems RJ, van Duijkeren E, Broens EM, Wagenaar JA, Lindsay JA, Vos MC (2015) What is the origin of livestock-associated methicillin-resistant *Staphylococcus aureus* clonal complex 398 isolates from humans without livestock contact? An epidemiological and genetic analysis. J Clin Microbiol 53:1836–1841
Li J, Feβler AT, Jiang N, Fan R, Wang Y, Wu C, Shen J, Schwarz S (2016) Molecular basis of rifampicin resistance in multiresistant porcine livestock-associated MRSA. J Antimicrob Chemother 71:3313–3315
Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nübel U, Fitzgerald JR (2009) Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci U S A 106: 19545–19550
Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339:520–532
McAdow M, Missiakas DM, Schneewind O (2012) *Staphylococcus aureus* secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infection. J Innate Immun 4:141–148
National Institute for Public Health and the Environment & Dutch Working Group on Antibiotic Policy (NethMap) (2021) NethMap 2021. Consumption of antimicrobial agents and

antimicrobial resistance among medically important bacteria in the Netherlands. https://www.rivm.nl/bibliotheek/rapporten/2021-0062.pdf

O'Dea M, Abraham RJ, Sahibzada S, Lee T, Jordan D, Laird T, Pang S, Buller N, Stegger M, Coombs GW, Trott DJ, Abraham S (2020) Antimicrobial resistance and genomic insights into bovine mastitis-associated *Staphylococcus aureus* in Australia. Vet Microbiol 250:108850

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM (2012) *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *mBio* 3:e00305–e00311

Richardson EJ, Bacigalupe R, Harrison EM, Weinert LA, Lycett S, Vrieling M, Robb K, Hoskisson PA, Holden MTG, Feil EJ, Paterson GK, Tong SYC, Shittu A, van Wamel W, Aanensen DM, Parkhill J, Peacock SJ, Corander J, Holmes M, Fitzgerald JR (2018) Gene exchange drives the ecological success of a multi-host bacterial pathogen. Nat Ecol Evol 2:1468–1478

Sahibzada S, Abraham S, Coombs GW, Pang S, Hernández-Jover M, Jordan D, Heller J (2017) Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. Sci Rep 7:5273

Sahibzada S, Pang S, Hernández-Jover M, Jordan D, Abraham S, O'Dea M, Heller J (2020) Prevalence and antimicrobial resistance of MRSA across different pig age groups in an intensive pig production system in Australia. Zoonoses Public Health 67:576–586

Schulz J, Friese A, Klees S, Tenhagen BA, Fetsch A, Rösler U, Hartung J (2012) Longitudinal study of the contamination of air and of soil surfaces in the vicinity of pig barns by livestock-associated methicillin-resistant *Staphylococcus aureus*. Appl Environ Microbiol 78:5666–5671

Shittu AO, Taiwo FF, Froböse NJ, Schwartbeck B, Niemann S, Mellmann A, Schaumburg F (2021) Genomic analysis of *Staphylococcus aureus* from the West African Dwarf (WAD) goat in Nigeria. Antimicrob Resist Infect Control 10:122

Sieber RN, Skov RL, Nielsen J, Schulz J, Price LB, Aarestrup FM, Larsen AR, Stegger M, Larsen J (2018) Drivers and dynamics of methicillin-resistant livestock-associated *Staphylococcus aureus* CC398 in pigs and humans in Denmark. MBio 9:e02142–e02118

Sieber RN, Urth TR, Petersen A, Møller CH, Price LB, Skov RL, Larsen AR, Stegger M, Larsen J (2020) Phage-mediated immune evasion and transmission of livestock-associated methicillin-resistant *Staphylococcus aureus* in humans. Emerg Infect Dis 26:2578–2585

Smith TC, Davis MF, Heaney CD (2018) Pig movement and antimicrobial use drive transmission of livestock-associated *Staphylococcus aureus* CC398. MBio 9:e02459–e02418

Spaan AN, van Strijp JAG, Torres VJ (2017) Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. Nat Rev Microbiol 15:435–447

Thammavongsa V, Kim HK, Missiakas D, Schneewind O (2015) Staphylococcal manipulation of host immune responses. Nat Rev Microbiol 13:529–543

Tong SY, Lilliebridge RA, Bishop EJ, Cheng AC, Holt DC, McDonald MI, Giffard PM, Currie BJ, Boutlis CS (2010) Clinical correlates of Panton-Valentine leukocidin (PVL), PVL isoforms, and clonal complex in the *Staphylococcus aureus* population of Northern Australia. J Infect Dis 202:760–769

van Alen S, Ballhausen B, Kaspar U, Köck R, Becker K (2018) Prevalence and genomic structure of bacteriophage phi3 in human-derived livestock-associated methicillin-resistant *Staphylococcus aureus* isolates from 2000 to 2015. J Clin Microbiol 56:e00140–e00118

van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, Verbrugh HA, Wertheim HF (2009) Reclassification of *Staphylococcus aureus* nasal carriage types. J Infect Dis 199:1820–1826

van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, Kluytmans JA (2011a) Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol 49:1030–1033

van Cleef BA, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, Zemlickova H, Skov RL, Vuopio-Varkila J, Cuny C, Friedrich AW, Spiliopouloum I, Pászti J, Hardardottir H,

Rossney A, Pan A, Pantosti A, Borg M, Grundmann H, Mueller-Premru M, Olsson-Liljequist B, Widmer A, Harbarth S, Schweiger A, Unal S, Kluytmans JA (2011b) Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerg Infect Dis 17:502–505

van Cleef BA, van Benthem BH, Verkade EJ, van Rijen M, Kluytmans-van den Bergh MF, Schouls LM, Duim B, Wagenaar JA, Graveland H, Bos ME, Heederik D, Kluytmans JA (2014) Dynamics of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* carriage in pig farmers: a prospective cohort study. Clin Microbiol Infect 20: O764–O771

van Rijen MM, Bosch T, Verkade EJ, Schouls L, Kluytmans JA, CAM Study Group (2014) Livestock-associated MRSA carriage in patients without direct contact with livestock. PLoS One 9:e100294

Verkade E, Kluytmans-van den Bergh M, van Benthem B, van Cleef B, van Rijen M, Bosch T, Schouls L, Kluytmans J (2014) Transmission of methicillin-resistant *Staphylococcus aureus* CC398 from livestock veterinarians to their household members. PLoS One 9:e100823

Viana D, Blanco J, Tormo-Más MA, Selva L, Guinane CM, Baselga R, Corpa J, Lasa I, Novick RP, Fitzgerald JR, Penadés JR (2010) Adaptation of *Staphylococcus aureus* to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. Mol Microbiol 77:1583–1594

Viana D, Comos M, McAdam PR, Ward MJ, Selva L, Guinane CM, González-Muñoz BM, Tristan A, Foster SJ, Fitzgerald JR, Penadés JR (2015) A single natural nucleotide mutation alters bacterial pathogen host tropism. Nat Genet 47:361–366

von Eiff C, Becker K, Machka K, Stammer H, Peters G (2001) Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med 344:11–16

Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M (2005) Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965–1966

Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ (2011) Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus* (ST398) in Dutch hospitals. Clin Microbiol Infect 17:316–319

Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA (2004) Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 364:703–705

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–762

Wilson GJ, Tuffs SW, Wee BA, Seo KS, Park N, Connelley T, Guinane CM, Morrison WI, Fitzgerald JR (2018) Bovine *Staphylococcus aureus* superantigens stimulate the entire T cell repertoire of cattle. Infect Immun 86:e00505–e00518

World Health Organization (WHO) (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1

Yebra G, Haag AF, Neamah MM, Wee BA, Richardson EJ, Horcajo P, Granneman S, Tormo-Más MÁ, de la Fuente R, Fitzgerald JR, Penadés JR (2021) Radical genome remodelling accompanied the emergence of a novel host-restricted bacterial pathogen. PLoS Pathog 17:e1009606

Yu F, Cienfuegos-Gallet AV, Cunningham MH, Jin Y, Wang B, Kreiswirth BN, Chen L (2021) Molecular evolution and adaptation of livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) sequence type 9. mSystems 6:e0049221

Zoonotic and Multidrug-Resistant Bacteria in Companion Animals Challenge Infection Medicine and Biosecurity

21

Birgit Walther, Katharina Schaufler, Lothar H. Wieler, and Antina Lübke-Becker

Contents

Enlarging the cake: zoonotic spread and continued adaptation of MDR bacteria across companion animal medicine and beyond

B. Walther
Advanced Light and Electron Microscopy (ZBS 4), Robert Koch Institute, Berlin, Germany
e-mail: WaltherB@rki.de

K. Schaufler
Institute of Pharmacy, University of Greifswald, Greifswald, Germany

Institute of Infection Medicine, Christian-Albrecht University and University Medical Center Schleswig-Holstein, Kiel, Germany
e-mail: katharina.schaufler@uni-greifswald.de

L. H. Wieler (✉)
Robert Koch Institute, Berlin, Germany
e-mail: Wielerlh@rki.de

A. Lübke-Becker
Institute of Microbiology and Epizootics, Center for Infection Medicine, Freie Universität Berlin, Berlin, Germany

Veterinary Centre for Resistance Research (TZR), Freie Universität Berlin, Berlin, Germany
e-mail: antina.luebke-becker@fu-berlin.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_17

Abstract

At present, various zoonotic and multidrug resistant (MDR) bacteria, including but not limited to *Staphylococcus aureus* and *Escherichia coli*, are well-equipped to survive and conquer new habitats, environments, and host species. These bacteria carry the ability to cross host species barriers through host-range broadening virulence factors and antimicrobial resistances, combined with mechanisms allowing them to spread and prosper, not only in clinical environments but also beyond their natural surroundings. This phenomenon can colloquially be summarized as an "enlarging the cake" strategy, meaning that from a long-term evolutionary perspective, generalist variants of bacterial species diverge into distinct habitats on their way to a specialized existence within a niche. While knowledge on the pathomechanisms behind bacterial diseases and infection sources are readily available, trans-sectoral research on the transmission of MDR bacteria across humans, animals, and the environment, although considered to be a prime example of the One Heath concept, lies still in its infancy, especially with respect to the role of companion animals. In addition, challenges such as the mobilization of novel antimicrobial resistance genes from the global resistome as well as incalculable external influences on this matter arising from both climate and landscape changes are predicted to arise in the near future. However, new opportunities to combat MDR bacteria in human and veterinary medicine lie within research conducted across the One Health framework: Novel technologies powered by bioinformatics that permit bacterial identification, typing, and source attribution on a nearly unlimited scale, allowing to unravel the natural forces driving bacterial evolution and enabling the development of suitable intervention strategies.

Keywords

Antimicrobial resistance · Companion animals · Antibiotics · Resistome · Infection medicine · Inter-species transmission · Methicillin resistant *Staphylococcus aureus* · MRSA · *Escherichia coli* · Extended-spectrum ß-lactamase · ESBL · Selective pressure · Drug resistance · Surveillance

21.1 Rising Awareness of Antimicrobial-Resistant and Zoonotic Bacteria in Human and Animal Medicine

Surviving under the selective pressure of an anti-infective agent to which the bacteria originally had been susceptible is referred to as antimicrobial resistance (AMR) (WHO 2017a). Acquired antimicrobial resistance is a result of either mutations within the bacterial genome or an uptake of foreign genetic material (Schwarz et al. 2017). Only recently AMR among common zoonotic bacteria has been recognized as an emerging threat for public health (Rozman et al. 2019). Infections associated with AMR pathogens often require extensive clinical care as well as *enhanced and/or sophisticated antibiotic treatment*, which in turn can lead to an increase of severe side *effects for both, human and animal patients. Prolonged patient* suffering or even death, enhanced medical expenses, as well as considerable economic costs are direct results of bacterial infections caused by resistant pathogens (Wozniak et al. 2019). A recent study investigated 471 million individual records and isolates world-wide with respect to AMR in human medicine. As a result, the authors estimated a global burden of 1.2 million deaths from infectious diseases being associated with AMR bacteria in 2019 alone (Murray et al. 2022). The leading causes behind fatal outcomes were, among others, infections with *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. As stated in the report (Murray et al. 2022), all of these bacteria had previously been categorized as priority pathogens by the World Health Organization (WHO 2017b). Since the early 2000s, reports on the occurrence and spread of bacteria exhibiting AMR or even multidrug resistance (MDR), many of which can spread between humans and animals such as methicillin resistant *Staphylococccus aureus* (MRSA) and extended-spectrum β-lactam (ESBL)-producing bacteria of livestock and companion animal origin, have increased the awareness of this particular topic and have highlighted its relevancy for public health (reviewed in Walther et al. 2017; Walther 2021).

A PubMed search run illustrates the continuing annual increase of publications tackling both leading topics during the last 20 years (Fig. 1).

While AMR entering the food-chain through animal products is monitored within the European Union and many other industrialized countries, few countries monitor AMR in bacteria of companion animal origin on a regular basis (Weese 2008). However, reports on bacteria exhibiting AMR are only expected to increase even further (Ogeer-Gyles et al. 2006).

A recent review suggests that the number of pathogens reported as causes behind hospital-acquired infections (HAIs) in human medicine is limited to only 12–17 microorganisms, which account for up to 87% of the reported cases (Haque et al. 2018). This group comprises Gram-positive bacteria like *S. aureus*, coagulase-negative staphylococci *Enterococcus* spp., Gram-negative enterobacteria, such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., as well as nonfermenters *P. aeruginosa* and *A. baumannii*. The authors estimated that 16–20% of all HAIs

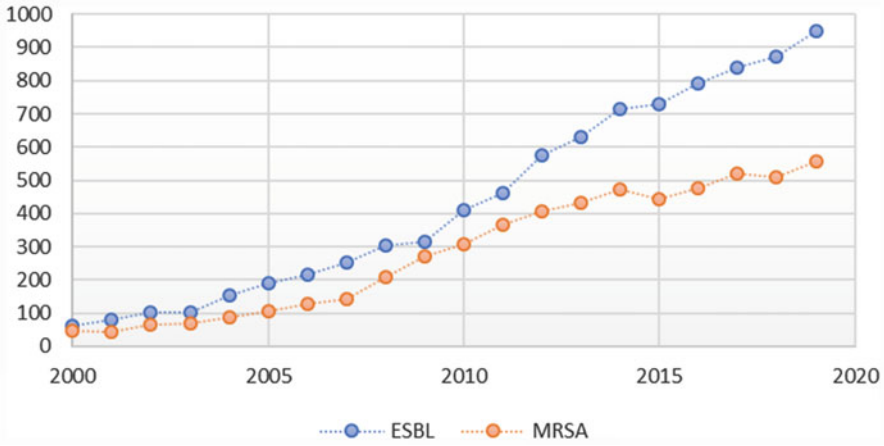

Fig. 1 Increase of publications on MDR bacteria associated with animals 2000–2019. PubMed search result (5 May 2021) of publications (total numbers (y)- and years on x-axis) on MRSA and ESBL-producing bacteria associated with animals using search query: [((animal) AND (extended-spectrum)) AND (lactamase) OR (ESBL)] and [(animal) AND ((aureus) AND (methicillin)) OR (MRSA) AND (animal) per year]

were caused by MDR bacteria including MRSA, vancomycin-resistant *E. faecium*, extended-spectrum β-lactam (ESBL)-producing *Enterobacteriaceae*, carbapenem-resistant enterobacteria, and Gram-negative nonfermenters (Haque et al. 2018).

Of note, bacteria exhibiting AMR often reside within human-, but also veterinary hospitals (Walther et al. 2017). Numerous reports on HAIs or even outbreaks associated with transmissible MDR bacteria are currently available not only from small animal clinics but also horse clinics (reviewed in Walther 2021). The most frequently reported HAIs associated with the companion animal health care sector are surgical site infections (SSI), blood stream infections, diarrhea, and urinary tract infections (Walther et al. 2014; Stull and Weese 2015). Due to the lack of systemic surveillance systems for recording infectious diseases across companion animals, including zoonotic pathogens and HAIs, reliable data on the occurrence and frequencies of various pathogens associated with outbreak scenarios in these hospitals are mostly absent (Walther 2021). However, ESBL-producing *Enterobacterales* and MRSA were among the most frequently reported causes of HAI in veterinary clinics (Wieler et al. 2015).

β-lactams, such as penicillin and cephalosporins, are known for their excellent pharmacokinetic properties which are commonly accompanied by a low toxicity for both humans and animals. Therefore, this group of well-tolerable anti-infective substances is among the first-line options for empirical antibiotic treatment of severe infectious diseases assumed to be caused by β-lactam susceptible bacterial species, including life-threatening scenarios such as septicemia (Walther 2021). Since β-lactam resistance is common among bacteria such as *Escherichia coli* and *Staphylococcus aureus* isolates from human as well as animal clinical origin, antibiotic options for severely ill patients are highly limited. Resistances toward other classes of antibiotics are frequently associated with β-lactam resistance

exhibiting bacteria such as ESBL-producing *E. coli* and MRSA (Geffers and Gastmeier 2011; Ruiz-Ripa et al. 2021) and can lead to a further reduction of therapeutic treatment options. Unsurprisingly, the development of specifically-tailored strategies to reduce the global burden of bacterial AMR is currently regarded as an urgent priority (Murray et al. 2022).

Understanding the evolutionary mechanisms and driving forces leading to the accumulation of AMR in bacteria, especially those considered to be transferable between humans and animals, is necessary in order to develop targeted and effective interventions for lowering the burden associated with both, the occurrence and spread of zoonotic AMR bacteria.

21.2 Mechanisms and Driving Forces Leading to the Emergence and Spread of Zoonotic and Antimicrobial Resistant Bacteria

21.2.1 The Accumulation of Mobile Antimicrobial Resistance in Bacteria Is Influenced by Selective Pressure

Several environmental bacteria and other microorganisms produce, beyond other metabolites, antibiotics to compete with co-habitants of their living space (Allen et al. 2010) (Fig. 2). As defensive measures, these microorganisms have developed

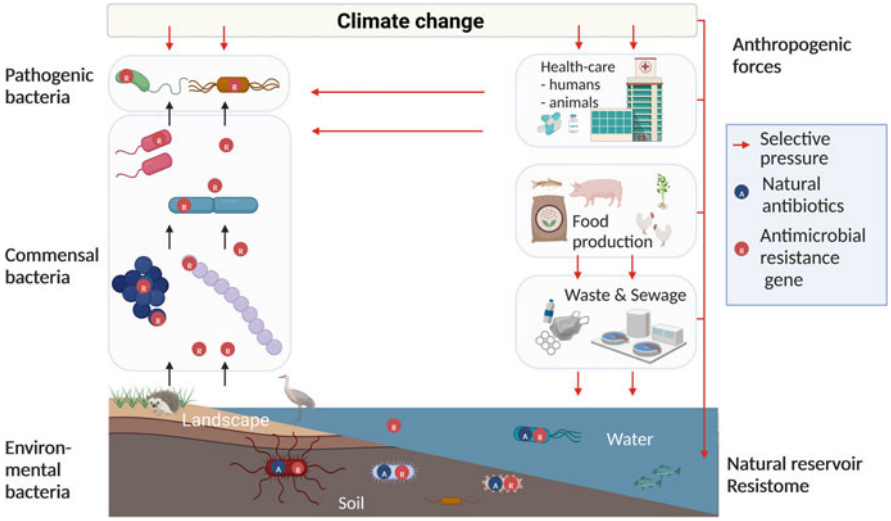

Fig. 2 Illustration of the mobile resistome: Evolutionary and ecological relationship of antimicrobial resistance genes (ARGs). (Adapted from an idea published in Walther (2021) illustration (created with BioRender.com, license BW (01.02.2021)). Natural and anthropogenic forces contribute to the selective pressure that ultimately mobilizes ARGs (Allen et al. 2010). Transmission of ARGs occurs through various pathways, summarized as horizontal DNA transfer (HDT) (reviewed in von Wintersdorff et al. 2016)

different mechanisms to exhibit AMR toward their own toxic agents (Davies and Davies 2010).

Some of these AMR encoding genes have gained mobility throughout prolonged periods of evolution, which in turn levelled the path for competing bacteria to also adapt resistances (D'Costa et al. 2011). Specific lineages of MRSA, for instance, emerged in European hedgehogs long before β-lactams were introduced for clinical usage (Larsen et al. 2022). Moreover, it has been demonstrated that the hedgehog dermatophyte *Trichophyton erinacei* produces two β-lactam antibiotics which provide a natural selective environment for MRSA in this particular host (Larsen et al. 2022).

All mobile genetic elements of pathogenic, commensal, as well as environmental bacterial origin constitute the global reservoir of antimicrobial resistance genes (ARGs) (referred to as the "mobile resistome," Fig. 2) from which susceptible bacteria can acquire resistance via horizontal gene transfer (HGT) (Schwarz et al. 2017). The prevalence of AMR in wild animals is in fact positively correlated with the level of human impact on the respective environment (Lagerstrom and Hadly 2021). While AMR among bacterial species residing in complex ecosystems is a natural and ancient phenomenon (D'Costa et al. 2011), accumulation of mobilized ARGs in bacteria is undisputable strongly anthropogenically influenced through artificial substances inducing a selective pressure on microorganisms in distinct life sectors (Wright 2007; Sultan et al. 2018) (Fig. 2). Thus, the use and misuse of antibiotics, heavy metals, and many other selective agents result in an increased spread of ARGs through mobile genetic elements (MGEs) from the environmental resistome to the commensal and finally to pathogenic bacteria (Fig. 2) (McEwen and Collignon 2018). The impact behind local and global changes in climate, especially temperature, and their association with increased antibiotic resistance and spread (Fig. 2) has recently shifted into focus of scientific interest (Rodriguez-Verdugo et al. 2020): many infectious diseases, including several vector- and water-borne diseases, are strongly influenced by climate variability resulting from large-scale environmental phenomena (Cavicchioli et al. 2019), and, to further complicate this issue, a recent prediction outlined that climate change will also increase rates of AMR in *Escherichia coli*, *Klebsiella pneumoniae,* and *Staphylococcus aureus* (MacFadden et al. 2018).

The emergence of AMR bacteria in companion animals is a complex field of research which is of increasing impact to both animal patient and public health issues (Weese 2008). In light of the emerging importance of ESBL-producing *E. coli* within farm and companion animals (Fig. 1), transmission of AMR pathogens (or resistance conferring genes) between distinct habitats including the environment, other animals, and humans, through manure (Schaufler et al. 2015; Kauter et al. 2021; Koeck et al. 2021) or close physical contact (Ewers et al. 2011; Wieler et al. 2011), for example, has gained scientific attention. ARG transfer between bacteria commonly residing within the intestines of humans and animal species, for instance, seems of high importance in respect to ARG accumulation of pathogens (Hu et al. 2016): Impressive networks for ARG transfer were identified for distinct genera such as Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria – bacteria that are prone to HDT-mediated uptake of AMR (Hu et al. 2016). Among these, the species

Escherichia coli, *Bacteroides fragilis,* and *Staphylococcus aureus* were found to share the highest level of ARG interconnectivity, with each of them having the ability to exchange resistance conferring genes with more than 260 bacterial species (Hu et al. 2016).

21.2.2 The Emergence of Bacteria in Novel Habitats and Host Species Followed by Subsequent On-Site Adaptation Depends on Evolutionary Rules

Throughout recent years, new insights into the driving forces and mechanisms behind the transmission of zoonotic infectious diseases were gained. Novel sequencing technologies, for instance, allow comparative genomics of microorganisms residing in completely different locations (habitats), including bacteria associated with specific hosts, such as plants, fungi, and animals, but also bacteria associated with non-living environmental habitats (Sriswasdi et al. 2017). As a result, most bacterial species on earth fulfil the criteria for specialists, i.e., bacteria that are well-adapted to a specific living space and its particular environmental conditions, while generalists are characterized by their ability to prosper in different habitats (Sriswasdi et al. 2017). A habitat is defined as a specific space in which an organism commonly occurs. It seems that the emergence of generalist species (Fig. 3) possessing the ability to cross habitat (and species) barriers is, compared to the across-the-board range of the more prevalent specialized species, likely a transition phase (Sriswasdi et al. 2017). This period has been proposed to begin with the sudden intrusion of a generalist species into a (naïve) habitat, accompanied by a significant local proliferation and tendency to spread into further habitats (Fig. 3).

Once a generalist species has gained access to a novel habitat, the process of adaptation begins immediately (Sriswasdi et al. 2017), since only rapid adaptation allows the intruding bacteria to survive the novel environmental challenges (Fig. 3) and putative co-habitants with respect to long-term survival on-site (Sriswasdi et al. 2017). To illustrate the mechanisms and evolutionary drivers behind the interspecies transmission of AMR resistant bacteria, a closer inspection of habitat generalist prime examples, such as *Escherichia coli* and *Staphylococcus aureus*, seems reasonable, although only few lineages of the respective generalist population may actually be more prone to cross multiple species barriers (Sheppard et al. 2018). Consequently, these extended host spectrum genotype (EHSG) lineages (Walther et al. 2009) are of highest concern with respect to zoonotic transfer and accumulation of MDR, including high-risk clonal lineages (Wieler et al. 2015).

Of course, many vital events and circumstances promote or hinder the emergence of a novel species within an unaffected living space (habitat), especially its accessibility (Peterson 2011). At present, the accessibility of naïve habitats for bacteria and other pathogens is largely influenced through direct and indirect anthropogenic forces (Allen et al. 2010). Moreover, zoonotic host diversity increases in human-dominated ecosystems (Gibb et al. 2020). First and foremost, the undamped encroachment of humankind into our pristine nature has at least two significant,

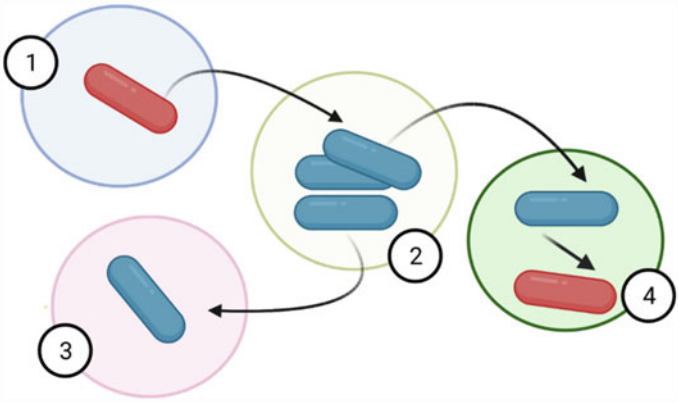

Fig. 3 Illustration of the generalist-specialist cycle of bacteria (Sriswasdi et al. 2017). A specialist (red) (**1**) develops into a generalist (blue), invades a novel habitat and proliferates (**2**). The generalist species (**2**) spreads into additional habitats (indicated by differently coloured circles) (**3** and **4**); if the generalist is not immediately become extinct within the novel living space (**4**), adaptive processes (ultimately) lead to a novel specialist fully equipped for long-term survival within the particular environment. The overall existence range of a habitat generalist seems, compared to the dominance of specialists, much shorter (Sriswasdi et al. 2017)

direct effects in regards to habitat accessibility of zoonotic bacteria: novel bacteria (and ARGs) are carried to locations they have never entered before and, on the other side, bacteria from these so far unexplored places are given access to the (general) population.

Prominent examples of such indirect forces include climate change, availability of essential supplies, selective pressure on bacterial habitats and, not at least, behavioural changes of humans and animals alike. Frequent, close physical contact, for instance, has become common between dogs and their owners (Wieler et al. 2015). A survey of a dog show event revealed that 68.5% of dog owners allowed their dog(s) to rest on the sofa, 39.8% allowed their dog(s) to lay on their bed, 93.5% let them lick their hands, and 52.8% let them lick their face (Walther et al. 2012). An additional study revealed that dog ownership not only significantly increases the shared skin microbiota in cohabiting adults, but dog-owning adults also share more "skin" microbiota with their own dogs than with other dogs (Song et al. 2013). Thus, close contact promotes the exchange of bacteria between humans and their companion animals, including horses, dogs, and cats (Bhat 2021). Access to novel habitats, as illustrated above, challenges bacteria with novel environmental conditions, resulting in either adaptation and survival or extinction. Examples of habitat generalists include prominent MDR bacterial species such as MRSA and *E. coli*; species which are of rising concern with respect to animal-human and human-animal transmission. Taken together, both, the invasive capabilities of bacteria as well as the consecutive process of on-site adaption challenged by novel habitats represents a force of nature (Sriswasdi et al. 2017) and therefore requires consideration in future research on the emergence of resistant and zoonotic bacteria in general.

21.3 A Closer Inspection of Prime Examples for Zoonotic and MDR Bacteria Reveals an Ongoing Adaptation Processes Toward Novel Environments and Host Species

21.3.1 Picturing Adaptation Strategies of MDR Bacteria Associated with Skin and Mucosal Surfaces: MRSA

21.3.1.1 *Staphylococcus aureus* Resides on Skin and Membranous Surfaces

Staphylococci are part of the commensal microorganisms residing on the skin and mucous surfaces of mammals and many other animals (Foster 2002). Classifying between coagulase-negative (CNS) and coagulase-positive (CPS) staphylococci is of high clinical relevancy: *S. aureus*, *S. pseudintermedius*, *S. coagulans*, *S. hyicus*, as well as some species of minor importance for healthcare (Foster 2002) are characterized by their ability to induce blood plasma coagulation via conversion of fibrinogen to fibrin (Pickering et al. 2021). The resulting clot acts as a mechanical shield, as it protects the bacteria from phagocytosis, enhances local adhesion opportunities and, not at least, provides a space for local population development (Foster 2002).

21.3.1.2 Clinical Importance of *S. aureus* in Human and Veterinary Medicine: A Brief Classification

While up to 40% of the general population occasionally carries *S. aureus*, 12–30% appear to be colonized permanently (Becker et al. 2017). Whenever outer barriers of the body, i.e., the skin or mucosal surfaces are vulnerable or damaged through either internal or external factors, *S. aureus* can gain access to the underlying tissues or the bloodstream and cause infection (Lee et al. 2018). The spectrum of illnesses caused by *S. aureus* ranges from toxin-associated diseases, i.e., food-poisoning, exfoliative/ulcerative skin diseases, and the toxic-shock syndrome, to severe invasive infections such as wound infections (especially surgical site infections), septicemia, pneumonia, and cellulitis. The reported incidence rates of *S. aureus* bacteremia range from 20 to 50 cases/100,000 population per year in different countries, and fatal outcomes were recorded for at least 10% of these cases (van Hal et al. 2012). An immediate suitable antibiotic intervention is essential in order to successfully combat *S. aureus* bloodstream infections, a fact that has been severely hampered due to the emergence of MRSA since the late 1970s. In recent years (2016–2020), the percentage of MRSA among *S. aureus* reported to the EARS/net in Europe has decreased, in some countries even below 5% (e.g., Spain, France, Germany). Other countries, however, still reported a rate above 25% (e.g., Greece, Italy, and Bulgaria) (www.atlas.ecdc.europa.eu/public) in 2020, highlighting the fact that MRSA remains an important pathogen, especially when combined with resistance toward further antimicrobial groups (EARS-Net 2021).

A German-wide representative study of 5229 samples from wound infections submitted by 1170 veterinary clinics illustrated the relevancy of *S. aureus* and MRSA for companion animal medicine. Swabs obtained from wound infections of dogs and cats revealed a *S. aureus* rate of 5.8% and 12.2%, respectively, and 22.8% for

samples of horse origin. The proportion of MRSA among these *S. aureus* was 62.7% (dogs), 46.4% (cats), and 41.3% (horses) (Vincze et al. 2014). Numerous studies performed comparative genomics on MRSA of companion animal origin. As a result, the majority of the isolates were identified to be part of phylogenetic lineages either dominating in human clinical cases ("epidemic lineages"), in livestock ("la") animals, or, to a much lesser extent, in free living animals and the environment (Cuny et al. 2010; Haenni et al. 2017; Larsen et al. 2022). MRSA currently dominating in European livestock animals and horses are of sequence type (ST) 398 or its variants and have successfully established a human/animal interface – this EHSG lineage (or generalist) is assumed to likely become a threat to public health through continued acquisition of virulence- and antibiotic resistance genes (Diene et al. 2017).

21.3.1.3 Adaptative Capabilities of *S. aureus*: Mechanisms Harnessed by a Habitat Generalist

Considering the general adaptation capabilities of *S. aureus*, recent research revealed that the species is a "master" with respect to its broad arsenal of immune evasion strategies (de Jong et al. 2019). Specific adaptations to evade host immune responses through the acquisition of MGEs harboring genes encoding immune evasion proteins were reported at least since 2013 (McCarthy and Lindsay 2013). Comparative analysis of the presence of genes mediating plasma-coagulation and the actual coagulation abilities of different CPS toward plasma of various host species (i.e., humans, birds, ruminants, horses, and pigs) has recently revealed that the *coa* gene encoding staphylocoagulase mediates plasma coagulation in *S. aureus*, while other CPS such as *S. pseudintermedius* depend on the presence of homologues of genes encoding the von Willebrand factor binding protein (*vwb*) (Pickering et al. 2021). It has been demonstrated that the ability to induce plasma coagulation was acquired on multiple occasions throughout the evolution of staphylococci (Pickering et al. 2021). Therefore, *S. aureus* harboring not only *coa* but also Staphylococcal Pathogenicity island (SaPI) encoded variants of the von Willebrand factor-binding protein (vWbp) are likely well-equipped to clot plasma of various host species (Viana et al. 2010) which indeed seems beneficial for "host jumping" bacteria.

Since activation of the complement system is essential for an effective immune response of mammals against invading pathogens, *S. aureus*-derived convertase inhibitors play a crucial role for the bacterial survival (Jongerius et al. 2007). The human-specific immune evasion complex (IEC) is commonly associated with temperate phages (van Wamel et al. 2006; Walther et al. 2018). The backbone of the IEC includes genes for a complement-protein cleaving Staphylokinase (SAK) and the Staphylococcal complement inhibitor (SCIN) known to interfere with the activation of complement factor C3 (McCarthy and Lindsay 2013). Of note, interfering with phagocytosis through inactivation of the C3 convertase complex in the alternative pathway of complement factor activation is among the most important immune evasion strategies of *S. aureus* (Ricklin et al. 2009). MRSA ST398 of horse origin harboring distinct allelic variants (*scn*, *scn*bov, *scn*eq) for encoding SCIN were reported across horses in different countries (Haenni et al. 2017; Walther et al. 2018). Functional analysis of the equine SCIN variant (SCINeq) revealed its general activity in plasma of multiple hosts, including horses, humans, and pigs (de Jong

et al. 2018). Since equine MRSA belonging to ST398 often harbour different combinations of *scn* variants (Walther et al. 2018), this lineage seems fully equipped to survive and prosper at least among humans, ruminants, pigs, and horses – a prime example of a host generalist (Walther 2021).

Of course, factors promoting host or habitat adaptation are not limited to immune evasion or plasma coagulation. Additional genes, often located on MGEs, encode adhesins, toxins, and further immunomodulatory factors known for their specific abilities to enhance survival in distinct hosts (Everitt et al. 2014; Diene et al. 2017; Walther 2021). Therefore, the broad host range associated with MDR MRSA, such as MRSA-ST398, have clear implications for biosafety and infection control (Walther 2021).

21.3.2 Adaptation Strategies of MDR Enterobacteria: *Escherichia coli*, the Classical "Jack of All Trades"

21.3.2.1 *Escherichia coli* Belongs to the Gut-Associated Microbiota in Humans and Animals

While staphylococci represent bacteria residing foremost on outer parts of the body such as skin and mucosal surfaces, *E. coli* are typical members of the gut microbiota, at least in mammals and birds (Guenther 2015). The commensal and thus usually apathogenic *E. coli* representatives are differentiated from intestinal (InPEC) and extra-intestinal pathogenic *E. coli* (ExPEC) (Pitout 2012). Pathogenic representatives can cause infectious diseases including sepsis, urinary tract and wound infections (UTI and WI), as well as diarrhea in humans and animals (Ewers et al. 2012). *E. coli* can be transmitted through the fecal-oral route either by direct or indirect contact via contaminated fluids, including surface water, food, and other carriers (de Graaf et al. 2017), regardless of their pathogenicity or AMR profile. *E. coli* are currently regarded as a significant indicator species for antimicrobial resistance in both veterinary and human medicine (Koeck et al. 2021). Overall, the gastrointestinal tract is regarded as an important reservoir for AMR Gram-negative bacteria in all mammals (Gibson et al. 2011), particularly the species *E. coli* which is among the key organisms with respect to acquired AMR in human and veterinary medicine, but also across extra-clinical environments (Guenther 2015).

21.3.2.2 Clinical Importance of *E. coli* in Human and Veterinary Medicine: A Brief Classification

E. coli is among the most frequently isolated organisms from clinical specimens in humans and animals. Besides its relevance as an etiological agent of diarrhea, *E. coli* is one of the leading causes of different extra-intestinal diseases in humans, with millions of cases resulting in billions of dollars of associated health care costs annually in the USA alone (Vila et al. 2016). On the other hand, considerable suffering of animals and substantial economic losses are caused by pathogenic *E. coli* across the various livestock sectors (Rhouma et al. 2017; Kathayat et al. 2021).

Beyond the clinical relevance of β-lactam resistance exhibited by ESBL-producing *E. coli,* these bacteria are often resistant against additional antibiotics such as

fluoroquinolones, tetracyclines, and aminoglycosides, and recently, against last-resort antibiotics like colistin and carbapenems, which exacerbates the overall tense clinical situation in case of infection (Koeck et al. 2021). The most common ESBL-genes in both humans and animals encode cefotaximases (CTX-M), such as $bla_{\text{CTX-M-1, 15, 14, 9}}$, followed by different TEM (from the Greek patient "Temoniera") and SHV (from "sulfhydryl variable") types (Ewers et al. 2012).

Considering the overall phylogenetic diversity of the *E. coli* species, comprising of more than 10,000 distinct STs, only a limited number of so-called international, high-risk clonal lineages dominate the global *E. coli* pool (Woodford et al. 2011). These clonal lineages (e.g., belonging to ST131, ST410, and ST648) are characterized by their broad dissemination across various host species, MDR and occurrence in human and veterinary medicine (Mathers et al. 2015; Schaufler et al., 2016b, 2019; Guenther et al. 2017).

A study from the United Kingdom summarized results on antimicrobial susceptibility testing (AST) for 29,330 canine and 8279 feline Enterobacterales isolates between April 2016 and July 2018 obtained from 2237 companion animal clinics: *E. coli* was the most commonly isolated Enterobacteriaceae in dogs (69.4%) and cats (90.5%). MDR was reported in 14.1% of canine and 12.0% of feline *E. coli* isolates (Singleton et al. 2021). Considering ESBL-types, $bla_{\text{CTX-M-15}}$ was most prevalent; and concerningly, the *E. coli* international high-risk clonal lineage ST131 predominated, further demonstrating the link between humans and companion animals in the context of AMR and successful, pathogenic clonal lineages (Singleton et al. 2021).

AMR *E. coli* are ubiquitous, not only in the context of clinical settings, animal husbandry, and companion animals, but also across wildlife of more remote areas (Guenther et al. 2011; Homeier-Bachmann et al. 2022). As illustrated above, anthropogenic forces largely impact both AMR mobilization from their environmental reservoirs as well as their consecutive spread (Allen et al. 2010), but also dissemination through migrating wildlife such as birds, wild boars, and other animals contribute to this (Guenther et al. 2011; Homeier-Bachmann et al. 2022). In more urban scenarios, rodents have been shown to further disseminate ESBL-producing *E. coli* (Guenther et al. 2010; Schaufler et al. 2018). However, a recent review revealed a striking paucity of information on *E. coli* in wild animals, despite clear evidence that these harbor pathogenic and antimicrobial-resistant *E. coli* among their gut microbiota they may even serve as melting pots for novel genetic combinations potentially harmful to human (and animal) health (Lagerstrom and Hadly 2021).

21.3.2.3 Occurrence and Distribution of an Important Multifaceted Generalist Species: *E. coli*

Previous studies illustrated the overall adaptive capabilities of the generalist *E. coli* to living "habitats" including the intra-macrophage environment, gastric passage and the intestine but also to host independent habitats such as, for instance, survival in sand, aquatic environments, and soil (Azevedo et al. 2016; Gekenidis et al. 2020; Rumball et al. 2020; Homeier-Bachmann et al. 2021). However, as mentioned above, only a limited number of clonal lineages are widely distributed across different habitats and hosts: To survive the adverse conditions of a competitive and demanding environment, such as the medical sector, tailored virulence, and the development of AMR are key

features of international high-risk clonal lineages (Beceiro et al. 2013), including Enterobacterales. These features are exemplified by the formation of biofilms as part of the host colonization capacities of *E. coli* ST648 or an enhanced iron acquisition and adaptation to conditions present in the urinary tract (e.g., the *pap* operon) of ST131. Rapid adaptation represents a key feature of Enterobacterales – as already outlined with regards to its genetic transfer network described above. Considering gut-associated bacteria, *E. coli* and *K. pneumoniae* seem to share the largest number of mobile ARGs (Hu et al. 2016), indicating the importance of HDT between these particular species. In some cases, a single event, such as a new plasmid acquisition, can result in an "ideal combination" of MDR and enhanced virulence for the survival and spread of the bacteria (Schaufler et al., 2016a).

At present, approximately 10% of horses admitted to clinical care in specialized veterinary clinics are already colonized with ESBL-producing *E. coli* upon hospital admission (Kauter et al. 2021). While raw meet diets for dogs were frequently contaminated with AMR bacteria, including ExPEC lineages which are of particular concern for human medicine (i.e., ST69 and clonal complex (CC) 648) (Nuesch-Inderbinen et al. 2019), herbivores such as horses are likely colonized through other sources. Contaminated water seems one plausible source, since ESBL-producing bacteria have been identified within bodies of water, such as wastewater and sewage, rivers, and lakes (Zarfel et al. 2013; Homeier-Bachmann et al. 2021). However, recent research also indicates that ESBL-producing Enterobacterales harbor genes promoting (i) their endophytic lifestyle and (ii) survival of acidic conditions. This includes (but is not limited to) international high-risk clonal lineages carrying ESBL CTX-M-15 enzymes (*E. coli* CC38 and CC648 and *K. pneumoniae* CC307) (Lopes et al. 2021). The authors further speculated that fresh vegetables may act as a figurative "Trojan horse" for the hidden spread of critical priority pathogens, since survival and growth in fresh vegetables and subsequent tolerance to gastric acidity may increase the chances of becoming colonized with these ESBL-producing Enterobacterales (Lopes et al. 2021).

Consequently, understanding the adaptation and persistence kinetics of AMR bacteria across different environmental habitats such as soil, water, and plants, including, but not limited to, ESBL-producing *E. coli*, aids in determining essential actions and strategies in order to mitigate their spread to people (Gekenidis et al. 2020) and, not at least, also to animals.

21.3.3 Challenges and Chances Associated with Zoonotic and Epidemic Bacteria Exhibiting AMR

21.3.3.1 Challenges Arising from the "Enlarging the Cake" Strategy of Zoonotic and MDR Bacteria Call for a Broader Research Perspective on the Subject

While the term "spillover" is widely used to describe the transmission of AMR bacteria from animals to humans and vice-versa, it might trivialise the dynamics associated with access and opportunities for these and other bacteria, including their ability to conquer novel habitats (i.e., hosts and environments). In fact, a zoonotic

spillover is defined as "processes that enable a pathogen from a vertebrate animal to establish infection in a human" (Plowright et al. 2017). These complex processes link the ecological dynamics within the reservoir host, pathogen development, survival and dissemination with the epidemiological and behavioural determinants of exposure and susceptibility of the recipient host (Plowright et al. 2017). A recent perspective on "Impacts of biodiversity and biodiversity loss on zoonotic diseases" highlights this aspect. The authors declare that "the current paradigm for research on spillover of zoonotic pathogens to humans emphasizes a single animal host species and an original spill over event, although, in reality, most zoonotic pathogens have multiple host species whose specific roles in transmission to and from humans are not known" (Keesing and Ostfeld 2021).

While the "generalist state" of bacteria is characterized by their – in comparison with the overwhelming majority of specialized bacterial species – short existence cycle (Sriswasdi et al. 2017), it allows for distinct pathogens (or even subtypes) to emerge and proliferate in completely different habitats, and, is therefore, often a characteristic feature of bacteria transmissible between humans and animals. Consequently, unidirectional views ("transmission from A to B") on the subject tend to ignore the "enlarging the cake" strategy of generalists: The existence of MDR bacteria fully equipped to conquer multiple habitats (hosts and environments), might appear transient from a long-term evolutionary perspective (Sriswasdi et al. 2017) but their emergence, on the other hand, is of highest importance in regards to research on reservoirs and pathways of inter-species transmission. Since only a limited number of lineages or subgroups within a specific transmissible bacterial population seem prone to cross habitat and/or species barriers (Sheppard et al. 2018), their particular adaptive capacity and AMR (including MDR) represent worthwhile research opportunities (Schaufler et al. 2016b; Huber et al. 2020). However, with respect to accumulation and spread of MDR bacteria, several hot spots have already been identified, including facilities providing health care for humans (Geffers and Gastmeier 2011; van Alen et al. 2017) as well as companion animals (Walther 2021). Consequently, staff of both human and veterinary medical facilities are frequently confronted with MDR bacteria. While hospital hygiene is an established clinical and research topic in human medicine, questions concerning biosecurity of veterinary personnel have emerged due to companion animals either being colonized or infected with MDR bacteria (Wieler et al. 2015; Walther et al. 2017). To ensure appropriate workplace safety and biosecurity with respect to transmissible MDR bacteria for people working in the field of veterinary medicine and, not at least, animal owners, initial hygiene recommendations acknowledging specific animal-depending work-place factors have been developed (Weese 2004; Gehlen et al. 2020).

21.3.3.2 Chances to Counteract AMR Bacteria Emerge from a Holistic Perspective on the Subject and Enhanced Interdisciplinary Efforts

To assess the risk of spillover, case studies should be developed that enable quantifying determinants of the complex process network involved in the transmission certain pathogens (Plowright et al. 2017).

While the importance of systematic and continuous data collection of the occurrence and spread of AMR bacteria (including their pathogenic traits) has been widely accepted, the European One Health action plan (2017) stated that initiatives need to be broadened, such as extending the One Health approach to include the environment and tackling AMR more comprehensively on the basis of improved data collection, monitoring, and surveillance (Union 2017). A tangible concept representing an interconnected and integrated One Health surveillance framework focusing on antimicrobial resistance as well as antimicrobial consumption has already been published (Queenan et al. 2016), but its implementation, especially the interconnectivity of various sectors, has not been achieved yet.

Moreover, the development of strategies involving new antimicrobials or non-antimicrobial compounds and novel point of care diagnostic methods that focus on high-risk clones and/or virulence markers may help to resolve the increasing problem of the association between virulence and resistance.

21.3.3.3 Tracking AMR in Commensal and Pathogenic Bacteria of Animal, Human, and Environmental Origin

Given the increasing knowledge of environmental reservoirs for resistances, it should now be possible to establish early warning systems of potential resistance mechanisms to new or old antibiotics and thus prepare for future problems in the clinic through a proactive manner (Martinez 2009). Monitoring of AMR in animal and human pathogens as well as in commensal bacteria enables to detect not only new resistance properties, but also potential transmission events and important reservoirs. The (still) ongoing development of DNA sequencing techniques allow fast and accurate genomics even on a population-wide scale. While novel DNA sequencing techniques have been made widely accessible and more user friendly, the data analysis and interpretation that follows still requires specialized bioinformatics expertise and appropriate computational and IT resources, as recently reviewed in (Maljkovic Berry et al. 2020). With respect to the early detection and surveillance of pathogens or even commensals harboring worrisome genes conferring AMR or enhanced pathogenicity (or a combination of both), automation with respect to pathogen detection, genome construction, and advanced analysis is required (Maljkovic Berry et al. 2020). However, gene calling and subsequent automated detection of virulence and resistance in an exploratory manner to gain insights into pathogenic potential, treatment options, and transmission patterns usually depend on the up-to-date state of different databases (Maljkovic Berry et al. 2020). Consequently, to detect important and/or novel factors enhancing pathogenicity or conferring AMR in bacteria, intelligent approaches are needed to connect clinical, phenotypical, and genomic data. Genome wide association studies (GWAS), for instance, provide opportunities to determine genes calling for a specific phenotype (Epping et al. 2021) without the drawbacks that are commonly associated with the use of reference databases. Moreover, host-specific traits of *E. coli* have recently been identified by using a GWAS-based approach and revealed, beyond others, genes that are strongly associated with sublineages dominating the human gut (Tiwari et al. 2022), indicating once more that

adaptation to a specific habitat is in fact a force of nature that drives the evolution of specific sublineages toward specialization, even though they may be part of a generalist species. Thus, GWAS generated data can be used with respect to risk analysis as well as diagnostic and monitoring purposes (Tiwari et al. 2022). Furthermore, culture-independent methods, such as metagenomics, represent unique opportunities for the surveillance of environmental resistance reservoirs (Danko et al. 2021).

Moreover, artificial intelligence (AI) represents a new paradigm to combat AMR (Lv et al. 2021), including AI-based AST prediction. However, as reviewed in (Lv et al. 2021), the lack of unified standardization and infrequent data updates in AMR databases currently hinders an efficient training of AI-based AMR predictive models.

21.4 Conclusion

The COVID-19 pandemic has certainly made the risks of zoonotic diseases a vivid and harrowing reality for every person on earth (Keesing and Ostfeld 2021).

In addition, due to the increased use and misuse of antibacterial agents and cleaners during the pandemic, a rise of bacterial resistance toward anti-infective agents is expected to emerge within the post-pandemic era (Mahoney et al. 2021), at least in some parts of the world. Although distinct sectors of the One Health framework (human, animal, and environmental health) are facing their own intrinsic issues associated with AMR, such as HAIs, lack of prudent use of antibiotics, environmental contamination by antibiotics and drug resistant bacteria, the rise of MDR bacteria in one sector always affects the other areas as well (Fig. 2). While the necessity and importance of inter-sectoral research (e.g., experts representing human, veterinary and environmental health, social and behavior science) is clear, a current lack of systematic research on the transmission of ARGs and/or AMR commensals/pathogens between different habitats restricts the early detection of presumptively clinically relevant strains, including, but not limited to, novel types and combinations of AMR and virulence factors. Moreover, it hinders the implementation of infection prevention strategies to halt further spread of zoonotic pathogens. In addition, after next-generation sequencing, we need to focus on "next-generation hazard perception" including up-to-date bioinformatic routines and tools for both primary diagnostics and research purposes. However, curating suitable databases for rapid and reliable species identification, virulence factor, and ARG detection is a challenging task that requires experienced microbiologists. Even approaches that integrate artificial intelligence to identify, for instance, novel resistance mechanisms or host-specific virulence factors will most likely depend on sufficient and reliable input data. Finally, yet importantly, epidemiology expertise and predictive mathematical models are fundamental to understand the course of transmission events and to plan and assess effective intervention and mitigation strategies.

21.5 Cross-References

- ▶ Dogs and Transmission of Infection to Man, "Respected Member of the Family?"
- ▶ Enterohemorrhagic *E. coli* (EHEC): Environmental-Vehicle-Human Interface
- ▶ Small Ruminants: Zoonotic Infections
- ▶ Zoonoses Transmitted by Poultry

References

Allen HK, Donato J et al (2010) Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 8(4):251–259

Azevedo M, Sousa A et al (2016) Trade-offs of *Escherichia coli* adaptation to an intracellular lifestyle in macrophages. PLoS One 11(1):e0146123

Beceiro A, Tomas M et al (2013) Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 26(2):185–230

Becker K, Schaumburg F et al (2017) *Staphylococcus aureus* from the German general population is highly diverse. Int J Med Microbiol 307(1):21–27

Bhat AH (2021) Bacterial zoonoses transmitted by household pets and as reservoirs of antimicrobial resistant bacteria. Microb Pathog 155:104891

Cavicchioli R, Ripple WJ et al (2019) Scientists' warning to humanity: microorganisms and climate change. Nat Rev Microbiol 17(9):569–586

Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) and the European Antimicrobial Resistance Surveillance Network (EARS-Net) (2021) Surveillance of antimicrobial resistance in Europe, 2020 data. WHO ECDC, Solna. ISBN 978-92-9498-556-9

Cuny C, Friedrich A et al (2010) Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. Int J Med Microbiol 300(2–3):109–117

D'Costa VM, King CE et al (2011) Antibiotic resistance is ancient. Nature 477(7365):457–461

Danko D, Bezdan D et al (2021) A global metagenomic map of urban microbiomes and antimicrobial resistance. Cell 184(13):3376–3393.e17

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433

de Graaf M, Beck R et al (2017) Sustained fecal-oral human-to-human transmission following a zoonotic event. Curr Opin Virol 22:1–6

de Jong NWM, Vrieling M et al (2018) Identification of a staphylococcal complement inhibitor with broad host specificity in equid *S. aureus* strains. J Biol Chem 293:4468–4477

de Jong NWM, van Kessel KPM et al (2019) Immune evasion by *Staphylococcus aureus*. Microbiol Spectr 7(2):0061

Diene SM, Corvaglia AR et al (2017) Prophages and adaptation of *Staphylococcus aureus* ST398 to the human clinic. BMC Genomics 18(1):133

Epping L, Walther B et al (2021) Genome-wide insights into population structure and host specificity of *Campylobacter jejuni*. Sci Rep 11(1):10358

Everitt RG, Didelot X et al (2014) Mobile elements drive recombination hotspots in the core genome of *Staphylococcus aureus*. Nat Commun 5:3956

Ewers C, Grobbel M et al (2011) Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted! Berl Munch Tierarztl Wochenschr 124(3–4):94–101

Ewers C, Bethe A et al (2012) Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18(7):646–655

European Commission (2017) A European One Health Action Plan Against Antimicrobial Resistance [online]. https://health.ec.europa.eu/system/files/2020-01/amr_2017_action-plan_0.pdf
Foster TJ (2002) *Staphylococcus aureus*. In: Sussmann M (ed) Molecular medical microbiology. Academic Press, Newcastle upon Tyne
Geffers C, Gastmeier P (2011) Nosocomial infections and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System). Dtsch Arztebl Int 108(6):87–93
Gehlen H, Simon C et al (2020) Biosecurity measures for equine clinics and ambulatory practice (Basis-Hygienemaßnahmen für den Pferdetierarzt in Praxis und Klinik). Berl Münch Tierärztl Wochenschr 133. https://doi.org/10.2376/1439-0299-2020-3
Gekenidis MT, Rigotti S et al (2020) Long-term persistence of blaCTX-M-15 in soil and lettuce after introducing extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* via manure or water. Microorganisms 8:11
Gibb R, Redding DW et al (2020) Zoonotic host diversity increases in human-dominated ecosystems. Nature 584(7821):398–402
Gibson JS, Morton JM et al (2011) Risk factors for multidrug-resistant *Escherichia coli* rectal colonization of dogs on admission to a veterinary hospital. Epidemiol Infect 139(2):197–205
Guenther S (2015) In: Centre for Infection Medicine (ed) Multi-resistant *Escherichia coli* from wildlife 2015, Berlin (Germany). Habilitation thesis, REFUBIUM Freie Universität Berlin, Berlin
Guenther S, Grobbel M et al (2010) Detection of pandemic B2-O25-ST131 *Escherichia coli* harbouring the CTX-M-9 extended-spectrum beta-lactamase type in a feral urban brown rat (*Rattus norvegicus*). J Antimicrob Chemother 65(3):582–584
Guenther S, Ewers C et al (2011) Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Front Microbiol 2:1–13
Guenther S, Semmler T et al (2017) Chromosomally encoded ESBL genes in *Escherichia coli* of ST38 from Mongolian wild birds. J Antimicrob Chemother 72(5):1310–1313
Haenni M, Chatre P et al (2017) Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in horses, cats, and dogs over a 5-year period in France. Front Microbiol 8:2493
Haque M, Sartelli M et al (2018) Health care-associated infections – an overview. Infect Drug Resist 11:2321–2333
Homeier-Bachmann T, Heiden SE et al (2021) Antibiotic-resistant Enterobacteriaceae in wastewater of abattoirs. Antibiotics 10:5
Homeier-Bachmann T, Schütz AK et al (2022) Genomic analysis of ESBL-producing *E. coli* in wildlife from north-eastern Germany. Antibiotics 11(2):123
Hu Y, Yang X et al (2016) The bacterial mobile resistome transfer network connecting the animal and human microbiomes. Appl Environ Microbiol 82(22):6672–6681
Huber C, Stamm I et al (2020) Silence as a way of niche adaptation: mecC-MRSA with variations in the accessory gene regulator (agr) functionality express kaleidoscopic phenotypes. Sci Rep 10(1):14787
Jongerius I, Kohl J et al (2007) Staphylococcal complement evasion by various convertase-blocking molecules. J Exp Med 204(10):2461–2471
Kathayat D, Lokesh D et al (2021) Avian pathogenic *Escherichia coli* (APEC): an overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens 10:4
Kauter A, Epping L et al (2021) Frequency, local dynamics, and genomic characteristics of ESBL-producing *Escherichia coli* isolated from specimens of hospitalized horses. Front Microbiol 12:671676
Keesing F, Ostfeld RS (2021) Impacts of biodiversity and biodiversity loss on zoonotic diseases. Proc Natl Acad Sci U S A 118:17
Koeck R, Herr C et al (2021) Multiresistant Gram-negative pathogens – a zoonotic problem. Dtsch Arztebl Int 118(35–36):579–589
Lagerstrom KM, Hadly EA (2021) The under-investigated wild side of *Escherichia coli*: genetic diversity, pathogenicity and antimicrobial resistance in wild animals. Proc Biol Sci 288(1948):20210399
Larsen J, Raisen CL et al (2022) Emergence of methicillin resistance predates the clinical use of antibiotics. Nature 602:135–141

Lee AS, de Lencastre H et al (2018) Methicillin-resistant *Staphylococcus aureus*. Nat Rev Dis Primers 4:18033

Lopes R, Fuentes-Castillo D et al (2021) Endophytic lifestyle of global clones of extended-spectrum beta-lactamase-producing priority pathogens in fresh vegetables: a Trojan horse strategy favoring human colonization? mSystems 6(1):e01125-20

Lv J, Deng S et al (2021) A review of artificial intelligence applications for antimicrobial resistance. Biosaf Health 3(1):22–31

MacFadden DR, McGough SF et al (2018) Antibiotic resistance increases with local temperature. Nat Clim Chang 8(6):510–514

Mahoney AR, Safaee MM et al (2021) The silent pandemic: emergent antibiotic resistances following the global response to SARS-CoV-2. iScience 24(4):102304

Maljkovic Berry I, Melendrez MC et al (2020) Next generation sequencing and bioinformatics methodologies for infectious disease research and public health: approaches, applications, and considerations for development of laboratory capacity. J Infect Dis 221(Suppl 3): S292–S307

Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc Biol Sci 276(1667):2521–2530

Mathers AJ, Peirano G et al (2015) *Escherichia coli* ST131: the quintessential example of an international multiresistant high-risk clone. Adv Appl Microbiol 90:109–154

McCarthy AJ, Lindsay JA (2013) *Staphylococcus aureus* innate immune evasion is lineage-specific: a bioinformatics study. Infect Genet Evol 19:7–14

McEwen SA, Collignon PJ (2018) Antimicrobial resistance: a One Health perspective. Microbiol Spectr 6(2):9

Murray CJL, Ikuta KS et al (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399(10325):629–655

Nuesch-Inderbinen M, Treier A et al (2019) Raw meat-based diets for companion animals: a potential source of transmission of pathogenic and antimicrobial-resistant Enterobacteriaceae. R Soc Open Sci 6(10):191170

Ogeer-Gyles JS, Mathews KA et al (2006) Nosocomial infections and antimicrobial resistance in critical care medicine. J Vet Emerg Crit Care 16(1):1–18

Peterson AT (2011) Ecological niche conservatism: a time-structured review of evidence. J Biogeogr 38(5):817–827

Pickering AC, Yebra G et al (2021) Evolutionary and functional analysis of coagulase positivity among the Staphylococci. mSphere 6(4):e0038121

Pitout JDD (2012) Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. Front Microbiol 3:9

Plowright RK, Parrish CR et al (2017) Pathways to zoonotic spillover. Nat Rev Microbiol 15(8): 502–510

Queenan K, Hasler B et al (2016) A One Health approach to antimicrobial resistance surveillance: is there a business case for it? Int J Antimicrob Agents 48(4):422–427

Rhouma M, Fairbrother JM et al (2017) Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Vet Scand 59(1):31

Ricklin D, Tzekou A et al (2009) A molecular insight into complement evasion by the staphylococcal complement inhibitor protein family. J Immunol 183(4):2565–2574

Rodriguez-Verdugo A, Lozano-Huntelman N et al (2020) Compounding effects of climate warming and antibiotic resistance. iScience 23(4):101024

Rozman V, Bogovič Matijašić B et al (2019) Antimicrobial resistance of common zoonotic bacteria in the food chain: an emerging threat. IntechOpen, London

Ruiz-Ripa L, Simon C et al (2021) *S. pseudintermedius* and *S. aureus* lineages with transmission ability circulate as causative agents of infections in pets for years. BMC Vet Res 17(1):42

Rumball NA, Mayer HC et al (2020) Selective survival of *Escherichia coli* phylotypes in freshwater beach sand. Appl Environ Microbiol 87(4):e02473-20

Schaufler K, Bethe A et al (2015) Putative connection between zoonotic multiresistant extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in dog feces from a veterinary campus and clinical isolates from dogs. Infect Ecol Epidemiol 5:25334

Schaufler K, Semmler T et al (2016a) Carriage of extended-spectrum beta-lactamase-plasmids does not reduce fitness but enhances virulence in some strains of pandemic *E. coli* lineages. Front Microbiol 7:336

Schaufler K, Semmler T et al (2016b) Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410 – another successful pandemic clone? FEMS Microbiol Ecol 92(1):fiv155

Schaufler K, Nowak K et al (2018) Clinically relevant ESBL-producing *K. pneumoniae* ST307 and *E. coli* ST38 in an urban West African rat population. Front Microbiol 9:150

Schaufler K, Semmler T et al (2019) Genomic and functional analysis of emerging virulent and multidrug-resistant *Escherichia coli* lineage sequence type 648. Antimicrob Agents Chemother 63(6):e00243

Schwarz S, Loeffler A et al (2017) Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. Vet Dermatol 28(1):82.e19

Sheppard SK, Guttman DS et al (2018) Population genomics of bacterial host adaptation. Nat Rev Genet 19(9):549–565

Singleton DA, Pongchaikul P et al (2021) Temporal, spatial, and genomic analyses of Enterobacteriaceae clinical antimicrobial resistance in companion animals reveals phenotypes and genotypes of One Health concern. Front Microbiol 12:700698

Song SJ, Lauber C et al (2013) Cohabiting family members share microbiota with one another and with their dogs. elife 2:e00458

Sriswasdi S, Yang CC et al (2017) Generalist species drive microbial dispersion and evolution. Nat Commun 8(1):1162

Stull JW, Weese JS (2015) Hospital-associated infections in small animal practice. Vet Clin North Am Small Anim Pract 45(2):217–233, v

Sultan I, Rahman S et al (2018) Antibiotics, resistome and resistance mechanisms: a bacterial perspective. Front Microbiol 9:2066

van Alen S, Ballhausen B et al (2017) In the centre of an epidemic: fifteen years of LA-MRSA CC398 at the University Hospital Munster. Vet Microbiol 200:19–24

van Hal SJ, Jensen SO et al (2012) Predictors of mortality in *Staphylococcus aureus* Bacteremia. Clin Microbiol Rev 25(2):362–386

van Wamel WJ, Rooijakkers SH et al (2006) The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J Bacteriol 188(4):1310–1315

Viana D, Blanco J, T et al (2010) Adaptation of Staphylococcus aureus to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. Mol Microbiol 77 (6):1583–1594

Vila J, Saez-Lopez E et al (2016) *Escherichia coli*: an old friend with new tidings. FEMS Microbiol Rev 40(4):437–463

Vincze S, Stamm I et al (2014) Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010–2012. PLoS One 9(1): e85656

von Wintersdorff CJH, Penders J et al (2016) Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 7:173

Walther B (2021) Emergence of antimicrobial resistant bacteria in and beyond companion animal medicine. Habiltation theisis, Freie Universität Berlin [online]. https://refubium.fu-berlin.de/bitstream/handle/fub188/34231/Walther_kumulative_Habilitation.pdf?sequence=4&isAllowed=y

Walther B, Monecke S et al (2009) Comparative molecular analysis substantiates a zoonotic potential of equine methicillin- resistant *Staphylococcus aureus* (MRSA). J Clin Microbiol 47:704–710

Walther B, Hermes J et al (2012) Sharing more than friendship – nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS One 7(4):e35197

Walther B, Lübke-Becker A et al (2014) Suspected nosocomial infections with multi-drug resistant *E. coli*, including extended-spectrum beta-lactamase (ESBL)-producing strains, in an equine clinic. Berl Munch Tierarztl Wochenschr 127(11–12):421–427

Walther B, Tedin K et al (2017) Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol 200:71–78

Walther B, Klein KS et al (2018) Equine methicillin-resistant sequence type 398 *Staphylococcus aureus* (MRSA) harbor mobile genetic elements promoting host adaptation. Front Microbiol 9:2516

Weese JS (2004) Barrier precautions, isolation protocols, and personal hygiene in veterinary hospitals. Vet Clin North Am Equine Pract 20(3):543–559

Weese SJ (2008) Antimicrobial resistance in companion animals. Anim Health Res Rev 9(2): 169–176

Wieler LH, Ewers C et al (2011) Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int J Med Microbiol 301(8):635–641

Wieler LH, Walther B et al (2015) Infections with multidrug-resistant bacteria: has the post-antibiotic era arrived in companion animals? In: Sing A (ed) Zoonoses – infections affecting humans and animals: focus on public health aspects. Springer, Dordrecht - Heidelberg - New York - London. ISBN 978-9401794565

Woodford N, Turton JF et al (2011) Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 35(5):736–755

World Health Organization (WHO) (2017a) In: WHO (ed) Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2016–2017. WHO, Geneva. ISBN: 978-92-4-151344-9

World Health Organization (WHO) (2017b) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO, Geneva. ISBN: 978-92-4-151344-9

Wozniak TM, Barnsbee L et al (2019) Using the best available data to estimate the cost of antimicrobial resistance: a systematic review. Antimicrob Resist Infect Control 8:26

Wright GD (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 5(3):175–186

Zarfel G, Galler H et al (2013) Comparison of extended-spectrum-beta-lactamase (ESBL) carrying *Escherichia coli* from sewage sludge and human urinary tract infection. Environ Pollut 173: 192–199

Part IV

Important Zoonoses in Non-food Animals

Influenza from a One Health Perspective: Infection by a Highly Versatile Virus

22

Leslie A. Reperant and Albert D. M. E. Osterhaus

Contents

Abstract

Influenza A viruses (IAVs) are among the most versatile viruses, in terms of host range, pathogenesis, route of transmission, transmissibility efficiency, and evolutionary dynamics. Several IAVs are recognized pathogens of a wide range of avian and mammalian species. On the one hand, AIVs that evolved in wild water birds, their natural reservoirs, cause mild or asymptomatic infections of their intestinal tract, resulting in annual epidemic cycles fueled by IAV fecal-oral transmission in watershed habitats. On the other hand, they may cause disease manifestations in avian and mammalian species, including humans, upon sporadic cross-species transmission—including spill-back transmission into wild birds—and during self-limiting outbreaks

L. A. Reperant
Pikado BV, Utrechtse Heuvelrug, The Netherlands
e-mail: reperant@alumni.princeton.edu

A. D. M. E. Osterhaus (✉)
University of Veterinary Medicine Hannover, Hannover, Germany
e-mail: albert.osterhaus@tiho-hannover.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_18

or large-scale airborne epidemics. In these spillover host species, clinical signs and symptoms range from inapparent to severe and often fatal respiratory and extra-respiratory conditions. A feature of IAVs in animal spillover hosts including humans is their ability to adapt to these new species to eventually be maintained independently of new introductions from their natural host reservoirs. In humans, IAVs of animal origin can be the precursors of pandemic influenza viruses. These pandemic viruses eventually evolve into seasonal influenza viruses that cause recurring epidemics of seasonal influenza. The unprecedented diversification and spread of highly pathogenic avian influenza viruses of the H5N1 and other H5Nx subtypes in wild birds in and across Asia, North America, Europe, and Africa, with spillover into wild mammals in Europe, over the past few years mark a worrying paradigm change in influenza epidemiology. Through their versatile nature, IAVs are a striking example of the flexible and ever-evolving nature of zoonotic threats and of the richness of avenues zoonotic pathogens can take to burden animal and public health.

Keywords

Influenza · Cross-species transmission · Adaptation · Zoonosis · Pandemic

22.1 Introduction

IAVs are orthomyxoviruses closely related to influenza B, C, and D viruses (Palese and Shaw 2007; Henritzi et al. 2019). IAVs are enveloped single-stranded negative-sense RNA viruses with eight gene segments. These include two genes coding for the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins and six genes coding for several other internal proteins, including the polymerase complex, matrix and nonstructural proteins. The surface glycoproteins HA and NA interact with cellular receptors and are important antigenic targets of the host's specific immunity mediated by neutralizing and other biologically active antibodies. Most internal proteins are involved in IAV structure or replication. Influenza B viruses (circulating in humans, pigs, and seals) have a similar genomic arrangement, while influenza C viruses (circulating in humans, pigs, cattle, dogs, and camels) and influenza D viruses (found in cattle, small ruminants, pigs, horses, camels, and wild boars) lack a neuraminidase and express a hemagglutinin esterase fusion surface protein (Henritzi et al. 2019; Liu et al. 2020).

The replication of IAV RNA genome lacks effective exonuclease proofreading capability and is known to introduce base mutations at relatively high rates. In addition, the segmented genome allows for reassortment during coinfection with different IAVs, resulting in new viruses containing gene segments of mixed parental origin. The high mutation rate and extensive reassortment have led to an unmatched diversity of IAV lineages (Chen and Holmes 2006; Joseph et al. 2017).

Based on the HA gene, IAVs may have emerged at least 4000 years ago, from a common ancestor with influenza B viruses, while the split between influenza A/B and influenza C viruses is estimated to have occurred over 8000 years ago

(Suzuki and Nei 2002). Influenza D virus, more closely related to the influenza C virus, appears to have a more recent evolutionary history, diverging from the other three influenza genera less than 1200 years ago (Yu et al. 2021). However, recent metagenomics studies of the diversity of RNA viruses in "lower" vertebrates demonstrated the presence of influenza-like viruses in amphibians, fish, and even jawless vertebrates. These viruses, forming basal sister groups with the four influenza types, share a common ancestor with a diverse group of invertebrate viruses, highlighting the largely hidden complexity and long evolutionary history of the influenza orthomyxoviruses (Wille and Holmes 2020; Harvey and Holmes 2022).

IAV HA and NA genes are grouped into 18 and 11 different subtypes, respectively. Although most IAV HA and NA subtypes likely split 2000 to 3000 years ago, IAV diversity within each subtype is fairly recent, dating from the past hundred to several hundred years (Suzuki and Nei 2002; Chen and Holmes 2006).

Several IAVs are recognized pathogens of a wide range of avian and mammalian species, and their natural history in their wide host range clearly illustrates the one health principle, whereby human health, animal health, and ecosystem health are closely interrelated. Wild water birds are considered the natural reservoirs of IAVs (Webster et al. 1992), yet IAV lineages, acquired upon cross-species transmission, have established themselves in domestic animal species. In turn, domestic animals, especially poultry and swine, are the main sources of zoonotic IAV infections. Zoonotic IAVs may spark influenza pandemics in humans, should they acquire efficient human-to-human transmissibility, which they typically lack upon cross-species transmission. Pandemic influenza viruses eventually evolve into human seasonal influenza viruses, causing annual epidemics that spread globally. While IAV cross-species transmission has occurred probably since IAV emergence several thousands of years ago, their versatile epidemiology and evolutionary dynamics in an expanding host range have been influenced by the expanding human species, especially during the past few hundred years. The relentless growth of domestic swine and poultry populations in the last decades has contributed to increased genetic diversity of IAVs circulating in wild and domestic animals and occasionally infecting humans, expanding the pool of IAVs with pandemic potential (for review see (Reperant and Osterhaus 2012)).

In this chapter, we will review IAVs population-level epidemiology, evolutionary dynamics, and associated host-level pathogenesis of infection in their wide host range. In addition, we will describe the adaptive changes associated with IAVs host switch and their sustained establishment in novel host species.

22.2 Population-Level Epidemiology and Evolutionary Dynamics

22.2.1 Natural Reservoirs

The majority of the currently known diversity of IAVs is maintained in avian hosts of the order Anseriformes, Charadriiformes, and Gruiformes, which encompass water birds such as geese, ducks, gulls, waders, and coots (for review, see (Olsen et al.

2006; Reperant et al. 2013; van Dijk et al. 2018)). Wild freshwater birds thus are traditionally considered IAVs natural reservoirs. IAVs harboring most of the 144 combinations of the first 16 and 9 subtypes of HA and NA genes, respectively, have been isolated from wild water birds (Munster et al. 2007). However, a sampling bias towards these orders is suspected as a wide diversity of IAV RNA has been detected in other avian orders across much of the world's ecosystems—and sufficiently sampled to allow the detection of a 1% AIV genetic prevalence (Caron et al. 2017). These diverse orders include the Psittaciformes (parrots), Apodiformes (swifts), Cuculiformes (cuckoos), Phoenicopteriformes (flamingos), Podicipediformes (grebes), Coraciiformes (kingfishers and bee-eaters), Passeriformes (passerines), Gaviiformes (loons), Columbiformes (doves and pigeons), Pelecaniformes (pelicans), Pteroclidiformes (sandgrouse), and Piciformes (woodpeckers and barbets) with a detected prevalence based on RT-PCR detection between 1% and 7%, and the Galliformes (quails and grouses), Accipitiformes (raptors), Strigiformes (owls), Ciconiiformes (egrets and storks), Sphenisciformes (penguins), Suliformes (gannets), Falconiformes (falcons), and Procellariiformes (shearwaters and petrels) with a detected prevalence below 1%. Overall, IAV RNA was more often detected in birds of the Indo-Maley and Afrotropic regions, followed by birds of the Palearctic, Neotropic, Antarctic, Nearctic, and Australasian regions. Avian IAV natural wide host range, counting over 110 wild bird species from 23 different orders, likely contributes to IAVs remarkable diversity in these species.

The recent discovery of IAVs H17N10 and H18N11 in little yellow-shouldered bats (*Sturnira lilium*) in Guatemala and Neotropical fruit bats (*Artibeus* spp.) in Peru, Bolivia, and Brazil and of a distinct H9N2 IAV in Egyptian fruit bats (*Rousettus aegyptiacus*) has unexpectedly revealed the maintenance of highly divergent IAVs by the flying mammals (Tong et al. 2012, 2013a; Kandeil et al. 2019; Ciminski et al. 2020). Structural and phylogenetic analyses demonstrated substantial divergence and ancient evolutionary relationship between these viruses and other IAVs (Tong et al. 2012, 2013a; Zhu et al. 2012, 2013; Kandeil et al. 2019). Future studies on IAVs in bats are needed to unveil their epidemiology and the role these mammalian hosts have played in IAV evolutionary history. Bats or birds may have been the ancestral hosts of the diversity of IAVs and at the origin of most ancient cross-species transmission events. It is however a mystery why not more bat influenza viruses have been discovered to date.

Other host species of IAVs are usually considered spillover host species, as they initially acquired IAVs upon cross-species transmission from their ancestral, natural reservoirs (see below). However, IAVs have established in domestic animal species, including poultry, pigs, horses, and dogs, and in humans. These species now maintain distinct IAVs and have thus become their natural reservoirs and potential or actual new players in IAVs cross-species transmission.

22.2.1.1 Epidemiology

IAV epidemiology in wild water birds is characterized by annual epidemic cycles. IAVs typically infect juvenile birds of the year, immunologically naïve to IAVs, or young individuals with limited history of past infections that congregate during one

stage of their life history (Olsen et al. 2006; van Dijk et al. 2018). Morbidity and mortality burdens associated with IAV infection in wild water birds are low, with little evidence of disease or death of infected birds. Some effects of IAV infection on wild birds' health or behavior have been proposed, such as reduced food intake and delayed or shortened migration, but are difficult to evidence (Kuiken 2013).

Avian IAV epidemiology has been particularly studied in three natural host systems: dabbling ducks, waders, and gulls. In dabbling ducks, such as mallards (*Anas platyrhynchos*) and common teals (*A. crecca*), annual epidemics generally occur in juvenile birds in fall, as they congregate after annual molt and prepare for migration (Olsen et al. 2006; Munster et al. 2007; van Dijk et al. 2018). The arrival of migrants from other populations into these congregating groups may play an important role in IAV spread (van Dijk et al. 2013, 2018). A seasonal IAV hotspot in waders occurs at the Delaware Bay, in North America, likewise when migrants congregate at this important stopover site during spring (Webster et al. 1992; Hanson et al. 2008). Large populations of waders timely refuel during this migratory stopover on spawning horseshoe crabs (*Limulus polyphemus*), creating unique conditions for IAV spread. However, such conditions have not been observed in Europe where IAV prevalence in waders is reported low throughout the year (Olsen et al. 2006; Munster et al. 2007). Lastly, annual IAV epidemics were recently demonstrated in young black-headed gulls (*Chroicocephalus ridibundus*) in northern Europe, when fledging birds from breeding colonies leave their nests in early summer (Verhagen et al. 2014). Targeted sampling of other gull species at the time of fledging may unveil similar IAV dynamics in this family of birds.

Juvenile birds may be particularly important in the epidemiology of avian IAVs as their immune system matures and transitions from the bursa of Fabricius to the thymus. This period may enable prolonged shedding and high viral titers (Joseph et al. 2017). Premigrational staging and high concentration of juveniles likely fuel transmission, while mounting population immunity reduces spread in wintering areas. Little is known of the epidemiological dynamics of IAVs in birds belonging to other avian orders and whether they may act as maintenance hosts.

The aquatic habitat of wild freshwater birds and their feeding and social behavior likely favor IAV transmission in these species (for review see (Olsen et al. 2006; Reperant et al. 2013; van Dijk et al. 2018)). IAV infect wild water birds via a fecal-oral and possibly fecal-cloacal route of transmission, which can be enhanced in watershed habitats heavily contaminated by large groups of birds. IAV can persist several months in water, especially at low temperatures and low salinity levels, and such environmental persistence may be important for IAV year-round persistence in wild water bird populations (Stallknecht and Brown 2009). The route of transmission in birds from other orders, in particular terrestrial birds, may differ. The presence of IAV RNA in tracheal swabs may point to the possibility of transmission via the respiratory route (Caron et al. 2017).

22.2.1.2 Evolutionary Dynamics

The remarkable diversity of wild bird IAV lineages is puzzling. Environmental transmission, supported by avian IAV durability and stability in watershed habitats,

may be a key driver of their genetic diversity, due to a so-called "storage effect" (Roche et al. 2014). Heterosubtypic immunity likely further drives AIV subtype dynamics of emergence and replacement.

High mutation rates and frequent reassortment characterize IAV evolutionary dynamics in wild bird reservoirs (Chen and Holmes 2006; Dugan et al. 2008; Lowen 2017) (Fig. 1). However, these dynamics largely differ between genes coding for internal and surface proteins.

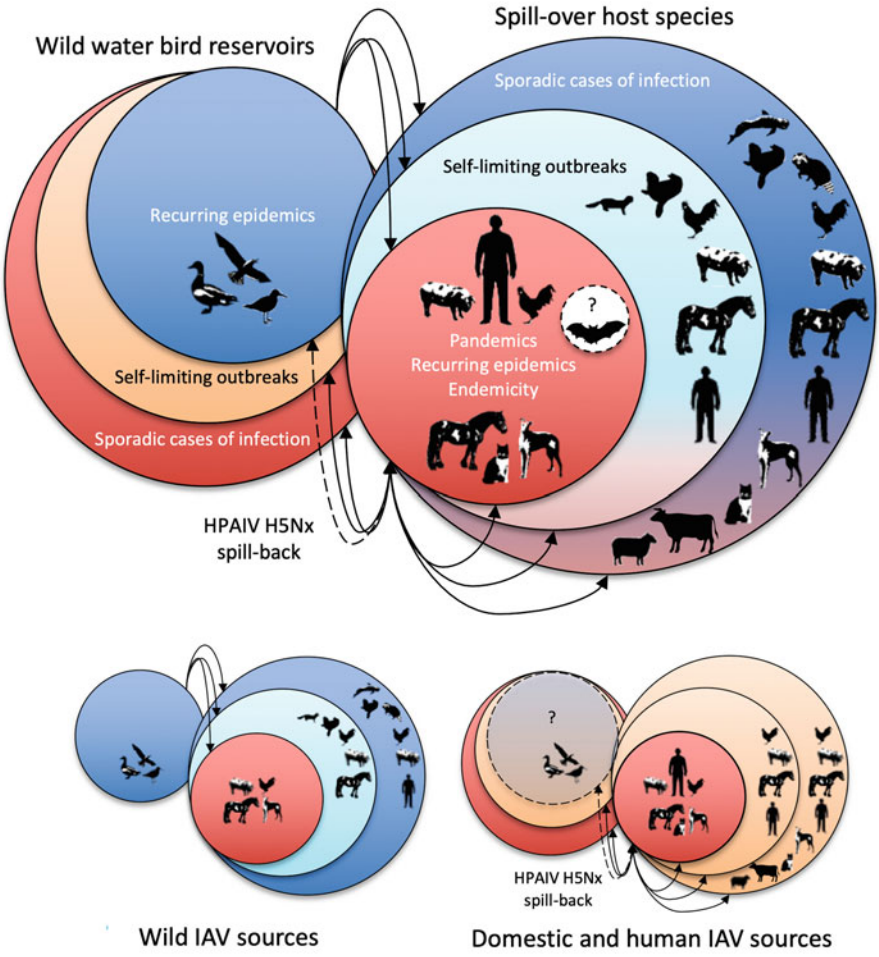

Fig. 1 Schematics of influenza A virus (IAV) diversity and evolutionary dynamics in natural reservoir and spillover host species (based on (Dugan et al. 2008)). IAV diversity in wild water birds is mainly characterized by co-circulation of a large number of subtypes and lineages and their frequent reassortment; in domestic animal species, by occasional IAV cross-species transmission, reassortment across subtypes, and genetic and antigenic drift; and in humans by rare IAV cross-species transmission, reassortment within subtype, and genetic and antigenic drift

IAV internal genes show high levels of genetic identity resulting in relatively conserved proteins. It suggests that these genes are under strong purifying selection, have reached fitness peaks, and may elicit immune response in wild birds. The conservation of these proteins allows widespread reassortment with exchange of functionally equivalent internal gene segments (Dugan et al. 2008; Lowen 2017). The NS gene may be under some form of balancing selection that has resulted in the co-circulation of two alleles that can be found in combination with any HA and NA subtype. In contrast, while the HA and NA genes within each subtype show high levels of genetic identity, the HA and NA genes of different subtypes are remarkably divergent. The absence of intermediate subtypes indicates strong natural selection likely resulting from cross-immunity. Frequent mixed infections are reported in wild birds, favoring reassortment between HA and NA subtypes, leading to many possible combinations. However, the current diversity of IAVs within each HA and NA subtypes in wild birds is recent and suggestive of population bottlenecks, during which IAV diversity may be periodically purged by the sweep of genes with high fitness, eliminating other lineages (Dugan et al. 2008).

IAVs circulating in gulls are generally distinct from those circulating in ducks and waders and mostly of the H13 and H16 subtypes. This demonstrates that for these subtypes, lineage divergence likely resulted from physical separation upon host switch. A limited number of IAV subtypes circulating in ducks have evolved in allopatry and geographical isolation, such as H15 present apparently exclusively in Australia. Most IAVs of wild water birds, however, can be grouped in large phylogenetic groups associated with broad geographical regions, corresponding to continents, hemispheres, or major water bird migration flyways, indicating limited geographical separation (Dugan et al. 2008). Reassortment is frequent between IAVs of different gene constellations within these groups but only occasional between IAVs of distinct geographical phylogenetic groups. Migratory birds breeding and wintering on distant continents can nonetheless disseminate avian IAVs between phylogeographical regions, as evidenced across the Bering Strait (Jeong et al. 2019).

22.2.2 Spillover Host Species

Several IAVs infect a wide range of host species other than wild water birds, including poultry, marine mammals, ungulates, carnivores, and humans, which all can be considered spillover host species (Table 1). In these species, IAVs may cause sporadic cases of infection, self-limiting outbreaks, pandemics, and recurring epidemics or even reach endemicity. IAVs infecting spillover host species, including those currently circulating independently of introductions from wild birds, have all originated from avian IAVs acquired upon cross-species transmission.

In contrast to what is observed in wild water birds, morbidity or mortality burdens can range from inapparent to high. Interestingly, most IAVs isolated from wild spillover host species cause sporadic cases of infection without further ongoing host-to-host transmission or cause self-limiting outbreaks that eventually die out in

Table 1 List of influenza A virus subtypes and lineages detected in spillover host species (based on following reviews: (Brown 2000; Swayne et al. 2019; Swayne 2007; Peiris 2009; Reperant et al. 2009; Taubenberger and Kash 2010; Reperant and Osterhaus 2012; Fereidouni et al. 2014; Yong-Feng et al. 2017; Borkenhagen et al. 2019, Philippon et al. 2020; Borland et al. 2020)

Pathotype	Influenza subtype	Infected species	Species of origin	Detection	Sustained
LP	H1	Raccoon	Avian	Serology	Unknown
LP	H1/H3/H7	Bothrops snake	Unknown	Serology	Unknown
LP	H1/H3/H7	Crotalus snake	Unknown	Serology	Unknown
LP	H1/H3/H7	Frog	Unknown	Serology	Unknown
LP	H1/H3/H7	Toad	Unknown	Serology	Unknown
LP	H1N1	Elephant seal	Human	Isolation	Yes
LP	H1N1	Human (1918–1957/ 1977–2009)	Unknown	Isolation	Yes
LP	H1N1	Human (1976)	Swine	Isolation	Yes
LP	H1N1	Human (2009–present)	Swine	Isolation	Yes
LP	H1N1	Swine	Avian	Isolation	Yes
LP	H1N1	Swine	Human	Isolation	Yes
LP	H1N1	Turkey	Human	Isolation	Yes
LP	H1N1	Turkey	Human	Isolation	Yes
LP	H1N1	Turkey	Swine	Isolation	Yes
LP	H1N1	Human	Swine	Isolation	No
LP	H1N1	Skunk	Human	Isolation	Unknown
LP	H1N1	Cattle	Swine	Isolation	Unknown
LP	H1N1	Cattle	Unknown	Serology	Unknown
LP	H1N1	Deer	Unknown	Serology	Unknown
LP	H1N1	Goat	Unknown	Serology	Unknown
LP	H1N1	Sheep	Unknown	Serology	Unknown
LP	H1N1	Dog	Human	Isolation	Yes
LP	H1N1r[a]	Dog	Swine	Isolation	Unknown
LP	H1N2	Mink	Swine	Isolation	Yes
LP	H1N2	Swine	Reassortant avian/ swine/human	Isolation	Yes
LP	H1N2	Human	Swine	Isolation	No
LP	H1N2r[a]	Dog	Swine	Isolation	Unknown
LP	H1N3	Balaenopterid whale	Avian	Isolation	Unknown
LP	H1N7	Swine	Reassortant equine/human	Isolation	Yes
LP	H2	Sheep	Unknown	Serology	Unknown
LP	H2N2	Human (1957–1968)	Reassortant Avian /human	Isolation	Yes
LP	H2N2	Cattle	Unknown	Serology	Unknown
LP	H3	Raccoon	Avian	Serology	Unknown

(continued)

Table 1 (continued)

Pathotype	Influenza subtype	Infected species	Species of origin	Detection	Sustained
LP	H3	Ringed seal	Unknown	Serology	Unknown
LP	H3	Seals	Unknown	Serology	Unknown
LP	H3N1	Swine	Reassortant avian/swine/human	Isolation	Yes
LP	H3N1	Dog	Human	Isolation	No
LP	H3N2/H1N1	Mink	Swine	Isolation	Yes
LP	H3N2	Human (1968–present)	Reassortant avian/human	Isolation	Yes
LP	H3N2	Dog	Avian	Isolation	Yes
LP	H3N2	Swine	Avian	Isolation	Yes
LP	H3N2	Dog	Avian	Isolation	Yes
LP	H3N2r[a]	Dog	Swine	Isolation	Unknown
LP	H3N2r[a]	Dog	Human	Isolation	Yes
LP	H3N2	Cat	Canine	Isolation	Yes
LP	H3N2	Mink	Swine	Isolation	Unknown
LP	H3N2	Swine	Human	Isolation	Yes
LP	H3N2	Swine	Reassortant avian/swine/human	Isolation	Yes
LP	H3N2	Human	Swine	Isolation	No
LP	H3N2	Cattle	Human	Isolation	Unknown
LP	H3N2	Cattle	Unknown	Serology	Unknown
LP	H3N2	Deer	Unknown	Serology	Unknown
LP	H3N2	Goat	Unknown	Serology	Unknown
LP	H3N2	Sheep	Unknown	Serology	Unknown
LP	H3N2	Water buffalo	Unknown	Serology	Unknown
LP	H3N2	Yak	Unknown	Serology	Unknown
LP	H3N3	Harbor seal	Avian	Isolation	Yes
LP	H3N3	Swine	Avian	Isolation	Yes
LP	H3N8	Horse	Avian	Isolation	Yes
LP	H3N8	Dog	Equine	Isolation	Yes
LP	H3N8	Harbor seal	Avian	Isolation	Yes
LP	H4	Seals	Unknown	Serology	Unknown
LP	H4N2	Raccoon	Avian	Serology	Unknown
LP	H4N5	Harbor seal	Avian	Isolation	Yes
LP	H4N6	Harbor seal	Avian	Isolation	Yes
LP	H4N6	Swine	Avian	Isolation	Yes
LP	H4N6	Raccoon	Avian	Serology	Unknown
HP	H5N1	Cat	Avian	Isolation	No
HP	H5N1	Dog	Avian	Isolation	No
HP	H5N1	Human	Avian	Isolation	No
HP	H5N1	Leopard	Avian	Isolation	No

(continued)

Table 1 (continued)

Pathotype	Influenza subtype	Infected species	Species of origin	Detection	Sustained
HP	H5N1	Owston's palm civet	Avian	Isolation	No
HP	H5N1	Stone marten	Avian	Isolation	No
HP	H5N1	Black-lipped pika	Avian	Isolation	No
HP	H5N1	Tiger	Avian	Isolation	No
HP	H5N6	Cat	Avian	Isolation	No
HP	H5N6	Human	Avian	Isolation	No
HP	H5N8	Grey seal	Avian	Isolation	No
HP	H5N8	Human	Avian	Isolation	No
LP	H6N1	Human	Avian	Isolation	No
LP	H7	Ringed seal	Unknown	Serology	Unknown
LP	H7	Seals	Unknown	Serology	Unknown
LP	H7N2	Human	Avian	Isolation	No
LP	H7N2	Cat	Avian	Isolation	Yes
LP	H7N2	Human	Feline	Isolation	No
LP	H7N3	Human	Avian	Isolation	No
HP	H7N3	Human	Avian	Isolation	No
LP	H7N4	Human	Avian	Isolation	No
LP	H7N7	Harbor seal	Avian	Isolation	Yes
LP	H7N7	Horse	Avian	Isolation	Yes
LP	H7N7	Human	Harbor seal	Isolation	No
HP	H7N7	Human	Avian	Isolation	No
LP	H7N7	Cattle	Unknown	Serology	Unknown
LP	H7N7	Ringed seal	Unknown	Serology	Unknown
LP	H7N7	Sheep	Unknown	Serology	Unknown
LP	H7N9	Human	Avian	Isolation	No
LP	H9N2	Swine	Avian	Isolation	Yes
LP	H9N2	Human	Avian	Isolation	No
LP	H9N2	Mink	Avian	Isolation	Yes
LP	H10N4	Mink	Avian	Isolation	Yes
LP	H10N5	Swine	Avian	Isolation	No
LP	H10N7	Human	Avian	Isolation	No
LP	H10N7	Harbour and gray seals	Avian	Isolation	Yes
LP	H10N8	Human	Avian	Isolation	No
LP	H12	Seals	Unknown	Serology	Unknown
LP	H13N2	Pilot whale	Avian	Isolation	Unknown
LP	H13N9	Pilot whale	Avian	Isolation	Unknown
LP	N1/N4/N6	Ringed seal	Unknown	Serology	Unknown
–	Unknown	Broad-snouted caiman	Avian	PCR	Unknown
–	Unknown	Chinese alligator	Avian	PCR	Unknown
–	Unknown	Nile crocodile	Avian	PCR	Unknown

(continued)

Table 1 (continued)

Pathotype	Influenza subtype	Infected species	Species of origin	Detection	Sustained
–	Unknown	Schneider's dwarf caiman	Avian	PCR	Unknown
–	Unknown	Baikal seal	Unknown	Serology	Unknown
–	Unknown	Beluga whale	Unknown	Serology	Unknown
–	Unknown	Caspian seal	Unknown	Serology	Unknown
–	Unknown	Chinese alligator	Unknown	Serology	Unknown
–	Unknown	Common mink whale	Unknown	Serology	Unknown
–	Unknown	Dall's porpoise	Unknown	Serology	Unknown
–	Unknown	Harp seal	Unknown	Serology	Unknown
–	Unknown	Hooded seal	Unknown	Serology	Unknown
–	Unknown	Fur seal	Unknown	Serology	Unknown
–	Unknown	Pacific walrus	Unknown	Serology	Unknown
–	Unknown	Reindeer	Unknown	Serology	Unknown
–	Unknown	Ringed seal	Unknown	Serology	Unknown
–	Unknown	Schneider's dwarf caiman	Unknown	Serology	Unknown
–	Unknown	Sea lion	Unknown	Serology	Unknown

[a]Reassortants with canine influenza virus

these species. In contrast, most IAV lineages that established and currently circulate in spillover host species, independently of cross-species transmission, emerged in domestic animals, including poultry, swine, horses, and dogs and in humans (Fig. 2). An exception warranting further research is bats, which may maintain distinct IAVs (Tong et al. 2013b; Ciminski et al. 2020). Domestic species and humans, which maintain distinct IAVs, have become their natural reservoirs and new players in their cross-species transmission.

22.2.2.1 Epidemiology

Nonestablished IAVs

Sporadic IAV infections or incidental serological evidence thereof have been reported in a wide range of avian and mammalian species, including humans, as well as in amphibians and reptiles (Table 1). Animal species that are in direct or indirect contact with IAV host reservoirs or spillover host species, e.g., due to predation, shared food and water resources, or overlapping habitat, may become infected with IAVs upon cross-species transmission (for review see (Reperant et al. 2009; Joseph et al. 2017)). IAV cross-species transmission between wild and domestic animal species and humans is not infrequent, including IAV transmission from humans to animals (Table 1). In humans, serological surveys demonstrated limited zoonotic exposure to IAVs in wild waterfowl hunters and bird banders (Gill et al.

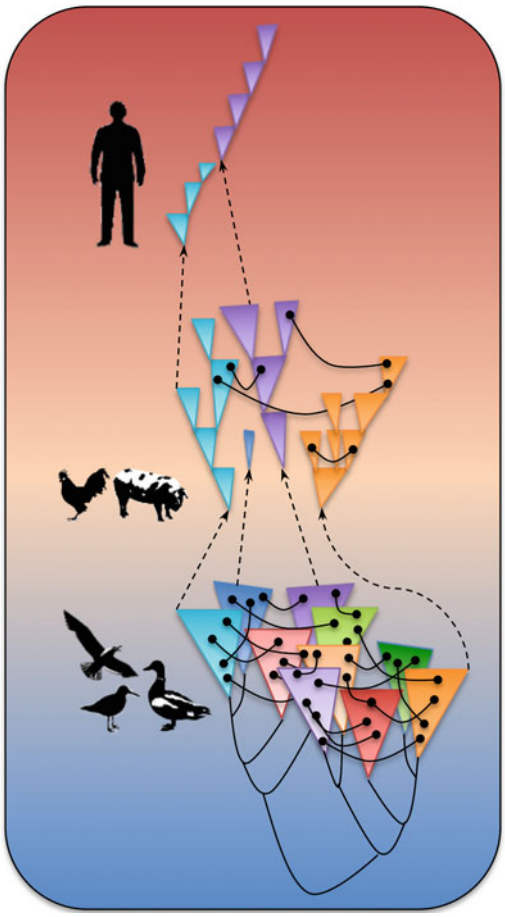

Fig. 2 Influenza A virus (IAV) epidemiological dynamics in natural reservoir and spillover host species. Wild water bird natural reservoirs sustain recurring IAV epidemics and may transmit these viruses to spillover host species, where they can cause sporadic cases of infection, self-limiting outbreaks, pandemics, and recurring epidemics or reach endemicity. Domestic animals and humans maintain adapted IAV, and bats may maintain evolutionary distinct IAV. Spillover species maintaining adapted IAV may also transmit these viruses to other host species, resulting in sporadic cases of infection and self-limiting and sustained outbreaks. These include spill-back infections and self-limiting outbreaks of highly pathogenic IAV (HPAIV) H5Nx in wild water birds

2006; Gray et al. 2011). Contact with domestic poultry and swine is associated with an increased likelihood of zoonotic IAV exposure (Gray et al. 2007). Outbreaks of zoonotic IAV infection in humans have increased in the past decades, in parallel with the relentless growth of domestic animal populations worldwide (for review see (Reperant and Osterhaus 2012; Parrish et al. 2015)). Subtypes known to have caused zoonotic human infections include swine H1N1, H1N2, and H3N2, avian H5N1, H5N6, H5N8, H6N1, H7N2, H7N3, H7N4, H7N7, H7N9, H9N2, H10N7 and H10N8, feline H7N2, and seal H7N7 (Borkenhagen et al. 2019**)**. Such infections may be at the basis of the emergence of novel pandemic IAVs (see below).

IAV cross-species transmission may lead to sustained spread of the virus, with ongoing host-to-host transmission in the new host species. This may result in self-limiting outbreaks that eventually die out or may spark large-scale epidemics that result in IAV establishment and continued circulation in the species concerned. Self-limiting outbreaks of IAV infection, following cross-species transmission, have been

reported in wild and domestic avian and mammalian species and in humans. The reasons behind the self-limiting nature of these outbreaks are little understood. IAVs may not acquire full adaptation to efficiently spread in a new host species upon cross-species transmission, and ongoing host-to-host transmission may remain limited below levels allowing sustained spread. In particular, adaptation of avian IAVs of wild bird origin to terrestrial poultry is thought necessary for their establishment in these species (Swayne 2007).

Alternatively, the number of immunologically naïve hosts in the population may be insufficient to sustain IAV transmission. This may be due to rapid depletion of the pool of susceptible hosts during outbreaks. For example, large self-limiting IAV outbreaks in wild harbor seals (*Phoca vitulina*) have resulted in high mortality and morbidity burdens, resulting in the death of up to 25% of local populations (Lang et al. 1981; Webster et al. 1981; Geraci et al. 1982; Hinshaw et al. 1984; Callan et al. 1995). A recent, similarly large yet self-limiting outbreak of AIV H10N7 infection erupted in harbor and grey seals (*Halichoerus grypus*) in North European waters in 2014 and killed over 10% of the seal population (Bodewes et al. 2015, Herfst et al. 2020). The depletion of the pool of susceptible hosts, associated with the patchy nature of seal populations and short annual breeding seasons—which replenish the pool of susceptible hosts only once a year—may explain the self-limiting nature of the outbreaks. There is currently no evidence of continued circulation of seal-adapted IAVs. Similarly, self-limiting outbreaks of avian IAVs in farmed American mink (*Vison vison*) (Klingeborn et al. 1985) and domestic swine (Brown 2000), resulting in high mortality or morbidity burdens, likely depleted the pool of susceptible hosts in the affected farms, and containment prevented further spread.

The presence of pre-existing immunity in spillover host populations may also contribute to the self-limiting nature of IAV outbreaks in domestic species and humans upon cross-species transmission. For example, IAV H3N8 of wild bird origin caused epidemics in horses in China during three consecutive years. Yet, the virus did not establish itself in the equine population (Guo et al. 1992). Circulation of a previously established equine IAV H3N8 and vaccination against this virus may have played a role in the eventual extinction of avian IAV H3N8 in horses. In humans, an outbreak of respiratory disease in a military camp in North America in 1976 caused by IAV H1N1 of swine origin turned out to be self-limiting (Goldfield et al. 1977; Top and Russell 1977). The simultaneous circulation of another IAV subtype—seasonal IAV H3N2—at the time of the outbreak and/or the presence of pre-existing immunity against AIV H1N1 may have contributed to limiting the spread of this virus in the human population (Gaydos et al. 2006).

Established IAV Lineages in New Animal Host Species

Occasionally, IAVs with efficient ongoing host-to-host transmission in a new host species may sustainably spread, reaching endemicity or causing recurring epidemics in the species concerned. A limited number of IAV lineages have adapted to domestic animals, including poultry, swine, horses, and dogs, and to humans. They are maintained independently of cross-species transmission from wild water

birds or other spillover host species. The conditions allowing IAV establishment and continued circulation in spillover host species remain little understood.

Domestic swine and poultry harbor the highest diversity of established IAVs in spillover host species, with several co-circulating subtypes and lineages (Brown 2000; Alexander 2007). A wide range of poultry species, including ducks, geese, chickens, and quails, are susceptible to IAV infection. Species diversity, high density and population turnover, and trade and mixing during co-raising or at animal markets are believed important determinants for the evolution, emergence, establishment, and diversification of swine and poultry IAV lineages (for review see (Reperant and Osterhaus 2012)). IAVs in swine and poultry typically are endemic over large geographical areas, if not globally, without displaying clear seasonal patterns of infection (Brown 2000; Alexander 2007; Henritzi et al. 2020). Endemicity in these species is likely maintained by the continued replenishment of susceptible hosts through trade or breeding. The growing diversity of swine and avian IAVs in livestock and of other IAVs in domestic species represents a major challenge to epidemic and pandemic preparedness (Parrish et al. 2015; Philippon et al. 2020; Anderson et al. 2021).

Endemic IAV infection may go unnoticed in swine and poultry, because of low morbidity and mortality burdens. However, IAV may cause detectable disease, especially in young animals immunologically naïve to IAV. In domestic poultry, IAV may also develop increased pathogenicity, defining IAV pathotype in these species. When IAV-associated mortality is low in poultry, IAVs are qualified as low pathogenic avian influenza viruses (LPAIV). In terrestrial poultry, mainly chickens and turkeys, LPAIV of the H5 and H7 subtypes can evolve into highly pathogenic avian influenza viruses (HPAIV), causing ravaging outbreaks with up to 100% mortality (Swayne 2007). High poultry density in industrial farms is thought to favor HPAIV evolution and emergence. Because of their high mortality burdens—also due to the rapid implementation of stamping out and other control measures—HPAIV may rapidly run out of susceptible hosts to infect, and outbreaks typically are self-limiting in adequately managed domestic poultry settings. HPAIV of the H5N1 subtype are a notorious exception. These viruses became endemic in poultry in Southeast Asia and Africa and rapidly diversified following their emergence more than 15 years ago (Chen et al. 2006; Li et al. 2010). They further have sprouted a growing diversity of HPAIV H5Nx subtypes (like H5N2, H5N3, H5N5, H5N6, H5N8, and H5N9) in poultry as well as in wild birds (see below; Pulit-Penaloza et al. 2020). While these viruses may silently circulate in aquatic poultry, they cause usually winter epidemics in terrestrial poultry, although most recently this pattern has changed to also include spring and summer (Pohlmann et al. 2019). Subclinically infected aquatic poultry may be crucial for the maintenance of HPAIV H5N1 and related viruses in domestic birds (Hulse-Post et al. 2005).

A limited diversity characterizes IAV circulating in horses and in domestic carnivores. Reports of equine epidemics of respiratory disease in past centuries suggest that equine IAVs have circulated in horses for several hundred years (Taubenberger and Morens 2009). However, only two IAV subtypes have been isolated from horses to date (for review see (Reperant et al. 2009)). Their emergence

likely resulted from IAV cross-species transmission from avian hosts. IAVs of the H7N7 subtype emerged in horses in the mid-1950s and have not been isolated since 1980. IAVs of the H3N8 subtype emerged in the early 1960s and currently cause recurring seasonal epidemics in equine populations worldwide, reminiscent of seasonal human influenza.

Equine IAVs H3N8 infected domestic dogs in the early 2000s and have subsequently become temporarily endemic in canine populations in North America and parts of Europe (Crawford et al. 2005; Daly et al. 2008). The canine H3N8 IAV eventually went extinct, perhaps because the density of the dog population is insufficient to indefinitely sustain host-to-host transmission (Wille and Homes 2020). In Southeast Asia, IAV H3N2 of avian origin have become endemic in canine populations in the late 2000s and have subsequently spilledover from dogs to domestic cats (Song et al. 2008; Song et al. 2011). These viruses may cause severe epidemics in both species. Canine H3N2 influenza emerged in North America in 2015, with multiple introductions and recurrent fade out of the epidemics (Voorhees et al. 2017; Voorhees et al. 2018). Ongoing studies are deciphering IAV evolutionary and epidemiological dynamics in domestic carnivores (Guo et al. 2021). They may represent new mixing vessels for the reassortment of novel IAVs (Parrish et al. 2015; Borland et al. 2020). The canine IAV H3N2 has reassorted multiple times with avian, swine, and human IAVs.

Established IAV Lineages in Humans

IAVs of animal origin with efficient human-to-human transmissibility can be at the origin of influenza pandemics in humans (Table 2). In particular, the high incidence of zoonotic IAV infections of any subtype in humans is feared to provide these viruses with opportunities to acquire efficient transmissibility, sparking an influenza pandemic. Outbreaks of sporadic IAV infections result from frequent cross-species transmission while ongoing host-to-host transmission remains limited. Recently, cross-species transmission of a number of swine and poultry IAVs to humans resulted in large outbreaks often associated with relatively high morbidity and mortality burdens. These include outbreaks of HPAIV H5N1, H5N6 and H7N7, swine IAV H3N2, and LPAIV H7N9 and H9N2, which have caused several hundred cases of human infection (Table 3). Evolution of these viruses by mutation and/or reassortment into a transmissible form may be at the basis of a novel pandemic IAV (Reperant et al. 2015).

Table 2 List of pandemic influenza A viruses in humans (for review see (Taubenberger and Kash 2010))

Influenza subtype	Lineage	Year of introduction	Years of circulation
H1N1	Avian	1918	1918–1957
H2N2	Reassortant avian/human	1957	1957–1968
H3N2	Reassortant avian/human	1968	1968–present
H1N1	Human	1977	1977–2009
H1N1	Swine	2009	2009–present

Table 3 List of recent zoonotic influenza A viruses causing large outbreaks in humans

Pathotype	Influenza subtype	Host of origin	Year	Number of cases	References
HPAIV	H7N7	Avian	2003	89	Koopmans et al. (2004)
HPAIV	H5N1	Avian	1997–present	>880	Philippon et al. (2020)
HPAIV	H5N6	Avian	2014–present	>20	Philippon et al. (2020)
LPAIV	H3N2	Swine	2011–present	>400	Anderson et al. (2021)
LPAIV	H7N9	Avian	2013–present	>1500	Philippon et al. (2020)
LPAIV	H9N2	Avian	2002–present	>50	Philippon et al. (2020)

Pandemic IAVs cause severe epidemic waves in humans, typically with high attack rates, morbidity, and mortality burdens. They infect a large proportion of the human population worldwide, with typically little pre-existing immunity against them (Taubenberger and Kash 2010; Saunders-Hastings and Krewski 2016). Introduction of a pandemic IAV in the human population results in the so-called antigenic shift, replacing one of the existing seasonal IAV subtypes. Pandemic waves in humans currently spread around the world within a matter of months. The viruses continue to circulate and evolve into seasonal influenza viruses after the pandemic that they caused, replacing one of the hitherto circulating seasonal IAVs. Such strain replacement suggests the existence of some levels of cross-immunity between IAVs of different subtypes. As a result, only a limited number of AIV subtypes have been established in the human population. The severity of the seasonal epidemics that follow pandemics typically declines with time, at least in part due to the building-up of specific immunity and the increasing inability of the IAVs to escape from the specific antibody landscape building up over time. However, ongoing seasonal drift can result in the emergence of immune-escape variants at the origin of more severe epidemics. As has been documented for IAV H3N2, seasonal waves of influenza tend to emerge in Southeast Asia, where multiple peaks of seasonal AIV infections are observed year-round. These viruses are seeded into countries of the northern hemisphere during the winter season, followed by their spread to countries of the southern hemisphere in the subsequent winter season there (Russell et al. 2008).

At least in part due to naïve, immature, or impaired immune responses, infants, the elderly, and individuals with comorbidities are particularly at risk of severe IAV infection that may be further complicated by bacterial infection (Taubenberger and Morens 2008). Because of limited pre-exposure and abundant social contacts, school-age children are considered major spreaders of IAVs in the human population. Serological surveys have established that most individuals of 7 years of age or older have been infected at least once by seasonal IAVs and are partially protected against IAV reinfection due to immune memory (Bodewes et al. 2011). Nevertheless,

healthy young adults may be severely affected during IAV pandemics, due to limited cross-protection from previous infections against a novel pandemic IAV (Saunders-Hastings and Krewski 2016).

Spilled-Over IAVs Spilling Back to Wild Bird Populations

IAVs circulating in spillover host species rarely spill back to wild bird populations. Adaptation of IAVs to spillover host species is thought to hinder their ability to infect wild water bird species. This has been demonstrated experimentally for a number of poultry IAVs, which inefficiently replicated in water birds (Swayne 2007). Yet, little is known about the extent of cross-species transmission of IAVs circulating in aquatic domestic poultry to related wild water bird species and their subsequent spread. LPAIV subtypes tend to be differently distributed in wild birds and poultry, suggesting different susceptibility to various subtypes (Verhagen et al. 2017).

Likewise, HPAIVs usually do not evolve in wild water birds, and if these birds become infected following cross-species transmission from poultry, the infection may not always be as severe as in domestic birds. However, since 2002, HPAIVs of the H5N1 subtype have spilled back from infected poultry to a wide range of wild bird species (Sturm-Ramirez et al. 2004), which can be considered spill-back hosts in this regard (for review see (Reperant et al. 2013)) (Fig. 2).

Infections with HPAIV H5N1 and related (reassortant H5Nx) viruses have caused inapparent to fatal disease in wild birds, following sporadic infection or during self-limiting outbreaks in Southeast Asia, the Middle East, Europe, Africa, and North America. Wild water birds, as well as birds from other orders, including corvids and birds of prey, can be severely affected. The routes of transmission of HPAIV H5Nx likely include the oral and respiratory routes, contrasting with the fecal-oral transmission of LPAIV. Predation or scavenging on infected carcasses may be a unique route of transmission of these viruses in corvids and birds of prey, as well as in wild carnivores, like seals, otters, and foxes.

Wild bird species little affected clinically by HPAIV H5Nx viruses may contribute to their geographical spread (Gilbert et al. 2006; Keawcharoen et al. 2008; Reperant et al. 2010; Lycett et al. 2016). In Europe, HPAIV H5N1 outbreaks in wild birds occurred during winter periods and were associated with the 0 °C isotherm, suggesting that congregation of water birds on open water bodies along freezing fronts fueled the epidemics (Reperant et al. 2010). For a long time, little evidence pointed to the maintenance of HPAIV H5Nx in wild bird populations, in the absence of repeated introductions from poultry. Yet in the past few years, successful clades of HPAIV H5Nx have repeatedly spread nearly globally with and among migratory and resident wild bird populations. This marks a major change in the epidemiology of AIVs in their natural reservoirs. Of note, HPAIV H5Nx introduced into North America in 2014 apparently went extinct after their control in poultry (Krauss et al. 2016).

The HPAIV H5N1 was first detected in a domestic goose in the Gangdong province of China in 1996. Over the following decades, it diversified into 10 genetically distinct virus clades numbered 0 to 9 and spread to over 80 countries in Asia,

Europe, Africa, and North America (Lee et al. 2017; Verhagen et al. 2021). HPAIV H5N1 caused mass mortality events in wild birds at Qinghai Lake in China in 2005, by members of the clade 2.2., and again in 2009, by members of the clade 2.3.2. Viruses of the clade 2.2 reached Europe from 2005 to 2009, following at least 3 separate introductions. Viruses of the clade 2.3.2.1c made incursions into European wild bird populations in 2010 and again in 2015. The HPAIV H5 clade 2.3.4—with first-time evidence of reassortments giving rise to H5N2, H5N5, and H5N8 subtypes—emerged in poultry in China in 2008 and further diversified into various subclades. Members of the subclade 2.3.4.4 reassorted with other clades of HPAIV H5N1 and with other local LPAIVs. In 2014, outbreaks of HPAIV H5N6 and H5N8 of the 2.3.4.4 subclade erupted in poultry and wild birds in China, Laos, and Vietnam and in Japan and Korea, respectively. Since 2014, these viruses and reassortants repeatedly spread with wild migratory birds from East Asia to North America, West Asia, and Europe. The number of outbreaks and the diversity of HPAIV H5Nx affecting wild birds and poultry populations in and across Asia, North America, Europe, and Africa since 2021 is unprecedented. Some of these viruses have spilled over to wild carnivores, including red foxes (*Vulpes vulpes*), Eurasian otters (*Lutra lutra*), and harbor and grey seals, with evidence of genetic adaptation to replication in mammals (Shin et al. 2019; Adlhoch et al. 2021; Postel et al. 2022). Serological evidence suggests exposure of wild boars (Schülein et al. 2021). The first local case of HPAIV H5N1 in a human in Europe was reported in the owner of Muscovy ducks affected by the disease at the end of 2021 in South West England (Oliver et al. 2022). These host jumps raise public health concerns as they may increase the risk of zoonotic transmission and of adaptation of HPAIV H5Nx to spreading in humans (Adlhoch et al. 2022). However, the mechanisms of maintenance and spread of these viruses in wild bird populations (comprising viruses of varying pathogenicity and virulence, bird species asymptomatically infected and highly susceptible species suffering high mortality) are little understood.

22.2.2.2 Evolutionary Dynamics

A limited number of IAV subtypes and lineages circulate in spillover host species, and most extant lineages emerged within the past hundred years. However, in domestic animals, like in humans, co-circulation of multiple IAV subtypes and lineages is reported. Although IAVs that are endemic in poultry and, to a lesser extent, in swine have diversified similarly to that in wild birds, the diversity in spillover host species is usually more restricted than that in wild water bird reservoirs (Fig. 1). Exceptions of growing concerns are the diversifying clades of HPAIV H5N1 and reassortants that circulate in poultry and wild birds. In humans, and to a lesser extent, in domestic animals, IAV lineages show evolutionary patterns associated with genetic and antigenic drift, whereby mutations in the antigenic sites of the surface glycoproteins allow IAVs to escape antibody mediated immunity that builds up in the population (Petrova and Russell 2018). IAV diversity is greatest in poultry and swine and results from repeated introductions of new IAVs, relatively frequent reassortment as well as genetic and antigenic drift in these species (Brown 2000; Olsen 2002; de Jong et al. 2007; Alexander 2007). In horses, two main lineages of

IAV H3N8 co-circulate (Lai et al. 2001). Distinct phylogenetic groups associated with broad geographical areas indicate some geographical isolation of IAVs of domestic species, despite international trade.

IAV antigenic drift is most pronounced in humans, probably due to a strong population herd immunity landscape associated with human population size, age structure, lifespan, and geographical structure (Taubenberger and Kash 2010; Petrova and Russell 2018). A limited number of combinations of amino-acid changes in HA antigenic sites of seasonal IAVs result in significant antigenic variations, leading to immune escape (Smith et al. 2004). Human seasonal IAVs antigenic drift is clustered, with such combinations of amino-acid changes occurring typically every 3 to 5 years—for yet unclear reasons. Single lineages usually predominate during human influenza seasons, with serial replacement of strains (Fig. 1). However, reassortment between IAV lineages within the same subtype appears more frequent than previously thought (Rambaut et al. 2008).

Although influenza vaccines have been available for over 80 years, most of those currently licensed still rely on decades-old technology, in particular on their production in embryonated chicken eggs, resulting in limitations in their effectiveness. Seasonal influenza vaccines furthermore need regular updating to attempt to best match upcoming circulating strains. Next-generation influenza vaccines building on alternative approaches for their design and production are currently being pursued to induce broader and longer-lasting immune responses to overcome seasonal influenza antigenic drift and to timely address the emergence of a new pandemic influenza virus (Becker et al. 2021).

22.3 Host-Level Pathogenesis of Infection

22.3.1 Natural Reservoirs

IAVs maintained in wild water bird reservoirs are typically of low pathogenic pathotype. However, the diversification, reassortment, and spread of HPAIV H5Nx in wild bird populations have lately challenged this paradigm (see below). LPAIVs typically cause inapparent intestinal tract infection in wild water birds. Cloacal shedding of high LPAIV loads lasts several days to a week, although prolonged shedding for weeks has been reported under experimental conditions. Wild water birds become infected following ingestion of water-borne IAVs that reach the intestine, or may contract the virus via the cloaca (Webster et al. 1992).

LPAIVs generally do not cause detectable damage in the intestinal tract of naturally or experimentally infected water birds, despite large numbers of infected cells detected by immunohistochemistry (for review see (Kuiken 2013)). Most infected intestinal epithelial cells are those of the intestinal crypts at the base of the villi of the large intestine and epithelial cells of the bursa of Fabricius. It has been suggested that the rapid turnover of intestinal epithelial cells coincides with the duration of LPAIV replication cycle (Daoust et al. 2011). Intestinal epithelial cells in the crypts continuously divide, pushing infected cells towards the tip of the intestinal

villi. Lytically infected cells may be released into the intestinal lumen, as they reach the tip of the villi and naturally shed into the lumen. Although visible intestinal damage may not occur, LPAIV infection of the gut may have clinical impact on wild water birds, such as impaired digestive functions. This may affect food intake, growth, migratory abilities, and reproductive success. Carefully designed studies are needed to address this unresolved issue (Kuiken 2013; Risely et al. 2018).

LPAIV infection can cause gross and histological lesions in other organs than the intestinal tract in experimentally infected water birds (for review see (Kuiken 2013)). In particular, tracheitis, pneumonia, and airsacculitis, associated with viral replication in respiratory epithelial cells in the airways and air sacs, have been described in several studies. Intranasal inoculation of mallards resulted in mild pharyngitis and tracheitis associated with LPAIV replication in respiratory epithelial cells, and this route of inoculation may reproduce respiratory infection of dabbling ducks during feeding. However, aerosol inoculation of domestic ducks with LPAIV resulted only in intestinal infection. Furthermore, neither lesions nor antigen-positive cells have been detected in the respiratory tract of naturally infected water birds, despite massive LPAIV infection of the intestinal tract. In accordance with these pathological findings, LPAIV pharyngeal shedding is typically limited in water birds.

Little is known about wild bird immune response to LPAI. The absence of lesions is associated with the absence of recruitment of inflammatory cells to the intestinal site of infection, suggesting a limited innate immune response (Daoust et al. 2011). However, immunoglobulins, including secretory IgX (equivalent of mammalian IgA), are produced and detectable within a few days following experimental infection in domestic ducks and may be present along the intestinal mucosa (Higgins et al. 1987; Magor et al. 1998). Avian adaptive immune responses against LPAIVs may protect at least in part against reinfection with IAVs of the same, and perhaps to a lesser extent, of different but related subtypes (Latorre-Margalef et al. 2013). The extent and duration of immunity against LPAIV reinfection in wild birds remain unknown yet may be essential in determining LPAIV epidemiological and evolutionary dynamics in their natural host reservoirs.

22.3.2 Spillover Host Species

22.3.2.1 Avian Spillover Host Species

LPAIVs of wild bird origin probably behave similarly in aquatic poultry as in wild water birds. In terrestrial poultry, LPAIVs of wild bird origin can cause clinical infection, principally of the respiratory tract and occasionally of the intestinal and urogenital tracts, with little mortality (Mo et al. 1997; Swayne et al. 2019). Infection with LPAIV results mainly in drop in egg production and respiratory signs, and occasionally, in intestinal and urogenital signs. Pharyngeal and cloacal shedding typically lasts several days to a week. Experimental inoculation of chickens demonstrated LPAIV replication in epithelial cells in the airways and lungs in association with inflammatory conditions like rhinitis, tracheitis, and pneumonia. LPAIV pathogenicity varies greatly between poultry species. Pneumonia may be complicated by

secondary bacterial infection. Rarely nephrosis and nephritis are observed. Intravenous inoculation of AIVs of wild bird origin has been shown to result in infection of renal tubular epithelial cells and intestinal epithelial cells in chickens (Swayne and Slemons 1990).

In naïve terrestrial poultry, LPAIVs of the H5 and H7 subtypes can evolve into HPAIVs. These viruses cause severe systemic infection resulting in more than 75% mortality, mainly in chickens and turkeys (Swayne et al. 2019; Swayne 2007). They are abundantly shed from both the respiratory and intestinal tracts. HPAIVs can infect endothelial cells and parenchymal cells in a wide range of organs, including respiratory tract, heart, pancreas, liver, intestine, kidneys, adrenal glands, and brain in poultry. HPAIV infection results in hemorrhages, coagulation failure and organ failure, and occasionally neurological signs, when birds survive the acute phase of the disease. However, gross and microscopic lesions of inflammation, necrosis, and hemorrhages may be rare, because sudden death can occur as early as 24 h after infection. Elevated pro-inflammatory immune responses are seen in chickens but not in ducks infected with HPAIVs; these may contribute to severe immune-pathology (Kuchipudi et al. 2014; Wang et al. 2021).

Most HPAIVs do not infect water birds or cause unapparent infection in these species. However, HPAIV H5N1 and related reassortant viruses have the ability to infect a wide range of avian hosts, including aquatic poultry and wild birds (for review see (Swayne 2007; Reperant et al. 2013; Alarcon et al. 2018)). HPAIV H5Nx pathogenicity in aquatic poultry and wild birds varies greatly and ranges from asymptomatic infection to fatal systemic disease, depending on the virus lineage and bird species involved. The timeframe of development of clinical signs also varies among wild bird species. Clinical disease may start a few days to more than a week after infection. Worryingly, recent HPAIVs H5Nx have caused unusually high mortality in ducks.

In contrast to LPAIV and HPAIVs of the H5N1 (clade 2.2) subtype were not found to infect the intestinal tract of water birds, and cloacal shedding was rare. HPAIV H5N1 was mainly shed from the pharynx of wild birds, and clinical signs included respiratory and neurological manifestations. Organs most often found infected were the respiratory tract, pancreas, liver, kidneys, adrenal glands, and brain in these species. Infected cells were parenchymal cells of most organs, and IAV antigen-expression in these cells was associated with lesions of inflammation and necrosis. In contrast to poultry, endothelial cells were not infected in most species of wild birds. Exceptions included whooper swans (*Cygnus cygnus*) and tufted ducks (*Aythya fuligula*), in which endothelial infection by HPAIV H5N1 and severe hemorrhages were reported (for review see (Reperant et al. 2013)).

Our current understanding of the immune response of poultry against LPAIV or HPAIV is incomplete (Suarez and Schultz-Cherry 2000; Swayne 2007). Neutralizing antibodies against the HA- and to a lesser extent NA-proteins provide protection against disease. The role of cellular immunity is less clear and may reduce viral shedding and help accelerate recovery upon infection. Overall, the immune response of terrestrial poultry against IAVs appears stronger than that of aquatic poultry. Vaccination of poultry against IAV elicits principally neutralizing antibodies against

the HA protein, but does not fully abrogate infection, and hence limited shedding can occur in vaccinated birds. Vaccination of poultry against HPAIV H5N1 may be an effective tool to reduce the virus prevalence in endemic countries, but stamping-out measures, combined with testing and reactive culling of poultry, are often necessary for the eventual eradication of HPAIVs.

22.3.2.2 Mammalian Spillover Host Species

IAVs that sporadically cross from avian to mammalian hosts, including zoonotic IAVs infecting humans, may cause from inapparent to severe respiratory and systemic infections. Clinical disease upon sporadic IAV infection is best documented in humans. In general, patients infected with zoonotic IAVs present with conjunctivitis or mild influenza-like illness (Peiris et al. 2007). However, in several cases, they may develop a severe or fatal lower respiratory tract infection characterized by necrotizing bronchiolitis, interstitial pneumonia, and diffuse alveolar damage.

Infections with HPAIVs of the H5N1 subtype display a unique pathogenesis. In addition to a respiratory route of infection, HPAIV H5N1 may infect mammalian species, including humans, via an intestinal route of infection. In most mammals, HPAIV H5N1 infections severely affect the lower respiratory tract as well as of other organs, including the liver, kidney, heart, pancreas, intestine, and brain (for review see (Reperant et al. 2009)). Lesions of inflammation and necrosis are associated with antigen-expression in parenchymal cells in these organs. Lungs, liver, and brain are the most frequently infected organs in most mammals, resulting in respiratory and neurological clinical signs. In humans, the lungs are the primary sites of infection, although evidence of HPAIV H5N1 replication in other organs has been reported, including in the brain and intestinal tract (Peiris et al. 2007). Lung lesions are typically severe, with diffuse epithelial necrosis, the presence of pulmonary edema and hemorrhage, and massive infiltration of inflammatory cells. Elevated levels of cytokines may contribute to the severity of the inflammatory response in fatal cases of human infection. These lesions can result in respiratory distress and death within 10 days.

The case-fatality rate of HPAIV H5N1 is above 50%. Severe respiratory infections similarly occur upon infection with HPAIV H5N6 (case-fatality rate of 70%), LPAIV H7N9 (case-fatality rate of 40%) and to a lesser extent LPAIV H9N2 (case-fatality rate of 2%). Interestingly, individuals most often infected with HPAIV H5N1 and LPAIV H9N2 are below 15 years of age, while those most often infected with LPAIV H7N9 are above 60. Bearing in mind the low number of cases, the highest incidence of cases of HPAIV H5N6 infection appears to be among the 30–44 age class (Philippon et al. 2020). It is not clear to what extent this is related to intrinsic age-related susceptibility or pre-existing immunity.

Most IAVs that efficiently transmit in mammalian species, including pandemic and seasonal IAVs in humans, typically cause both upper and lower respiratory tract infection, resulting in rhinitis, tracheobronchitis, bronchiolitis, and interstitial pneumonia (Kuiken and Taubenberger 2008). Clinical signs range from mild nasal secretions and coughing to severe pneumonia, which may be complicated by bacterial infection, acute respiratory distress, and death. In general, infection is more severe and located along the entire respiratory tract in individuals with

immature or impaired immune responses, with little history of past infections or with history of past infections by IAVs of a different subtype. These include infants and the elderly or individuals with comorbidity; individuals of a species occasionally infected by IAVs, like harbor seals and farmed mink; and individuals infected by a novel IAV subtype, like pandemic IAV in humans or a novel IAV strain in domestic species. In contrast, the infection is usually less severe and more localized to the upper region of the respiratory tract in individuals with optimal immune functions or a history of past infections with the same or related virus strains. These include healthy individuals infected with seasonal or endemic IAVs.

The pathogenicity of IAV infection in mammals appears also largely virus strain dependent. In general, IAVs that recently crossed the species barrier and some drift variants that escape population immunity tend to cause more severe disease, with the infection being located in both upper and lower respiratory tract in immunologically naïve individuals (Kuiken and Taubenberger 2008). In contrast, IAVs that recurrently circulate in mammalian populations tend to cause less severe disease, with the infection located in the upper regions of the respiratory tract. However, since IAV pathogenicity depends on both host and viral factors, the disease outcome upon IAV infection is highly variable. In humans, mild (e.g., 1968 and 2009), moderately severe (e.g., 1957), and severe (e.g., 1918) pandemics have occurred, with the severity of certain seasonal IAV epidemics matching that of mild pandemics (Taubenberger and Morens 2009).

In mammals, neutralizing immunoglobulins and secondary cellular immune responses, which tend to be stronger and longer-lived in the deeper regions of the respiratory tract, provide protection against reinfection with the same or closely related IAV (Doherty et al. 2006; Petrova and Russell 2018). While neutralizing immunoglobulins are largely strain specific, secondary cellular immune responses likely provide some cross-protection against heterosubtypic strains (Rimmelzwaan and Osterhaus 1995; Rimmelzwaan et al. 2007). Pre-existing immunological memory in mammalian populations may not only protect against severe lower respiratory disease upon reinfection but also exert selective pressures on IAVs, possibly resulting in changes in tropism patterns along the host respiratory tract (Reperant et al. 2012a).

22.4 Crossing Species, Crossing Scales: Adaptive Changes and Gain in Efficient Transmissibility

22.4.1 Host Switch

IAV crossing species barriers from natural host reservoirs to other avian or mammalian spillover hosts can be accompanied by differences in epidemiological and evolutionary signatures and different pathogenesis patterns. This may include changing tissue tropism patterns and variable severity of resulting disease. IAV tissue tropism and disease patterns are determined by the ability of the IAV to infect and replicate in target cells and stimulate host immune responses. These IAV replication

processes involve virus-host interactions that occur at molecular and cellular levels and govern the extent of IAV adaptation to avian and mammalian hosts.

22.4.1.1 IAV Adaptation to Avian Hosts

IAV tissue tropism and patterns of infection in avian hosts are largely determined by the distribution of IAV receptors in the avian intestinal and respiratory tracts and by IAV pathotype. The HA proteins of IAVs mediate their attachment to sialylated glycans of a variety of structural conformations, expressed on the surface of host cells. The HA proteins of IAVs circulating in wild water birds and in most poultry species have a preferred receptor binding affinity for sialic acids with α2,3 linkage to galactose (SAa2,3Gal) (for review see (Taubenberger and Kash 2010; Reperant et al. 2012b; Joseph et al. 2017)) and recognize both *N*-acetylneuraminic acids (Neu5Ac) and *N*-glycolylneuraminic acids (Neu5Gc) (Suzuki et al. 2000). In ducks and terrestrial poultry, these receptors predominate and are abundantly expressed on the surface of intestinal and respiratory epithelial cells (Kuchipudi et al. 2009; Pillai and Lee 2010).

Likewise, the NA proteins of IAVs circulating in avian hosts typically have sialidase specificity for SAa2,3Gal (Joseph et al. 2017). The frequent reassortment of IAV HA and NA genes in wild water birds indicates that these genes are generally compatible and balanced across subtypes and do not point to specific HA/NA combination with higher fitness (Dugan et al. 2008). In terrestrial poultry, an in-frame deletion of approximately 20 amino acids from the stalk region of the NA protein is associated with reduced enzymatic activity and may represent a common adaptive change in IAVs circulating in these species (Taubenberger and Kash 2010; Joseph et al. 2017).

In some terrestrial poultry species, such as quails and turkeys, the HA proteins of circulating IAVs can have a dual receptor binding affinity for both SAa2,3Gal and sialic acids with α2,6 linkage to galactose (SAa2,6Gal). The latter are expressed on intestinal and respiratory epithelial cells of terrestrial poultry but are absent or rare on those of ducks (Wan and Perez 2006; Kimble et al. 2010; Pillai and Lee 2010). Because SAa2,6Gal receptors are preferentially recognized by IAVs that spread efficiently in the human population, independently of cross species transmission (see below), terrestrial poultry may contribute to the generation of IAVs with pandemic potential.

The localized and systemic nature of LPAIV and HPAIV infection, respectively, in terrestrial poultry (and in other avian hosts for HPAIV H5N1) is determined by the HA protein cleavage site, mediating HA cleavage and fusion of the viral and cellular envelopes (for review see (Swayne 2007; Taubenberger and Kash 2010; Reperant et al. 2012b)). The HA protein cleavage site of most avian IAV and poultry LPAIV is composed of a conserved Q/E-X-R motif that requires the presence of extra-cellular trypsin-like proteases to be cleaved. Tryspin-like proteases are secreted in the intestine and are present in the respiratory tract of birds. In contrast, the HA cleavage site of HPAIV is characterized by the insertion of multiple basic amino acids, resulting in a R-X-R/K-R motif. It is recognized by ubiquitous intra-cellular furin-like proteases present in cells of many organs. HPAIV multi-basic cleavage site thus

contributes to the ability of these viruses to cause systemic infection beyond the intestinal and respiratory tracts in birds.

Little is known about the determinants of efficient replication of avian IAV in birds. However, avian IAV tends to be more sensitive to low temperatures than mammalian IAV. Avian IAV replicates efficiently at bird body temperature of 41 °C, while their replication at 33 °C is reduced in vitro. A glutamic acid residue at position 627 in the PB2 protein in part governs IAV cold-temperature sensitivity (Massin et al. 2001). The PB2 627E residue is highly conserved in avian IAVs, although a lineage of HPAIV H5N1 with substitution E627K in the PB2 protein has evolved and circulates in bird populations.

Upon infection, many host factors interact with the viral ribonucleoprotein to support viral replication. The polymerase activity of avian influenza viruses lately was shown to be supported by the host factor ANP32A (belonging to the acidic nuclear phosphoprotein 32 family), which has a special 33 amino acid deletion in birds (Zhang et al. 2019, 2021; Yu et al. 2022). The avian ANP32A as a result supports the polymerase activity of avian IAVs only. Structural studies demonstrated that the structure of this host factor allows it to bridge two asymmetric viral polymerases and mediates the assembly of the influenza virus replicase. Interestingly, the avian ANP32 proteins cannot support the activity of influenza B virus polymerase, which might explain why birds are rarely naturally infected with these viruses (Yu et al. 2022).

22.4.1.2 IAV Adaptation to Mammalian Hosts

The HA proteins of IAVs circulating in mammals have variable receptor binding affinity for SAa2,3Gal, SAa2,6Gal, or both types of receptors (for review see (Taubenberger and Kash 2010; Reperant et al. 2012b; Joseph et al. 2017)). In most mammalian species, the HA proteins of circulating IAVs recognize both Neu5Ac and Neu5Gc, except for human IAVs, which only recognize Neu5Ac (Suzuki et al. 2000). Intriguingly, bat IAVs H17N10 and H18N11 use major histocompatibility complex class II molecules (MHC-II) and not sialic acids for cell entry. The neuraminidase of these bat IAVs lacks sialidase activity and its function remains enigmatic (it may downregulate the surface expression of MHC-II molecules, facilitating viral release; Ciminski et al. 2020).

In general, in mammals, SAa2,3Gal receptors are abundantly expressed on respiratory epithelial cells located in the deeper regions of the respiratory tract, including bronchioles and alveoli (Shinya et al. 2006; van Riel et al. 2007; van Riel et al. 2010). The affinity of avian IAVs for these receptors coincides with the location of avian IAV infection in the lower respiratory tract of mammals, including humans, and in part determines the severity of the resulting disease.

Similar to avian IAVs, most IAVs circulating in seals, horses, and dogs, as well as the bat IAV H9N2, have a preferred receptor binding affinity for SAa2,3Gal (Connor et al. 1994; Matrosovich et al. 2000; Kandeil et al. 2019). In horses and dogs, SAa2,3Gal receptors are abundantly expressed on respiratory epithelial cells in the trachea and bronchi, as well as in the bronchioles and alveoli (Suzuki et al. 2000; Maas et al. 2007; Ning et al. 2012). The canine IAV H3N2 has a mutation in the HA

protein that may also increase its binding affinity for specific receptors with Neu5Aca2–3Galb1–4(Fuca-) or Neu5Aca2–3Galb1–3(Fuca-)-like structures that are present in dogs (Yang et al. 2013). In contrast, SAa2,6Gal are more rarely expressed. The abundance of IAV receptors in both upper and lower respiratory tract may partly determine the relative severity of IAV infection and frequent development of bronchiolitis and pneumonia in these species. However, the bat found infected with IAV H9N2 was asymptomatic.

Seals have both SAa2,3Gal and SAa2,6Gal distributed along their respiratory tract, especially in the bronchioles and alveoli. In these marine mammals, many self-limiting outbreaks have been caused by avian IAVs with a preferred affinity for SAa2,3Gal. However, avian IAVs of the H3N8 and H10N7 subtypes that emerged in seals in 2011 and 2014, respectively, demonstrated increased affinity for SAa2,6Gal (Karlsson et al. 2014; Herfst et al. 2020). These viruses accumulated mutations that may mark their adaptation to the marine mammals. While avian and early seal IAV H10N7 preferentially bond to SAa2,3Gal, later isolates demonstrated a decreased avidity for SAa2,3Gal and an increased affinity for SAa2,6Gal.

The HA proteins of IAVs circulating in humans typically have a preferred receptor binding affinity for SAa2,6Gal and in swine a dual receptor binding affinity for both SAa2,3Gal and SAa2,6Gal (for review see (Taubenberger and Kash 2010; Reperant et al. 2012b)). Some pandemic IAVs also have been shown to have dual receptor binding affinity; however, most seasonal IAVs, which evolved from pandemic IAVs, have predominant receptor binding affinity for SAa2,6Gal. This preferred affinity is associated with the widening of the binding pocket of the IAV HA protein. The receptor binding site of avian IAVs is narrower and typically results in a steric hindrance of the SAa2,6Gal (Joseph et al. 2017). The widening of the HA binding pocket—and potentially the creation of a hydrophobic environment—is mediated by IAV subtype-specific mutations in the HA protein, within and outside the receptor binding site. In parallel, an increase in NA enzymatic specificity for SAa2,6Gal typically accompanies the changes in HA affinity. It notably occurred over time in the human N2 neuraminidase, from the emergence of pandemic IAV H2N2 in humans in 1957 to recent seasonal IAV H3N2 (Kobasa et al. 1999).

In both swine and humans, SAa2,3Gal and SAa2,6Gal are expressed on the surface of different respiratory epithelial cells (van Riel et al. 2007, 2010; Van Poucke et al. 2010; Trebbien et al. 2011). SAa2,3Gal predominate in the deeper regions of the respiratory tract. They are expressed mainly on non-ciliated respiratory epithelial cells and type II pneumocytes, which are the most abundant cell types in the bronchioles and alveoli, respectively. In contrast, SAa2,6Gal predominate in the upper regions of the respiratory tract. They are mainly expressed on ciliated respiratory epithelial cells and type I pneumocytes. Ciliated respiratory epithelial cells are the most abundant cell type in the nasal cavity, trachea, and bronchi. A similar distribution of SAa2,3Gal and SAa2,6Gal receptors is described in the respiratory tract of the ferret, which provides a most relevant animal model of human IAV pathogenesis.

While the abundance of avian IAV receptors in mammalian lower respiratory tract correlates with the severity of avian IAV infection in mammals, the predominance of

SAa2,6Gal in the upper respiratory tract of humans, swine, and ferrets correlates with milder disease, as described for seasonal and/or endemic IAVs in humans, ferrets and swine. Remarkably, the presence of IAV receptors—whether SAa2,3Gal or SAa2,6Gal—in the upper regions of the respiratory tract, i.e., nasal cavity, trachea, and bronchi, is common to those mammalian species that sustain epidemic spread of IAVs. Infection of the upper respiratory tract is considered essential for ongoing host-to-host transmission of IAVs in mammals (for review see (Taubenberger and Kash 2010; Sorrell et al. 2011; Reperant et al. 2012b)) (see below).

The HA proteins of IAVs that efficiently spread in mammalian species all have a cleavage site with a single arginine motif, corresponding to LPAIV in poultry, and are cleaved by extracellular trypsin-like proteases. Such proteases are present in the respiratory tract of mammals (for review see (Reperant et al. 2012b)). In contrast, the multi-basic cleavage site of HPAIVs H5N1 contributes to the systemic nature of the infection in mammals.

The replication in mammalian cells of IAVs circulating in mammals is typically more efficient than that of avian IAVs. Mammalian IAVs typically replicate efficiently at low temperatures corresponding to those recorded in the mammalian upper respiratory tract. Substitution E627K in the PB2 protein is conserved among many mammalian IAVs and may confer resistance to cold temperatures (Massin et al. 2001). This PB2 substitution introduced in a range of IAV genetic backgrounds, including of avian and mammalian origin, increases IAV replication and pathogenicity in mammals (for review see (de Wit et al. 2008; Taubenberger and Kash 2010; Reperant et al. 2012b; Joseph et al. 2017)). It improves binding of PB2 and NP proteins to assemble into viral ribonucleoproteins (vRNP) in mammalian cells, increasing viral transcription, replication, and production. Most intriguingly, it was recently shown that this substitution overcomes the restriction of the human ANP32 proteins in supporting the replication of avian influenza viruses (Zhang et al. 2019). The human ANP32A and ANP32B host factors facilitate human IAV RNA synthesis but cannot efficiently support the polymerase activity of avian influenza viruses. Swine in contrast have an ANP32A host factor with a unique amino acid evolutionary pathway that allows it to support both avian and swine influenza viruses, in accordance with their known role of "mixing vessel" (Zhang et al. 2021; Yu et al. 2022).

Interestingly, however, the PB2 E627K substitution is not present in equine IAVs of the H7N7 subtype and in equine and canine IAVs of the H3N8 subtype, suggesting that other host factors likely affect the evolution of this mutation (Joseph et al. 2017). More surprisingly, this substitution is also absent in the pandemic IAV H1N1 of 2009, and introducing the mutation in this virus genome did not result in enhanced replication or increased pathogenicity of the virus (Herfst et al. 2010).

The nuclear transport of some mammalian IAV vRNPs is mediated by different importins-α than those mediating transport of avian IAV vRNPs (Gabriel et al. 2011). Six isoforms of importin-α, which recognize vRNP nuclear localization signals as part of the classical nuclear import pathway, have been described in humans and chickens. While avian IAV vRNP nuclear import was shown to be dependent upon importin-α1 and importin-α3, increased use of importin-α1 and a switch from

importin-α3 to importin-α7 correlated with efficient IAV replication in mammalian cells and impaired replication in avian cells. Interestingly, importin-α7 (encoded by the KPNA6 gene) interacts with the ANP32 proteins and affects polymerase activity (Yu et al. 2022). The changes in importin-α usage are associated with substitution D701N in the PB2 protein and N319K in the NP protein in a mouse-adapted strain of avian IAV H7N7. Substitution D701N in the PB2 protein is found in many mammalian IAVs, including in the seal IAV H3N8 of avian origin, and in avian IAVs that caused severe disease in humans, including HPAIV H5N1. It is considered one of several genetic markers differentiating avian from human IAV isolates. However, and again quite surprising, this substitution, like E627K, is absent in pandemic IAV H1N1 of 2009, and its introduction into the genome of this virus did not enhance replication nor increase pathogenicity (Herfst et al. 2010).

Elevated levels of pro-inflammatory cytokines have been reported in fatal human cases of 1918 pandemic IAV H1N1 infection and in fatal and severe cases of HPAIV H5N1 infection (for review see (de Wit et al. 2008; Taubenberger and Kash 2010; Reperant et al. 2012b). The NS1 proteins of these viruses are potent inhibitors of the antiviral effect of the innate immune response. Escape from host antiviral responses may lead to the development of a so-called cytokine storm, further contributing to the severe respiratory symptoms of these infections. However, NS1 mutations associated with such escape from host antiviral responses are not present in most IAVs circulating in humans and thus do not appear to be markers of IAV host adaptation.

22.4.2 Transmissibility

Although several markers of IAV adaptation to avian or mammalian hosts, associated with sustained IAV circulation in these species, have been identified, the determinants of the effective transmission of progeny viruses produced during infection to a new individual host are incompletely understood (Sorrell et al. 2011).

Receptor binding affinity for SAa2,6Gal and high replication levels in the upper respiratory tract appear essential for transmission of IAVs among humans. Only two amino acids, changing IAV H1N1 receptor binding affinity from SAa2,6Gal to SAa2,3Gal, abrogated contact transmission of 1918 pandemic IAV H1N1 in the ferret model (Tumpey et al. 2007). Conversely, the introduction of one mutation in the HA gene of avian IAV H9N2 conferred receptor binding affinity for SAa2,6Gal and improved contact transmission of the virus in ferrets (Wan et al. 2008). To date, specific residues of IAV HA proteins of the H1, H2, H3, H4, H5, H7, and H9 subtypes affecting their receptor binding affinity for SAa2,3Gal or SAa2,6Gal have been described (for review see (Reperant et al. 2012b)). However, receptor binding affinity for SAa2,6Gal is not sufficient to confer IAV transmissibility. A number of mutations have been found to decrease binding affinity of HPAIV H5N1 for SAa2,3Gal and increase their binding affinity for SAa2,6Gal; however, none of the mutated viruses efficiently spread by contact or aerosols in the ferret model (Chutinimitkul et al. 2010; Herfst et al. 2012).

Additional mutations, notably in the vRNP and matrix genes, affect IAV transmissibility in animal models. In particular, mutations improving IAV replication in mammalian cells were shown to confer or improve IAV transmissibility. In combination with the HA and NA proteins of the 1918 pandemic IAV H1N1 (with binding affinity for SAa2,6Gal), PB2 protein with E627K substitution allowed aerosol transmission of an avian IAV in ferrets (Van Hoeven et al. 2009). Conversely, substitution K627E in the PB2 protein of human seasonal IAV H3N2 impaired its aerosol transmission in guinea pigs (Steel et al. 2009). Substitution D701N in the PB2 protein also improved contact transmission of HPAIV H5N1 and aerosol transmission of human seasonal IAV H3N2 in guinea pigs (Steel et al. 2009). Lastly, the M gene was recently shown critical for the high transmissibility of 2009 pandemic IAV H1N1 in guinea pigs (Chou et al. 2011).

A set of mutations in the HA and PB2 proteins were found to allow aerosol transmission of HPAIV H5N1 in the ferret model (Herfst et al. 2012). These included two mutations in the HA gene known to change receptor binding affinity of HPAIV H5N1 from SAa2,3Gal to SAa2,6Gal; two additional mutations in the HA gene, of which one affected glycosylation of the protein; and PB2 E627K substitution. Reassortment of HPAIV H5N1 with 2009 pandemic IAV H1N1 also resulted in viruses that efficiently transmitted via respiratory droplets in ferrets and guinea-pigs (Imai et al. 2012; Zhang et al. 2013). In most of these reassortant viruses, mutations in the HPAIV H5N1 gene conferring SAa2,6Gal were present, while several genes of pandemic H1N1 origin were shown to improve transmissibility, including PA, NP, NA, M, and NS genes.

Importantly, thermostability of the HA and to pH of fusion may be additional prerequisites for aerosol or respiratory droplet transmissibility. In particular, compensatory mutations in the HA emerged and improved stability after the affinity switch from SAa2,3Gal to SAa2,6Gal in the seal H10N7 IAV (Herfst et al. 2020). Similar mutations improved respiratory transmission of HPAIV H5N1 and of the 2009 pandemic H1N1 virus. It is speculated that the HA stability phenotype may affect the stability of HA in aerosols, resistance to drought, stability in mucus, or resistances to changes in pH in the host environment (Herfst et al. 2020).

22.5 Conclusions

The highly diverse populations of IAVs circulating in wild bird species exist as a large pool of functionally equivalent and so often interchangeable gene segments that form transient gene constellations (Dugan et al. 2008). Occasionally, some of these gene constellations may lead to IAV infection of other avian or mammalian species, including humans. The ability of these viruses to productively replicate and sustainably spread among these new host species still poses numerous questions, likely to be addressed by studying more members of the wide diversity of IAVs in an increasing number of host species. IAV host switch and adaptation to novel host species, transmissibility, and pathogenicity are each dependent on complex multifactorial, interrelated, and mutual interactions between virus and host and are likely under

largely different selective pressures (Taubenberger and Kash 2010). Striking similarities though, in pathogenesis and transmissibility of IAV infections in newly invaded avian and mammalian species, including humans, may point to common pathways that lead to adaptation and sustained presence in newly "colonized" species.

References

Adlhoch C, Fusaro A, Gonzales JL, Kuiken T, Marangon S, Niqueux É, Staubach C, Terregino C, Aznar I, Muñoz Guajardo I, Baldinelli F (2021) Avian influenza overview September – December 2021. EFSA J 19(12):e07108. https://doi.org/10.2903/J.EFSA.2021.7108

Adlhoch C, Baldinelli F, Fusaro A, Terregino C (2022) Avian influenza, a new threat to public health in Europe? Clin Microbiol Infect 28(2):149–151. https://doi.org/10.1016/j.cmi.2021.11.005

Alarcon P, Brouwer A, Venkatesh D, Duncan D, Dovas CI, Georgiades G, Monne I, Fusaro A, Dan A, Śmietanka K, Ragias V, Breed AC, Chassalevris T, Goujgoulova G, Hjulsager CK, Ryan E, Sánchez A, Niqueux E, Tammiranta N, . . . Brown IH (2018) Comparison of 2016–17 and previous epizootics of highly pathogenic avian influenza H5 Guangdong lineage in Europe. Emerg Infect Dis. https://doi.org/10.3201/eid2412.171860

Alexander DJ (2007) An overview of the epidemiology of avian influenza. Vaccine 25(30):5637–5644

Anderson TK, Chang J, Arendsee ZW, Venkatesh D, Souza CK, Kimble JB, Lewis NS, Davis CT, Vincent AL (2021) Swine influenza a viruses and the tangled relationship with humans. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a038737

Becker T, Elbahesh H, Reperant LA, Rimmelzwaan GF, Osterhaus ADME (2021) Influenza vaccines: successes and continuing challenges. J Infect Dis. https://doi.org/10.1093/infdis/jiab269

Bodewes R, de Mutsert G, van der Klis FR, Ventresca M, Wilks S, Smith DJ, Koopmans M, Fouchier RA, Osterhaus AD, Rimmelzwaan GF (2011) Prevalence of antibodies against seasonal influenza A and B viruses in children in Netherlands. Clin Vaccine Immunol 18(3): 469–476

Bodewes R, Bestebroer TM, Van Der Vries E, Verhagen JH, Herfst S, Koopmans MP, Fouchier RAM, Pfankuche VM, Wohlsein P, Siebert U, Baumgärtner W, Osterhaus ADME (2015) Avian influenza a(H10n7) virus–associated mass deaths among harbor seals. Emerg Infect Dis. https://doi.org/10.3201/eid2104.141675

Borkenhagen LK, Salman MD, Ma MJ, Gray GC (2019) Animal influenza virus infections in humans: a commentary. Int J Infect Dis. https://doi.org/10.1016/j.ijid.2019.08.002

Borland S, Gracieux P, Jones M, Mallet F, Yugueros-Marcos J (2020) Influenza A virus infection in cats and dogs: a literature review in the light of the "one health" concept. Front Public Health. https://doi.org/10.3389/fpubh.2020.00083

Brown IH (2000) The epidemiology and evolution of influenza viruses in pigs. Vet Microbiol 74(1–2):29–46

Callan RJ, Early G, Kida H, Hinshaw VS (1995) The appearance of H3 influenza viruses in seals. J Gen Virol 76(1):199–203

Caron A, Cappelle J, Gaidet N (2017) Challenging the conceptual framework of maintenance hosts for influenza A viruses in wild birds. J Appl Ecol. https://doi.org/10.1111/1365-2664.12839

Chen R, Holmes EC (2006) Avian influenza virus exhibits rapid evolutionary dynamics. Mol Biol Evol 23(12):2336–2341

Chen H, Smith GJ, Li KS, Wang J, Fan XH, Rayner JM, Vijaykrishna D, Zhang JX, Zhang LJ, Guo CT, Cheung CL, Xu KM, Duan L, Huang K, Qin K, Leung YH, Wu WL, Lu HR, Chen Y, Xia NS, Naipospos TS, Yuen KY, Hassan SS, Bahri S, Nguyen TD, Webster RG, Peiris JS, Guan Y (2006) Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc Natl Acad Sci U S A 103(8):2845–2850

Chou YY, Albrecht RA, Pica N, Lowen AC, Richt JA, Garcia-Sastre A, Palese P, Hai R (2011) The M segment of the 2009 new pandemic H1N1 influenza virus is critical for its high transmission efficiency in the Guinea pig model. J Virol 85(21):11235–11241

Chutinimitkul S, van Riel D, Munster VJ, van den Brand JM, Rimmelzwaan GF, Kuiken T, Osterhaus AD, Fouchier RA, de Wit E (2010) In vitro assessment of attachment pattern and replication efficiency of H5N1 influenza A viruses with altered receptor specificity. J Virol 84 (13):6825–6833

Ciminski K, Pfaff F, Beer M, Schwemmle M (2020) Bats reveal the true power of influenza A virus adaptability. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1008384

Connor RJ, Kawaoka Y, Webster RG, Paulson JC (1994) Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. Virology 205(1):17–23

Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, Chen L, Smith C, Hill RC, Ferro P, Pompey J, Bright RA, Medina MJ, Johnson CM, Olsen CW, Cox NJ, Klimov AI, Katz JM, Donis RO (2005) Transmission of equine influenza virus to dogs. Science 310(5747):482–485

Daly JM, Blunden AS, Macrae S, Miller J, Bowman SJ, Kolodziejek J, Nowotny N, Smith KC (2008) Transmission of equine influenza virus to English foxhounds. Emerg Infect Dis 14(3):461–464

Daoust PY, Kibenge FS, Fouchier RA, van de Bildt MW, van Riel D, Kuiken T (2011) Replication of low pathogenic avian influenza virus in naturally infected Mallard ducks (*Anas platyrhynchos*) causes no morphologic lesions. J Wildl Dis 47(2):401–409

de Jong JC, Smith DJ, Lapedes AS, Donatelli I, Campitelli L, Barigazzi G, Van Reeth K, Jones TC, Rimmelzwaan GF, Osterhaus AD, Fouchier RA (2007) Antigenic and genetic evolution of swine influenza A (H3N2) viruses in Europe. J Virol 81(8):4315–4322

de Wit E, Kawaoka Y, de Jong MD, Fouchier RAM (2008) Pathogenicity of highly pathogenic avian influenza virus in mammals. Vaccine 26(S4):D54–D58

Doherty PC, Turner SJ, Webby RG, Thomas PG (2006) Influenza and the challenge for immunology. Nat Immunol 7(5):449–455

Dugan VG, Chen R, Spiro DJ, Sengamalay N, Zaborsky J, Ghedin E, Nolting J, Swayne DE, Runstadler JA, Happ GM, Senne DA, Wang R, Slemons RD, Holmes EC, Taubenberger JK (2008) The evolutionary genetics and emergence of avian influenza viruses in wild birds. PLoS Pathog 4(5):e1000076

Fereidouni S, Munoz O, von Dobschuetz S, de Nardi M (2014) Influenza virus infection of marine mammals. In EcoHealth. https://doi.org/10.1007/s10393-014-0968-1

Gabriel G, Klingel K, Otte A, Thiele S, Hudjetz B, Arman-Kalcek G, Sauter M, Shmidt T, Rother F, Baumgarte S, Keiner B, Hartmann E, Bader M, Brownlee GG, Fodor E, Klenk HD (2011) Differential use of importin-alpha isoforms governs cell tropism and host adaptation of influenza virus. Nat Commun 2:156

Gaydos JC, Top FH Jr, Hodder RA, Russell PK (2006) Swine influenza A outbreak, Fort Dix, New Jersey, 1976. Emerg Infect Dis 12(1):23–28

Geraci JR, St Aubin DJ, Barker IK, Webster RG, Hinshaw VS, Bean WJ, Ruhnke HL, Prescott JH, Early G, Baker AS, Madoff S, Schooley RT (1982) Mass mortality of harbor seals: pneumonia associated with influenza A virus. Science 215(4536):1129–1131

Gilbert M, Xiao X, Domenech J, Lubroth J, Martin V, Slingenbergh J (2006) Anatidae migration in the western Palearctic and spread of highly pathogenic avian influenza H5NI virus. Emerg Infect Dis 12(11):1650–1656. https://doi.org/10.3201/eid1211.060223

Gill JS, Webby R, Gilchrist MJ, Gray GC (2006) Avian influenza among waterfowl hunters and wildlife professionals. Emerg Infect Dis 12(8):1284–1286

Goldfield M, Bartley JD, Pizzuti W, Black HC, Altman R, Halperin WE (1977) Influenza in New Jersey in 1976: isolations of influenza A/New Jersey/76 virus at Fort Dix. J Infect Dis 136 (Suppl):S347–S355

Gray GC, Trampel DW, Roth JA (2007) Pandemic influenza planning: shouldn't swine and poultry workers be included? Vaccine 25(22):4376–4381

Gray GC, Ferguson DD, Lowther PE, Heil GL, Friary JA (2011) A national study of US bird banders for evidence of avian influenza virus infections. J Clin Virol 51(2):132–135

Guo Y, Wang M, Kawaoka Y, Gorman O, Ito T, Saito T, Webster RG (1992) Characterization of a new avian-like influenza A virus from horses in China. Virology 188(1):245–255

Guo F, Roy A, Wang R, Yang J, Zhang Z, Luo W, Shen X, Chen RA, Irwin DM, Shen Y (2021) Host adaptive evolution of avian-origin H3N2 canine influenza virus. Front Microbiol 12:1435. https://doi.org/10.3389/FMICB.2021.655228/BIBTEX

Hanson BA, Luttrell MP, Goekjian VH, Niles L, Swayne DE, Senne DA, Stallknecht DE (2008) Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? J Wildl Dis 44(2):351–361

Harvey E, Holmes EC (2022) Diversity and evolution of the animal virome. Nat Rev Microbiol. https://doi.org/10.1038/s41579-021-00665-x

Henritzi D, Hoffmann B, Wacheck S, Pesch S, Herrler G, Beer M, Harder TC (2019) A newly developed tetraplex real-time RT-PCR for simultaneous screening of influenza virus types A, B, C and D. Influenza Other Respir Viruses 13(1):71–82. https://doi.org/10.1111/IRV.12613

Henritzi D, Petric PP, Lewis NS, Graaf A, Pessia A, Starick E, Breithaupt A, Strebelow G, Luttermann C, Parker LMK, Schröder C, Hammerschmidt B, Herrler G, Beilage E große, Stadlbauer D, Simon V, Krammer F, Wacheck S, Pesch S, . . . Harder TC (2020) Surveillance of European domestic pig populations identifies an emerging reservoir of potentially zoonotic swine influenza A viruses. Cell Host Microbe 28(4):614–627.e6. https://doi.org/10.1016/J.CHOM.2020.07.006

Herfst S, Chutinimitkul S, Ye J, de Wit E, Munster VJ, Schrauwen EJ, Bestebroer TM, Jonges M, Meijer A, Koopmans M, Rimmelzwaan GF, Osterhaus AD, Perez DR, Fouchier RA (2010) Introduction of virulence markers in PB2 of pandemic swine-origin influenza virus does not result in enhanced virulence or transmission. J Virol 84(8):3752–3758

Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de Wit E, Munster VJ, Sorrell EM, Bestebroer TM, Burke DF, Smith DJ, Rimmelzwaan GF, Osterhaus AD, Fouchier RA (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. Science 336(6088):1534–1541

Herfst S, Zhang J, Richard M, McBride R, Lexmond P, Bestebroer TM, Spronken MIJ, de Meulder D, van den Brand JM, Rosu ME, Martin SR, Gamblin SJ, Xiong X, Peng W, Bodewes R, van der Vries E, Osterhaus ADME, Paulson JC, Skehel JJ, Fouchier RAM (2020) Hemagglutinin traits determine transmission of Avian A/H10N7 influenza virus between mammals. Cell Host Microbe. https://doi.org/10.1016/j.chom.2020.08.011

Higgins DA, Shortridge KF, Ng PL (1987) Bile immunoglobulin of the duck (*Anas platyrhynchos*). II. Antibody response in influenza A virus infections. Immunology 62(3):499–504

Hinshaw VS, Bean WJ, Webster RG, Rehg JE, Fiorelli P, Early G, Geraci JR, St Aubin DJ (1984) Are seals frequently infected with avian influenza viruses? J Virol 51(3):863–865

Hulse-Post DJ, Sturm-Ramirez KM, Humberd J, Seiler P, Govorkova EA, Krauss S, Scholtissek C, Puthavathana P, Buranathai C, Nguyen TD, Long HT, Naipospos TS, Chen H, Ellis TM, Guan Y, Peiris JS, Webster RG (2005) Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proc Natl Acad Sci U S A 102(30):10682–10687. https://doi.org/10.1073/pnas.0504662102

Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K, Zhong G, Hanson A, Katsura H, Watanabe S, Li C, Kawakami E, Yamada S, Kiso M, Suzuki Y, Maher EA, Neumann G, Kawaoka Y (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. Nature 486(7403):420–428

Jeong S, Lee DH, Kim YJ, Lee SH, Cho AY, Noh JY, Tseren-Ochir EO, Jeong JH, Song CS (2019) Introduction of Avian influenza A(H6N5) virus into Asia from North America by wild birds. Emerg Infect Dis. https://doi.org/10.3201/eid2511.190604

Joseph U, Su YCF, Vijaykrishna D, Smith GJD (2017) The ecology and adaptive evolution of influenza A interspecies transmission. Influenza Other Respir Viruses 11(1):74–84. https://doi.org/10.1111/IRV.12412

Kandeil A, Gomaa MR, Shehata MM, El Taweel AN, Mahmoud SH, Bagato O, Moatasim Y, Kutkat O, Kayed AS, Dawson P, Qiu X, Bahl J, Webby RJ, Karesh WB, Kayali G, Ali MA (2019) Isolation and characterization of a distinct influenza A virus from Egyptian bats. J Virol. https://doi.org/10.1128/jvi.01059-18

Karlsson EA, Ip HS, Hall JS, Yoon SW, Johnson J, Beck MA, Webby RJ, Schultz-Cherry S (2014) Respiratory transmission of an avian H3N8 influenza virus isolated from a harbour seal. Nat Commun. https://doi.org/10.1038/ncomms5791

Keawcharoen J, van Riel D, van Amerongen G, Bestebroer T, Beyer WE, van Lavieren R, Osterhaus AD, Fouchier RA, Kuiken T (2008) Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). Emerg Infect Dis 14(4):600–607

Kimble B, Nieto GR, Perez DR (2010) Characterization of influenza virus sialic acid receptors in minor poultry species. Virol J 7:365. https://doi.org/10.1186/1743-422X-7-365

Klingeborn B, Englund L, Rott R, Juntti N, Rockborn G (1985) An avian influenza A virus killing a mammalian species – the mink. Arch Virol 86:347–351

Kobasa D, Kodihalli S, Luo M, Castrucci MR, Donatelli I, Suzuki Y, Suzuki T, Kawaoka Y (1999) Amino acid residues contributing to the substrate specificity of the influenza A virus neuraminidase. J Virol 73(8):6743–6751

Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, Meijer A, van Steenbergen J, Fouchier R, Osterhaus A, Bosman A (2004) Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet 363(9409):587–593

Krauss S, Stallknecht DE, Slemons RD, Bowman AS, Poulson RL, Nolting JM, Knowles JP, Webster RG (2016) The enigma of the apparent disappearance of Eurasian highly pathogenic H5 clade 2.3.4.4 influenza A viruses in North American waterfowl. Proceedings of the National Academy of Sciences of the United States of America. https://doi.org/10.1073/pnas.1608853113

Kuchipudi SV, Nelli R, White GA, Bain M, Chang KC, Dunham S (2009) Differences in influenza virus receptors in chickens and ducks: implications for interspecies transmission. J Mol Genet Med 3(1):143–151

Kuchipudi SV, Tellabati M, Sebastian S, Londt BZ, Jansen C, Vervelde L, Brookes SM, Brown IH, Dunham SP, Chang KC (2014) Highly pathogenic avian influenza virus infection in chickens but not ducks is associated with elevated host immune and pro-inflammatory responses. Vet Res. https://doi.org/10.1186/s13567-014-0118-3

Kuiken T (2013) Is low pathogenic avian influenza virus virulent for wild waterbirds? Proc Biol Sci 280(1763):20130990. https://doi.org/10.1098/rspb.2013.0990

Kuiken T, Taubenberger JK (2008) Pathology of human influenza revisited. Vaccine 26S:D59–D66

Lai AC, Chambers TM, Holland RE Jr, Morley PS, Haines DM, Townsend HG, Barrandeguy M (2001) Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. Arch Virol 146(6):1063–1074

Lang G, Gagnon A, Geraci JR (1981) Isolation of an influenza A virus from seals. Arch Virol 68(3–4):189–195

Latorre-Margalef N, Grosbois V, Wahlgren J, Munster VJ, Tolf C, Fouchier RA, Osterhaus AD, Olsen B, Waldenstrom J (2013) Heterosubtypic immunity to influenza A virus infections in mallards may explain existence of multiple virus subtypes. PLoS Pathog 9(6):e1003443. https://doi.org/10.1371/journal.ppat.1003443

Lee DH, Bertran K, Kwon JH, Swayne DE (2017) Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. In. J Vet Sci. https://doi.org/10.4142/jvs.2017.18.S1.269

Li Y, Shi J, Zhong G, Deng G, Tian G, Ge J, Zeng X, Song J, Zhao D, Liu L, Jiang Y, Guan Y, Bu Z, Chen H (2010) Continued evolution of H5N1 influenza viruses in wild birds, domestic poultry, and humans in China from 2004 to 2009. J Virol 84(17):8389–8397. https://doi.org/10.1128/JVI.00413-10

Liu R, Sheng Z, Huang C, Wang D, Li F (2020) Influenza D virus. Curr Opin Virol. https://doi.org/10.1016/j.coviro.2020.08.004

Lowen AC (2017) Constraints, drivers, and implications of influenza A virus reassortment. Ann Rev Virol. https://doi.org/10.1146/annurev-virology-101416-041726

Lycett SJ, Bodewes R, Pohlmann A, Banks J, Bányai K, Boni MF, Bouwstra R, Breed AC, Brown IH, Chen, H., Dán Á, DeLiberto TJ, Diep N, Gilbert M, Hill S, Ip HS, Ke CW, Kida H, Killian ML, … Kuiken T (2016) Role for migratory wild birds in the global spread of avian influenza H5N8. Science https://doi.org/10.1126/science.aaf8852

Maas R, Tacken M, Ruuls L, Koch G, van Rooij E, Stockhofe-Zurwieden N (2007) Avian influenza (H5N1) susceptibility and receptors in dogs. Emerg Infect Dis 13(8):1219–1221

Magor KE, Warr GW, Bando Y, Middleton DL, Higgins DA (1998) Secretory immune system of the duck (*Anas platyrhynchos*). Identification and expression of the genes encoding IgA and IgM heavy chains. Eur J Immunol 28(3):1063–1068

Massin P, van der Werf S, Naffakh N (2001) Residue 627 of PB2 is a determinant of cold sensitivity in RNA replication of avian influenza viruses. J Virol 75(11):5398–5404

Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, Castrucci MR, Donatelli I, Kawaoka Y (2000) Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. J Virol 74(18): 8502–8512

Mo IP, Brugh M, Fletcher OJ, Rowland GN, Swayne DE (1997) Comparative pathology of chickens experimentally inoculated with avian influenza viruses of low and high pathogenicity. Avian Dis 41(1):125–136

Munster VJ, Baas C, Lexmond P, Waldenstrom J, Wallensten A, Fransson T, Rimmelzwaan GF, Beyer WE, Schutten M, Olsen B, Osterhaus AD, Fouchier RA (2007) Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS Pathog 3(5): e61

Ning ZY, Wu XT, Cheng YF, Qi WB, An YF, Wang H, Zhang GH, Li SJ (2012) Tissue distribution of sialic acid-linked influenza virus receptors in beagle dogs. J Vet Sci 13(3):219–222

Oliver I, Roberts J, Brown CS, Byrne AM, Mellon D, Hansen RDE, Banyard AC, James J, Donati M, Porter R, Ellis J, Cogdale J, Lackenby A, Chand M, Dabrera G, Brown IH, Zambon M (2022) A case of avian influenza A(H5N1) in England, January 2022. Eur Secur 27(5). https://doi.org/10.2807/1560-7917.ES.2022.27.5.2200061

Olsen CW (2002) The emergence of novel swine influenza viruses in North America. Virus Res 85 (2):199–210

Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus AD, Fouchier RA (2006) Global patterns of influenza a virus in wild birds. Science 312(5772):384–388

Palese P, Shaw ML (2007) Orthomyxoviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology. Lippincott, Williams & Wilkins, pp 1647–1690

Parrish CR, Murcia PR, Holmes EC (2015) Influenza virus reservoirs and intermediate hosts: dogs, horses, and new possibilities for influenza virus exposure of humans. J Virol. https://doi.org/10.1128/jvi.03146-14

Peiris M (2009) Avian influenza viruses in humans. Rev Sci Tech 28(1):161–174

Peiris JS, de Jong MD, Guan Y (2007) Avian influenza virus (H5N1): a threat to human health. Clin Microbiol Rev 20(2):243–267

Petrova VN, Russell CA (2018) The evolution of seasonal influenza viruses. Nat Rev Microbiol. https://doi.org/10.1038/nrmicro.2017.118

Philippon DAM, Wu P, Cowling BJ, Lau EHY (2020) Avian influenza human infections at the human-animal interface. J Infect Dis. https://doi.org/10.1093/infdis/jiaa105

Pillai SP, Lee CW (2010) Species and age related differences in the type and distribution of influenza virus receptors in different tissues of chickens, ducks and turkeys. Virol J 7:5. https://doi.org/10.1186/1743-422X-7-5

Pohlmann A, Hoffmann D, Grund C, Koethe S, Hüssy D, Meier SM, King J, Schinköthe J, Ulrich R, Harder T, Beer M (2019) Genetic characterization and zoonotic potential of highly pathogenic avian influenza virus A(H5N6/H5N5), Germany, 2017–2018. Emerg Infect Dis. https://doi.org/10.3201/eid2510.181931

Postel A, King J, Kaiser F, Kennedy J, Lombardo M, Reineking W, De le Roi M, Harder T, Pohlmann A, Gerlach T, Rimmelzwaan G, Rohner S, Striewe L, Gross S, Schick L, Klink J, Kramer K, Osterhaus A, Beer M, … Becher P (2022) Infections with highly pathogenic avian influenza A virus (HPAIV) H5N8 in harbor seals at the German North Sea coast, 2021. Emerg Microb Infect

Pulit-Penaloza JA, Brock N, Pappas C, Sun X, Belser JA, Zeng H, Tumpey TM, Maines TR (2020) Characterization of highly pathogenic avian influenza H5Nx viruses in the ferret model. Sci Rep. https://doi.org/10.1038/s41598-020-69535-5

Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC (2008) The genomic and epidemiological dynamics of human influenza A virus. Nature 453(7195):615–619. https://doi.org/10.1038/nature06945

Reperant LA, Osterhaus ADME (2012) Chapter 4: Avian and animal influenza. In: Van-Tam J, Sellwood C (eds) Introduction to pandemic influenza, 2nd edn. CABI, Wallingford, Oxfordshire, UK

Reperant LA, Rimmelzwaan GF, Kuiken T (2009) Avian influenza viruses in mammals. Rev Sci Tech 28(1):137–159

Reperant LA, Fuckar NS, Osterhaus AD, Dobson AP, Kuiken T (2010) Spatial and temporal association of outbreaks of H5N1 influenza virus infection in wild birds with the 0 degrees C isotherm. PLoS Pathog 6(4):e1000854

Reperant LA, Kuiken T, Grenfell BT, Osterhaus ADME, Dobson AP (2012a) Linking influenza virus tissue tropism to population-level reproductive fitness. PLoS One 7(8):e43115

Reperant LA, Kuiken T, Osterhaus AD (2012b) Adaptive pathways of zoonotic influenza viruses: from exposure to establishment in humans. Vaccine 30(30):4419–4434

Reperant LA, Osterhaus ADME, Kuiken T (2013) Avian influenza. In: Gavier-Widen D, Duff P, Meredith A (eds) Infectious diseases of wild mammals and birds in Europe. Wiley-Blackwell, Chicester, West Sussex, UK

Reperant LA, Grenfell BT, Osterhaus ADME (2015) Quantifying the risk of pandemic influenza virus evolution by mutation and re-assortment. Vaccine. https://doi.org/10.1016/j.vaccine.2015.10.056

Rimmelzwaan GF, Osterhaus AD (1995) Cytotoxic T lymphocyte memory: role in cross-protective immunity against influenza? Vaccine 13(8):703–705

Rimmelzwaan GF, Fouchier RA, Osterhaus AD (2007) Influenza virus-specific cytotoxic T lymphocytes: a correlate of protection and a basis for vaccine development. Curr Opin Biotechnol 18(6):529–536

Risely A, Klaassen M, Hoye BJ (2018) Migratory animals feel the cost of getting sick: a meta-analysis across species. J Anim Ecol. https://doi.org/10.1111/1365-2656.12766

Roche B, Drake JM, Brown J, Stallknecht DE, Bedford T, Rohani P (2014) Adaptive evolution and environmental durability jointly structure phylodynamic patterns in avian influenza viruses. PLoS Biol. https://doi.org/10.1371/journal.pbio.1001931

Russell CA, Jones TC, Barr IG, Cox NJ, Garten RJ, Gregory V, Gust ID, Hampson AW, Hay AJ, Hurt AC, de Jong JC, Kelso A, Klimov AI, Kageyama T, Komadina N, Lapedes AS, Lin YP, Mosterin A, Obuchi M, Odagiri T, Osterhaus AD, Rimmelzwaan GF, Shaw MW, Skepner E, Stohr K, Tashiro M, Fouchier RA, Smith DJ (2008) The global circulation of seasonal influenza A (H3N2) viruses. Science 320(5874):340–346

Saunders-Hastings PR, Krewski D (2016) Reviewing the history of pandemic influenza: understanding patterns of emergence and transmission. Pathogens. https://doi.org/10.3390/pathogens5040066

Schülein A, Ritzmann M, Christian J, Schneider K, Neubauer-Juric A (2021) Exposure of wild boar to Influenza A viruses in Bavaria: analysis of seroprevalences and antibody subtype specificity before and after the panzootic of highly pathogenic avian influenza viruses A (H5N8). Zoonoses Public Health. https://doi.org/10.1111/zph.12841

Shin DL, Siebert U, Lakemeyer J, Grilo M, Pawliczka I, Wu NH, Valentin-Weigand P, Haas L, Herrler G (2019) Highly pathogenic avian influenza A(H5N8) virus in gray seals, Baltic Sea. Emerg Infect Dis. https://doi.org/10.3201/eid2512.181472

Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y (2006) Avian flu: influenza virus receptors in the human airway. Nature 440(7083):435–436. https://doi.org/10.1038/440435a

Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Rimmelzwaan GF, Osterhaus AD, Fouchier RA (2004) Mapping the antigenic and genetic evolution of influenza virus. Science 305(5682):371–376

Song D, Kang B, Lee C, Jung K, Ha G, Kang D, Park S, Park B, Oh J (2008) Transmission of avian influenza virus (H3N2) to dogs. Emerg Infect Dis 14(5):741–746

Song DS, An DJ, Moon HJ, Yeom MJ, Jeong HY, Jeong WS, Park SJ, Kim HK, Han SY, Oh JS, Park BK, Kim JK, Poo H, Webster RG, Jung K, Kang BK (2011) Interspecies transmission of

the canine influenza H3N2 virus to domestic cats in South Korea, 2010. J Gen Virol 92(Pt 10): 2350–2355
Sorrell EM, Schrauwen EJA, Linster M, de Graaf M, Herfst S, Fouchier RAM (2011) Predicting 'airborne' influenza viruses: (trans)mission impossible? Curr Opin Virol. https://doi.org/10.1016/j.coviro.2011.07.003
Stallknecht DE, Brown JD (2009) Tenacity of avian influenza viruses. Rev Sci Tech 28(1):59–67
Steel J, Lowen AC, Mubareka S, Palese P (2009) Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. PLoS Pathog 5(1):e1000252
Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, Dyrting K, Rehg JE, Poon L, Guan Y, Peiris M, Webster RG (2004) Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. J Virol 78(9):4892–4901
Suarez DL, Schultz-Cherry S (2000) Immunology of avian influenza virus: a review. Dev Comp Immunol 24(2–3):269–283
Suzuki Y, Nei M (2002) Origin and evolution of influenza virus hemagglutinin genes. Mol Biol Evol 19(4):501–509
Suzuki Y, Ito T, Suzuki T, Holland RE Jr, Chambers TM, Kiso M, Ishida H, Kawaoka Y (2000) Sialic acid species as a determinant of the host range of influenza A viruses. J Virol 74(24):11825–11831
Swayne DE (2007) Understanding the complex pathobiology of high pathogenicity avian influenza viruses in birds. Avian Dis 51(1 Suppl):242–249
Swayne DE, Suarez DL, Sims LD (2019) Influenza. Dis Poultry, 210–256. https://doi.org/10.1002/9781119371199.CH6
Swayne DE, Slemons RD (1990) Renal pathology in specific-pathogen-free chickens inoculated with a waterfowl-origin type A influenza virus. Avian Dis 34(2):285–294
Taubenberger JK, Kash JC (2010) Influenza virus evolution, host adaptation, and pandemic formation. Cell Host Microbe 7(6):440–451. https://doi.org/10.1016/j.chom.2010.05.009
Taubenberger JK, Morens DM (2008) The pathology of human influenza infections. Ann Rev Pathol: Mechan Disease 3:499–522
Taubenberger JK, Morens DM (2009) Pandemic influenza--including a risk assessment of H5N1. Rev Sci Tech 28(1):187–202
Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle AS, Moran D, Rogers S, Shi M, Tao Y, Weil MR, Tang K, Rowe LA, Sammons S, Xu X, Frace M, Lindblade KA, Cox NJ, Anderson LJ, Rupprecht CE, Donis RO (2012) A distinct lineage of influenza A virus from bats. Proc Natl Acad Sci U S A 109(11): 4269–4274. https://doi.org/10.1073/pnas.1116200109
Tong S, Zhu X, Li Y, Shi M, Zhang J et al (2013a) New world bats harbor diverse influenza A viruses. PLoS Pathog 9(10):e1003657
Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J, Chen LM, Johnson A, Tao Y, Dreyfus C, Yu W, McBride R, Carney PJ, Gilbert AT, Chang J, Guo Z, Davis CT, Paulson JC, Stevens J, Rupprecht CE, Holmes EC, Wilson IA, Donis RO (2013b) New world bats harbor diverse influenza a viruses. PLoS Pathog 9(10):e1003657. https://doi.org/10.1371/journal.ppat.1003657
Top FH Jr, Russell PK (1977) Swine influenza A at Fort Dix, New Jersey (January-February 1976) IV Summary and speculation. J Infect Dis 136(Suppl):S376–S380
Trebbien R, Larsen LE, Viuff BM (2011) Distribution of sialic acid receptors and influenza A virus of avian and swine origin in experimentally infected pigs. Virol J 8:434. https://doi.org/10.1186/1743-422X-8-434
Tumpey TM, Maines TR, Van Hoeven N, Glaser L, Solorzano A, Pappas C, Cox NJ, Swayne DE, Palese P, Katz JM, Garcia-Sastre A (2007) A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. Science 315(5812):655–659
van Dijk JGB, Hoye BJ, Verhagen JH, Nolet BA, Fouchier RAM, Klaassen M (2013) Juveniles and migrants as drivers for seasonal epizootics of avian influenza virus. J Anim Ecol. https://doi.org/10.1111/1365-2656.12131
van Dijk JG, Verhagen JH, Wille M, Waldenström J (2018) Host and virus ecology as determinants of influenza A virus transmission in wild birds. Curr Opin Virol. https://doi.org/10.1016/j.coviro.2017.10.006

Van Hoeven N, Pappas C, Belser JA, Maines TR, Zeng H, Garcia-Sastre A, Sasisekharan R, Katz JM, Tumpey TM (2009) Human HA and polymerase subunit PB2 proteins confer transmission of an avian influenza virus through the air. Proc Natl Acad Sci U S A 106(9):3366–3371

Van Poucke SG, Nicholls JM, Nauwynck HJ, Van Reeth K (2010) Replication of avian, human and swine influenza viruses in porcine respiratory explants and association with sialic acid distribution. Virol J 7:38. https://doi.org/10.1186/1743-422X-7-38

van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T (2007) Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals. Am J Pathol 171(4):1215–1223

van Riel D, den Bakker MA, Leijten LME, Chutinimitkul S, Munster V, de Wit E, Rimmelzwaan GF, Fouchier RAM, Osterhaus ADME, Kuiken T (2010) Seasonal and pandemic human influenza viruses attach better to human upper respiratory tract epithelium than avian influenza viruses. Am J Pathol 176:1614–1618

Verhagen JH, Majoor F, Lexmond P, Vuong O, Kasemir G, Lutterop D, Osterhaus AD, Fouchier RA, Kuiken T (2014) Epidemiology of influenza A virus among Black-headed Gulls, the Netherlands, 2006–2010. Emerg Infect Dis 20(1):138–141. https://doi.org/10.3201/eid2001.130984

Verhagen JH, Lexmond P, Vuong O, Schutten M, Guldemeester J, Osterhaus ADME, Elbers ARW, Slaterus R, Hornman M, Koch G, Fouchier RAM (2017) Discordant detection of avian influenza virus subtypes in time and space between poultry and wild birds, towards improvement of surveillance programs. PLoS One. https://doi.org/10.1371/journal.pone.0173470

Verhagen JH, Fouchier RAM, Lewis N (2021) Highly pathogenic avian influenza viruses at the wild–domestic bird interface in europe: future directions for research and surveillance. Viruses. https://doi.org/10.3390/v13020212

Voorhees IEH, Glaser AL, Toohey-Kurth K, Newbury S, Dalziel BD, Dubovi EJ, Poulsen K, Leutenegger C, Willgert KJE, Brisbane-Cohen L, Richardson-Lopez J, Holmes EC, Parrish CR (2017) Spread of canine influenza a(H3N2) virus, United States. Emerg Infect Dis. https://doi.org/10.3201/eid2312.170246

Voorhees IEH, Dalziel BD, Glaser A, Dubovi EJ, Murcia PR, Newbury S, Toohey-Kurth K, Su S, Kriti D, Van Bakel H, Goodman LB, Leutenegger C, Holmes EC, Parrish CR (2018) Multiple incursions and recurrent epidemic fade-out of H3N2 canine influenza A virus in the United States. J Virol. https://doi.org/10.1128/jvi.00323-18

Wan H, Perez DR (2006) Quail carry sialic acid receptors compatible with binding of avian and human influenza viruses. Virology 346(2):278–286. https://doi.org/10.1016/j.virol.2005.10.035

Wan H, Sorrell EM, Song H, Hossain MJ, Ramirez-Nieto G, Monne I, Stevens J, Cattoli G, Capua I, Chen LM, Donis RO, Busch J, Paulson JC, Brockwell C, Webby R, Blanco J, Al-Natour MQ, Perez DR (2008) Replication and transmission of H9N2 influenza viruses in ferrets: evaluation of pandemic potential. PLoS One 3(8):e2923

Wang B, Su Q, Luo J, Li M, Wu Q, Chang H, Du J, Huang C, Ma J, Han S, Yuan G, He Y, Guo M, Zhang Q, He H (2021) Differences in highly pathogenic H5N6 avian influenza viral pathogenicity and inflammatory response in chickens and ducks. Front Microbiol. https://doi.org/10.3389/fmicb.2021.593202

Webster RG, Hinshaw VS, Bean WJ, van Wyke KL, Geraci JR, St Aubin DJ, Petursson G (1981) Characterization of an influenza A virus form seals. Virology 113:712–724

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y (1992) Evolution and ecology of influenza A viruses. Microbiol Rev 56(1):152–179

Wille M, Holmes EC (2020) The ecology and evolution of influenza viruses. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a038489

Yang G, Li S, Blackmon S, Ye J, Bradley KC, Cooley J, Smith D, Hanson L, Cardona C, Steinhauer DA, Webby R, Liao M, Wan XF (2013) Mutation tryptophan to leucine at position 222 of haemagglutinin could facilitate H3N2 influenza A virus infection in dogs. J Gen Virol. https://doi.org/10.1099/vir.0.054692-0

Yong-Feng Z, Fei-Fei D, Jia-Yu Y, Feng-Xia Z, Chang-Qing J, Jian-Li W, Shou-Yu G, Kai C, Chuan-Yi L, Xue-Hua W, Jiang SJ, Zhi-Jing X (2017) Intraspecies and interspecies transmission of mink H9N2 influenza virus. Scient Rep 7:1, 7(1):1–7. https://doi.org/10.1038/s41598-017-07879-1

Yu J, Li F, Wang D (2021) The first decade of research advances in influenza D virus. J Gen Virol. https://doi.org/10.1099/JGV.0.001529

Yu M, Sun L, Zhang Z, Zhang Y, Zhang H, Na L, Wang X (2022) KPNA6 is a cofactor of ANP32A/B in supporting influenza virus polymerase activity. Microbiol Spectrum. https://doi.org/10.1128/SPECTRUM.02073-21

Zhang Y, Zhang Q, Kong H, Jiang Y, Gao Y, Deng G, Shi J, Tian G, Liu L, Liu J, Guan Y, Bu Z, Chen H (2013) H5N1 hybrid viruses bearing 2009/H1N1 virus genes transmit in Guinea pigs by respiratory droplet. Science. https://doi.org/10.1126/science.1229455

Zhang H, Zhang Z, Wang Y, Wang M, Wang X, Zhang X, Ji S, Du C, Chen H, Wang X (2019) Fundamental contribution and host range determination of ANP32A and ANP32B in influenza A virus polymerase activity. J Virol. https://doi.org/10.1128/jvi.00174-19

Zhang M, Liu M, Bai S, Zhao C, Li Z, Xu J, Zhang X (2021) Influenza A virus–host specificity: an ongoing cross-talk between viral and host factors. Front Microbiol. https://doi.org/10.3389/fmicb.2021.777885

Zhu X, Yang H, Guo Z, Yu W, Carney PJ, Li Y, Chen LM, Paulson JC, Donis RO, Tong S, Stevens J, Wilson IA (2012) Crystal structures of two subtype N10 neuraminidase-like proteins from bat influenza A viruses reveal a diverged putative active site. Proc Natl Acad Sci U S A 109 (46):18903–18908. https://doi.org/10.1073/pnas.1212579109

Zhu X, Yu W, McBride R, Li Y, Chen LM, Donis RO, Tong S, Paulson JC, Wilson IA (2013) Hemagglutinin homologue from H17N10 bat influenza virus exhibits divergent receptor-binding and pH-dependent fusion activities. Proc Natl Acad Sci U S A 110(4):1458–1463. https://doi.org/10.1073/pnas.1218509110

Important Zoonoses in Animals: Parapoxviruses (PPV)

23

Local Infection and Occupational Zoonosis, Vector for Vaccinology

Mathias Büttner and Hanns-Joachim Rziha

Contents

M. Büttner (✉)
Faculty Veterinary Medicine, Institute of Immunology, University of Leipzig, Leipzig, Germany
e-mail: mathias.buettner@uni-leipzig.de

H.-J. Rziha
Interfaculty Institute for Cell Biology, Department of Immunology, University of Tübingen, Tübingen, Germany

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_19

Abstract

The current review summarizes features of Parapoxviruses (PPV): virus properties, host range, epidemiology, clinical manifestation, diagnosis, immunology, and countermeasures. The zoonotic potential in transmission to man resulting in self-limiting local skin lesions is discussed and shown by case pictures. Successful usage of PPV, especially Orf virus, as vector vaccines is demonstrated. Future aspects such as PPV application for oncolytic therapy are discussed.

Keywords

Parapoxviruses · Zoonosis · Diagnosis · Vector vaccines · Oncolytic therapy

23.1 Introduction

The PPV species are assembled in the genus *Parapoxvirus* of the subfamily *Chordopoxvirinae* within the family *Poxviridae*. Currently five species of PPV are established: orf virus (ORFV), bovine papular stomatitis virus (BPSV), pseudocowpoxvirus (PCPV), PPV of red deer (previously red deer in New Zealand, PVNZ), and PPV of grey seal. There are other tentative species attributable to the genus such as camel and chamois contagious ecthyma virus. The prototype member of the PPV genus, *Parapoxvirus ovis* or ORFV, is endemic in most sheep and goat raising countries in the world causing contagious ecthyma (CE) or "Orf," a term probably derived from the old Icelandic word "hurf" meaning a wound scab. The worldwide awareness of *Parapoxvirus* (PPV) infections is high; however due to regional differences in occurrence, morbidity, and mortality (generally low) and the limitation of zoonotic infections to occupational contact, mostly clinical cases find a high degree of attention. On the other hand, PPV represent important examples for the demonstration of escape mechanisms in the pathogenicity of local skin and mucosal infections. In contrast, the complexity and multi-protein composition of the large PPV particles turned out to show beneficial immune modulation when applied systemically. A different field of attention is the use of PPV, especially of the prototype ORFV, as a potent viral vector for foreign gene expression in vaccinology. Finally, a more recent research area is oncolytic therapy using PPV.

23.2 Virus Properties

The ovoid PPV virion measures 220–300 nm in length and 147–170 nm in width and is surrounded by a tubule-like spiral structure resulting in the characteristic and distinct "ball-of-wool" appearance. The virion harbors a single linear, double-stranded DNA molecule of 130–140 kbp with an exceptional high G + C content of approximately 64%. The genomic termini are cross-linked by single-stranded hairpin loops and contain inverted terminal repeats (ITR) of 2.6 to 3.5 kbp in size, which can be enlarged by terminal genomic rearrangements after multiple cell culture passages. To date,

complete sequence information of 14 strains of ORFV, BPSV, and PCPV is available (Rziha and Büttner 2021). The genomes encode 124–134 genes, 88 are conserved in all *Chordopoxvirinae*. Central parts of the genome are highly conserved and contain genes essential for virus replication, particle packaging, and export (Rziha and Büttner 2021). Although the 132 putative genes in ORFV are present in different isolates, substantial sequence variations can occur (Mercer et al. 2006). Highest sequence differences among PPV species are found in the near terminal ends of the right and left genomic ends. PPV genomes show a remarkable plasticity when the viruses are subject to cell culture passages; already after six passages, gene deletions can occur (Rziha and Büttner 2021). After serial cell culture passages and adaption to cell lines gene, duplications, rearrangements, as well as major deletions of nonessential genomic regions can occur, which are accompanied by virus attenuation (Cottone et al. 1998; Rziha et al. 2019).

23.3 Immunomodulatory, Immune Evasion Genes of PPV

Poxviruses share many properties and particularly viral genes involved in evasion from and modulation of the immune response. An increasing number of genes encoding those immunomodulatory proteins (IMPs) become revealed in the PPV genome, which are also involved in regulation of virulence, pathogenesis, or host range (Fleming et al. 2015). These IMP genes are predominantly located close to the genomic termini, are early expressed, and are dispensable for virus replication. The first virulence determinant of PPV was described in ORFV, a functional homolog of the mammalian, angiogenic vascular endothelial growth factor (vegf), designated as vegf-e (Fleming et al. 2015; Meyer et al. 1999). The vegf-e gene is unique for PPV among *Chordopoxvirinae* and is suggested to be acquired by the virus from its mammalian host. It is responsible for extensive vascular proliferation beneath parapoxvirus lesions (Meyer et al. 1999). The construction of various apathogenic ORFV recombinants was enabled by the deletion of the vegf-e gene (Rziha et al. 2019). As shown in Fig. 1, no more formation of pustules and inflammatory vascularization was detectable after removal of the vegf-e gene from the attenuated ORFV strain D1701-B but could be restored after restoration of the vegf-e gene. The ORFV-encoded viral interleukin 10 (vIL-10) is an orthologue of the ovine IL-10 and might also be acquired by ORFV from its host. ORFV lacking the vIL-10 gene has reduced pathogenicity in sheep (Fleming et al. 2015). Two other secreted IMP have been discovered, a chemokine-binding protein (CBP) and the soluble protein inhibitor of ovine granulocyte-monocyte colony-stimulating factor and interleukin-2 (GIF), which both are important for infection, clinical course, and disease progression in sheep (Fleming et al. 2015; Sharif et al. 2019; Martins et al. 2021). Furthermore, ORFV encodes an interferon (IFN)-resistance gene (VIR or IFNR), which inhibits antiviral activity of interferon as its vaccinia virus homolog E3L (Fleming et al. 2015). Programmed cell death (apoptosis) represents a common innate host strategy to eliminate virus-infected cells. The ORFV-encoded Bcl2-like inhibitor of apoptosis can lead to the survival of infected cells (Fleming et al. 2015; Li et al. 2018). Signaling pathways of nuclear factor kappa B (NF-κB) represent

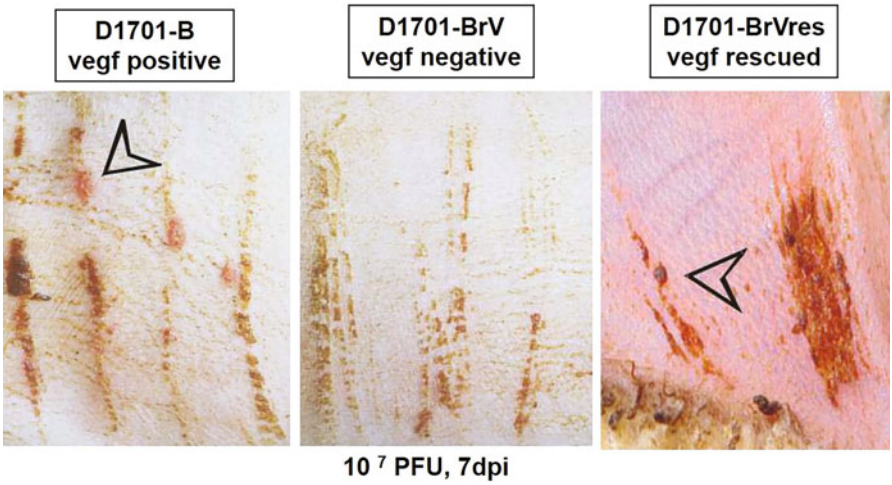

Fig. 1 Lesion development in sheep skin after scarification and application of 10^7 plaque-forming units (PFU) of the attenuated ORFV strain D1701 – day 7 after infection. The vegf-e gene deleted mutant (BrV) lacks induction of pustule formation and inflammatory vascularization as seen in the parental (D1701-B) and the vegf-e rescued virus (arrows). Derived from: M. Henkel, 2007, Parapockenvirus ORF-Virus D1701: Attenuierung und Herstellung einer Vektorvakzine gegen die Borna'sche Krankheit, Dissertation Eberhard-Karls-Universität, Tübingen, Germany

important regulators of innate immune response including apoptosis. For PPV until now, five encoded NF-κB inhibitors are known, some of them also shown to be involved in viral virulence (Khatiwada et al. 2017). A functional dUTPAse is encoded at the left genomic terminus of PPV, which is missing in some attenuated ORFV strains (Cottone et al. 2002). Poxviruses growing in the epidermis including ORFV encode a so-called *Poxvirus* APC/C regulator (PACR) involved in virus growth and replication (Fleming et al. 2015). Finally, as many other poxviruses also PPV contain several genes that express proteins comprising the so-called ankyrin repeat (ANK) consensus motifs (Rziha et al. 2003; Chen et al. 2017).

23.4 Host Range and Epidemiology

PPV infections and clinical signs occur worldwide but are predominantly restricted to domestic and wildlife ruminants. Natural infection has been reported in many wildlife and domestic small and larger ruminants such as bighorn, and thinhorn sheep, domestic and rocky mountain goat, Dall's sheep, chamois, ibex, Himalayan thar, muskox, reindeer, caribou, steenbok, and camelids from which all are transferable to humans. BPSV and PCPV both establish infection in cattle and humans, but all other species tested, including sheep, are resistant. Both, ORFV and PCPV can affect reindeer where ORFV infection caused the more severe lesions (Rziha and Büttner 2021). PVNZ originally found in New Zealand is also present in Europe is now consolidated to PPV of red deer, it induces only very mild lesions in sheep. The

new established species PPV of seals has been reported in different pinniped species, i. e., *Phocidae* (true seals), *Otariidae* (eared seals), and *Odobenidae* (walrus), and led to the inclusion of PPV of grey seal in the actual taxonomy. Recent sequence data propose the classification of all pinniped PPV under a species named "pinniped PPV" within the genus (Costa et al. 2021). PPVs do not infect poultry and do not produce lesions on the chorioallantoic membrane of the chicken embryo.

23.5 Zoonosis

The zoonotic transmission to man can be caused by all PPV species known so far. It leads to localized erythema, papules, or pustules commonly called milker's nodule (MN), paravaccina, or pseudocowpox (PCP). In general, a precondition for successful infection and manifestation of PPV lesion in man as well as in animals is broken skin or micro-wounds. PPV infections are occupational zoonoses mostly affecting veterinarians, farmers, hunters, butchers, abattoir workers, and all persons in close contact to sheep, goats, cattle, or infected wildlife. Frequent infections are reported at mass slaughtering of sheep or goats during religious feasts. Due to the frequent benign resolution of the lesions as well as the lack of specific etiology reports, cases are often underestimated. However, more dramatic disease with painful lesions can occur in severely immune-compromised persons, e.g., after burn accidents (Baj et al. 2020). As in animals, human infections with PPV occur worldwide, especially ORFV transmission is common in countries with intense sheep and goat breeding. The rate of subclinical infections in domestic small and large ruminants is unknown, but it is often reported that the contact animal(s) had no visible lesions. Nowadays, ORFV transmission from sheep and goats to humans dominates the zoonotic infections (Fig. 2). Little is known about immune reactions in PPV-infected persons. It can be assumed that immune responses in affected humans are similar as reported for sheep. In man cutaneous infiltrates have been characterized by immunohistochemistry showing an influx of CD3-positive T-lymphocytes of which the majority was CD4-positive. On infiltrated lymphocytes CD30 was detected as a marker for stimulated cells indicating a Th2 rather than a Th1 immune response (Rose et al. 1999). In humans, inter-individual transmission, e.g.. by close contact from a diseased individual to another is not evident.

For treatment of severe lesions (giant orf) in human PPV patients, cyclic nucleoside analogs, such as acyclovir ACV or cidofovir CDV, selectively interacting with poxviral DNA polymerase are effective. Topical treatment with CDV resulted in complete regression of lesions even in immunocompromised patients (Rziha and Büttner 2021).

23.6 Clinical Features and Pathology

PPVs cause inflammatory and/or proliferative lesions that are confined to the skin and oral mucosa with no evidence of systemic spread. Infection is initiated in abrasions and generally proceeds through an afebrile, self-limiting lesion that

resolves within 3–9 weeks without leaving a scar. Orf lesions are most generally seen around the mouth and nares; hence, the infection is commonly referred to as scabby mouth or sore mouth. Lesions are also observed on other parts of the body, for example, the coronet, udder, or vulva. Following experimental inoculation of scarified skin, lesions progress through erythema, papule, vesicle, pustule, and necrotic

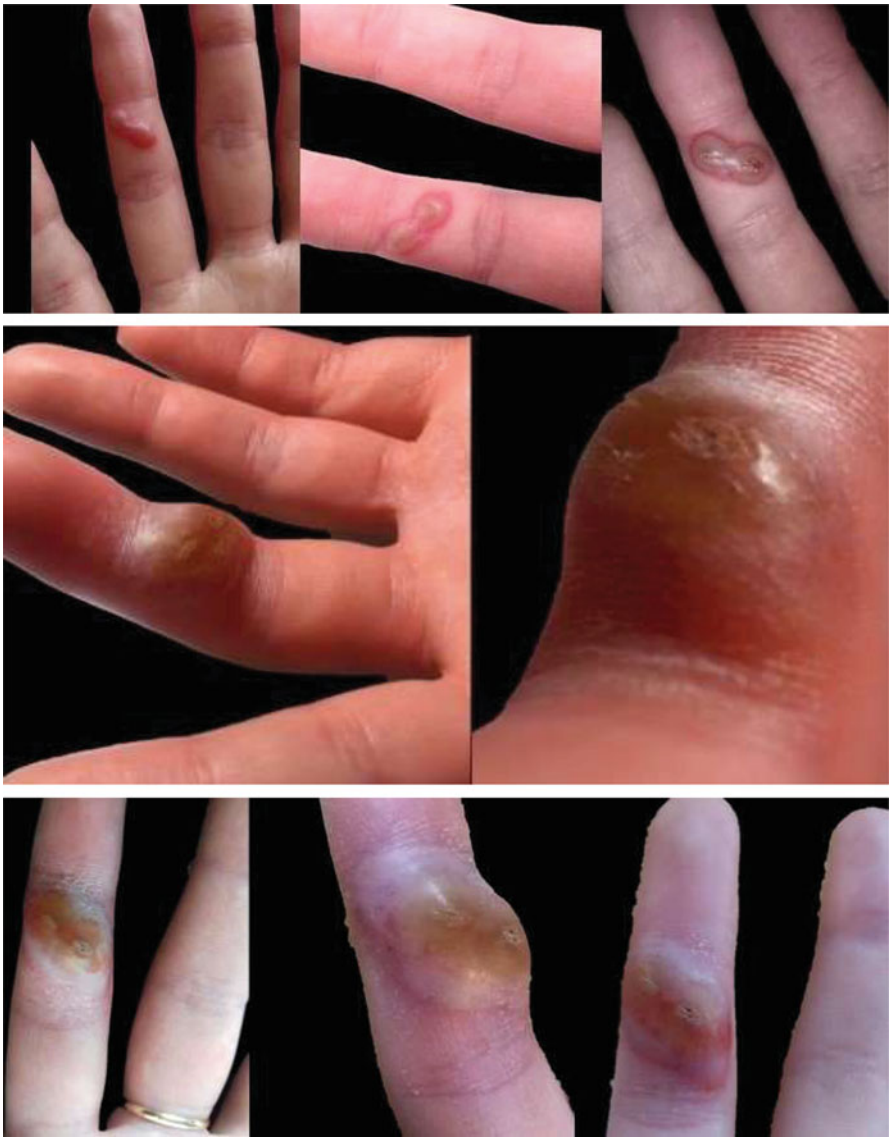

Fig. 2 Series of orf manifestation at the fingers of a veterinary student handling infected sheep in Chile. Progress of lesions and beginning of wound healing. Courtesy of Carolina Madariaga, Universidad Santo Tomás, Chile and Carlos A. Flores Olivares, Universidad Pedro de Valdivia, Chile

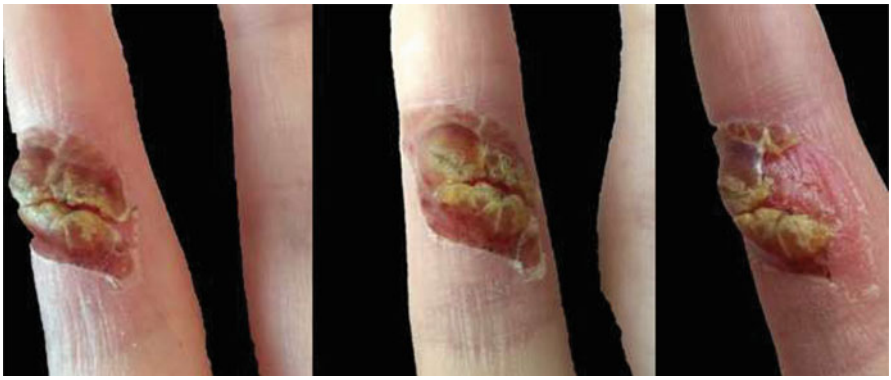

Fig. 2 (continued)

scab before resolving. Large, proliferative, tumor-like lesions can be observed. It is likely that such lesions are a result of an immune impairment and bacterial super infection of the host animal.

23.7 Orf, Scabby Mouth, Contagious Pustular Dermatitis (CPD), Ecthyma Contagiosum

Lesions around the mouth can interfere with feeding or suckling and especially in young animals result in failure to thrive. Teating lesions can have similar effects through the inhibition of suckling. Lesions in sheep and goat can develop into tumor-like, cauliflower proliferative erosions usually accompanied by secondary bacterial infection (Fig. 3). Edema of the head and swelling of the regional lymph nodes are common but rather nonspecific signs of severe progression. In severe cases, glossitis phlegmonosa and ulcerosa with secondary bacterial infections can lead to starvation especially in young animals. Most common is the *labial form* that impressed the designation of the disease. Blisters and yellowish pustules that may reach pea size are formed on the lips and at the corners of the mouth and can extend up to the nose, ears, and eyelids. The mild labial form heals within 3–6 weeks. Pustules can develop on the udders of ewes shortly before the lambing period. Secondary bacterial infections cause complications. In sheep severe lesion development is known as bloody lesion that is probably linked the stimulation of vascular endothelial cells and to proliferation leading to a cauliflower tumor-like clinical outcome. The *podal form* (scabby foot) can occur simultaneously with the labial manifestation or independently. Lesions develop at the coroner edges of the hooves, at the pasterns and in the hoof gaps. The end of the digits are painful and lead to lameness and the refusal to stand. *The genital form* is less common. Typical pustule and crust formation occur on the udder mostly developing into mastitis. Skin lesions also can occur on the inner leg, the labia, or the prepuce.

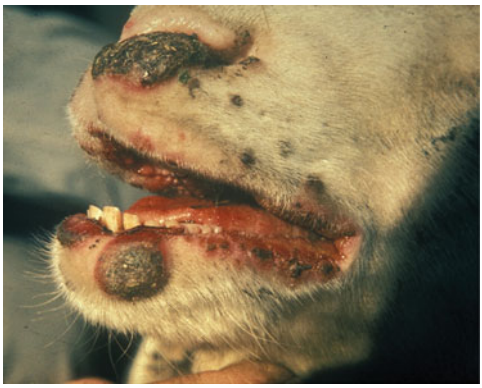

Fig. 3 Orf (ecthyma contagiosum, scabby mouth) in a lamb: proliferative lesions complicated by bacterial superinfections

23.8 Milker's Nodule, Paravaccinia, Pseudocowpox

It is highly likely that all PPVs from susceptible domestic and wildlife animals can be transmitted to humans. Most frequent infections originate from contact with sheep or goats (human orf) followed by virus transmission from diseased cattle or reindeer (milker's nodule, paravaccinia, pseudocowpox**)** and wildlife. From human patients complete genome sequence is available from few ORFV an PCPV isolates (Friederichs et al. 2014; Andreani et al. 2019). The highest risk of infection is reported for handling of sheep fleece or wool and ritual slaughter of affected sheep, e.g., at religious feasts. Progression of the lesions is essentially as seen in sheep and cattle such that the infection is benign and confined to localized pustular lesions on the skin mainly at the hand and fingers at the points of virus entry (Fig. 2). *Restitutio ad integrum* without leaving a scar usually occurs after a few weeks post infection. More severe progressive disease can occur in immune-compromised individuals showing strong cellular proliferation, called giant orf. Such cases have also been recorded in otherwise normal individuals after burn events and in cases of atopic dermatitis. *Erythema multiforme* reactions in the form of rashes on the backs of the hands and on the legs and ankles are common.

23.9 Bovine Papular Stomatitis (BPS)

Bovine popular stomatitis normally is a mild form of inflammation around the muzzle and mucous membrane of the mouth in large ruminants (Fig. 4a, b). However, more severe lesions can occur resulting in extension of confluent inflammation to the hard gum, the tongue, and far down to the esophagus. Lesions on the teats (pseudocowpox) are less frequently seen but are painful and lead to rejection of the suckling calf. Pseudocowpox virus (PCPV) represents a unique species with molecular characteristics. Interestingly, BPSV and PCPV can be found associated with ticks infesting cattle in Africa indication possible longevity of the virus in skin (Ouedraogo et al. 2020). Pustular vulvovaginitis caused by PCPV is a rare and

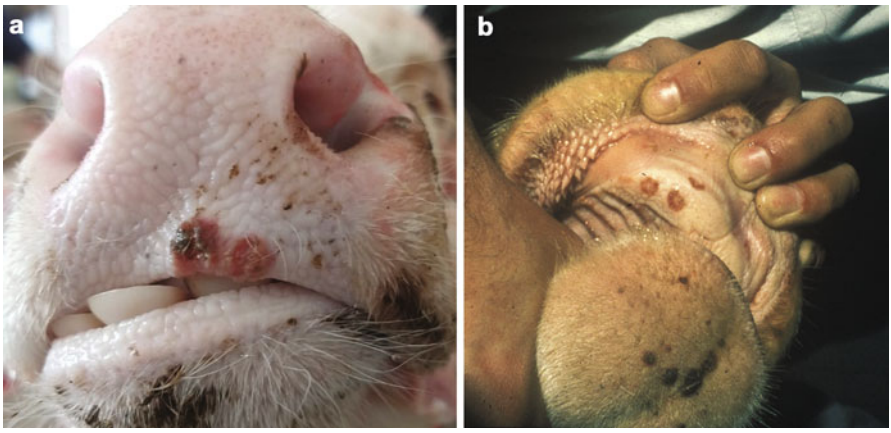

Fig. 4 Bovine popular stomatitis, local inflammatory circular lesion on the muzzle. (**a**) Planar herds of inflammation and necrosis at the muzzle of a cow from an outbreak of bovine papular stomatitis (BPS) in Argentina. (Micheloud JF et al. Tropical Animal Health and Production 2020, 52: 453–459. https://doi.org/10.1007/s11250-019-02006-w). Kindly provided by A. Peralta, Instituto de Agrobiotecnología y Biología Molecular (IABIMO), INTA, Consejo Nacional de investigaciones Científicas y Tecnológicas (CONICET), Hurlingham, Argentina. (**b**) BPS inflammatory lesions at the hard gum and tongue of a cow (arrows). In severe progressive cases, the lesions can spread deep down to the esophagus. Note the unprotected hand/fingers facilitating virus transmission to man

sporadic occurring disease. Signs of BPS may arise spontaneously in apparently healthy animals (especially young calves) without reports about skin damage or injury. Like infections with PPV of red deer, subclinical persistence of the virus cannot be excluded. The trigger of clinical manifestation can be either unusual virulence of BPSV or immune suppression or both in synergy.

23.10 Parapox of Red Deer

Initially described in red deer of New Zealand, it became evident that red deer in Europe and probably worldwide can become infected with this unique species of PPV. In Italy, clinical manifestation in red deer was seen as inflammation of the mouth whereas in Germany subclinical infection was diagnosed by viral DNA presence and virus isolation in cell culture (Rziha and Büttner 2021). For red deer in New Zealand, it is reported that PPV infection in growing deer antlers can affect antler growth and severely affect marketability of the product.

23.11 Patho-histology

Keratinocytes are key target cells in ORFV infection where in distinct areas a cytopathic effect (CPE) is seen as ballooning degeneration of the cells; vacuolization in the supra-basal layers and typical intra-plasmatic eosinophilic inclusion bodies

can be found (Muhsen et al. 2019). Ballooning in context with poxvirus infections is characterized by cell swelling and rounding of the cytoplasm and is caused by the rearrangement of the intermediate filaments of the cytoskeleton.

23.12 Diagnosis

Clinical diagnosis of BPS and Orf in sheep is complicated by different similar inflammatory disease manifestations in the skin and mucous membranes caused by other viruses such as foot and mouth disease (FMD), bluetongue (BT), bovine viral diarrhea and border disease (BVD, BD), and bovine herpesvirus (BHV-1) rhinotracheitis. Shortly after infection nonspecific clinical signs like head edema make it difficult to find a diagnosis. However, rapid and reliable differential diagnosis for such communicable and economically important diseases is essential.

Electron microscopy is a method of choice when enough virus particles can be expected (10^5 or more particles) in a sample (biopsy material, skin specimen). In samples from affected persons, it is extremely difficult to find PPV virions in electron microscopy because of a lack of sufficient tissue and frequent pre-diagnostic treatment of lesions. Therefore, currently electron microscopy has been replaced or at least is accompanied by molecular diagnosis, predominantly polymerase chain reaction (PCR). Several sensitive real-time PCR protocols became established for rapid and reliable detection of PPV genome presence in tissue samples. Conventional PCR followed by electrophoresis of amplified DNA fragments still is of importance for further molecular analysis, e.g., sequencing. The latter is used to differentiate PPV species and to perform molecular epidemiology. The most popular target region for this purpose is the open reading frame (ORF) 011 containing the B2L gene, an orthologue of the vaccinia virus Copenhagen F13L gene, that encodes the major envelope protein p37K (Sullivan et al. 1994).

Whole genome sequencing (WGS) techniques become increasingly popular and affordable, and direct WGS out of the sample is forward-looking. Nevertheless, for intense further studies, PPV laboratory strains are essential, although cell culture isolation of PPV out-of-field samples is tedious and time consuming. Primary ruminant or human fibroblasts are the most permissive cells followed by Vero cells that have been successfully used for virus propagation. Long incubation (up to 7 days) of inoculated cells and blind passages can become necessary for successful PPV isolation.

Serology is of little value in PPV diagnosis. Antibody development is readily induced, mainly directed against the major envelope protein (B2L gene), but antibody screening in ruminants is of poor significance due to the high and widespread subclinical infection prevalence in the population. There are no commercial antibody assays available. Therefore, only experimental ELISA protocols have been developed that can also be used to test human sera. Virus-neutralizing antibodies can only be generated by multiple experimental injections to produce hyper-immune sera or monoclonal antibodies.

23.13 Immune Reactions

PPV infections lead to antibody development that bind to virus components (Western Blot and ELISA reactive), but antibodies partially neutralizing the virus are only found after repeated infections or several booster injections. The ewe's colostrum does not protect her lambs from infection. Vaccines for sheep have been developed long time ago beginning with scarification trials using scab material, and nowadays attenuated cell culture-adapted ORFV strains are used as live vaccines, but with limited success. At least in sheep, vaccination can prevent the severity of pustule development in lambs and protect them for a certain time from reinfection in a flock. There are no special studies that evaluate the prevention of virus spread by consequent and repeated vaccination. The resistance of PPV, especially of the mature enveloped particles, against environmental influences must be taken in account about contaminated pastures, fodder, and wool of the animals.

23.14 Immune Stimulation

Following iatrogenic application, e.g., by intramuscular or subcutaneous injection, ORFV can exert a strong immune-stimulatory but well-balanced effect. This has been exploited especially in treatment of nonpermissive animals in veterinary medicine to counteract infectious diseases or stress situations at the innate level of immune defense. Whether the early expressed immune evasion genes of PPV are functional in vivo in nonpermissive cells is unknown, but the complex proteins and lipoproteins of the large particles stimulate a battery of cellular and humoral innate immune reactions, e.g., secretion of cytokines such as type I interferons (Büttner et al. 1995; Friebe et al. 2004; McGuire et al. 2012). In laboratory animals a beneficial effect of inactivated ORFV was reported in infection and even in tumor models. The nonspecific side effect of ORFV application also supports its extraordinary efficacy as a vector vaccine (see below) comprising its own adjuvant.

23.15 Biomedical Potency (Vector, Oncotherapy)

23.15.1 ORFV Vector Platform

The utility of poxviruses as expression vectors was first described in the early 1980s. Since then, poxviruses have been widely used as vaccine delivery platforms in human and veterinary medicine. Meanwhile also ORFV evolved as a novel poxvirus vector platform for delivering heterologous antigens without the risk of a vigorous immune response against the vector backbone. Additional advantages of the ORFV vector are the restricted host range, the lack of systemic spread even in immuno-compromised animals, and the induction of strong, balanced humoral and cellular immune responses against expressed foreign antigens (Reguzova et al. 2020). Importantly, ORFV-based

vaccines induce strong immune responses also in hosts nonpermissive for PPV. The ORFV inherent properties comprise the stimulation of strong innate immune responses. Dendritic cell activation via toll-like receptor 9 also potentiates the ORFV-induced immune response (von Buttlar et al. 2014). Those mechanisms can explain the potency of ORFV-based vaccines without the need of adjuvants.

For the first time, ORFV recombinants were generated from the attenuated strain D1701 by replacing the nonessential virulence vegf-e gene by foreign antigens (Rziha et al. 2016, 2019). This finding was the kickoff for the development of the ORFV strain D1701-based vaccine vector platform resulting in numerous protective vaccines (Rziha et al. 2019). One special feature of this platform is the use of early ORFV promoters**,** which regulate the expression of the heterologous antigens. Consequently, the successful expression and immune recognition of the foreign antigens needs neither replication nor production of the inoculated recombinant vaccines representing an additional safety aspect. Lately, new additional genomic loci or genes were described in ORFV D1701-V that are suitable for insertion and expression of foreign genes. This allows the generation of multivalent recombinant vaccines (Rziha et al. 2019). Just recently for the purification of D1701-V recombinants, a new strategy of a downstream process was reported combining filtration and chromatographic methods, which is adequate for safe application and within the regulatory limits for contaminant levels (Lothert et al. 2020).

The use of ORFV recombinants for delivery of heterologous proteins must not be restricted to apathogenic, attenuated virus strains. For instance, replacing one of the abovementioned IMP genes resulted in protective recombinant ORFV vaccines with reduced pathogenicity in sheep and goat (Joshi and Diehl 2021).

23.15.2 Oncolytic Potency

Oncolytic viruses that are selected or engineered for selective or preferential infection of tumor cells can be promising candidates for cancer treatment. Several members of *Poxviridae* were successfully used in oncolytic virotherapy, and PPV including ORFV, PCPV, and BPSV could be shown to possess oncolytic properties (Ricordel et al. 2018). In addition to immune stimulation by inactivated ORFV, also replicating ORFV was discovered to destroy human cancer cells in vitro and to reduce the tumor burden in different mouse tumor models including breast, lung, or colon cancer (Rintoul et al. 2012; Chen et al. 2021).

23.15.3 Prospects

PPV are deeply rooted in biocenosis. The species within the genus have adopted to successful infection, replication, and manifestation in the skin and mucous membranes. Although the animal host range of PPVs is limited, their transmission to man can be achieved under the precondition of micro-wounds or skin damage but is limited to the entry site. As a typical local infection, PPVs represent a major

difference in pathogenicity to members of the *Orthopoxvirus* genus. Thus, the impact of PPV as a zoonosis is of limited danger in comparison to manifestation of systemic infections with some *Orthopoxvirus*, e.g., cowpox virus. With respect to clinical outcome, some disturbance can arise concerning speculations about a possible PPV etiology probably flanked by unclear electron microscopical pictures. Recently, emergence of a new poxvirus in red squirrels (McInnes 2006) and the occurrence of skin pathology in horses led to the assumption of a PPV etiology. The equine skin disease turned out to be caused by a molluscum contagiosum-like virus (Ehmann et al. 2021). Nowadays progress in molecular diagnostics including WGS can rapidly clarify such uncertainties. The plasticity of the ORFV genome is demonstrated in vitro after serial cell culture passages and by growth adaptation to certain cell lines. It always led to attenuation and stability of genome alterations in laboratory strains providing a safe platform for its use as vector, as an immune-stimulating agent and probably a tool for oncotherapy.

References

Andreani J, Fongue J, Bou Khalil JY, David L, Mougari S, Le Bideau M, Abrahão J, Berbis P, La Scola B (2019) Human infection with Orf virus and description of its whole genome, France, 2017. Emerg Infect Dis 25(12):2197–2204. https://doi.org/10.3201/eid2512.181513

Baj J, Korona-Głowniak I, Buszewicz G, Forma A, Sitarz M, Teresinski G (2020) Viral infections in burn patients: a state-of-the-art review. Viruses 12(1315):1–18. https://doi.org/10.3390/v12111315

Büttner M, Czerny C-P, Lehner K-H, Wertz K (1995) Interferon induction in peripheral blood mononuclear leukocytes of man and farm animals by poxvirus vector candidates and some poxvirus constructs. Vet Inununol Immunopathol 46:237–250

Chen D-Y, Fabrizio J-A, Wilkins SE, Dave KA, Gorman JJ, Gleadle JM, Fleming SB, Peet DJ, Mercer AA (2017) Ankyrin repeat proteins of Orf virus influence the cellular hypoxia response pathway. J Virol 91(1):e01430–e01416

Chen D, Wang R, Long M, Li W, Xiao B, Deng H, Weng K, Gong D, Liu F, Luo S, Hao W (2021) Identification of in vitro and in vivo oncolytic effect in colorectal cancer cells by Orf virus strain NA1/11. Oncol Rep 45(2):535–546

Costa H, Klein J, Breines EM, Nollens HH, Matassa K, Garron M, Duignan PJ, Schmitt T, Goldstein T, Tryland M (2021) A comparison of Parapoxviruses in North American Pinnipeds. Front Veterinary Sci 8(5):1–8. https://doi.org/10.3389/fvets.2021.653094

Cottone R, Büttner M, Bauer B, Henkel M, Hettich E, Rziha HJ (1998 Jul) Analysis of genomic rearrangement and subsequent gene deletion of the attenuated Orf virus strain D1701. Virus Res 56(1):53–67

Cottone R, Büttner M, McInnes CJ, Wood AR, Rziha H-J (2002) Orf virus encodes a functional dUTPase gene. J Gen Virol 83(5):1043–1048

Ehmann R, Brandes K, Antwerpen M, Walter M, v. Schlippenbach K, Stegmaier E, Essbauer S, Bugert J, Teifke JP, Meyer H (2021) Molecular and genomic characterization of a novel equine molluscum contagiosum-like virus. J Gen Virol 102:001357. https://doi.org/10.1099/jgv.0.001357

Fleming SB, Wise LM, Mercer AA (2015) Molecular genetic analysis of Orf virus: a Poxvirus that has adapted to skin. Viruses 7:1505–1539

Friebe A, Siegling A, Friederichs S, Volk H-D, Weber O (2004) Immunomodulatory effects of inactivated parapoxvirus ovis (ORF virus) on human peripheral immune cells: induction of cytokine secretion in monocytes and Th1-like cells. J Virol 78:9400–9411

Friederichs S, Krebs S, Blum H, Wolf E, Lang H, von Buttlar H, Büttner M (2014) Comparative and retrospective molecular analysis of Parapoxvirus (PPV) isolates. Virus Res 181:11–21

Joshi LR, Diehl DG. 2021 Poxvirus vectors. In Vanniasinkam T, Tikoo, Samal SK (eds) Viral vectors in veterinary vaccine development. Springer Nature Switzerland AG 2021. https://doi.org/10.1007/978-3-030-51927-8

Khatiwada S, Delhon G, Nagendraprabhu P, Chaulagain S, Luo S, Diel DG, Flores EF, Rock DL (2017) A parapoxviral virion protein inhibits NF-κB signaling early in infection. PLoS Pathog 13(8):e1006561

Li W, Chen H, Deng H, Kuang Z, Long M, Chen D, Liao X, Li M, Rock DL, Luo S, Hao W (2018) Orf virus encoded protein ORFV119 induces cell apoptosis through the extrinsic and intrinsic pathways. Front Microbiol 9:1056. https://doi.org/10.3389/fmicb.2018.01056

Lothert K, Pagallies F, Eilts F, Sivanesapillai A, Hardt M, Moebus A, Feger T, Amann R, Wolff MW (2020) A scalable downstream process for the purification of the cell culture derived Orf virus for human or veterinary applications. J Biotechnol 323:221–230

Martins M, Rodrigues FS, Joshi LR, Jardim JC, Flores MM, Weiblen R, Flores EF, Diel DG (2021) Orf virus ORFV112, ORFV117 and ORFV127 contribute to ORFV IA82 virulence in sheep. Vet Microbiol 257:109066

McGuire MJ, Johnston SA, Sykes KF (2012) Novel immune-modulator identified by a rapid, functional screen of the parapoxvirus ovis (Orf virus) genome. Proteome Sci 10(1):4

McInnes CJ (2006) Genomic characterization of a novel poxvirus contributing to the decline of the red squirrel (Sciurus vulgaris) in the UK. J Gen Virol 87(8):2115–2125

Mercer AA, Ueda N, Friederichs S-M, Hofmann K, Fraser KM, Bateman T, Fleming SB (2006 Mar) Comparative analysis of genome sequences of three isolates of Orf virus reveals unexpected sequence variation. Virus Res 116(1–2):146–158

Meyer M, Clauss M, Lepple-Wienhues A, Waltenberger J, Augustin HG, Ziche M, Lanz C, Büttner M, Rziha H-J, Dehioa C (1999) A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases. EMBO J 18(2):363–374

Muhsen M, Protschka M, Schneider LE, Müller U, Köhler G, Magin TM, Büttner M, Alber G, Siegemund S (2019) Orf virus (ORFV) infection in a three-dimensional human skin model: characteristic cellular alterations and interference with keratinocyte differentiation. PLoS One 14(1):e0210504. https://doi.org/10.1371/journal.pone.0210504

Ouedraogo A, Luciani L, Zannou O, Biguezoton A, Pezzi L, Thirion L, Belem A, Saegerman C, Charrel R, Lempereur L (2020) Detection of two species of the genus Parapoxvirus (bovine papular stomatitis virus and Pseudocowpox virus) in ticks infesting cattle in Burkina Faso. Microorganisms 8:644. https://doi.org/10.3390/microorganisms8050644

Reguzova A, Ghosh M, Müller M, Rziha H-J, Amann R (2020) Orf virus-based vaccine vector D1701-V induces strong CD8+ T cell response against the transgene but not against ORFV-derived epitopes. Vaccine 8(2):295

Ricordel M, Foloppe J, Pichon C, Findeli A, Tosch C, Cordier P, Cochin S, Quémeneur E, Camus-Bouclainville C, Bertagnoli S, Erbs P (2018) Oncolytic properties of non-vaccinia poxviruses. Oncotarget 9(89):35891–35906

Rintoul JL, Lemay CG, Tai L-H, Stanford MM, Falls TJ, de Souza CT, Bridle BW, Daneshmand M, Ohashi PS, Wan Y, Lichty BD, Mercer AA, Auer RC, Atkins HL, Bell JC (2012) ORFV: a novel oncolytic and immune stimulating Parapoxvirus therapeutic. Mol Therap 20(6):1148–1157

Rose C, Starostik P, Bröcker EB (1999) Infection with Parapoxvirus induces CD30-positive cutaneous infiltrates in humans. J Cutan Pathol 26(10):520–522

Rziha H-J, Büttner M (2021) Parapoxviruses (Poxviridae). In: Bamford DH, Zuckerman M (eds) Encyclopedia of virology, vol 2, 4th edn. Academic, Oxford, pp 666–674. https://doi.org/10.1016/B978-0-12-814515-9.00058-8

Rziha H-J, Bauer B, Adam K-H, Röttgen M, Cottone R, Henkel M, Dehio C, Büttner M (2003) Relatedness and heterogeneity at the near-terminal end of the genome of a parapoxvirus bovis 1 strain (B177) compared with Parapoxvirus ovis (Orf virus). J Gen Virol 84(5):1111–1116

Rziha H-J, Rohde J, Amann R (2016) Generation and selection of Orf virus (ORFV) recombinants. Methods Mol Biol 1349:177–200

Rziha H-J, Büttner M, Müller M, Salomon F, Reguzova A, Laible D, Amann R (2019) Genomic characterization of Orf virus strain D1701-V (Parapoxvirus) and development of novel sites for multiple transgene expression. Viruses 11(2):127

Sharif S, Ueda N, Nakatani Y, Wise LM, Clifton S, Lateef Z, Mercer AA, Fleming SB (2019) Chemokine-binding proteins encoded by Parapoxvirus of Red Deer of New Zealand display evidence of gene duplication and divergence of ligand specificity. Front Microbiol 10:1421. https://doi.org/10.3389/fmicb.2019.01421

Sullivan JT, Mercer AA, Fleming SB, Robinson AJ (1994) Identification and characterization of an Orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K. Virology 202(2):968–973

von Buttlar H, Siegemund S, Büttner M, Alber G (2014) Identification of toll-like receptor 9 as Parapoxvirus ovis-sensing receptor in plasmacytoid dendritic cells. PLoS One 9(8):e106188. https://doi.org/10.1371/journal.pone.0106188

Zoonotic Orthopoxviruses: Innocuous Rash or Global Public Health Threat?

24

Jesse Bonwitt, Jeffrey B. Doty, Andrea M. McCollum, and Yoshinori Nakazawa

Contents

Abstract

The *Orthopoxvirus* genus is a group of large DNA viruses with importance to both animal and human health. These viruses show a range of clinical presentations from localized lesions to generalized rashes; and have various degrees of complexity in their natural history involving one or multiple hosts. *Variola virus* is the most notable member of this genus and is the causative agent of smallpox, a disease that

J. Bonwitt
Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA

Department of Anthropology, Durham University, Durham, UK
e-mail: jesse.bonwitt@durham.ac.uk

J. B. Doty · A. M. McCollum (✉) · Y. Nakazawa
Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA
e-mail: uwb7@cdc.gov; azv4@cdc.gov; inp7@cdc.gov

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_62

was declared eradicated in 1980. Zoonotic orthopoxviruses include viruses known to infect multiple mammal species and pose a risk to animal (domestic and wildlife) and human health such as *Monkeypox virus* (endemic to Central and West African countries), *Cowpox virus* (endemic to Eurasia), and *Vaccinia virus* (endemic to South America and parts of Asia, also used in the smallpox vaccine). Recently described orthopoxviruses, *Alaskapox virus*, and *Akhmeta virus*, highlight the possibility of existence of unknown members of this genus with zoonotic potential. Circulation of cryptic orthopoxviruses, *Volepox virus*, *Racoonpox virus*, *Skunkpox virus*, exemplify viruses that appear to have more restricted ability to infect multiple hosts. Prompt identification followed by prevention and control of these viruses will prevent additional spread in animals and humans.

Keywords

Orthopoxvirus · Zoonosis · Akhmeta virus · Alaskapox virus · Cowpox virus · Monkeypox virus · Vaccinia virus · Camelpox virus · Veterinary public health · Diagnosis · Clinical presentation · Epidemiology · Prevention · Control

24.1 Introduction

Viruses in the genus *Orthopoxvirus* (family *Poxviridae*) are distinguished by their diversity in disease ecology, epidemiology, and natural history; and, subsequently, in the approach toward prevention, control, and treatment. Orthopoxviruses (OPXVs) are present throughout the globe and have a diverse host range, including both humans and animals; these viruses can cause a spectrum of disease ranging from localized skin lesions to fatal disease. OPXVs are double-stranded DNA viruses that contain large genomes, which includes all genetic material required for their replication once they enter a host cell. These viruses are capable of causing infections in a broad range of mammal hosts through multiple routes; furthermore, OPXVs employ strategies to evade the host's immune response (Reynolds et al. 2018).

Members of the *Orthopoxvirus* genus include *Abatino macacapox virus*, *Akhmeta virus*, *Camelpox virus*, *Cowpox virus*, *Ectromelia virus*, *Monkeypox virus*, *Raccoonpox virus*, *Skunkpox virus*, *Taterapox virus*, *Vaccinia virus*, *Variola virus*, and *Volepox virus* (ICTV 2022). In 2019, *Alaskapox virus* became the most recently described species within this genus (Gigante et al. 2019). Human infections originating from exposure to infected animals have been confirmed for *Alaskapox virus*, *Akhmeta virus*, *Cowpox virus* (CPXV), *Monkeypox virus* (MPXV), and *Vaccinia virus* (VACV). OPXVs have historically been named after the animal species from where they were first identified (i.e., MPXV, CPXV, *Skunkpox virus*, etc.), which has subsequently led to some misunderstandings about their reservoir species. For example, the name MPXV comes from the discovery of this distinct OPXV after it caused an outbreak in wild caught primates shipped to Europe for laboratory studies. Only since has the term become a misnomer, as MPXV is a pathogen of primates, but evidence does not support primates to be the reservoir host for the virus (Reynolds et al. 2012).

Variola virus, the causative agent of smallpox, was one of the most significant public health threats in history with high transmission and case fatality rates (20–60%), and permanently debilitating sequalae among some survivors (Breman and Henderson 2002). The host specificity of *Variola virus* and development of effective smallpox vaccines ensured that it became the first infectious disease to be eradicated. The eradication of *Variola virus* was made possible thanks to early efforts of Edward Jenner, who exploited observations that milk maids who were previously infected with CPXV did not develop smallpox after being exposed to smallpox patients. Anti-OPXV antibodies, as the result of infection or vaccination, engender significant immunological cross-protection against infection by other members of the genus. Jenner created the first smallpox vaccines using infectious material from cows and horses thought to be infected with CPXV or possibly *Horsepox virus*. By the twentieth century, VACV was used in pharmaceutical vaccine products as the smallpox vaccine during the global eradication campaign (Schrick et al. 2017; Li et al. 2007a).

Multiple strains of VACV exist following repeated cell passage and attenuation for creating smallpox vaccines. Some of these strains are thought to have escaped back into the wild via inoculation from an active vaccination site or when domesticated bovines were inoculated with VACV for propagation of the smallpox vaccine. VACV is currently endemic in parts of Asia and South America where it contributes to animal and human disease (Oliveira et al. 2017).

The cessation of smallpox vaccination in the 1980s has led to waning population-level immunity to OPXVs, raising concerns that an immunologically naïve population will no longer be afforded cross-protection against OPXV infections. These predictions have been most stark with regards to monkeypox, an emerging zoonotic disease in Central and Western Africa that causes relatively high mortality and morbidity in humans (Durski et al. 2018). MPXV is particularly concerning due to its unique ability among extant OPXVs to cause extended human-to-human transmission, with reported transmission chains of up to 7 generations (Nolen et al. 2016). Its recent resurgence in Nigeria (Yinka-Ogunleye et al. 2018); its high incidence rates in the Democratic of the Congo (Rimoin et al. 2010; Whitehouse et al. 2021); and increasing number of exportation events in humans and animals (Hutson et al. 2007; Mauldin et al. 2022) outside of its zone of endemicity draw even more attention to its global relevance.

24.1.1 Orthopoxvirus Relationships

OPXVs are brick-shaped viruses (220–450 nm) with large genomes (170,000–200,000 base pairs) that encode approximately 200 proteins with a core central region that is highly conserved (low genetic variability) across the genus (Hendrickson et al. 2010). The infectious particle (mature virion) is composed of a lipoprotein membrane that surrounds a core nucleoprotein containing the DNA. During infection, the virion fuses with the host cell membrane and it is brought into the cytoplasm through endocytosis (Bengali et al. 2012). Replication of OPXV

DNA occurs in the cellular cytoplasm, where intracellular mature virions and intracellular enveloped virions are produced and transported to the cell membrane, exiting the cell as extracellular enveloped virions (Smith and Law 2004).

Based on phylogenetic inferences, OPXVs have been grouped into New World (e.g., *Volepox virus*, *Raccoonpox virus*, *Skunkpox virus*) and Old World OPXVs (e.g., *Variola virus*, CPXV, VACV, MPXV) (Smithson et al. 2017; Carroll et al. 2011). New World OPXVs – also referred to as North American OPXVs – form a distinct group that appears to be ancestral to all other OPXVs and are thought to be endemic to North America; these viruses are not known to cause infections in humans. Similar to other OPXVs, they were named after the animal species in which they were initially identified (i.e., vole, skunk, raccoon); however, several aspects of their geographic distribution, host range, and transmission remain largely unknown. *Alaskapox virus*, a recently described OPXV from the Fairbanks region of Alaska, appears to naturally occur in this state, causes infections in humans, and is genetically more similar to Old World OPXVs than to those from North America, which poses interesting questions about the evolution of these viruses (Gigante et al. 2019). *Akhmeta virus* is another recently described OPXV that is genetically similar to Old World OPXVs. This virus was first detected in lesions from cattle herders from the Democratic Republic of Georgia near the town Akhmeta (Vora et al. 2015).

Among Old World OPXVs, CPXV, MPXV, and VACV are known to be zoonotic and have the largest impact on human and animal health. CPXV is known to have a broad geographic distribution across Eurasia. Recent phylogenetic analyses suggest that the group of viruses called CPXV are not necessarily the same virus and that genetic differences between them could support splitting this group into at least five different species (Mauldin et al. 2017). One of these proposed groups contains viruses that are genetically similar to VACV and *Horsepox virus*, which supports hypotheses of the origin of VACV strains used as smallpox vaccines (Esparza et al. 2017).

VACV products were used as smallpox vaccines during smallpox eradication campaigns in the twentieth century by inducing a cross-OPXV immune response; however, the origin of smallpox vaccines is obscured by propagation practices used in early stages of its use (Fenner 1993). Individual strains were replicated in laboratories around the world using standard practices to produce attenuated viruses to reduce the potential of adverse events from the use of the vaccine (Fenner et al. 1988). At the end of the 1990s, Brazil started reporting cases of VACV infections in humans and cows that eventually expanded to several states and regions of the country (Oliveira et al. 2017). Phylogenetic analyses of VACV isolates from Brazil suggest that the virus had been circulating in the wild before the smallpox eradication campaigns in the 1960s and 1970s, which may point to viruses brought into the country through early practices of arm-to-arm inoculations prior to eradication campaigns (Trindade et al. 2007). VACV isolates from Brazil are further divided into two groups (Groups 1 and 2) based on genetic differences (Oliveira et al. 2017). Colombia is another country from which zoonotic infections of VACV have been reported (Usme-Ciro et al. 2017), including one case involving an immunocompromised individual (Laiton-Donato et al. 2020). VACV infections have also been reported in parts of Asia (India and Bangladesh), where buffaloes are involved in its transmission (referred as Buffalopox) (Singh et al. 2006).

MPXV has two distinct genetic clades: Congo Basin and West Africa. West African MPXV has been described to have a more aggressive clinical presentation (Likos et al. 2005). The Congo Basin clade includes isolates from the Democratic Republic of the Congo, the Republic of Congo, Cameroon, and the Central African Republic, while the West Africa clade has been reported in Sierra Leone, Liberia, Côte d'Ivoire, Ghana, Nigeria, and northern Cameroon (Durski et al. 2018).

24.1.2 Ecology and Epidemiology

Animal and human infections occur throughout the endemic range of their respective reservoir hosts. CPXV spillover infections are restricted to Eurasia, VACV in South America (often referred to as bovine vaccinia), VACV in buffaloes and other bovine species on the Indian subcontinent (buffalopox), and MPXV is a significant human infection in Central and Western Africa (Oliveira et al. 2017; Durski et al. 2018; Eltom et al. 2020; Baxby et al. 1994). Camelpoxvirus (CMLV) occurs in areas with major camel production operations, except in Australia (Duraffour et al. 2011). OPXV cases have occurred outside of endemic areas due to travel and the movement of animals and people. This transboundary movement is best exemplified by MPXV, which caused an outbreak in humans and animals in the United States in 2003 due to a shipment of infected wild mammals from Ghana. The mammals were imported for the exotic pet trade and co-housed with prairie dogs (*Cynomys* sp.) that were subsequently adopted as pets and transmitted the infection to humans. This outbreak led to 47 confirmed and probable human cases, including veterinary staff and animal handlers (Croft et al. 2007; Reynolds et al. 2007). More recently, there have been a total of eight instances of travelers who left Nigeria and then sought care in the United Kingdom (4), USA (2), Singapore (1), and Israel (1) due to MPXV infections; secondary human-to-human transmission has occurred in two of these instances in the UK (healthcare worker and family members) (Mauldin et al. 2022; Vaughan et al. 2020; Hobson et al. 2021).

The incidence of OPXVs is not well understood because the infections are often not notifiable to animal or public health authorities, diagnostic capabilities are lacking in many endemic countries, and the viruses are responsible for relatively rare infections that often cause limited disease presentation (with the exception of MPXV) where care by veterinarians or physicians is often not sought by owners or patients. Moreover, OPXV incidence may be increasing across the globe due to cessation of routine smallpox vaccination and waning vaccine-derived immunity in humans. Traditional smallpox vaccines utilize a version of live VACV to elicit an immune response sufficient against OPXVs, including *Variola virus.* In the absence of vaccinations (lack of vaccine-derived immunity), marked increases in human monkeypox incidence and the discovery of new OPXVs (*Akhmeta, Alaskapox viruses*) have occurred (Durski et al. 2018; Vora et al. 2015; Springer et al. 2017); several other reasons for these observations could include changes in environmental and ecological factors that increase interactions between humans and animal hosts (Reynolds et al. 2012; Durski et al. 2018).

OPXVs are commonly transmitted through direct or indirect contact with an infectious host. Skin lesions are infectious through all lesion stages (including crusts); bodily fluids may be infectious (blood, urine, feces); contaminated household items (e.g., clothing, bedding, toys), healthcare items used for patient care, and farm equipment (e.g., milking machines, scratching posts, troughs) have all been implicated as fomites that may contain infectious virus (Duraffour et al. 2011; Prkno et al. 2017; Borges et al. 2017, 2014). The portal of entry is either via direct inoculation through the skin (e.g., wounds, abrasions, scratch, bite) or mucous membranes (e.g., ingestion of contaminated feed or water, or a splash of infected bodily fluids in the eye or mouth), or through inhalation of respiratory droplets (Oliveira et al. 2017; Prkno et al. 2017).

The epidemiology of zoonotic OPXVs is characterized by point-source exposure to reservoirs hosts (via direct contact with infected bodily fluids, skin lesions, or infected materials such as bedding or contaminated surfaces). The resulting transmission events will depend on the virus and strain involved, host susceptibility, and contact patterns between individuals. Animal crowding and species-mixing can lead to extensive multi-species outbreaks. For example, rodent-to-cat-to-human transmission of CPXV is frequently limited to a single individual or household, but can cause outbreaks if susceptible animals are housed together, as has been the case with infected pet rats (Campe et al. 2009). Another illustrative example involves an outbreak of MPXV in a zoo following the introduction of infected giant anteaters (*Myrmecophaga tridactyla*) that infected more than seven species of non-human primates (Peters 1966). Similarly, the importation of infected African small mammals for the pet trade in the United States resulted in transmission to at least 4 species of African mammals, 4 species of North American mammals, and 47 humans (Hutson et al. 2007).

CPXV has been isolated from a wide array of domestic and wild animals. This virus is maintained in nature by bank voles (*Myodes glareolus*), field voles (*Microtus agrestis*), and woodmice (*Apodemus sylvaticus*) (Bennett et al. 1997). Susceptible species include non-human primates, cats, dogs, horses, llamas, foxes (*Vulpes vulpes*), Patagonian cavies (*Dolichotis patagonum*), banded mongoose (*Mungos mungos*), jaguarundis (*Herpailurus yagouaroundi*), okapis (*Okapia johnstoni*), giant anteaters (*Myrmecophaga tridactyla*), African and Asian elephants (*Loxodonta africana*, *Elephas maximus*), rhinoceroses (*Ceratotherium simum*), and several species of big cats (Prkno et al. 2017; Baxby et al. 1982; Kik et al. 2006; Marennikova et al. 1977; Martina et al. 2006; Tryland et al. 2011; Zwart et al. 1971; Abrahao et al. 2010; Kurth et al. 2009). CPXV has been implicated in epizootics in captive wildlife and caused extensive multi-species outbreaks and deaths in zoo animals (Marennikova et al. 1977; Zwart et al. 1971; Kurth et al. 2009). Sequencing data confirmed that wild rats (*Rattus norvegicus*) were the source of the outbreak in a primate sanctuary and in a circus, and epidemiologic investigations indicated feeder rodents as the infection source in two zoo outbreaks (Marennikova et al. 1977; Martina et al. 2006; Kurth et al. 2008). In Europe, case investigations have demonstrated that pet cats have often acted as intermediate hosts between wild reservoir hosts and humans, with diagnoses and severe disease having

occurred in both the cats and humans (Eis-Hubinger et al. 1990; Haase et al. 2011). Human-to-human transmission has not been documented.

In contrast to CPXV, VACV is primarily a disease of bovids and horses in South America, and water buffalo in the Indian subcontinent (buffalopox), although it does not appear to cause outbreaks in horses (Borges et al. 2018). In the Indian subcontinent, however, bovine herds affected by VACV have had documented attack rates as high as 50% or more, causing significant morbidity and agricultural loss (Bhanuprakash et al. 2010; Gurav et al. 2011).

MPXV is capable of infecting a wide range of mammalian taxa, including rodents, marsupials, and primates, including chimpanzees (*Pan troglodytes*), orangutans (*Pongo pygmaeus*), sooty mangabeys (*Cercocebus atys*), baboons (*Papio cynocephalus*), cynomolgus monkeys (*Macaca fascicularis*), and marmosets (*Callithrix jacchus*), with cases also likely in gorillas (*Gorilla gorilla*), gibbons (*Hilobates lar*), squirrel monkeys (*Saimiri sciurea*), and owl-faced monkeys (*Cercopithecus hamlyni*) (Reynolds et al. 2018; Hutson et al. 2007; Arita and Henderson 1968). Since its initial discovery in wild caught primates, MPXV has caused significant outbreaks among captive chimpanzees housed at two independent sanctuaries in Cameroon and among wild chimpanzees in Ivory Coast, highlighting the threat of emerging infectious diseases to endangered wildlife. The source of these outbreaks is unknown (Patrono et al. 2020) (unpublished reports).

24.1.3 Zoonotic and Human-to-Human Transmission

As with most zoonotic infections, individuals most at risk of OPXV infection include people with close and frequent animal contact. This includes pet owners, animal workers (e.g., farmers, milkers, zookeepers, veterinarians, wildlife biologists), hunters, and people handling raw meat or unprocessed animal products. Laboratory workers, healthcare staff, and household members are also at risk of disease from secondary transmission, especially where personal protective equipment is inadequate. Additional risk factors may include daily exposure to an infected animal, cleaning cages and bedding of an infected animal, and providing clinical care to an infected animal (Croft et al. 2007; Reynolds et al. 2007).

Zoonotic transmission of CPXV and VACV are relatively well understood and invariably follow close contact with symptomatic animals, for example, during milking, providing veterinary care, or through close interactions with pets. Epidemiological and laboratory investigations have demonstrated zoonotic CPXV infection following contact with cats, wild and pet rats (Campe et al. 2009; Wolfs et al. 2002), and captive elephants (Hemmer et al. 2010). Veterinary staff are noted in several case reports after treating animals with cowpox, including one alarming self-report and warning for practicing veterinarians (Hall and Stevens 1987; Vestey et al. 1991; Glatz et al. 2010; Lawn 2010).

Animal infections with VACV have led to human cases among handlers and milkers (Bhanuprakash et al. 2010; Gurav et al. 2011; Venkatesan 2010; Trindade et al. 2006). Lesions often appear on the arms and hands of handlers who reported

touching the infectious lesions of ill bovids, which are often on the teats and in or around the mouth. Although human-to-human transmission is rarely noted for VACV, it can occur among close contacts (Oliveira et al. 2017). VACV has been isolated in dairy products, suggesting the potential for foodborne transmission, although epidemiologic evidence is lacking (de Oliveira et al. 2015, 2018). There has been a suggestion of milk-associated transmission to humans with no animal contact after drinking of unpasteurized buffalo milk (Gurav et al. 2011); this observation warrants additional attention and careful investigation for future cases.

Akhmeta virus isolates have been recovered from wild caught rodents of the genus *Apodemus*, which likely serve as the animal reservoir and maintains its circulation in nature (Doty et al. 2019). Zoonotic transmission of this virus probably follows a similar dynamic as VACV, requiring contact with infected bovines, but the evidence is extremely limited given that only three human cases have ever been reported (Vora et al. 2015). Risk factors for zoonotic transmission of *Alaskapox virus* are less clear. Only four unrelated laboratory-confirmed cases have been reported in humans. All the cases live within the same area of Alaska, all reported outdoor activities, and several reported contact with asymptomatic cats and residing in residences with wild rodents (Springer et al. 2017) (CDC unpublished data). The discovery of *Alaskapox virus* in rodents (*Myodes rutilus*) trapped in proximity to one of the cases suggests potential cat-borne exposures or contact with an environmental surface that has been contaminated by infected small mammals (CDC unpublished reports).

MPXV transmission via fomites, direct contact, and respiratory droplet (without direct contact) was demonstrated in the prairie dog model, and aerosol transmission was documented in cynomolgus macaques (Hutson et al. 2011). Although similar risk factors likely apply in the natural setting, there is no conclusive evidence for how zoonotic MPXV infections occur. Risk factors for acquisition of the virus in central Africa include school aged males, those who hunt, and those who have frequent contact with uncooked wild animal meat (Nolen et al. 2015). However, without knowledge of the specific wild animal species that are involved in maintaining the virus, definitive risk factors for zoonotic infection remain an open question in the field. There may indeed be a much wider role for wild animal products beyond wild animal meat, such as peri-domestic small mammal contamination of residences, or specific cultural behaviors that place individuals at greater risk of exposure and subsequent infections in endemic areas (Bonwitt et al. 2017; Friant et al. 2022). These factors may also explain the epidemiology of outbreaks in Nigeria, where virus sequencing data suggest multiple zoonotic introductions; probably as a result of an undetected epizootic, but where cases occurred in urban settings without reports of animal exposures (Yinka-Ogunleye et al. 2018).

CMLV is host-restricted, and its zoonotic potential is disputed. In a comprehensive study involving 465 herdsmen handling CMLV-infected dromedaries, not a single human skin lesion sample of 335 tested positive for the virus (Jezek et al. 1983). Epidemiologic, clinical, and non-specific laboratory findings supported zoonotic transmission, but not definitive confirmation of viral infection in three camel herders handling infected animals (Bera et al. 2011). Given the high incidence in camel herds and rarity (or absence) of human cases, CMLV is not known to present a major public health concern.

24.2 Clinical Features

In general, OPXV infection results in a febrile prodrome followed by the appearance of a typical pox exanthem on the skin and mucosa. The characteristic lesions of OPXV infections have similar appearance across species; photographs of the lesions are important tools for clinical consultation (Fig. 1). The exanthem progresses through macules (flat discoloration), papules (slightly raised, firm swelling), vesicles (fluid-filled sac), pustules (pus-filled blisters often with central umbilication), before crusting, desquamation, and healed, healthy skin. Extensive lesions present in the oral cavity and throat may cause pain and impact food and water intake (Reynolds et al. 2017). Lesions on the teats of bovids may impact milking or feeding; secondary infection and mastitis are concerns (Gurav et al. 2011). Viral multiplication occurs in the draining lymph nodes, and the lymph nodes are often enlarged early in the course of illness in humans and non-human primates. In severe cases, viraemia leads to dissemination to internal organs (including heart, lungs, gastrointestinal tract, liver, lymph nodes, placental and fetal tissues) causing systemic signs (Reynolds et al. 2017). In humans, with the exception of MPXV and *Variola virus*, OPXV infections cause localized lesions; however, individuals with immunosuppressive or dermatologic conditions (e.g., atopic dermatitis, eczema) are at risk for a disseminated rash and severe illness (Laiton-Donato et al. 2020; Lawn 2010).

The severity of disease depends on the viral species and strain, the susceptibility of the host species, and host immune status. Complications of the skin may include secondary infection of the skin and cutaneous necrosis; fluid loss may be a concern due to extensive skin perturbation. Ocular infections can lead to permanent corneal scarring and vision impairment. Fetuses of pregnant females are at risk of infection and death in humans (Reynolds et al. 2017; Mbala et al. 2017; Ferrier et al. 2021; Franke et al. 2016).

Clinicians should also consider parapoxviruses, cutaneous anthrax, varicella, herpes simplex, treponema, and rickettsial pox as differential diagnoses that may have presentation characteristics similar to OPXVs. Laboratory confirmation may be pursued to determine the cause of infection and pathological analyses can be performed from necropsy or biopsy specimens.

24.2.1 CPXV

CPXV exhibits fetal tissue tropism and can cause abortion, even in asymptomatic dams; this has also occurred in an elephant and two foals (*Elephas maximus*) (Franke et al. 2016; Ellenberger et al. 2005; Wisser et al. 2001). Infected domestic cats present with pyrexia, inappetence, depression, and mild respiratory infection in 20% of cases. Skin lesions typically begin at the bite site and 20% of cases have oral lesions. Infection is usually self-limiting and healing is complete within 6 weeks. Severe cases have been associated with immunosuppression, and these animals have presented with secondary bacterial infection, cellulitis, and necrotizing pneumonia, which is nearly always fatal (Bennett et al. 1990; Hoare et al. 1984; McInerney et al. 2016). Horses appear relatively refractory to infection; of 3 reported cases, two

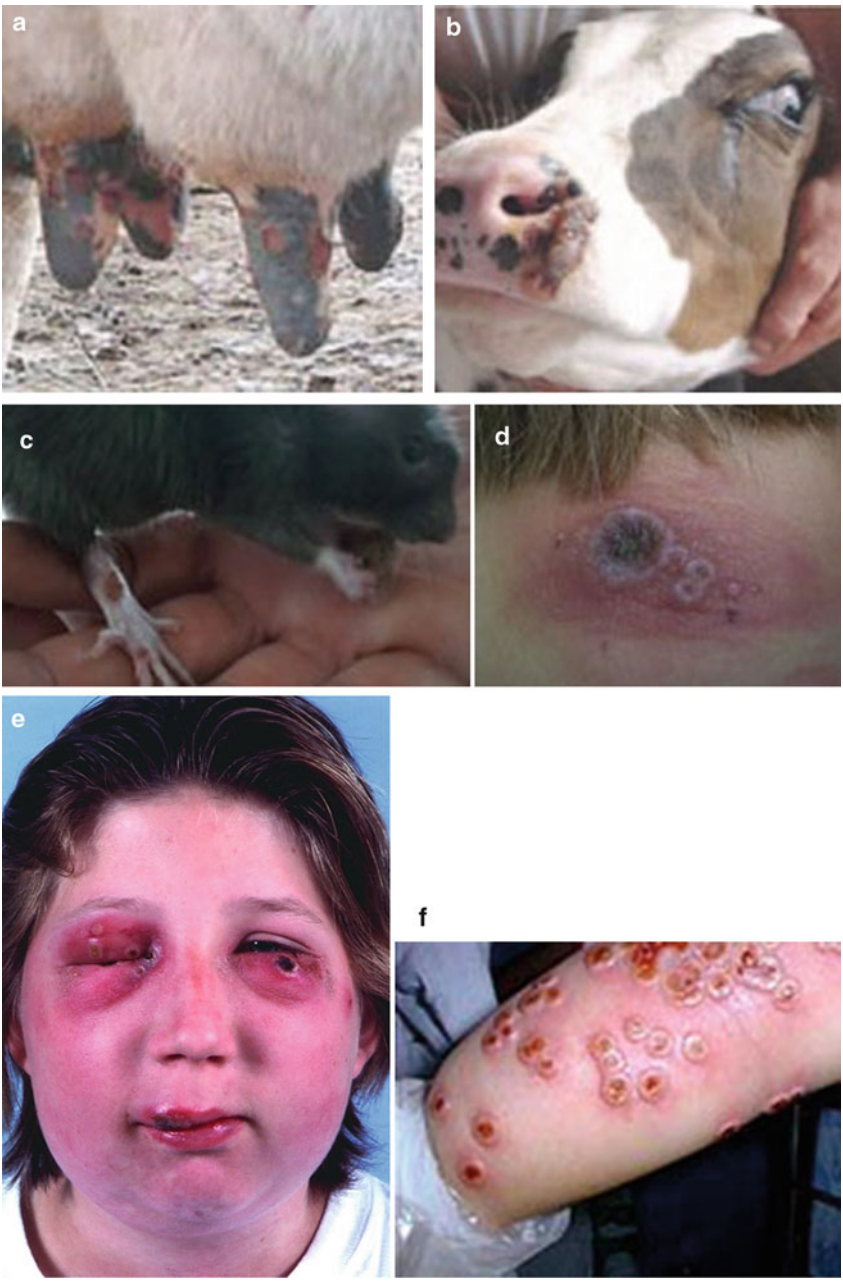

Fig. 1 Vaccinia virus lesions on the teats (**a**) and muzzle of a cow (**b**) (Leite et al. 2005); Cowpox virus lesions on a pet rat (**c**) and neck (**d**) of a human (Campe et al. 2009); Cowpox virus lesions with ocular manifestations (**e**) (Wolfs et al. 2002); and disseminated lesions from a Cowpox virus infection in a pediatric patient with a history of atopic dermatitis (**f**) (Pelkonen et al. 2003)

involved aborted or neonatal foals without involvement of the dams (Franke et al. 2016; Ellenberger et al. 2005). Contemporary case reports do not include cattle or other bovids.

Alpacas and llamas are susceptible to infection with CPXV; signs include alopecia, local or generalized exanthem, and keratoconjunctivitis (Prkno et al. 2017; Cardeti et al. 2011). In susceptible wildlife species, ulcerated lesions are common and lesions in the oropharynx can manifest as anorexia, hypersalivation, and dysphagia. Wild felids and elephants are especially susceptible to infection and a number of deaths have been reported. In felids, disease severity ranges from ulcerated skin lesions to necrotizing pneumonia (Marennikova et al. 1977). Infection is particularly lethal in cheetahs (*Acinonyx jubatus*) (Baxby et al. 1982; Stagegaard et al. 2017). Zoo outbreaks in elephants used to be frequent in European zoos but have been largely brought under control thanks to routine vaccination with VACV (i.e., smallpox vaccine) (Kurth and Nitsche 2011).

In humans, CPXV is usually self-limiting and individuals have limited lesions notably at the site of existing disruptions to the dermal barrier, or at the location of animal bites or scratches. For patients who own pet rats, lesions have been noted to occur on the neck or face (Campe et al. 2009). Severe infections, including deaths, have been noted in individuals with underlying atopic dermatitis, immunosuppression, and pregnancy (Lawn 2010; Ferrier et al. 2021; Pelkonen et al. 2003); and complicated ocular infections have occurred (Schwarzer et al. 2013; Kiernan and Koutroumanos 2021).

24.2.2 VACV, Alaskapox Virus, Akhmeta Virus

VACV can infect a wide range of hosts but mostly causes disease in bovines. With high attack rates (as high as 80–100%) and significant morbidity, outbreaks in dairy herds can cause significant economic loss (Oliveira et al. 2017). After a 3–7 day incubation period, VACV causes vesiculation followed by an ulcerative exanthem that mostly affects teats and udders, but that can also progress to skin and mucous membranes (Leite et al. 2005). In water buffalos (*Bubalus bubalis*), clinical signs include a typical pox exanthema that appear on the face, proximal and distal limbs, udder, teats, and scrotum (Bhanuprakash et al. 2010; Gurav et al. 2011). Suckling calves of infected cows can present with oronasal lesions (Leite et al. 2005). Production losses occur due to secondary mastitis, reluctance to being milked, and cows avoiding suckling calves. In buffalo herds, infection can result in a 42–70% reduction in milk yield (Eltom et al. 2020). Systemic disease may result in a relatively high mortality (up to 11%) in buffalos (Gurav et al. 2011).

A VACV outbreak has been recorded in Brazilian horses, involving 14 horses and foals causing extensive coalescing pox lesions on the muzzle, external nares, and external and internal lips, but without systemic signs (Brum et al. 2010; Campos et al. 2011). VACV has also been isolated from non-human primates, cats, dogs, equids, marsupials, lagomorphs, rodents, and bats in South America (Oliveira et al.

2017; Costa et al. 2017, 2018). Unlike CPXV, however; infection in wildlife or other domestic animals were not accompanied by clinical signs.

Although *Akhmeta virus* has never been isolated from domestic animals, infection in cattle probably follows a similar course to CPXV or VACV. In the Democratic Republic of Georgia, 10 of 71 dairy cows with epidemiologic links to laboratory-confirmed human cases of *Akhmeta virus* presented with characteristic pox lesions on their udder and teats. All cows fully recovered except one, in which teat contracture resulted in reduced milk production (Vora et al. 2015).

In humans, zoonotic VACV infection often manifests as a self-limiting infection with few lesions, often appearing at the site of exposure (via milking or other handling), the hands, or the face (Gurav et al. 2011; Megid et al. 2012). A recent report documenting a severe form of VACV infection, progressive vaccinia, in a dairy cattle handler from Colombia highlights the severity of such an infection in an individual with underlying immunosuppression caused by HIV and subsequent AIDS (Laiton-Donato et al. 2020). Further, VACV is easily transferred from a contaminated surface or infected individual (via contaminated hands or contact with lesions) to another person and autoinoculation can occur to other sites of a patient's body. Nosocomial transmission of VACV has occurred in Pakistan (Zafar et al. 2007) and there is a body of literature documenting the risks of inoculation from VACV lesions via smallpox vaccination.

24.2.3 MPXV

Monkeypox (MPX) disease presentation is very similar in humans and non-human primates (Arita and Henderson 1968). After a prodromal period of fever, fatigue, respiratory symptoms, and often lymphadenopathy (cervical, maxillary, and/or inguinal), the characteristic rash is apparent, often first appearing in the mouth or face followed by dissemination to other parts of the body. A MPX rash is distinct in that it is generalized and often includes lesions in the palms of the hands and soles of the feet. This rash will slowly progress through different stages until desquamation and final healing of the skin. Severe disease can occur as indicated above, and death occurs in approximately 11% of humans without prior smallpox vaccination (Jezek et al. 1987). Respiratory complications can include bronchopneumonia and airway obstruction due to significant lymphadenopathy; the latter has contributed to deaths in chimpanzees infected with MPXV (personal communication) (Reynolds et al. 2017). Exposure via an animal bite or scratch versus a less invasive exposure has been associated with more severe forms of disease (Reynolds et al. 2006).

In-vivo bioluminescent imaging of infected prairie dogs has shown widespread dissemination in internal organs (lymph nodes, intestines, heart, lungs, kidneys, and liver) prior to the disseminated rash (Weiner et al. 2019). Separate studies have isolated MPXV from most internal organs of infected animals (Hutson et al. 2009; Marennikova et al. 1972; Guarner et al. 2004; Langohr et al. 2004). Higher doses cause a more severe presentation with widespread rash lesions, generalized hemorrhagic manifestations, and faster symptom onset in laboratory infected marmosets

(*Callithrix jacchus*) and prairie dogs (*Cynomys ludovicianus*) (Falendysz et al. 2014; Mucker et al. 2015). Susceptibility to MPXV varies across species, as evidenced during a multi-species outbreak in a zoo where clinical signs occurred along a spectrum of severity ranging from pox exanthems to anorexia, lymphadenopathy, dyspnea, nasal discharge, depression, secondary infections, and death (Peters 1966).

24.2.4 CMLV

In contrast to other zoonotic OPXVs, CMLV is thought to have a narrow host range, only affecting bactrian (*Camelus bactrianus*) and dromedary (*C. dromedarius*) camels. It is highly contagious and causes significant production losses (Duraffour et al. 2011). After a 9–13 day incubation period, camels may present with pox lesions on the rostrum, eyelids, margins of the ears, and oronasal mucosa. In severe cases, lesions may cover the entire body. Systemic signs include fever, anorexia, lymphadenopathy, and mucopurulent discharge (WOAH, 2019). Abortion rates can be as high as 87% (Al-Zi'abi et al. 2007). Mortality can exceed 30% in adults and up to 100% in calves (Jezek et al. 1983; Krizn 1982).

24.3 Laboratory Diagnosis

OPXV infections can be difficult to diagnose clinically; thus, laboratory diagnostic evaluation is essential for confirming infection in humans and animals, which can be achieved using a variety of techniques (Table 1). Different methods have a range of veterinary and public health utilities and require different sample types; however, OPXV diagnostic assays are largely the same for animals and humans. Understanding disease progression and transmission is key to identifying the appropriate diagnostic sample type and timing of sample collection in relation to disease onset or exposure; however, for some enigmatic viruses, little is known about disease progression and transmission routes. Lesion material is consistently the best diagnostic sample type and the primary clinical feature used to identify OPXV infections. The presence of characteristic pox-like lesions on the surface of the skin, in the oral cavity, or on the surface of organs often suggests possible infection with an OPXV. Samples from these lesions provide a relatively simple and less invasive sample to collect for acute (active) infections in living patients.

The most reliable way to diagnose and differentiate among OPXV species is the detection of viral nucleic acids, for which polymerase chain reaction (PCR)-based tests are the gold standard. PCR assays target a specific piece of viral DNA to amplify for detection and/or sequencing, and can use a conventional approach where a PCR product is visualized on an electrophesis gel, or they can utilize a real-time approach that is capable of quantifying viral DNA in a sample (Li et al. 2006, 2007b, 2010). Most PCR assays that are used to differentiate OPXV species and strains are real-time assays which target variable regions of the genome where only nucleic acids from one species of virus will be amplified. Skin lesions are the optimal specimen type for PCR

Table 1 List of diagnostic assays, preferred sample types, and utility

Assay	Target	Specimen type	Species specific	Utility
PCR	Nucleic acid	Lesion material	Yes	Gold standard for diagnostics, including conventional or real-time PCR and may include species-specific assays
Viral DNA sequencing	Nucleic acid	Lesion material	Yes	Can be used for diagnostics when no species-specific PCR assay exists, but generally utilized to examine evolution of viruses or other genetic features
Serology (ELISA, PRNT, Western blot)	Antibodies (IgG or IgM) and proteins	Serum or plasma	No	Retrospective case identification or serosurveys, can complement PCR
Immuno-histochemistry	Antigen	Lesion material; tissue biopsy	No	May be used to identify or rule out a biological agent in differential diagnosis
Viral culture	Viable infectious viral particles	Lesion material	No	May be used to generate sufficient material for whole genome sequencing, requires strict biosafety measures
Electron microscopy	Viral particles	Lesion material	No	Can identify poxvirus particles when poxvirus is not a primary differential during diagnosis of unknown pathogens

as they contain viral particles and nucleic acids. Blood is not an optimal specimen type, as the period of viremia in the blood is often short relative to the entire course of clinical presentation (Hutson et al. 2009; Nitsche et al. 2007).

Viral sequencing is a tool that has been increasingly used in recent years and can be used to determine the strain and species of a virus in a particular sample. Additionally, analysis of these sequences allows investigators to determine the relationships among viral strains and species, assess geographic variations, examine evolutionary diversification, and may provide insights into the epidemiology of the virus or the clinical presentation (Gigante et al. 2019; Likos et al. 2005; Gruber et al. 2018).

Serological assays are used for antibody detection in serum and plasma samples. Common assays include enzyme-linked immunosorbent assay (ELISA), plaque-reduction neutralization test (PRNT), Western blot, and hemagglutination-inhibition. ELISA is the most commonly used serologic assay and can detect either IgM or IgG antibodies. Serology is not recommended as a primary diagnostic tool because it lacks the ability to distinguish infections caused by different OPXV species; rather, they allow investigators to evaluate antibody response to OPXVs on the whole. In humans, this will include individuals who received prior smallpox vaccination with VACV. The utility and interpretation of antibody detection will depend on the intended use of the assay. Serological assays can be used as a tool to identify

retrospective cases, assess immune responses to smallpox vaccination, and are useful for a variety of research purposes (Doshi et al. 2019; Doty et al. 2017; Guagliardo et al. 2020; Townsend et al. 2013).

Antibody responses may vary from one individual to the next and may vary significantly based on the viral species of exposure (Gilchuk et al. 2016). Generally, IgM antibodies are present a few days after exposure and may be detectable for approximately 2 months. Conversely, IgG antibody production will rise following the acute phase of illness, suggesting it may not be detectable until a few weeks after exposure, but may remain at detectable levels for years. Given this information, the presence of IgM antibodies indicates a recent OPXV infection or exposure, whereas IgG antibodies indicate an exposure months to years before the serum samples were collected.

Immunohistochemistry (IHC) is conducted on lesion material from affected skin, including crusts and lesion biopsies. Staining is used to detect antigen within cells using an OPXV antibody that is cross-reactive with many (if not all) viruses in the genus. While this can be a powerful tool for pathological samples, the method is labor and reagent intensive, and requires specialized equipment (Sejvar et al. 2004).

Diagnostic electron microscopy was widely used for smallpox diagnosis until its eradication, with decreased use in favor of molecular diagnostic techniques (i.e., PCR); however, it still remains an important tool for the identification of novel OPXVs that may be divergent enough from known ones not detected by other diagnostic assays (Gelderblom and Madeley 2018).

While the majority of diagnostic assays require laboratories with specialized equipment and experienced laboratorians, novel point-of-care assays are currently being developed and tested for field deployment. While these assays are generally being developed for human diagnostics, they could potentially be used for animals in certain situations where OPXV diagnosis may be important for public health or veterinary outbreak response situations such as monkeypox infections in primate sanctuaries in monkeypox endemic regions).

24.4 Animal and Public Health Control Measures

Interventions against animal and human OPXV infections hinge on preventing epizootics in domestic and captive wild animals, reducing the risk of zoonotic exposures, and preventing human-to-human transmission. Given the broad host range of OPXVs, interrupting disease transmission between members of a same species, as well as across species (i.e., spillover transmission), is the cornerstone of a cohesive strategy to prevent infection in animals and humans alike. The survival of OPXV on surfaces and prolonged survival of virus in lesion material for years (McCollum et al. 2014) means that environmental disinfection is paramount.

In animals, prevention and control can only be achieved in domestic animals and captive wildlife. Infection control in free-ranging wildlife (reservoirs or spillover hosts) is currently impossible due to the absence of a licensed vaccine, logistical challenges of vaccine delivery, and broad host range. In domestic pets, infection

prevention can be achieved by reducing contact with susceptible hosts where the reservoir hosts are known; for example, by keeping cats indoors in areas endemic for CPXV. In herds, zoos, and wildlife sanctuaries, standard biosecurity precautions may be instituted to prevent pathogen introduction, including quarantine of new arrivals (21 days), and routine rodent control measures. Rodents fed to captive mammals should be bred in a closed system and feeding using wild rodents should be avoided. Off-label smallpox vaccination has been used for susceptible wildlife species kept in captivity, especially in outbreak situations (Kurth et al. 2009) or where prior cases have occurred indicating endemic circulation in wild rodents. Modified VACV Ankara (MVA) vaccine has been used in European zoos for captive elephants, rhinoceroses, and felids at risk of infection (Stagegaard et al. 2017; Wolters and van Bolhuis 2008). Researchers have demonstrated that MVA can provide protection against MPXV infection in non-human primates (Stittelaar et al. 2005), although its use and effectiveness at preventing outbreaks in captive non-human primates has not been assessed.

Symptomatic animals may be immediately isolated to prevent animal-to-animal and zoonotic transmission. Biosecurity measures for isolation pens may address the potential of fomite transmission via animal bedding, shared equipment (e.g., food troughs, milking machines), and animal handlers, as well as the potential for droplet transmission to susceptible species. OPXVs can be shed via infectious lesions and bodily fluids, including urine, feces, and milk, thus infected animals should be isolated from susceptible animals. Special attention should be paid to routine disinfection of the environment where the ill animals reside and rodent control to prevent further spread to wild rodents (Guedes et al. 2013; Rehfeld et al. 2017). Preventing infection in susceptible animals that have frequent and close contact with humans (e.g., cats) also serves to reduce zoonotic transmission and is especially important for individuals at higher risk of disease. Rodent control in houses (rodent proofing, removing rodent harborage and attractants) may be implemented to reduce animal-human contact and environmental contamination.

People at risk of severe disease from OPXV infection (including individuals with a history of atopic dermatitis or eczema, pregnant women, individuals with an immunosuppressive condition or taking medications that may cause immunosuppression) should not handle infected animals. Personal protective equipment is effective at preventing transmission: gloves, gown, rubber boots, eye and mouth protection (mask or face shield). MPXV infections may produce infectious respiratory droplets and additional PPE should also include a N95 or filtering respirator (CDC 2021a). Preventing zoonotic transmission of MPXV is challenging, as the reservoir species are unknown and the endemic range overlaps with areas of food insecurity where people principally rely on wild animals as a source of protein. In this setting, avoiding hunting rodents and animals found sick or dead is a measure to reduce the risk of zoonotic transmission. There are, however, a number of cases in urban environments of Nigeria with no known wild animal exposure (Yinka-Ogunleye et al. 2018), further complicating our understanding of MPXV control.

Control of human-to-human OPXV transmission relies on hygiene practices to avoid transmission from skin lesions, contaminated items, and surfaces, and for MPXV, respiratory droplets. Healthcare providers and family members should adopt

standard contact precautions with personal protective equipment with the addition of a N95 or filtering respirator for MPXV. MPXV patients should be isolated in a healthcare or home-based care environment (CDC 2021a). For patients with localized lesions (and not a disseminated rash), lesions may be covered with a non-adhesive dressing to prevent spread to close contacts. Coverage of an infectious lesion also reduces the risk of self-inoculation which can lead to significant complications such as an ocular infection. Clothing, linen, and towels carry a risk of fomite transmission and should be treated as biohazard; they should either be destroyed or washed using standard laundry detergents at the highest temperature setting. Surfaces and shared household items can be disinfected using household chlorine bleach or quaternary ammonium products (CDC 2021a).

Except for MPXV, human-to-human transmission of zoonotic OPXVs is rare but cannot be discounted, especially among immunosuppressed individuals, in healthcare settings, or among close contacts. For all OPXV infections, infection prevention measures should be maintained until all crusts separate and a fresh layer of skin forms at former lesion sites, at which time they are no longer infectious. Given the broad range of susceptible animals and potential introduction of OPXVs in new geographic areas, humans should avoid direct contact with mammals (e.g., pets and wild rodents) during their period of infectiveness until all lesions have resolved and a fresh layer of skin has formed, to prevent further spread and reverse zoonosis (CDC 2021a).

Smallpox vaccination has been shown to be effective against MPXV infection (Jezek et al. 1988) and is recommended in the United States for prevention of OPXV infections in specific occupational groups (Petersen et al. 2016). Traditional smallpox vaccines use attenuated VACV to deliver a controlled dose to stimulate an immune response via vaccination. More recently, a third-generation vaccine utilizing MVA (which does not replicate efficiently in human cells) has been approved in the United States and European Union for the prevention of monkeypox in adults at high risk of infection. Further studies are needed to evaluate its effectiveness in outbreak response and duration of immunity. Studies of its pre-exposure use are underway in areas endemic for MPXV in humans (Petersen et al. 2018).

Finally, disease reporting to animal and public health authorities is critical for localized and international control efforts. Many countries may deem OPXV infections in humans reportable under the International Health Regulations (IHR); animal infections may be reported to the World Organization for Animal Health. The presence of transboundary events and viral spread to non-endemic areas may lead to a Public Health Emergency of International Concern under the IHR. These events can be serious and have led to traveler's health notices (CDC 2021b) and the ban of African rodent importation to the United States (Bernard and Anderson 2006; CDC 2015).

24.5 Future Perspectives & Conclusions

Following decades of research, our understanding of OPXVs is extensive, but many features of their ecology, epidemiology, and natural history continue to elude us. Enhanced human and animal surveillance, including confirmation by laboratory

diagnostics, will be critical to detect emerging zoonotic OPXVs, especially in the face of a global waning smallpox immunity, increased susceptibility to infection as result of immunosuppressive conditions (e.g., HIV/AIDS pandemic, immunosuppressive therapies, etc.), and increasing movement of humans and animals. Equally, animal surveillance will be key to monitor OPXV infections that threaten animal production and welfare in the context of livestock practices. OPXV surveillance is challenging, as many occur in areas with poor laboratory capacity or are considered relatively benign and are therefore not reportable. Similarly, detailed epidemiological investigations, including relevant zoonotic parameters, need to be pursued to elucidate the role of atypical transmission (e.g., via zootherapy and dairy products) and that of unrecognized domestic or peri-domestic reservoir hosts (e.g., dogs, cats, and coatis) (Costa et al. 2018). By harnessing the high degree of cross-reactivity and long duration of immunity, vaccine research is critical to protect persons at risk, reduce animal production losses, and hedge against disease emergence and bioterrorism. It is no exaggeration to say that poxviruses have shaped and are being shaped by human behaviors and practices. In all certainty, they will continue their evolutionary trajectory with humans and animals, and will continue to surprise us for years to come, for better or for worse.

24.6 Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

References

Abrahao JS et al (2010) Human vaccinia virus and Pseudocowpox virus co-infection: clinical description and phylogenetic characterization. J Clin Virol 48(1):69–72

Al-Zi'abi O, Nishikawa H, Meyer H (2007) The first outbreak of camelpox in Syria. J Vet Med Sci 69(5):541–543

Arita I, Henderson DA (1968) Smallpox and monkeypox in non-human primates. Bull World Health Organ 39(2):277–283

Baxby D et al (1982) An outbreak of cowpox in captive cheetahs: virological and epidemiological studies. J Hyg (Lond) 89(3):365–372

Baxby D, Bennett M, Getty B (1994) Human cowpox 1969–1993: a review based on 54 cases. Br J Dermatol 131:598–607

Bengali Z, Satheshkumar PS, Moss B (2012) Orthopoxvirus species and strain differences in cell entry. Virology 433(2):506–512

Bennett M et al (1990) Feline cowpox virus infection. J Small Anim Pract 31(4):167–173

Bennett M et al (1997) Cowpox in British voles and mice. J Comp Pathol 116:35–44

Bera BC et al (2011) Zoonotic cases of camelpox infection in India. Vet Microbiol 152(1–2):29–38

Bernard SM, Anderson SA (2006) Qualitative assessment of risk for monkeypox associated with domestic trade in certain animal species, United States. Emerg Infect Dis 12(12):1827–1833

Bhanuprakash V et al (2010) Zoonotic infections of buffalopox in India. Zoonoses Public Health 57(7–8):e149–e155

Bonwitt J et al (2017) At home with Mastomys and Rattus: human-rodent interactions and potential for primary transmission of Lassa virus in domestic spaces. Am J Trop Med Hyg 96(4):935–943
Borges IA et al (2017) Dairy production practices and associated risks for bovine vaccinia exposure in cattle, Brazil. New Microbes New Infect 20:43–50
Borges IA et al (2018) Serological evidence of Orthopoxvirus circulation among equids, Southeast Brazil. Front Microbiol 9:402. https://doi.org/10.3389/fmicb.2018.00402
Breman JG, Henderson DA (2002) Diagnosis and management of smallpox. N Engl J Med 346(17): 1300–1308
Brum MC et al (2010) An outbreak of orthopoxvirus-associated disease in horses in southern Brazil. J Vet Diagn Investig 22(1):143–147
Campe H et al (2009) Cowpox virus transmission from pet rats to humans, Germany. Emerg Infect Dis 15(5):777–780
Campos RK et al (2011) Assessing the variability of Brazilian vaccinia virus isolates from a horse exanthematic lesion: coinfection with distinct viruses. Arch Virol 156(2):275–283
Cardeti G et al (2011) Cowpox virus in llama, Italy. Emerg Infect Dis 17(8):1513–1515
Carroll DS et al (2011) Chasing Jenner's vaccine: revisiting cowpox virus classification. PLoS One 6(8):e23086
CDC (2015) African Rodent Importation Ban. [cited 2022]. Available from: https://www.cdc.gov/poxvirus/monkeypox/african-ban.html
CDC (2021a) Monkeypox. [cited 2022]. Available from: https://www.cdc.gov/poxvirus/monkeypox/index.html
CDC (2021b) Monkeypox in Nigeria. [cited 2022]. Available from: https://wwwnc.cdc.gov/travel/notices/watch/monkeypox-nigeria
Costa GB et al (2017) Detection of vaccinia virus in urban domestic cats, Brazil. Emerg Infect Dis 23(2):360–362
Costa GB et al (2018) Vaccinia virus among domestic dogs and wild coatis, Brazil, 2013–2015. Emerg Infect Dis 24(12):2338–2342
Croft DR et al (2007) Occupational risk during a monkeypox outbreak, Wisconsin, 2003. Emerg Infect Dis 13(8):1150–1157
de Oliveira TM et al (2015) Detection of vaccinia virus in milk: evidence of a systemic and persistent infection in experimentally infected cows. Foodborne Pathog Dis 12(11):898–903
de Oliveira TML et al (2018) Vaccinia virus detection in dairy products made with milk from experimentally infected cows. Transbound Emerg Dis 65(1):e40–e47
Doshi RH et al (2019) Epidemiologic and ecologic investigations of Monkeypox, Likouala Department, Republic of the Congo, 2017. Emerg Infect Dis 25(2):281–289
Doty JB et al (2017) Assessing monkeypox prevalence in small mammals at the human-animal interface in the Democratic Republic of the Congo. Viruses 9(10):283. https://doi.org/10.3390/v9100283
Doty JB et al (2019) Isolation and characterization of Akhmeta Virus from Wild-Caught Rodents (Apodemus spp.) in Georgia. J Virol 93:e00966–19. https://doi.org/10.1128/JVI.00966-19.
Duraffour S et al (2011) Camelpox virus. Antivir Res 92(2):167–186
Durski KN et al (2018) Emergence of Monkeypox – West and Central Africa, 1970–2017. MMWR Morb Mortal Wkly Rep 67(10):306–310
Eis-Hubinger AM et al (1990) Fatal cowpox-like virus infection transmitted by cat. Lancet 336 (8719):880
Ellenberger C et al (2005) Cowpox virus infection associated with a streptococcal septicaemia in a foal. J Comp Pathol 132(1):101–105
Eltom KH et al (2020) Buffalopox virus: an emerging virus in livestock and humans. Pathogens 9(9):676. https://doi.org/10.3390/pathogens9090676
Esparza J et al (2017) Equination (inoculation of horsepox): an early alternative to vaccination (inoculation of cowpox) and the potential role of horsepox virus in the origin of the smallpox vaccine. Vaccine 35(52):7222–7230
Falendysz EA et al (2014) Evaluation of Monkeypox virus infection of black-tailed prairie dogs (*Cynomys ludovicianus*) using in vivo bioluminescent imaging. J Wildl Dis 50(3):524–536

Fenner F (1993) Smallpox: emergence, global spread, and eradication. Hist Philos Life Sci 15(3): 397–420
Fenner F et al (1988) Smallpox and its eradication, vol 6. World Health Organization, Geneva
Ferrier A et al (2021) Fatal cowpox virus infection in human fetus, France, 2017. Emerg Infect Dis 27(10):2570–2577
Franke A et al (2016) Fatal cowpox virus infection in an aborted foal. Vector Borne Zoonotic Dis 16 (6):431–433
Friant S et al (2022) Zootherapy as a potential pathway for zoonotic spillover: a mixed-methods study of the use of animal products in medicinal and cultural practices in Nigeria. One Health Outlook 4(1):5
Gelderblom HR, Madeley D (2018) Rapid viral diagnostics of Orthopoxviruses by electron microscopy: optional or a must? Viruses 10:142
Gigante CM et al (2019) Genome of Alaskapox Virus, a novel orthopoxvirus isolated from Alaska. Viruses 11(8)
Gilchuk I et al (2016) Cross-neutralizing and protective human antibody specificities to poxvirus infections. Cell 167(3):6840694
Glatz M et al (2010) Human cowpox in a veterinary student. Lancet Infect Dis 10(4):288
Gruber C et al (2018) Whole genome characterization of orthopoxvirus (OPV) abatino, a zoonotic virus representing a putative novel clade of old world orthopoxviruses. Viruses 10(10): 546. https://doi.org/10.3390/v10100546
Guagliardo SAJ et al (2020) Asymptomatic orthopoxvirus circulation in humans in the wake of a Monkeypox outbreak among chimpanzees in Cameroon. Am J Trop Med Hyg 102(1):206–212
Guarner J et al (2004) Monkeypox transmission and pathogenesis in prairie dogs. Emerg Infect Dis 10(3):426–431
Guedes MI et al (2013) Detection of vaccinia virus in blood and faeces of experimentally infected cows. Transbound Emerg Dis 60(6):552–555
Gurav YK et al (2011) Buffalopox outbreak in humans and animals in Western Maharashtra, India. Prev Vet Med 100(3–4):242–247
Haase O et al (2011) Generalized cowpox infection in a patient with Darier disease. Br J Dermatol 164(5):1116–1118
Hall CJ, Stevens JD (1987) Ocular cowpox. Lancet 1(8524):111
Hemmer CJ et al (2010) Human cowpox virus infection acquired from a circus elephant in Germany. Int J Infect Dis 14(Suppl 3):e338–e340
Hendrickson RC et al (2010) Orthopoxvirus genome evolution: the role of gene loss. Viruses 2(9): 1933–1967
Hoare CM et al (1984) Cowpox in cats. Vet Rec 114(1):22
Hobson G et al (2021) Family cluster of three cases of monkeypox imported from Nigeria to the United Kingdom, May 2021. Euro Surveill 26(32):pii=2100745. https://doi.org/10.2807/1560-7917.ES.2021.26.32.2100745
Hutson CL et al (2007) Monkeypox zoonotic associations: insights from laboratory evaluation of animals associated with the multi-state US outbreak. Am J Trop Med Hyg 76(4):757–768
Hutson CL et al (2009) A prairie dog animal model of systemic orthopoxvirus disease using West African and Congo Basin strains of Monkeypox virus. J Gen Virol 90:323–333
Hutson CL et al (2011) Monkeypox disease transmission in an experimental setting: prairie dog animal model. PLoS One 6(12):e28295
ICTV (2011) dsDNA Viruses. Virus Taxonomy: The Classification and Nomenclature of Viruses. The 9th Report of the ICTV (2011) [cited 2022]. Available from: https://talk.ictvonline.org/ictv-reports/ictv_9th_report
Jezek Z, Kriz B, Rothbauer V (1983) Camelpox and its risk to the human population. J Hyg Epidemiol Microbiol Immunol 27(1):29–42
Jezek Z et al (1987) Human monkeypox: clinical features of 282 patients. J Infect Dis 156(2):293–298
Jezek Z et al (1988) Human monkeypox: secondary attack rates. Bull World Health Organ 66(4): 465–470
Kiernan M, Koutroumanos N (2021) Orbital Cowpox. N Engl J Med 384(23):2241

Kik MJ, Liu PL, van Asten JA (2006) Cowpoxvirus infection in the Patagonian cavy (*Dolichotis patagonum*) emerging disease in an educational animal park the first reported case. Vet Q 28(2): 42–44
Krizn B (1982) A study of camelpox in Somalia. Journal of Comparative Pathology Volume 92(1):1–8. https://doi.org/10.1016/0021-9975(82)90037-8.
Kurth A, Nitsche A (2011) Cowpoxin zoo animals. In: Miller RE, Fowler ME (eds) Fowler's zoo and wild animal medicine current therapy. Elsevier Saunders, pp 32–37
Kurth A et al (2008) Rat-to-elephant-to-human transmission of cowpox virus. Emerg Infect Dis 14 (4):670–671
Kurth A et al (2009) Cowpox virus outbreak in banded mongooses (*Mungos mungo*) and Jaguarundis (*Herpailurus yagouaroundi*) with a time-delayed infection to human. PLoS One 4(9): e6883. https://doi.org/10.1371/journal.pone.0006883
Laiton-Donato K et al (2020) Progressive Vaccinia in a patient with HIV/AIDS acquired by zoonotic transmission, Colombia. Emerg Infect Dis 26(3):601
Langohr IM et al (2004) Extensive lesions of monkeypox in a prairie dog (*Cynomys* sp). Vet Pathol 41(6):702–707
Lawn R (2010) Risk of cowpox to small animal practitioners. Vet Rec 166(20):631
Leite JA et al (2005) Passatempo virus, a vaccinia virus strain, Brazil. Emerg Infect Dis 11(12): 1935–1938
Li Y et al (2006) Detection of Monkeypox virus with real-time PCR assays. J Clin Virol 36(3): 194–203
Li Y et al (2007a) On the origin of smallpox: correlating variola phylogenics with historical smallpox records. Proc Natl Acad Sci U S A 104(40):15787–15792
Li Y et al (2007b) Orthopoxvirus pan-genomic DNA assay. J Virol Methods 141(2):154–165
Li Y et al (2010) GC content-based pan-pox universal PCR assays for poxvirus detection. J Clin Microbiol 48(1):268–276
Likos AM et al (2005) A tale of two clades: Monkeypox viruses. J Gen Virol 86:2661–2672
Marennikova SS et al (1972) Poxviruses isolated from clinically ill and asymptomatically infected monkeys and a chimpanzee. Bull World Health Organ 46(5):613–620
Marennikova SS et al (1977) Outbreak of pox disease among Carnivora (*Felidae*) and Edentata. J Infect Dis 135(3):358–366
Martina BE et al (2006) Cowpox virus transmission from rats to monkeys, the Netherlands. Emerg Infect Dis 12(6):1005–1007
Mauldin MR et al (2017) Cowpox virus: what's in a name? Viruses 9(101). https://doi.org/10.3390/v9050101
Mauldin MR et al (2022) Exportation of Monkeypox virus from the African continent. J Infect Dis 225(8):1367–1376. https://doi.org/10.1093/infdis/jiaa559
Mbala PK et al (2017) Maternal and fetal outcomes among pregnant women with human Monkeypox infection in the Democratic Republic of Congo. J Infect Dis 216(7):824–828
McCollum AM et al (2014) Poxvirus viability and signatures in historical relics. Emerg Infect Dis 20(2):177–184
McInerney J et al (2016) Pulmonary cowpox in cats: five cases. J Feline Med Surg 18(6):518–525
Megid J et al (2012) Vaccinia virus zoonotic infection, Sao Paulo State, Brazil. Emerg Infect Dis 18 (1):189–191
Mucker EM et al (2015) Susceptibility of marmosets (*Callithrix jacchus*) to Monkeypox virus: a low dose prospective model for Monkeypox and smallpox disease. PLoS One 10(7):e0131742
Nitsche A, Kurth A, Pauli G (2007) Viremia in human cowpox virus infection. J Clin Virol 40(2): 160–162
Nolen LD et al (2015) Introduction of Monkeypox into a community and household: risk factors and zoonotic reservoirs in the Democratic Republic of the Congo. Am J Trop Med Hyg 93(2): 410–5. https://doi.org/10.4269/ajtmh.15-0168.
Nolen LD et al (2016) Extended human-to-human transmission during a Monkeypox outbreak in the Democratic Republic of the Congo. Emerg Infect Dis 22(6):1014–1021
Oliveira JS et al (2017) Vaccinia virus natural infections in Brazil: the good, the bad, and the ugly. Viruses 9(11)

Patrono LV et al (2020) Monkeypox virus emergence in wild chimpanzees reveals distinct clinical outcomes and viral diversity. Nat Microbiol 5(7):955–965
Pelkonen PM et al (2003) Cowpox with severe generalized eruption, Finland. Emerg Infect Dis 9 (11):1458–1461
Peters JC (1966) An epizootic of monkey pox at Rotterdam Zoo. Int Zoo Yearbook 6(1):274–275
Petersen B et al (2016) Use of vaccinia virus smallpox vaccine in laboratory and health care personnel at risk for occupational exposure to orthopoxviruses – recommendations of the Advisory Committee on Immunization Practices (ACIP), 2015. MMWR Morb Mortal Wkly Rep 65(10):257–262
Petersen BW et al (2018) Vaccinating against monkeypox in the Democratic Republic of the Congo. Antivir Res 162:171–177. https://doi.org/10.1016/j.antiviral.2018.11.004
Prkno A et al (2017) Epidemiological investigations of four Cowpox Virus outbreaks in Alpaca Herds, Germany. Viruses 9(344). https://doi.org/10.3390/v9110344
Rehfeld IS et al (2017) Subclinical bovine vaccinia: an important risk factor in the epidemiology of this zoonosis in cattle. Res Vet Sci 114:233–235
Reynolds MG et al (2006) Clinical manifestations of human monkeypox influenced by route of infection. J Infect Dis 194(6):773–780
Reynolds MG et al (2007) Spectrum of infection and risk factors for human monkeypox, United States, 2003. Emerg Infect Dis 13(9):1332–1339
Reynolds MG, Carroll DS, Karem KL (2012) Factors affecting the likelyhood of monkeypox's emergence and spread in the post-smallpox era. Curr Opin Virol 2012(2):335–343
Reynolds MG et al (2017) Improving the care and treatment of Monkeypox patients in low-resource settings: applying evidence from contemporary biomedical and smallpox biodefense research. Viruses 9(12):E380. https://doi.org/10.3390/v9120380.rrrrr
Reynolds MG et al (2018) Understanding orthopoxvirus host range and evolution: from the enigmatic to the usual suspects. Curr Opin Virol 28:108–115
Rimoin AW et al (2010) Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. Proc Natl Acad Sci U S A 107(37):16262–16267
Schrick L et al (2017) An early American smallpox vaccine based on Horsepox. N Engl J Med 377 (15):1491–1492
Schwarzer H et al (2013) Severe ulcerative keratitis in ocular cowpox infection. Graefes Arch Clin Exp Ophthalmol 251(5):1451–1452
Sejvar JJ et al (2004) Human monkeypox infection: a family cluster in the Midwestern United States. J Infect Dis 190(10):1833–1840
Singh RK et al (2006) An outbreak of buffalopox in buffalo (*Bubalus bubalis*) dairy herds in Aurangabad, India. Rev Sci Tech 25(3):981–987
Smith GL, Law M (2004) The exit of vaccinia virus from infected cells. Virus Res 106(2):189–197
Smithson C et al (2017) The genomes of three north American orthopoxviruses. Virus Genes 53(1): 21–34
Springer YP et al (2017) Novel orthopoxvirus infection in an Alaska Resident. Clin Infect Dis 64(12):1737–1741
Stagegaard J et al (2017) Seasonal recurrence of cowpox virus outbreaks in captive cheetahs (*Acinonyx jubatus*). PLoS One 12(11):e0187089
Stittelaar KJ et al (2005) Modified vaccinia virus Ankara protects macaques against respiratory challenge with Monkeypox virus. J Virol 79(12):7845–7851
Townsend MB et al (2013) Humoral immunity to smallpox vaccines and Monkeypox virus challenge: proteomic assessment and clinical correlations. J Virol 87(2):900–911
Trindade GS et al (2006) Short report: isolation of two vaccinia virus strains from a single bovine vaccinia outbreak in rural area from Brazil: implications on the emergence of zoonotic orthopoxviruses. Am J Trop Med Hyg 75(3):486–490
Trindade GS et al (2007) Brazilian vaccinia viruses and their origins. Emerg Infect Dis 13(7): 965–972

Tryland M et al (2011) Orthopoxvirus DNA in Eurasian lynx, Sweden. Emerg Infect Dis 17(4): 626–632
Usme-Ciro JA et al (2017) Detection and molecular characterization of zoonotic poxviruses circulating in the Amazon region of Colombia, 2014. Emerg Infect Dis 23(4):649–653. https://doi.org/10.3201/eid2304.161041
Vaughan A et al (2020) Human-to-human transmission of Monkeypox virus, United Kingdom, October 2018. Emerg Infect Dis 26(4):782–785
Venkatesan G (2010) Emerging and re-emerging zoonotic buffalopox infection: a severe outbreak in Kolhapur (Maharashtra), India. Vet Ital 46(4):439
Vestey JP, Yirrell DL, Aldridge RD (1991) Cowpox/catpox infection. Br J Dermatol 124(1):74–78
Vora NM et al (2015) Human infection with a zoonotic orthopoxvirus in the country of Georgia. N Engl J Med 372(13):1223–1230
Weiner ZP et al (2019) Characterization of Monkeypox virus dissemination in the black-tailed prairie dog (*Cynomys ludovicianus*) through in vivo bioluminescent imaging. PLoS One 14(9): e0222612
Whitehouse ER et al (2021) Clinical and epidemiological findings from enhanced Monkeypox surveillance in Tshuapa Province, Democratic Republic of the Congo during 2011–2015. J Infect Dis 223(11):1870–1878
Wisser J et al (2001) Cowpox virus infection causing stillbirth in an Asian elephant (*Elephas maximus*). Vet Rec 149:244–246
Wolfs TF et al (2002) Rat-to-human transmission of cowpox infection. Emerg Infect Dis 8(12): 1495–1496
Wolters M, van Bolhuis H (2008) Cowpox virus fact sheet. In: Kaandorp J (ed) Transmissible disease handbook. European Association of Zoo and Wildlife Veterinarians, Berne
World Organisation for Animal Health (2019). Chapter 3.5.1. Camelpox. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organisation for Animal Health, Paris, 1655–1668.
Yinka-Ogunleye A et al (2018) Reemergence of human Monkeypox in Nigeria, 2017. Emerg Infect Dis 24(6):1149–1151. https://doi.org/10.3201/eid2406.180017
Zafar A et al (2007) Nosocomial buffalopoxvirus infection, Karachi, Pakistan. Emerg Infect Dis 13(6):902–904
Zwart P, Gispen R, Peters JC (1971) Cowpox in okapis Okapia johnstoni at Rotterdam Zoo. Br Vet J 127(1):20–24

Elimination of Rabies: A Missed Opportunity

25

Thomas Müller, Charles C. Rupprecht, Anthony R. Fooks, Leo Both, Samuel P. Smith, Andrew P. Gibson, Frederic Lohr, Anna Fahrion, and Conrad M. Freuling

Contents

T. Müller (✉) · C. M. Freuling
Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany
e-mail: Thomas.Mueller@fli.de

C. C. Rupprecht
LYSSA LLC, Cumming, GA, USA

A. R. Fooks
Animal and Plant Health Agency (APHA), Surrey, UK

L. Both · S. P. Smith
St George's Medical School, University of London, London, UK

A. P. Gibson · F. Lohr
Mission Rabies, Cranborne, UK

A. Fahrion
Institute of International Animal/One Health, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_21

Abstract

Rabies is the oldest diseases known to mankind. Occurring on all continents, except Antarctica, rabies remains one of the neglected tropical diseases (NTD) that predominantly affects the poor of the poor particularly in Asia and Africa still causing tens of thousands of lives lost every year. This chapter 'Elimination of Rabies—A Missed Opportunity' provides an up-to-date review of information known about the etiology and epidemiology of the disease, the rabies situation worldwide, human rabies prevention, prophylaxis and experimental therapy, rabies control in wildlife and dogs as well as road blocks on the way to global elimination of dog mediated rabies from a "One Health" perspective.

Keywords

Control · Dogs · Epidemiology · Humans · Prevention · Prophylaxis · One health · Rabies · Wildlife

25.1 Rabies – Fascinating Backgrounds of a Deadly Disease[1]

Ancient world writings from as early as Mesopotamian and Greek times suggest that rabies (lyssa, hydrophobia) is the oldest recognized zoonosis, perhaps as old as mankind. The first written record of rabies is in the Mesopotamian Codex of Eshnunna of Babylon (circa 1930 BC) and mentioned preventive measures to be taken by owners of dogs supposed of having contracted rabies and further detailed heavy sentences for dog owners in case another person being bitten by their rabid dog later died (Dunlop and Williams 1996). Rabies is defined as an acute, progressive, incurable viral encephalitis that is transmitted following bites of infected mammals. While the name rabies is derived from the Latin name for "madness," the old Greeks derived the word lyssa from lud or "violent"; this root is used in the name of the genus lyssavirus (Rupprecht et al. 2020a).

For millennia, the disease has been considered a scourge for its prevalence as well as a dual public horror and biomedical travesty (Rupprecht et al. 2008). There is probably no other zoonosis known today, which has been eliciting such anxiety to people, has been so intensively studied, and about which so many common and scientific reports have been published. It is a tragedy that despite the pioneering work of Louis Pasteur, more than 150 years ago, who with his first vaccination trials in man paved the way for today's efficient pre- and post-exposure rabies prophylaxis in humans and preventive vaccination in animals, rabies still is an imminent danger for humans and animals alike.

25.1.1 Virological Background and Diversity of Lyssaviruses

For a long time, rabies was believed to be caused by a single virus and it was not until the first discovery of bat-associated lyssaviruses in the second half of the last century that this perception changed. Today it is commonly accepted that rabies as a disease is caused by a plethora of different negative-strand RNA viruses of the Lyssavirus genus, family Rhabdoviridae of the Mononegavirales order (Fooks et al. 2017; Fisher et al. 2018).

Currently, the genus has been subdivided into 17 recognized and 2 putative virus species (Walker et al. 2020; Amarasinghe et al. 2018). Intriguingly, *Chiroptera* appear to be the reservoir for almost all lyssaviruses thus putting rabies in the list of the most significant viral zoonosis associated with bats (Luis et al. 2013). While all lyssaviruses share certain morphological and structural characteristics, on the basis of their genetic and antigenic relatedness as well as their biological properties, e.g., pathogenicity, induction of apoptosis, cell receptor recognition, and immunogenicity, lyssaviruses can be further segregated into at least three phylogroups (Badrane et al. 2001). The great majority of lyssaviruses are considered members of phylogroup I, including the prototypic rabies virus (RABV), European bat

[1] Authors: Thomas Müller and Conrad M. Freuling

lyssavirus-1 (EBLV-1), EBLV-2, Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus (KHUV), Irkut virus (IRKV), Bokeloh bat lyssavirus (BBLV), Duvenhage virus (DUVV), Gannuruwa bat lyssavirus (GBLV), and Taiwan bat lyssavirus (TWBLV). The African lyssaviruses Lagos bat virus (LBV), Mokola virus (MOKV), and Shimoni bat lyssavirus (SHIBV) were assigned to phylogroup II, while the genetically more diverse lyssaviruses including West Caucasian bat virus (WCBV), Ikoma virus (IKOV) and Lleida bat lyssavirus (LLEBV) are likely representatives of other phylogroups (Walker et al. 2020; Kuhn et al. 2020). Recent discoveries of one further novel lyssavirus, Kotalahti bat lyssavirus (KBLV) and two sequences from a potentially novel lyssavirus named Matlo bat lyssavirus (MBLV) in European and African bats, respectively (Fooks et al. 2021), clearly indicate that there is reason to believe that with reinforced and enhanced surveillance, particularly in bats, the diversity of lyssaviruses will further evolve. Interestingly, the recent detection of lyssavirus-like sequences in frogs and anoles challenges the host restriction to mammals (Horie et al. 2020). Further research is needed to corroborate such findings and assess their relevance for, e.g. virus origin.

Although lyssaviruses are capable of infecting all mammals, onward transmission in a new host population requires adaptation of the virus, in a number of stages with both host and virus factors determining the outcome. Within each lyssavirus species, genetic diversity may vary more or less within sublineages corresponding to distinct variants circulating in specific geographical regions and/or particular reservoir hosts in complex ecological communities. It is assumed that limited diversity of a specific variant corresponds to a dynamic equilibrium (or genetic stasis) resulting from a relatively conserved and long-term virus-host coevolution and coadaptation (Mollentze et al. 2014). Such stability is observed frequently among bat lyssavirus variants. While in general, the immune status of the host, the nature of exposure and strain differences influence infection and transmission dynamics (Fisher et al. 2018), modeling studies suggest that increased virulence in a novel host might act as a limiting factor preventing onward transmission (Mollentze et al. 2020). Interestingly, RABV has successfully crossed species barriers and established infectious cycles in new hosts to become the global multi-host pathogen it is today, while other lyssaviruses appear very restricted in hosts, suggesting that RABV is the exception but not the rule among lyssaviruses (Marston et al. 2018).

25.1.2 Reservoir Hosts of Classical Rabies

While all mammals are susceptible for the prototypic RABV, yet its lyssavirus reservoir in its entire complexity is cryptic (Fisher et al. 2018). Intriguingly, RABV is the only lyssavirus known to have primary reservoirs in species of the orders *Chiroptera* (bats) and *Carnivora*, where lineages of RABV circulate independently. Particularly canine species (family Canidae) are reservoir hosts of RABV in most parts of the world (Müller and Freuling 2020a). Next to wild carnivores, rabies virus maintained and transmitted by domestic dogs (*Canis familiaris*) by far poses the most serious threat to public health. Dog-mediated rabies is responsible for

more than 95% of the tens of thousands of human rabies casualties reported every year and results in millions of exposure contacts that require costly medical intervention. Mainly developing countries from Asia and Africa suffer from the burden of dog-mediated rabies (Hampson et al. 2015).

25.1.3 Americas

The Americas are the only continents where just one single lyssavirus species is present – the classical rabies virus (RABV); the reason for this paradox remains elusive. In North America, terrestrial rabies is a multispecies reservoir problem. Here, independent infectious cycles exist in raccoons (*Procyon lotor*), skunks (*Memphitis ssp*), red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), and arctic foxes (*Alopex lagopus*) (Ma et al. 2021) with the latter contributing to the circumpolar transmission of arctic variants of RABV (Mork and Prestrud 2004; Hanke et al. 2016; Mansfield et al. 2006b).

Historically, it has not been fully elucidated up to now whether rabies was present in the New World prior to the arrival of the first European settlers in the mid of the previous millennium (Vos et al. 2011; Rupprecht et al. 2017). It is certain, however, that canine RABV was introduced to the Americas during European colonization causing many human casualties. The same applies to the small Indian mongoose (*Herpestes auropunctatus*), a small terrestrial carnivorous mammal introduced during the latter part of the nineteenth century, which is now implicated as the principal wildlife reservoir for rabies on many Caribbean islands (Seetahal et al. 2013, 2018; Zieger et al. 2014). While enzootic rabies elimination from dogs has been almost achieved in many countries in the Americas (Vigilato et al. 2013a; Velasco-Villa et al. 2017a), the disease seemed to have reemerged in wild terrestrial carnivores (Velasco-Villa et al. 2008). In South America, other species including the Crab-eating fox (*Cerdocyon thous*), the Hoary fox (*Lycalopex vetulus*) (Antunes et al. 2018; Bernardi et al. 2005; Silva et al. 2009), and the Peruvian fox (*Lycalopex sechurae*) have been identified as wildlife reservoirs maintaining species adapted RABV variants (Velasco-Villa et al. 2017a). Interestingly, recent detections of a new RABV variant in marmosets (*Callithrix jacchus*) suggest the first independent infectious cycle in monkeys (Favoretto et al. 2001).

The Americas are also unique for their chiropteran rabies reservoirs as almost any indigenous species of bats on both continents ranging from insectivorous, frugivorous, nectarivorous to hematophagous bats has been identified to harbor distinct well-adapted RABV variants (Banyard et al. 2020b). While RABV in insectivorous bats only causes sporadic spillovers, vampire bat (*Desmodus rotundus*) transmitted rabies poses a serious threat to public and animal health in Central and South America (Johnson et al. 2014). It has been hypothesized that an ancient chiropteran origin of RABV might be the most likely explanation for the existence of the independently evolved "indigenous American" virus lineages as well as for the absence of RABVs in related bat species in the Old World (Rupprecht et al. 2017). Indeed, phylogenetic analysis suggests that RABV first evolved as a bat virus and subsequently crossed the

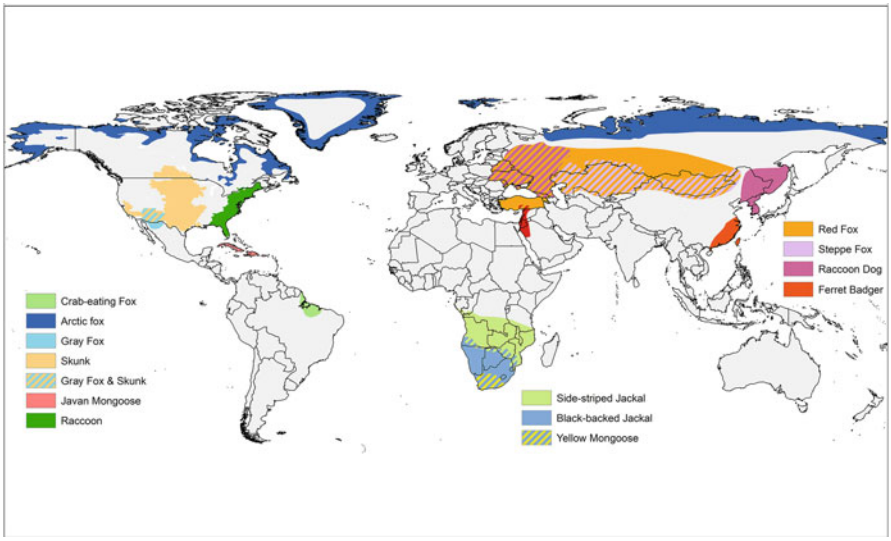

Fig. 1 Distribution of terrestrial wildlife rabies caused by RABV in different reservoir. The individual ranges are based on data provided by the IUCN

species barrier from Chiroptera to the Carnivora order (Badrane and Tordo 2001). In fact, both the raccoon as well as the skunk variant of RABV in Southern USA have been shown to descent from an ancestral bat origin (Velasco-Villa et al. 2017b). Even today, cross-species transmission events are documented regularly. While most of these spill-over events lead to dead-end infections, molecular inferences suggest multiple sustained spillovers of RABV from bats to mesocarnivores in North America as documented for foxes (Daoust et al. 1996); skunks (Leslie et al. 2006), and gray foxes (Kuzmin et al. 2012), hence, confirming the possibility of such historical events (Marston et al. 2017, 2018) (Fig. 1).

25.1.4 Africa

Next to the diversity of lyssaviruses found in Mega- and Microchiroptera, the current epidemic of classical rabies probably is a result of a more recent event because both historical records and phylogenetic analysis prove that the cosmopolitan lineage of canine RABV was introduced during European colonization (Rupprecht et al. 2020a). Apart from independent introductions in space and time, translocation, emergence among feral dogs, and adaptation to wildlife species are considered important dissemination mechanisms of canine rabies that are supposed to have peaked early in the twentieth century (Rupprecht et al. 2008). Classical rabies cycles are sustained by many carnivorous hosts of the family Canidae. Alongside the domestic dog, which is the major host reservoir of classical RABV in Africa, evidence is mounting that other wildlife canids such as jackals (*Canis adustus* and

C. mesomelas) and bat-eared foxes (*Otocyon megalotis*) are reservoirs for RABV in Southern Africa too (Bingham et al. 1999; Sabeta et al. 2003). While usually RABV variants are independently maintained in these species, in some areas common dog-jackal transmission cycles have been reported resulting in a rather complex epidemiological situation (Bellan et al. 2012). There has been no sustained progress yet in eliminating dog-mediated rabies on the continent (Haselbeck et al. 2021).

Also, members of the family Herpestidae appear to be responsible for the transmission cycle of a distinctive variant of RABV (Nel et al. 2005) as suggested for the Yellow mongoose (*Cynictis penicillata*) and the Slender mongoose (*Galerella sanguinea*) (King et al. 1993; Foggin 1988). Uniquely, for a long period of time independent horizontal transmission of the cosmopolitan RABV lineage among kudu antelopes (*Tragelaphus strepsiceros*) has been suspected, a phenomenon exclusively found in Namibia (Scott et al. 2012). However, phylogenetic and experimental research suggest that jackal and kudu may form part of the same epidemiological cycle of rabies in Namibian wildlife (Mansfield et al. 2006a) and that rabies epidemics in this herbivorous species is rather a combination of spill-over events and perhaps locally restricted horizontal transmission (Hassel et al. 2018).

25.1.5 Europe

The earliest references to rabies coming from ancient sources clearly indicate that terrestrial rabies has been endemic in Europe for centuries (Neville 2004). Until the nineteenth century, historical records mainly refer to dog-mediated rabies, while anecdotal reports also mention outbreaks of rabies in wolves and foxes (Blancou 2004; Rupprecht et al. 2020a). So, there is reason to believe that variants of RABV proceeded to involve not only dogs, but also other wild canids over vast areas. While dog-mediated rabies has been successfully controlled in Europe (Müller and Freuling 2018; Rupprecht et al. 2020a), Turkey remains the only country, where dog-mediated rabies persists (Johnson et al. 2010).

In the mid-twentieth century, wildlife rabies emerged in and was maintained by the red fox (*Vulpes vulpes*) as the new main reservoir host, spreading the disease throughout the continent within a few decades (Wandeler 2004, 2000). While due to oral vaccination of foxes large parts of Western and Central Europe have been declared free from fox-mediated rabies (Müller et al. 2012, 2015), it is still endemic in Eastern parts of the continent (Müller and Freuling 2018). Besides the red fox, the raccoon dog (*Nyctereutes procyonoides*), an alien species to Europe is supposed to be another wildlife rabies reservoir. Rabies cases have also been reported in golden jackals (*C. aureus*) on the Balkan Peninsula. However, it is not yet clear whether the raccoon dog or the golden jackal represent independent rabies reservoir hosts (Johnson et al. 2008; Müller et al. 2015). In the northernmost parts of Europe, conspecifics of the Arctic fox (*Alopex lagopus*) are involved in the circumpolar transmission cycle of arctic rabies (Mork and Prestrud 2004; Johnson et al. 2007).

Also, other alien species including the North American raccoon (*Procyon lotor*) and the small Indian mongoose (*Herpestes auropunctatus*) are now firmly established in parts of Europe often reaching higher population densities compared to their areas of origin (Cirovic et al. 2011; Vos et al. 2012). Sustained introductions of RABV variants into these species would pose a significant challenge in terms of rabies control (Vos et al. 2012; Müller et al. 2015).

25.1.6 Asia

Domestic dogs represent the major reservoir and vector for the disease in large parts of Asia (Müller and Freuling 2020a), and dog transmitted RABV causes thousands of victims per year (Song et al. 2014; Sudarshan et al. 2007). The veterinary public health focus on dogs may mask the presence of rabies in wildlife. In fact, the disease has also been reported from wildlife species in many Asian countries (Müller and Freuling 2020a). In Russia and other countries of the former Soviet Union, rabies is also maintained by wild canids, particularly foxes, while local dog epizootics occurred only sporadically in some territories. The red fox serves as the main reservoir, but the steppe fox (*Vulpes corsac*) and the golden jackal participate in RABV circulation in the steppe and desert territories (Kuzmin et al. 2004; Shulpin et al. 2018). Phylogenetic data indicate that except for arctic foxes in the polar region and raccoon dogs in Far East Russia an association of RABV variants with host species was less obvious (Shulpin et al. 2018). In Central Asia, fragmentary surveillance data indicate that the majority of cases are reported from dogs. Although wildlife rabies has been reported only scarcely, it seems to play a role in the epidemiology of the disease (Gruzdev 2008; Sultanov et al. 2016). In the Middle East region, besides dogs, also wild canid species, i.e., golden jackals and fox species are considered reservoirs. Turkey is a country, where a host switch from dogs into red foxes was reported that lead to an epidemic in the Aegean region of Turkey (Johnson et al. 2006; Vos et al. 2009; Marston et al. 2017). Sri Lanka may also serve as an example of the coexistence of dog-mediated and wildlife-mediated rabies, with the latter associated with mongoose and jackals (Karunanayake et al. 2014). Also, in China and Taiwan, rabies is associated with the Ferret badger (Lan et al. 2017; Zhang et al. 2009).

25.1.7 Other Lyssaviruses

Intriguingly, all recognized and proposed lyssavirus species within the genus *Lyssavirus* have reservoirs in Chiroptera, except for MOKV and IKOV (Banyard et al. 2020a)

Bats have particular traits that may promote the maintenance and transmission of lyssaviruses. It is hypothesized that all lyssaviruses originated from a precursor bat virus and therefore, bats with their more than 1100 recognized species to date (more than 16% of mammalian species) are the ultimate historical source of carnivore

rabies infections, based in part upon epidemiological, ecological, and phylogenetic inferences (Badrane and Tordo 2001; Calisher et al. 2006). In Europe, bat rabies is caused by various lyssaviruses. EBLV-1 accounts for more than 95% of all bat rabies cases in Europe and is mainly found in Serotine bats (*Eptesicus serotinus*) and Isabelline bats (*E.isabellinus*) (Schatz et al. 2013). In contrast, EBLV-2 has only been detected sporadically, always associated with Myotis bats (*M. daubentonii* and *M. dascyneme*) in the Netherlands, United Kingdom, Switzerland, Germany, Norway, and Finland (McElhinney et al. 2018). Bat rabies cases in Europe are seemingly less frequent than in the Americas; however, in many European countries bat rabies surveillance is still inadequate, despite international recommendations (Schatz et al. 2013). Discoveries of novel bat lyssaviruses WCBV (2001 from *Miniopterus schreibersii*), BBLV (2010 from *M. nattererii*), LLEBV (2012 from *M. schreibersii*), and KBLV (2019 from *M. brandtii*) (Freuling et al. 2011; Aréchiga Ceballos et al. 2013; Nokireki et al. 2018; Kuzmin et al. 2005) indicate the diversity of lyssaviruses in Europe that is likely to expand in the future.

In Africa, bat-associated LBV and DUVV have been associated with several taxa, including Eidolon, Epomophorus, Miniopterus, and Nycteris species (Hayman et al. 2012; Markotter et al. 2020). SHIBV was isolated from an insectivorous Hipposideros bat in Kenya (Arai et al. 2003; Botvinkin et al. 2003), and recently a novel virus, tentatively named Matlo bat lyssavirus (MBLV), that is closely related to WCBV, was detected in Natal long-fingered bats (*Miniopterus natalensis*) in South Africa (Coertse et al. 2020).

There is increasing knowledge on the diversity of lyssaviruses known to circulate also in Asian bat populations. Single representatives of ARAV and KHUV were isolated from Eurasian Microchiroptera, i.e., *Myotis blythi*, and *M. mystacinus* (Arai et al. 2003; Botvinkin et al. 2003). IRKV was isolated from infected *Murina leucogaster* in Irkut (Kuzmin et al. 2005) and China (Liu et al. 2013b). Also, one human rabies case after IRKV infection was reported in the Russian Far East (Leonova et al. 2009). More recently, rabies cases in the Japanese pipistrelle bat (*Pipistrellus abramus*) in Taiwan were characterized as the novel lyssavirus TWBLV (Hu et al. 2018). Also, genetic analyses confirmed that rabies cases in Indian flying foxes (*Pterpus medius*) in Sri Lanka were caused by the novel lyssavirus GBLV (Gunawardena et al. 2016). In Australia, variants of ABLV exist among several species of Pteropus, as well as in insectivorous Yellow-bellied sheath-tailed bats (*Saccolaimus flaviventris)* (Foord et al. 2006; Iglesias et al. 2021) (Fig. 2).

Despite MOKV being isolated from a variety of mammal species including shrews (*Crocidura* spp.), rodents (*Lophuromys* spp.), unvaccinated and vaccinated domestic cats and dogs (Sabeta and Phahladira 2013), and IKOV from an African civet (*Civettictis civetta*) from Tanzania (Marston et al. 2012), these species are the only lyssaviruses never to have been isolated from bats. Given the fact that C*hiroptera* are considered key reservoir species for lyssaviruses, there is reason to believe that the reservoir hosts of those lyssaviruses are bats too. However, such reservoirs for MOKV and IKOV still remain to be identified.

Now that it is understood that each lyssavirus has evolved to fit a particular ecological niche and that continuing evolutionary progression yields new variants

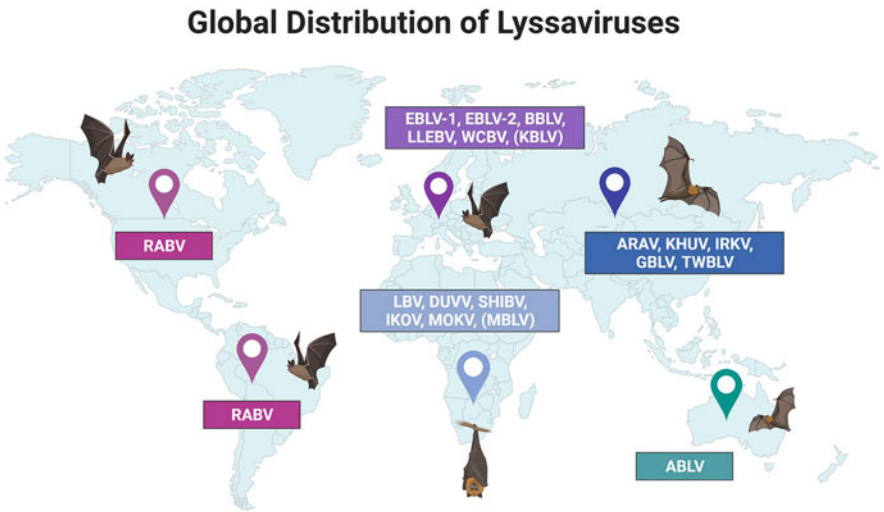

Fig. 2 Lyssaviruses associated with bats. Of note: Mokola virus (MOKV) and Ikoma lyssavirus (IKOV) were isolated from terrestrial animals and the reservoir host is unknown. Figure created with Biorender.com

and new threats to human and animal health, concerted global actions to combat rabies in terrestrial animals become increasingly important.

25.1.8 Self-Made Problems

The underlying diversity of the neurotropic, negative-stranded RNA viruses responsible for infection, combined with adaptation to the central nervous system in a broad spectrum of abundant, widely distributed mammalian hosts, may seem to hinder serious contentions for disease abatement (Rupprecht et al. 2008; Rupprecht and Salahuddin 2019). For a long time, unfortunately, the global rabies situation has not changed dramatically, despite local successes in rabies control in particular reservoir hosts, such as dogs or foxes in Europe (Müller et al. 2012), Latin America (Vigilato et al. 2013a) and North America (Ma et al. 2020). Hence, disease distribution still encompasses all continents, with the exception of Antarctica. There is ample evidence that for centuries human sociocultural evolution as well as population growth and human-related activities (e.g., the introduction of alien wildlife species, livestock, the translocation of domestic and wild animals) has affected its epidemiology and control. It is likely that in the future these factors will also affect the (re) emergence of rabies (for review see (Freuling et al. 2013). Furthermore, ineffective rabies control measures at the animal source may well create new rabies-related problems in wildlife. Mongoose rabies on the Caribbean islands and emergence of fox rabies in Turkey, for example, are considered a result of independent spillover infections from rabid dogs (Nadin-Davis et al. 2006b; Johnson et al. 2003;

Vos et al. 2009). The latter phenomenon is observed in many other countries in Asia, Africa, and the Americas as well.

It is speculated that growing urbanization, man-made environmental changes (e.g., deforestation), or climate change will put human populations at risk of exposure to zoonotic pathogens including lyssaviruses (Allen et al. 2017; Grange et al. 2021).

25.2 Human Rabies Prevention, Prophylaxis, and Experimental Therapy[2]

Rabies is a highly neglected, but also a vaccine-preventable, zoonotic disease (Briggs 2012; Fooks et al. 2017). For practical purposes, rabies should be considered as universally fatal, once clinical symptoms manifest (Fooks et al. 2014). However, progress is being made in the basic understanding of the design and application of biologics and anti-viral drugs to prevent, and in the future potentially treat, clinical rabies (Ledesma et al. 2020; Du Pont et al. 2020; Smith et al. 2020; Smith et al. 2019a; Rupprecht et al. 2006). Vaccines may be applied to at-risk populations prior to exposure (pre-exposure immunization or PrEP), or biologics are provided through post-exposure prophylaxis (PEP), administered to individuals after viral exposure, but before the onset of illness (Tables 1 and 2).

Not all animal exposures are "rabies-prone" and human rabies prevention should occur via an integrated bite case management approach (Undurraga et al. 2017). The administration of PEP occurs only after a thorough risk assessment, predicated in part by viral pathogenesis, the local epidemiology of rabies, the mammalian species involved, and the circumstances of each specific exposure (Table 2). Exposure is defined as occurring either via the bite (any penetration of the skin by the teeth of a rabid animal) or non-bite (transdermal or mucosal contact with virus-infected material, such as brain tissue) routes. Almost all human cases are caused after the bite from a rabid mammal. After such an event, PEP begins by thorough washing of the wound with soap and water. In previously unvaccinated persons, rabies immune globulin (RIG) is infiltrated into and around the bite, as an initiation of passive immunization. The RIG may be human in origin (HRIG), heterologous (obtained from other species), or monoclonal antibody (mAb)-based therapies. The first of several doses of rabies vaccine is also administered at the same time as RIG. The use of RIG on day 0 bridges the time after viral exposure, but before the active induction of rabies virus neutralizing antibodies (VNA) from vaccine administration (Rupprecht et al. 2020b).

While cases of PEP failure exist (Tinsa et al. 2015; Shantavasinkul et al. 2010b; Wilde 2007), many are attributed to errors in PEP administration (Wilde 2007; Wilde et al. 1989) or possible direct inoculation of RABV into the CNS or other neural tissues (Bharti et al. 2019). Therefore, survivorship is almost certain if prophylaxis is

[2] Authors: Charles E. Rupprecht, Anthony R. Fooks, Leo Both, and Samuel P. Smith

Table 1 Rabies Immunization Recommendations

Criteria for pre-exposure immunization			
Exposure category	Nature of risk	Typical populations	Pre-exposure regimen
"Continuous"	Virus present continuously, usually in high concentrations. Specific exposures may be unrecognized. Bite, non-bite, or aerosol exposures	Rabies research workers.[c] Rabies biologics production workers	Primary course. Serologic testing every ~6 months. Booster immunization if antibody titer falls below "acceptable" level[c,d]
Frequent	Exposure usually episodic, with source recognized, but exposure also may be unrecognized. Bite, non-bite, or aerosol exposures	Rabies diagnostic workers,[c] cavers, veterinarians and staff, and animal control and wildlife workers in areas where rabies is enzootic. All persons who handle bats	Primary course. Serologic testing every ~2 years. Booster vaccination if antibody titer is below "acceptable" level
Infrequent (greater than population-at-large)	Exposure nearly always episodic with source recognized. Bite or non-bite exposures	Veterinarians and animal control staff working with terrestrial animals in areas where rabies is uncommon to rare. Veterinary students. Travelers visiting areas where rabies is enzootic and immediate access to appropriate medical care including biologics is limited	Primary course. No serologic testing or booster vaccination
Rare (population-at-large)	Exposure always episodic. Bite or non-bite exposure	Population-at-large, including individuals in rabies-epizootic areas	No vaccination necessary, unless exposed

[a] Adapted from recent WHO and US Advisory Committee on Immunization Practices (ACIP) guidelines

[b] Pre-exposure immunization. Pre-exposure immunization consists of three doses of cell culture vaccine, ID or IM (i.e., deltoid area), one each on days 0, 7, and 21 or 28. Administration of routine booster doses of vaccine depends on exposure risk category as noted above

Post-exposure immunization. All PEP should begin with immediate thorough cleansing of all wounds with soap and water. Persons not previously immunized: Three PEP regimens are available depending on severity of exposure. Category I exposures only require washing exposed skin. RIG (40 IU/kg for ERIG, and 20 IU/mL for HRIG) is only indicated in category III exposures. Currently, vaccination regimens and recommendations do not differ between category II and III exposures and one of three vaccination regimes may be adhered to; first, a 1-week, two-site ID regimen involves ID injection of vaccine on days 0, 3, and 7; second, a 2-week IM PEP regimen (Essen regimen) involves a single site IM injection of vaccine on days 0, 3, 7, and between day 14–28; lastly, a 3-week IM PEP regimen (Zagreb) includes delivery into two intramuscular sites on day 0, and delivery into a single intramuscular site on days 7 and 21. For persons previously immunized: only wound washing is required for category I exposures and RIG is not indicated for any category of exposure. Furthermore, three vaccination regimes are suggested; first, a single ID vaccination on days 0 and 3; second, vaccination administered at four sites on day 0; third, a single-site IM vaccination on days 0 and 3. These vaccination regimes do not differ between category II and category III exposures. Furthermore, immediate vaccination is not needed if the individual has received a complete PEP course within the last 3 months. Pre-exposure immunization with modern cell culture vaccine; prior PEP with modern cell culture vaccine; or persons previously immunized with any other type of rabies biologic and a documented history of an "acceptable" rabies virus neutralizing antibody response to the prior vaccination

[c] Assessment of relative risk and any extra monitoring of immunization status of laboratory workers is the responsibility of the laboratory supervisor (as an example, see guidelines in the current edition of the United States Department of Health and Human Services' Biosafety in Microbiological and Biomedical Laboratories)

[d] Routine Pre-exposure booster immunization consists of one dose of modern cell culture vaccine, ID or IM (i.e., deltoid area). An acceptable antibody level is 0.5 IU/ml. Boost if the VNA titer falls below this level, as long as the person remains at risk of viral exposure

Table 2 Rabies risk assessment and prophylaxis considerations

Animal type	Evaluation and disposition of animal	Post-exposure prophylaxis recommendations
Dogs and cats	Healthy and available for 10 days observation	Should not begin PEP unless animal develops signs of rabies[a]
	Rabid or suspected rabid	Initiate PEP immediately[b]
	Unknown (escaped)	Consult public health officials
Bats, foxes, mongoose, raccoons, skunks, other carnivores	Regard as rabid unless geographic area is known to be free of rabies or until animal is proven negative by diagnostic tests	Initiate PEP.[c] Consider factors such as provocation, suggestive clinical signs, severity of wounds, type of exposure, and timeliness of diagnostic results (24–48 h) for decisions regarding immediate initiation, or to delay pending test results
Livestock, insectivores, most rodents, and lagomorphs (rabbits and hares)	Consider individually	Consult public health officials; bites of rats, mice, hamsters, guinea pigs, gerbils, chipmunks, squirrels, shrews, and other small mammals almost never require PEP

Adapted from recent WHO and US Advisory Committee on Immunization Practices (ACIP) guidelines

[a]If clinical signs compatible with rabies develop during a ~ 10-day confinement and observation period, the animal should be euthanized and tested immediately. Depending on circumstances, initiation of PEP may be delayed pending a laboratory report, if results may be obtained promptly in ~24–48 h

[b]If the bite was unprovoked or resulted in severe wounds, prophylaxis of the bitten person should begin immediately with rabies immune globulin and modern cell culture vaccine. Rabies PEP may be discontinued if the test is negative

[c]If available, the animal should be euthanized and tested as soon as possible. Holding for an observation period is not recommended since the potential viral shedding period prior to clinical signs has only been determined for dogs, cats, and ferrets

begun in a timely and appropriate manner after exposure. However, there is no proven treatment after the manifestation of rabies as an acute progressive encephalomyelitis in humans or other animals.

25.2.1 Preventive Vaccination

Vaccination of certain occupational groups, such as first responders in rabies management, is recommended due to the higher risk of viral exposure in comparison to the population-at-large (Table 1). PrEP simplifies PEP, priming the immune response (Mills et al. 2021) and potentially providing a degree of protection against minor, unrecognized exposures. Such exposures, if proper personal protection equipment (PPE) is used, appropriate, well recognized techniques are employed, and exposure protocols are followed, should by definition, be negligible. If recipients of PrEP are exposed to RABV, booster doses of vaccine are used to induce an anamnestic

response. RIG is unnecessary under such circumstances due to the preexistence of VNAs and additional RIG may form immune complexes and interfere with development of a normal anamnestic response. However, individuals who receive PrEP should remain vigilant in recognizing potential viral exposures and seek appropriate PEP ad hoc.

If exposures occur, but are unrecognized, the recipient of PrEP may still succumb to rabies after exposure, albeit very rarely. One documented case of mortality despite previous vaccination occurred in a Peace Corps volunteer in Kenya. This volunteer had completed a standard three-dose intradermal vaccination regimen using human diploid cell rabies vaccine, but upon being bitten by her own puppy died of a disease compatible with rabies (Bernard et al. 1985). Travelers should be advised to consider PrEP based upon their planned activities and destination, as certain critical biologics may not be always readily available (Jentes et al. 2013).

25.2.2 PrEP of Children?

An estimated 10–16 million people undergo PEP worldwide each year following exposure to proven or suspected rabid animals (Both et al. 2012). Of special importance is the pediatric population (Kessels et al. 2017). Of note, the first ever patient to receive Pasteur's rabies vaccine was a child presenting with multiple deep bite wounds during July 1885. This case exemplifies that children in particular are at a high risk of exposure to rabid dogs. On average, 50% of rabies deaths are estimated to occur in children under 18 years of age. Children 5–10 years of age are particularly exposed, because they are often unable to discern abnormal animal behavior, they may be watched less by their parents than younger children, they enjoy playing with dogs, and, due to their size, they are frequently bitten on the head and neck, which carries a higher risk of contracting rabies (Ichhpujani et al. 2008; Knobel et al. 2005). Because of the large numbers of affected children, rabies is the seventh most relevant global infectious disease with regard to the years of life lost (Jackson 2008).

Surveys have confirmed the disproportionate toll of rabies among children, resulting from an insufficient supply of rabies biologics, including RIG and modern cell-culture vaccines (Pancharoen et al. 2001a; Wilde et al. 1996; Sriaroon et al. 2006; Pancharoen et al. 2001b; Kularatne et al. 2016; Ngugi et al. 2018; Kessels et al. 2017). Studies in Tanzania have shown that up to 55% of victims are children (Mazigo et al. 2010; Hampson et al. 2008). In a survey from South African, 49% of rabies exposures were children <10 years and 22% were 11–20 years (Weyer et al. 2011). One study in Kenya between 2011 and 2016 revealed 36% of all recorded cases affected children <14 years of age, and a 17% incidence in ages 15–24 (Ngugi et al. 2018). A study across eight Asian countries revealed that 43% of all patients were children and teenagers (Dodet et al. 2008). A study focusing on rabies in China demonstrated that children under 15 years of age constituted 25% of the nation's rabies deaths (Song et al. 2009). Another study in Cambodia showed that, among 44 human rabies cases, 37% were 15 years old or younger, and RIG was administered free of charge only for children with wounds on their upper arms and faces (Ly et al. 2009).

The need for RIG can be avoided by the use of PrEP, as previously vaccinated bite victims only receive two booster doses with vaccine and no RIG is required (O'Brien et al. 2019). PrEP may be useful for certain populations at high risk of RABV infections, e.g., veterinarians, animal handlers, diagnostic workers, vaccine producers, research scientists, cavers, wildlife biologists, children in endemic areas containing many unvaccinated stray dogs, or those in very remote areas, such as the Amazon who suffer routine exposure to vampire bat bites (Fooks et al. 2017). PrEP may also be considered for certain international travelers (Fooks et al. 2003). In the UK, an annual number of 3,700–5,700 rabies vaccine prescriptions were dispensed between 2009 and 2011. The field of rabies immunization benefits from over a century of classical scientific insights, but also lingers in the legacy of Pasteur toward certain conservative tendencies, particularly related to the basic use, types, doses, routes, and schedules of biologics (Wu et al. 2011). As background, several publications have reviewed in depth the history, basic approach, available products, and biological basis for rabies immunization (World Health Organization 2013; Smith et al. 2019a; Ertl 2019; World Health 2018; Fooks et al. 2019). For other updates, specific details and current recommendations on rabies immunization may be found at the websites (http://www.who.int/rabies/en/) of the World Health Organization (WHO) and the Advisory Committee on Immunization Practices (ACIP), United States (http://www.cdc.gov/vaccines/acip/recs/index.html).

The etiological agents of rabies consist of diverse RNA viruses in the Family Rhabdoviridae, Genus Lyssavirus (Kuzmin et al. 2009). While all lyssaviruses cause rabies, historical and rabies biologics are produced only using RABV strains. Modern rabies biologics will protect against all known RABV variants As there is evidence for broad spectrum cross-neutralization and cross-protection within lyssavirus phylogroups, current commercial rabies vaccines are known to cross protect against all members of phylogroup I (Brookes et al. 2006; Fekadu et al. 1988; Hanlon et al. 2005; Liu et al. 2013a; Malerczyk et al. 2009, 2014). However, little to no protection is evident against phylogroup II lyssaviruses (Servat et al. 2019; Malerczyk et al. 2014; Hanlon et al. 2005; Fekadu et al. 1988; Banyard et al. 2018; Horton et al. 2014). Cross-protection against phylogroups I and II has been achieved in a variety of experimental studies by either creating chimeric lyssavirus glycoproteins (Evans et al. 2018; Fisher et al. 2020) or the sequential insertion of glycoproteins within a viral backbone (Kgaladi et al. 2017; Weyer et al. 2008).

The original anti-rabies biologics of the late nineteenth and early twentieth century consisted of RABVs propagated in mammalian nervous tissues, such as in rabbits, small ruminants, nonhuman primates, or suckling mice. These nerve tissue origin (NTO) vaccines are no longer recommended for human immunization, although such products are still in use in a few developing countries, such as in Ethiopia. Due in part to poor potency, over the course of a month, 14–21 administrations of NTO vaccines occurred by the intracutaneous route applied over the abdominal region. Such NTO biologics were gradually replaced by safer and more potent cell culture vaccines during the late twentieth century. Modern rabies cell culture vaccines include the human diploid cell vaccine (HDCV), purified chick embryo cell vaccine (PCEC), purified duck embryo vaccine (PDEV), and purified

Vero cell rabies vaccine (PVRV). Besides an improvement in overall quality, the use of HDCV, PCEC, PDEV, and PVRV allowed for a decrease in the total number of vaccine doses applied to ~ 4–5 by the end of the twentieth century, and the application via the intramuscular (IM) route at a volume of 0.5–1.0 mL.

Rabies vaccines and RIG are listed among the WHO Essential Medicines for both adults and children, but access across the developing world is often insufficient (Li et al. 2019). In fact, shortages of rabies vaccine and especially RIG are common in developing countries. Bite victims may need to travel long distances to obtain any PEP and may present to medical personal with substantial delays. Also, costs are a restriction, since the price for modern tissue-culture vaccine vials ranges from $7–20 in many low-income countries and multiple vials are required per patient depending upon the PEP regimen used (Hampson et al. 2011; Quiambao et al. 2005). To save costs compared to IM vaccination, intradermal (ID) vaccination makes use of less vaccine, with smaller amounts of vaccine being injected into the skin at multiple sites on the first day of the course to elicit a potent immune response (0.1 ml for each ID injection versus a single 0.5 or 1 ml vial for each IM injection) without limitations in safety, immunogenicity, or efficacy for both PrEP as well as in use for PEP (Shantavasinkul et al. 2010a, c; Khawplod et al. 2012; Warrell 2012; Shantavasinkul and Wilde 2011; Gongal and Sampath 2019; Denis et al. 2019; Kessels et al. 2019).

The disadvantage of ID regimens is that vaccine leftovers in partially used vials must be discarded after several hours to avoid the risk of contamination. Additionally, ID vaccination is technically more demanding, is subject to pharmaceutical regulations, and may result in more frequent local adverse events. However, where feasible, due to a high turnover of animal bite victims, local health clinics, and well-trained staff, switching from IM to ID immunization will make rabies PEP more accessible and affordable (Hampson et al. 2011; Gongal and Sampath 2019). Besides alternative routes, shorter vaccine schedules, based in part upon the evidence provided in animal models, the basic immunological response to rabies vaccines, epidemiological investigations, and human clinical trial data have been investigated (Rupprecht et al. 2009, 2010; Robertson et al. 2010; Khawplod et al. 2012). While some studies have focused on the reduction of PrEP regimens to 7 days (Soentjens et al. 2019) other studies have addressed reductions in PEP regimens (Warrell 2019a, b).

After vaccination, antibody titers serve as surrogates of response, but do not directly correlate with absolute protection against a fatal productive infection, because other immunological factors also play a role in prevention of disease (Briggs 2011). Hence, there is no known absolute "protective antibody" level for all humans. Minimum arbitrary standards are based empirically on presumed activity of rabies virus-specific antibodies, e.g., VNA, for a given exposure scenario and on repeatable values for paired sera. For example, a VNA titer of 0.5 IU/mL by WHO standards is evidence of adequate immunization in persons at either constant or frequent risk of exposure, at 6-month or 2-year intervals, respectively, as a measure of baseline immunity. A single booster vaccine dose is administered if the VNA level is lower than recommended, based on a determination of risk (Table 1). After a century of use, several generalizations are apparent in the application and use of human rabies

Table 3 Ten generalized observations on human rabies immunization in the twenty-first century

Antibodies to the viral glycoprotein appear to be the most important in rabies immunization
Anti-IgG antibodies begin to appear within ~7–14 days of rabies vaccination in healthy subjects
A basic prime-boost strategy seems most effective in rabies pre-or post-exposure vaccination
Modern cell culture vaccines are superior to nerve tissue origin products in safety and immunogenicity
Intradermal vaccine regimens provide comparative effectiveness compared to intramuscular use
Routine serologic monitory is unnecessary except in the immune-compromised or after major deviations from standard recommendations
Host factors, such as age, genetic background, etc., will affect an ideal immune response to vaccine
The role of cell-mediated immunity is not well documented in rabies prophylaxis
Timely and appropriate wound care, infiltration of rabies immune globulin, and administration of potent cell culture vaccines virtually assure human survivorship, even after severe bite exposures
Most people die of rabies because they do not receive appropriate access to modern rabies biologics

Adapted from (Rupprecht and Plotkin 2013)

biologics today (Table 3). Future alterations in the methods used to measure basic rabies vaccine potency are anticipated to reduce the dependency on the use of animals and to more effectively resolve a cumulative understanding of human response to precise doses of vaccine by more comparative techniques (Stokes et al. 2012).

25.2.3 Improvements to PEP

While current biologics to prevent rabies are extremely effective, progressive development of other products is necessary. Modern inactivated cell culture vaccines and RIG are vastly improved over historical NTO vaccines, but such products are expensive (especially in the developing world where they are most needed), are often in scarce supply, and may carry a perceived theoretical risk of adventitious agents. Hence a major focus in rabies prevention has concentrated on the need for potent, inexpensive PEP, especially different routes, fewer vaccine doses, shorter schedules, and replacement of costly RIG, while retaining activity against a wide variety of diverse lyssaviruses (Both et al. 2012). While advances have been made in rabies PEP regimens as discussed previously (Warrell 2019a, b), PEP remains costly and is still in short supply in areas that need it most.

Besides HRIG, there are other products to provide passive immunity. In contrast to the relatively poor-quality equine rabies immunoglobulin serum used in the past that resulted in high adverse reactions, such as serum sickness in up to 40% of human recipients, modern purified equine rabies immune globulin (ERIG) products are safer, more potent, and more affordable than older cruder products, and are less expensive than HRIG. For example, in Bhutan, prophylaxis of an adult with HRIG is approximately 20 times more expensive than equivalent treatment with

ERIG (Tenzin et al. 2012). Due to its potency and apparent lack of significant local or systemic effects, purified ERIG products have traditionally been seen only as immediate alternatives to HRIG, if supply should be threatened by shortages, contamination, or other limitations (Quiambao et al. 2009). However, ERIG has been used effectively in conjunction with vaccine in human rabies PEP, particularly in developing countries, and no preference between HRIG and ERIG is currently given by WHO recommendations (Sparrow et al. 2019). The use of such heterologous HRIG/ERIG products may be considered a temporary antecedent until the widespread and commercial availability of more novel replacements, such as mAbs (Both et al. 2013a, b). Particularly, countries with chronic shortages of RIG would benefit greatly from replacement of these scarce and expensive polyclonal preparations, especially in Africa which suffers from an estimated 24,000 rabies deaths, with less than 2% of exposed patients receiving RIG (Knobel et al. 2005). Studies into RIG alternatives have revealed the potential of neutralizing camelid variable domain on a heavy chain (VHH) antibodies, mAb, and scFv proteins. VHHs, also called nanobodies, are the antigen-binding variable domain of *Camelidae* antibodies and are able to recognize more epitopes, are more thermostable, and have faster tissue penetration than traditional antibodies (Harmsen and Haard 2007). One VHH cocktail of note is Rab-E8/H7, a combination of two monovalent VHHs (E8 and H7). E8 and H7 were discovered when two phage-display libraries were constructed after the immunization of llamas with an inactivated HDCV vaccine (Hultberg et al. 2011). Further study of Rab-E8/H7 has revealed its effectiveness in preventing the onset of disease if given as either PrEP or PEP when given, like RIG, in the context of vaccination (Terryn et al. 2014, 2016).

Hybridomas that secreted RABV antigen-specific mAbs have been generated during the 1970s. The resulting mAbs were selected on the basis of isotype, antigen and epitope specificity, virus strain specificity, affinity, and neutralizing activity. The administration of mAbs have various theoretical advantages over RIG: Firstly, mAbs need comparatively small volumes for equivalent active protein content as specific neutralizing activity per mass of protein is higher, so mAbs may be optimal for lessening the trauma and pain of local wound infiltration with a source of passive antibodies. Second, safety issues arising from the possibility of adventitious agents associated with human or animal blood products would be alleviated by bulk production under modern GMP conditions in cell culture. In a WHO consultation during 2002, a number of mAbs were proposed for inclusion into an antibody cocktail (Müller et al. 2009) and several mAb combinations were designed based on stringent criteria (Table 4). Fifteen years later, the Strategic Advisory Group of Experts reviewed its recommendations for the inclusion of anti-Rabies mAbs into PEP. This, in concert with the publishing of a WHO position paper during 2018, encouraged the use of mAbs within PEP where available (Meeting of the Strategic Advisory Group of Experts on immunization, October 2017 – conclusions and recommendations 2017; World Health Organization 2018a; Sparrow et al. 2019). Since 2002, these RABV-specific mAbs have

Table 4 Criteria for antibody selection and testing by the WHO Rabies Collaborating Centres

a) **Criteria for mAb selection**
The history of hybridomas, including relative risk of contamination with certain agents (e.g., FMDV, TSE agents) and use of FCS (fetal calf serum) should be available
A production of a minimum of 100 IU per ml of crude hybridoma supernatant should be obtained
Stability expressed as loss of antibody secretion production should not exceed 10% upon passaging
In-vitro cross-reactivity should be measured by RFFIT or FAVN on a selection of RABV and phylogroup I lyssaviruses isolated from major of reservoir host species and geographical areas, including dogs from Asia, Africa, and the New World
At least two broadly cross-reactive mAbs should be selected recognizing different G protein epitopes
The mAbs should not interfere with each other during neutralization testing
The mAbs should not be inferior to RIG with regard to viral reactivity
b) **In vitro testing of candidate mAbs**
History of hybridomas should be established in writing
Use of FCS should be avoided. Low serum or no serum media must be preferred
Each laboratory should establish a mini master cell bank with a minimum of 10 vials
Tests for mycoplasma, bacteria, etc., in T25 cm^2
Thirty passages must be performed (with freezing aliquots after every 10 passages)
Culture batch (500 ml) on roller bottles
Purify IgG on protein A column and determine IU/mg.
Compare supernatant of passages 0, 10, 20, 30 (a 30% variation +/- for naturalization from test to test is acceptable) and determine isotype at passages 0 and 30
Purify IgG at a concentration of at least 1000 IU/ml

(WHO consultation, 2002)

been under investigation and some are either in advanced stages of clinical development or have been licensed for use in humans.

Two mAbs have now been licensed for use: Rabishield and Twinrab (Rabimab) in India. Rabimabs is a cocktail of two mAbs antibodies docaravimab (62-71-3) and miromavimab (M777-16-3). These mAbs bind two distinct nonoverlapping epitopes and have the ability to neutralize a wide variety of RABVs and related escape mutants (Müller et al. 2009). In a Phase 3 noninferiority trial, Twinrab™ was assessed at 40 IU/kg (in combination with rabies vaccination during PEP) in 124 patients who had a category III exposure and were aged ≥5. No deaths or serious adverse events were recorded, and no statistically significant difference was observed between HRIG and Twinrab™ (Kansagra et al. 2020).

Rabishield consists of a single IgG1 mAb,17C7. Developed by Massachusetts Biologic Laboratories, 17C7 was generated using transgenic mice expressing human antibody genes (Bakker et al. 2008; Sloan et al. 2007). Multiple preclinical studies have shown that mAb 17C7 was able to neutralize street RABVs and can confer protection equivalent to HRIG in hamster challenge models, tested either alone or in combination with rabies vaccine (Sloan et al. 2007; Wang et al. 2011b). The 17C7 mAb, also known as RAB1 or SIIR mAb, has also been investigated in randomized, dose-escalation phase 1 safety study in adults. Rabishield was found to be well

tolerated and reached comparable antibody titers to vaccine and HRIG cohorts (Gogtay et al. 2012). In phase 2/3 trials with patients (> age 5) suffering from a category III exposure, no deaths, severe adverse events, or anti-mAb antibodies were recorded. Rabishield elicited rabies virus neutralizing activity that was noninferior to HRIG (Gogtay et al. 2018).

25.2.4 Treatment of Clinical Rabies?

Prevention of viral exposures is still the optimal approach in rabies prevention, or when this fails, application of modern PEP before onset of illness. However, in some circumstances, alternatives regarding experimental treatment of clinical rabies may be warranted (Willoughby 2007). Unfortunately, the use of many different products such as cytosine or adenine arabinoside, interferon-α, ketamine, amantadine, minocycline, acyclovir, antithymocyte globulin, steroids, vidarabine, ribavirin, favipiravir, and inosine pranobex have been unsuccessful (Jackson et al. 2003; Appolinario and Jackson 2015). Historically, there have been <30 cases of rabies recovery. Of the cases where patients were treated until recovery (or died within recovery) and the outcome is known, only 18% completely recovered while 82% of patients were left with mild to severe sequelae (Nadeem and Panda 2020). From human case studies and findings of apparent natural acquired immunity, these data suggest that human rabies may not be uniformly fatal but rather behave as a continuum (Feder et al. 2012; Gilbert et al. 2012). Furthermore, the observation that over 90% of human rabies survivors receive at least one of either antivirals, vaccination, or RIG, suggests that host defenses may be further exploited for the treatment of symptomatic rabies.

One classical example in aiding the host immune response for the treatment of symptomatic rabies is in the treatment of a 15-year-old female Wisconsin resident who was bitten by a bat on her hand during 2004. The small wound on her finger was cleaned; however, PEP was not administered. Approximately a month later, she developed generalized fatigue and paresthesia of her left hand, diplopia, ataxia, nausea, and vomiting. On the fourth day of illness, blurred vision, left leg weakness, and ataxia were noted, and later fever, slurred speech, nystagmus, and tremors of her left arm, and she was admitted to a pediatric facility. On the second day of care, the presence of rabies virus-specific antibodies in the patient's CSF and serum were documented. However, attempts to isolate virus, detect viral antigens, or amplify viral nucleic acids from skin biopsies and saliva samples were unsuccessful. An experimental treatment, termed the "Milwaukee Protocol," was initiated, which combined anti-excitatory and antiviral drugs, including ketamine, ribavirin, and amantadine, in conjunction with supportive intensive care (Willoughby et al. 2005). Neither rabies vaccine nor RIG was administered because of the patient's VNA response and the theoretical potential for harm from an altered immune response. After more than 70 days of hospitalization, the patient recovered, with only minor neurologic sequelae (Hu et al. 2007). She became the first person to survive clinical rabies without a history

of prior vaccination. While promising, this experimental protocol has been attempted in approximately 40 patients with only 6 documented survivors (Ledesma et al. 2020).

While there have been no recent advances in the treatment of symptomatic rabies in humans, significant advancement has been made during in vivo studies. This has included the use of either live attenuated vaccine viruses (LAVVs) or mAbs. As reviewed extensively much progress has been made in the attenuation of live RABVs by the insertion of either nonviral elements (Smith et al. 2019a) or the multiplication (Faber et al. 2002, 2009) of attenuated RABV glycoproteins. Many of these are effective in causing a strong influx of T cells, B cells, and APCs into the CNS and brain after inducing permeabilization of the blood–brain barrier (Smith et al. 2019a). Studies have investigated the use of LAVVs in preventing mortality when given intracranially up to 6 days after peripheral lethal rabies virus challenge. These studies have used either TriGAS (also known as SPBAANGAS-GAS-GAS) (Faber et al. 2009), SRV9 (Huang et al. 2014; Huang et al. 2015), or a GM-CSF expressing LBNSE rabies virus vector (Wang et al. 2011a). All attenuated viruses used in these studies were effective in preventing mortality in a minimum of 20% of mice peripherally lethally challenged with RABV. In addition to LAVVs use as alternatives to inactivated rabies vaccines (Smith et al. 2019a), these studies also indicated that LAVVs represent a potential avenue for the post-clinical onset treatment of rabies. However, these LAVVs were only administered prior to the onset of clinical signs, with diminishing effectiveness as the number of days after lethal challenge increased, so further research may be needed before they are used for true post-onset treatment.

One area that has seen notable improvement in post-onset treatment is mAb therapy in murine in vivo models. For example, one study was able to successfully treat rabies in mice using a combination of two human monoclonal mAbs (RVC20 and RVC58) (Melo et al. 2020). The RVC20, able to bind antigenic site I of the RABV glycoprotein, neutralizes almost all phylogroup I lyssaviruses in addition to Shimoni bat virus (SHBV) and Ikoma lyssavirus (IKOV) from phylogroups II and III, respectively. The RVC58, able to bind antigenic site III, was also able to potently neutralize all phylogroup I lyssaviruses tested. By combining RVC20 and RVC58 into a single 1:1 cocktail these were able to neutralize 100% of non-rabies lyssavirus phylogroup I viruses tested. For comparison, HRIG was able to neutralize only 38% of non-rabies lyssavirus phylogroup I viruses tested (Benedictis et al. 2016). Firstly, the ability of the RVC20/58 cocktail to prevent the onset of rabies if administered peripherally was evaluated. Here, two doses were assessed via IM injection (2 and 20 mg/kg); however, while earlier timepoints were more effective, even the higher dose was only able to protect 1/5 mice when administered 6 days after lethal challenge. These data suggest that peripheral immunity alone is unable to effectively prevent clinical rabies. The study was repeated with daily intracerebral ventricular (IVC) mAb cocktail infusions alongside a single IM dose, starting at 6/7/8 days after lethal challenge, where data had suggested that RABV had already accessed the CNS, was impacting motor performance, or was resulting in clinical signs. Here, mice were equipped with automated microinfusion pumps, which administered 2 mg/kg/day IVC for 20 days of each mAb. A second IM injection was also included after the cessation of IVC mAb infusion. This

treatment regime was able to prevent mortality in 100% of mice when begun on day 6 (virus in CNS) and 55% of mice when treatment began on day 7 (affected motor performance). Most importantly, when treatment began after the onset of clinical signs, 33% of mice recovered from infection. However, of the 10/15 mice that did not survive when treatment was initiated on day 8, three of these died during IVC infusion, and one that died after treatment had ended presented low viral loads in their brain, suggesting that viral clearance had already started. They also explored the necessity of the Fc portion of mAbs through the use of antibody LALA mutations, which have been shown to abrogate Fc-gamma receptor binding (Saunders 2019). Here, LALA-mutation mAbs promoted lower survival rates when administered at 7 and 8 days after lethal challenge, where only 20% and 0% of animals were protected, respectively. In summary, this regime of IM and IVC administration of mAbs represented a possible avenue for future rabies treatments in humans (Melo et al. 2020).

Despite recent advances in the field of post-onset treatment, no established therapies exist for patients who develop rabies. Efforts should continue on basic viral pathogenesis research, design of anti-viral compounds, development of relevant surrogate animal models and protocols that mimic supportive intensive care, and experimental applications in human cases where ethical/legal approvals and modern teams and facilities exist for thorough application and evaluation (Willoughby et al. 2008, 2009; Smith et al. 2011; Franka and Rupprech 2011; Lingappa et al. 2013; Jackson 2013; O'Sullivan et al. 2013)

25.3 Rabies Control in Wildlife – An Innovative and Demanding Idea

Historically, rabies control in wildlife had long been disregarded since dog rabies control was given priority. It was only during the second half of the twentieth century that the existence of wildlife reservoirs for rabies was slowly becoming acceptable. In Europe and North America, at around the same time when dog-mediated rabies was about to be eliminated, the disease emerged in wildlife spreading quickly through vast parts of the continents and since then has been maintained in multiple species of mesocarnivores (Müller et al. 2012; Blanton et al. 2012).

Theoretically, wildlife rabies can be controlled either by drastic decimation or mass vaccination of the primary reservoirs, and thus reducing the number of susceptible animals below an endemic threshold, where disease transmission is interrupted to $R_{eff}<1$ (Aubert 1992). Early attempts aiming exclusively at a drastic decimation of reservoir populations (e.g., by hunting, trapping, poisoning, and gassing) failed (Aubert 1999; Rosatte 2013). In fact, elimination of a reservoir species outweighs any advantages as it is impractical, expensive, and ethically and ecologically unacceptable (Rupprecht et al. 2001). Parenteral vaccination of trapped wild animals works in principle (Aubert 1994; Rosatte et al. 1990) and has been part of common campaign tactics such as trap–vaccinate–release (TVR) and point infection control (PIC). However, TVR and PIC are labor-intensive and the most

expensive tactics per unit area and therefore, are no options for large-scale control of wildlife rabies (Sterner et al. 2009). Clearly, given their biodiversity, distribution, and abundance, novel methods were necessary to consider meaningful control of rabies in these wildlife reservoir species.

25.3.1 Innovative Approach

The discovery that foxes could be effectively immunized orally using highly attenuated RABV vaccines (Baer et al. 1971) along with the first proof-of-concept field trial (Steck et al. 1982) made oral rabies vaccination (ORV) a preferential and evolving rabies control technology for use in wildlife. This involves large-scale distribution of machine-made baits containing orally immunogenic vaccines across the landscape, thereby targeting wildlife to establish population immunity and prevent spread or eliminate specific rabies variants (Johnston and Tinline 2002).

During the past four decades ORV programs had been implemented in 30 European countries, Canada, and the USA (Müller and Freuling 2018; Fehlner-Gardiner 2018; Freuling et al. 2013) with more than 1.28 billion baits containing different oral rabies vaccines distributed in Europe and North America (Müller and Freuling 2020b). Recent ORV successes include the elimination of fox-mediated rabies in Ontario, Canada, and in almost the entire territory of the European Union (Robardet et al. 2019; MacInnes et al. 2001) as well as of the domestic dog-coyote variant of rabies from the USA (Maki et al. 2017). Due to ORV raccoon rabies has also been successfully eliminated in Ontario and prevented incursions of raccoon RABV variants from neighboring US states (Stevenson et al. 2016). Furthermore, in the USA, ORV programs stopped the expansion of a gray fox-mediated rabies outbreak and the westward spread of raccoon rabies into naïve raccoon populations (Sidwa et al. 2005; Sterner et al. 2009). ORV campaigns in wildlife were also conducted in Turkey (Ün et al. 2012) and Israel (Linhart et al. 1997).

25.3.2 Challenges

Although control and elimination of RABV variants in certain reservoir species is possible, one has to confess that wildlife-mediated rabies is not a candidate for eradication. Despite numerous success stories, diverse complexities and challenges are commonplace when applying ORV to control rabies in wild meso-carnivores (Slate et al. 2009).

Given the huge areas that would need to be covered with vaccine baits to eliminate wildlife rabies in Europe, Asia, North America, and other parts of the world, the planning, implementation, and management of ORV programs may require considerable long-term expenditures. This would affect the USA, Canada, and Russia in particular, but also other bigger countries in Asia and Africa where wildlife rabies is commonplace. Costs may even arise when areas have been freed from wildlife rabies because vaccination belts would need to be established to prevent reintroduction from adjacent areas where wildlife rabies is still endemic

until ORV campaigns are being implemented there (Freuling et al. 2008; Robardet et al. 2019). Novel cost-effective vaccination strategies to be applied under different ecological conditions are therefore required.

Generally, RABV spillover events and subsequent establishment in several sympatric mesocarnivores as well as disease emergence in the absence of effective oral vaccines in the new reservoir host pose another major challenge in achieving tangible objectives in wildlife rabies control (Rupprecht et al. 2008; Slate et al. 2009; Müller et al. 2015). Notable examples are maintenance of the Arctic variant of RABV in striped skunks (Nadin-Davis et al. 2006a) and sustained spillover infections of bat-associated RABV variants into skunks in Arizona (Leslie et al. 2006). While the control and elimination of fox- and raccoon dog-mediated rabies seems to be relatively simple, effective, and easy to implement, it is evident that other wildlife reservoir hosts for rabies including raccoons and skunks appear more refractory to vaccination by the oral route, even when high virus titers were administered. The biological background for these species-specific differences in vaccine efficiency is poorly understood. However, recent results of a comparative study in various reservoir species support a model in which the susceptibility to oral live RABV vaccine infection of lymphatic tissue is a major determinant in vaccination efficacy (Te Kamp et al. 2020). This might explain why the effectiveness of current commercial oral rabies vaccines appears to be adequate to prevent further expansion of rabies in raccoons but may not support disease elimination in this species in the near future (Slate et al. 2009). Apart from efficient oral rabies vaccines, the development of species-specific baits is often underestimated but of utmost importance because a highly potent oral rabies vaccine is useless without an attractive bait. Also, a bait specifically developed for one reservoir species might not be necessarily attractive for other species. For example, ORV campaigns in raccoons require a multiple of commercial baits per unit area as does the control of fox rabies (Rosatte 2013). While current oral rabies vaccines are safe and immunogenic in the small Indian mongoose (Berentsen et al. 2020, 2021; Vos et al. 2013; Ortmann et al. 2018) suitable baits would still need to be developed. In contrast, there are no effective oral rabies vaccines and baits available for skunks yet. Adaptive reservoir species-specific bating strategies for enhanced effectiveness in rabies control require attention to a broad range of research needs. This includes an understanding of the ecology of reservoir species, the target and nontarget species foraging behaviors, community dynamics of the meso-carnivore complex, bait uptake relative to a suite of species-specific spatiotemporal variables, and model development to support ORV decision-making (Slate et al. 2009)

25.3.3 Rabies Control in Bats?

Although bats are considered the true reservoirs for lyssaviruses, considering the sheer abundance of bat species across the world (Agnarsson et al. 2011) the question is not whether the plethora of lyssavirus infection can be controlled. In addition, control measures would be per se limited by the accessibility and often protected status of bats in many parts of the world. Especially from a nature conservation

perspective, targeted population control measures to reduce the spread and incidence of lyssavirus infections in bats are inappropriate and should therefore be critically questioned, if not banned, if they are still used (Brass 1994). Rather, in keeping with conservation rules and measures, activities should focus on (i) the establishment of adequate rabies surveillance, (ii) increasing public awareness, and (iii) targeted pre- and post-exposure prophylaxis (PEP).

One exception often discussed is vampire bat rabies – a medically and economically important zoonosis in Latin America. The ecology of vampire bats as obligate hematophagous bats provides a unique transmission route for RABV to humans and animals alike (Johnson et al. 2014; Streicker et al. 2012). Culling of parts of the vampire population is the policy currently implemented in North, Central, and South America to control vampire bat–mediated rabies. While this strategy is considered to reduce the burden of bat bites on humans and livestock, the real effects of this measure on rabies transmission and the population dynamics remain highly controversial. Oral vaccination may be an alternative to control the disease as vampire bats respond well to experimental oral rabies vaccinations (Stading et al. 2017; Setien et al. 1998; Aguilar-Setien et al. 2002). Also, linking field studies with fluorescent biomarkers to mathematical models indicated that spreadable vaccines, e.g. vaccines that autonomously transfer among individuals of inaccessible wildlife populations asuc as bats, may provide substantial advantages over culling vampire bats (Bakker et al. 2019); however, from a scientific and practical point of view this strategy is far from becoming a reality in the near future.

25.4 Elimination of Dog-Mediated Rabies – Possible But Unlikely?![3]

Canine-mediated rabies is a driver of inequality and poverty in developing countries around the world, posing a threat to over 6.2 billion people in 122 countries (Wallace et al. 2017). The close, interwoven ecology of domestic dogs and humans in many parts of the world allows for frequent opportunity for zoonotic transmission of the virus, accounting for 99% of human rabies deaths (Knobel et al. 2005). The risk of death from canine-transmitted rabies is drastically skewed by factors of geographic isolation and economic hardship due to the need for prompt post-exposure prophylaxis following a bite from a rabid animal, making canine rabies elimination a complex sociopolitical issue to solve (Shwiff et al. 2013). A century of scientific toil is yet to translate into widespread political momentum for national and multinational control interventions; however renewed international focus on rabies in the early twenty-first century offers hope for new inroads to combat the disease at scale (Cleaveland and Hampson 2017; Rohde and Rupprecht 2020; Umeno and Doi 1921).

[3] Authors: Andy P. Gibson and Frederic Lohr

25.4.1 Intractable Neglect

The protracted incubation period and relatively low transmissibility of the RABV result in a chronic, slow-moving endemic picture, with canine rabies cases occurring sporadically in any community or even city over a period of months or years. For example, in the rabies endemic Central African Republic capital city of Bangui, long periods of canine RABV absence were observed even though the region had never experienced mass dog vaccination (Bourhy et al. 2016). This natural fluctuation in disease incidence and heterogeneous epidemiological picture makes the disease inconspicuous to individuals and communities at large, as if it is rarely present. It is only when rabies incidence is monitored throughout a region that the continuous, widespread impact of the disease on a district, state, and nation as a whole becomes apparent (Colombi et al. 2020; Shwiff et al. 2018). Without robust data on dog rabies incidence, it is impossible to communicate a clear narrative of the true impact and extent of the disease to politicians, decision makers, funders, and the public alike. News headlines appear in sporadic bursts of activity covering events of individual rabies cases, which soon abate to the characteristic chronic periodic endemic pattern even in the absence of control intervention. This fleeting spotlight on canine-transmitted rabies in the media and public at large only propagates political torpor on the subject.

Political inaction is exacerbated by the weighted impact of the virus falling on marginalized communities lacking visibility and influence in society to lobby for change. The suffering, death, and economic consequences of rabies for people of low socioeconomic background go undocumented and unreported to health authorities and therefore remain invisible to high-level policy makers (Hampson et al. 2008). Unlike diseases of production animals, rabies affects a species of no direct economic value; however, many studies have revealed the true substantial economic impact of rabies resulting from loss of workforce, impact on livestock, and provision of post-exposure treatment (Anderson and Shwiff 2013; Hampson et al. 2015; Shwiff et al. 2013). While pilot initiatives exploring mass dog vaccination implementation must forge ahead without delay, systems to effectively monitor rabies incidence will be critical in shining a spotlight on the true burden of disease to stimulate and sustain political support for widespread control.

The global efforts to eliminate smallpox, polio, and rinderpest, and the more recent action to control COVID-19, have shown that sustained political commitment stimulates and enables scientific advancement to meet priorities. The scientific community continues to gain a deeper theoretical understanding of rabies transmission and control; however, until large-scale government-led initiatives are actioned, the most relevant and critical gaps in knowledge needed to eliminate the disease will remain opaque (Bardosh et al. 2014; Filla et al. 2021; Mpolya et al. 2017; Zinsstag 2013).

Despite these constraints, progress has been made toward greater prioritization of rabies in international agendas through high-level consensus among the scientific community, political advocacy, and core partnerships, culminating in the formation

of the Zero by 30 Global Strategic Plan in 2018 (World Health Organization 2018b). This unified international strategy serves as a single point of reference and guidance as countries consider rabies control as a viable national undertaking.

25.4.2 The One Health Case

The close connection between dogs, their owners, and the communities in which they live make canine-transmitted rabies control exemplary of the One Health concept. Effective control of the RABV both requires and benefits human health, animal health, and environmental management sectors; however, this multi-disciplinary approach presents administrative and managerial challenges at the point of implementation (Coetzer et al. 2016; Lechenne et al. 2017). Funding, operational, and reporting systems are most often structured vertically within government departments, making joint-departmental complex to realize. Success has been seen in the formation of taskforces or intersectoral zoonotic units, which simplify administrative processes for such undertakings (Belotto 2004; Buregyeya et al. 2020).

Domestic dog populations have remained dependent on human habitation, surviving and reproducing due to the resources provided either intentionally or unintentionally by people (Butler and Bingham 2000; Perry 1993). As a result, there is a predictable association between human and dog populations, with expansion in the former providing opportunity for growth in the latter. The size of the canine rabies reservoir species is therefore expected to rise over the coming decades and unless measures are taken to control rabies in dogs, the disease will pose an increasing threat to people. This growing disease burden will also have the potential to drive emergence and re-emergence in wildlife species, as has been seen in mongoose populations in the Caribbean and foxes in Turkey, respectively (Nadin-Davis et al. 2006b; Vos et al. 2009).

The importance of the sustained collective contribution and support from a broad majority of society must not be underestimated in the success of mass immunization programs. Top-down, government-led initiatives can carry the risk of misalignment with the health and social priorities of people most needed to contribute. National dog vaccination campaigns require the mobilization of a huge workforce who must be united in their understanding of the purpose and benefit of the initiative to be able to deliver sustained success. Furthermore, the perceived importance and benefit of such a visibly enormous undertaking to address the singular issue of rabies must be perceived as worthwhile by the general population, whose contribution in facilitating dog vaccination is critical for achieving high vaccination coverage (Bardosh et al. 2014). Grounding the planning and implementation of rabies control efforts within the wider social and cultural context of the local area is imperative. Building relationships and trust with leaders and gatekeepers at the community level provides the opportunity for two-way information exchange, not only helping to improve contribution from important groups of society, but in garnering grassroots feedback to iteratively adjust the campaign strategy in response to concerns and issues.

25.4.3 An Integrated Approach to Bite Management and Rabies Surveillance

In most instances the point of rabies transmission to people is not occult, it involves the violent event of a bite from an infected animal. The requirement for immediate prophylactic intervention at the time of the exposure creates a scenario in which the One Health concept has a direct tangible impact at this interface between animal and human disease.

Bites inflicted by rabid dogs may not constitute a high proportion of bite presentations at medical clinics, making up just 3% of bites in a study in Haiti (Medley et al. 2017). Therefore, health systems indiscriminately administering PEP to all dog bite presentations result in dispensation of vaccine to individuals at no risk of rabies, while people with high-risk exposures may go without treatment due to stock shortages (Lushasi et al. 2020). Methods of integrated bite case management (IBCM) combine veterinary assessment of the biting animal with the human PEP decision-making for post-exposure to improve the prioritization of vaccine to those at risk of rabies infection. This approach has not only been shown to be cost-effective, but also improved compliance in individuals with high-risk exposures to complete the full course of treatment (Etheart et al. 2017; Lushasi et al. 2020; Undurraga et al. 2020).

In addition to the benefits to human health outcomes and economics, the veterinary components of IBCM contribute to the objectives of canine rabies control. In the first instance, active removal of rabid dogs from the population during the animal investigation prevents continued viral transmission and supports the hastened control of the disease in dogs (Laager et al. 2019; Wallace et al. 2015). Additionally, the data generated on canine rabies incidence and distribution provides insight into the burden of disease and forms a basis on which to plan and adapt mass dog vaccination and community engagement strategies. Finally, the increased submission of field samples from suspect rabies cases provides demand and incentive for sufficient laboratory capacity to be developed for timely rabies diagnosis (Lushasi et al. 2020).

Progress has been made to increase access to post-exposure treatment, to reduce human deaths from rabies; however challenges remain in reaching many at-risk individuals (Madjadinan et al. 2020; Sudarshan and Ashwath Narayana 2019). Dose-sparing intra-dermal regimens requiring fewer clinic visits not only reduce the cost to the individual seeking treatment, but also enable existing stocks of vaccine to treat more people (WHO Rabies Modelling Consortium 2019; Hampson et al. 2019; World Health Organization 2018a). These advances in PEP usage, along with improved mechanisms of IBCM will amplify the potential impact of the inclusion of rabies vaccination in the 2021–2025 strategy of Gavi, the Vaccine Alliance (Gavi - the Vaccine Alliance 2021).

25.4.4 Prospects for Rapid Field Diagnosis

Rabies surveillance is a core tenet to rabies control with significance to understanding disease burden, monitoring the efficacy of vaccination activities, and in

validating rabies freedom status (Office International des Epizooties 2021; World Health Organization 2018b). Establishing functional processes for reporting, investigation, and diagnosis of suspect cases are central to the success of rabies surveillance.

Establishing and sustaining the laboratory infrastructure needed for rabies diagnostic tests has been a major barrier to effective surveillance across much of the rabies endemic world. The need for experienced laboratory personnel, specialized equipment, and costly reagents are barriers to building subnational laboratory capacity. Innovations in tests such as direct rapid immunohistochemical test (dRIT) and real time PCR overcome the need for rabies-specific equipment or expertise, respectively (Office International des Epizooties 2021); however, developing regional laboratory testing capacity remains an expensive and resource intensive undertaking.

In addition to laboratory constraints, ensuring the logistical feasibility of sample collection and safe transport to the laboratory is fraught with challenges. From maintaining a secure cold chain during the journey to timely transport links from remote areas, the chances of samples being of diagnostic quality on arrival at the laboratory is minimal. The considerable effort needed to safely take brainstem samples is only worth undertaking if there is a high confidence that a rabies diagnosis will result. And so, when many submitted samples fail to provide timely diagnosis, the rate of submission will invariably be low, which in turn reduces the justification for maintaining laboratory capacity for rabies diagnostic testing. The recent development of cadaver-side lateral-flow diagnostic tests present the possibility of increasing confirmation of the presence of RABV in resource limited settings and in turn may increase the chances of sample submission to laboratories (Yale et al. 2019).

Lateral flow assays (LFAs), also known as rapid immunochromatographic diagnostic tests, are inexpensive, are easy to perform, and do not require expensive equipment (Mauti et al. 2020). Virus is inactivated by the LFA buffer solution and fixed in the test strip during the test and can therefore be shipped to laboratories at ambient temperature for molecular confirmation and genotyping where available. A number of recent evaluations of these tests, however, have highlighted concerns over unsatisfactory sensitivity of all devices tested, with wide variation between manufacturers and batches (Eggerbauer et al. 2016; Klein et al. 2020). Two field evaluations of the Anigen, Rapid Rabies Ag Test Kit manufactured by Bionote Inc, Republic of Korea, reported sensitivities of 95.3% and 96% (Léchenne et al. 2016; Yale et al. 2019). If consistent performance can be demonstrated, LFAs are likely to increase sampling and submission of suspect rabid animals due to the opportunity to gain immediate information and improving the ease of shipping (Léchenne et al. 2016; Yale et al., 2019).

25.4.5 The Complexities of a Dog Population Reservoir

The frequent, unrestricted interaction between free-roaming dogs provides sufficient opportunity for the RABV to transmit through contact during the infectious period and to propagate within the dog population. This epidemiology was exploited in the elimination of canine rabies from the UK by 1902 through enforcement of strict dog

confinement laws (Carter 1997); however such measures are impossible to implement to the degree required to eliminate the virus in modern day endemic settings.

Rabies virus transmission dynamics are influenced by myriad factors of dog demography, ecology, and human behavior; however the basic reproductive number (R_0) for canine rabies, that is, the average number of secondary cases from an infectious individual in a naïve population, is consistently low across numerous settings (Bourhy et al. 2016; Coleman and Dye 1996; Hampson et al. 2009; Hou et al. 2012; Kitala et al. 2001; Zinsstag et al. 2009). Estimated values for R_0 vary between 1.2 and 2.4, as compared to R_0 estimates of 6.9 for smallpox, 15.7 for measles, and 2.87 for SARS-CoV-2 (however these vary considerably by location) (Billah et al. 2020; Eichner and Dietz 2003; Guerra et al. 2017). The proportion of the population that must be vaccinated to impact RABV transmission is therefore low in comparison to other diseases, estimated to be 30–40% of the dog population. Nevertheless, high rates of population turnover rapidly diminish vaccination coverage achieved during a single pulse vaccination effort and human-mediated transport of dogs poses high risk of viral reintroduction from endemic regions, adding complexity to the potential for canine rabies elimination (Hampson et al. 2007; Laager et al. 2019; Layan et al. 2021).

Contact rates between dogs vary within dog populations at the community level, with some dogs posing a greater potential for RABV spread through their increased connectivity within the population (Castillo-Neyra et al. 2017; Hudson et al. 2019; Laager et al. 2019; Leung and Davis 2017). Dog ownership and confinement practices have been shown to influence dog contact networks and therefore may be of significance to both RABV transmission and dog vaccination campaign strategies (Warembourg et al. 2021). It is yet to be determined whether targeting vaccination efforts at subpopulations of dogs estimated to be more connected has a positive impact on RABV elimination or even whether such a campaign would be feasible to implement at scale (Hou et al. 2012; Laager et al. 2019; Leung and Davis 2017).

The role of dog density in sustaining and driving RABV transmission is still unclear. Although the areas of high density may have the highest canine rabies incidence (Laager et al. 2019), there are several examples where these regions have been demonstrated not to be the sole drivers of RABV transmission (Bourhy et al. 2016; Laager et al. 2019; Zinsstag et al. 2017). Furthermore, endemic RABV transmission is supported even at low population densities, with efforts to eliminate the virus through population reduction invariably failing (Hossain et al. 2011; Tenzin et al. 2015; Windiyaningsih et al. 2004). As a result, interventions focused on culling and dog population control are unlikely to be effective at controlling rabies (Townsend et al. 2013); however increasing the lifespan of dogs through dog population management may reduce the loss of immunity and therefore, benefit control efforts (Laager et al. 2019).

25.4.6 Canine Rabies Control at Source

Elimination of the canine RABV has been demonstrated through annual vaccination of 70% of the dog population (Cleaveland et al. 2003; Cleaveland and Dye 1995;

Kitala et al. 2001), which is advocated for as the target for mass dog vaccination campaigns (World Health Organization 2018b) Several examples have reported substantial reductions in incidence following two effective annual campaigns; however the time taken to achieve elimination is dependent upon the dog population size, distribution, connectivity, demography, and vaccination coverage (Brunker et al. 2020; Cleaveland et al. 2003; Ferguson et al. 2015; Zinsstag et al. 2017).

Large-scale, effective campaigns require sustained support over many years to develop from concept and intention through to enduring high-coverage interventions across large expanses of a country (Mpolya et al. 2017; Vigilato et al. 2013a; Wallace et al. 2017). The early stages of development involve field studies generating data about the dog population, refining the campaign strategy, and creating training processes. This is followed by a progressive scale-up of methods in which logistical, administrative, and operational challenges must be overcome. Finally these activities are sustained with continued evaluation to impact of RABV transmission over a large area (Wallace et al. 2017).

25.4.7 Approaches to Mass Dog Vaccination

Parenteral dog vaccination approaches include central point (CP), door-to-door vaccination (DDV), and capture-vaccinate-release (CVR). From CP, to DDV and CVR, the approaches increase in intensity from aspects of cost per vaccination team unit, campaign logistical complexity, and human resource requirement. Each approach has advantages in accessing particular demographics within the dog population and so the approach, or combination of approaches selected for a mass dog vaccination campaign, must be matched to the makeup of the local dog population to achieve the desired immunization coverage at the lowest cost and effort (Undurraga et al. 2020). Tools have been developed to aid campaign planners in the exercise of programmatic alchemy required to design the optimal campaign strategy (Mazeri et al. 2021; Wallace et al. 2019). A vaccination campaign that fails to immunize a sufficient proportion of the dog population to interrupt enzootic rabies transmission has limited benefit other than to build expertise, experience, and infrastructure. Therefore, campaign implementation should remain an iterative process of reflection and refinement, adapting and intensifying in areas where insufficient coverage is achieved or surveillance data indicate that rabies is persisting (Mazeri et al. 2021).

Dog ownership offers an opportunity to confine dogs at periods, both to limit the risk of RABV transmission and also to avail of opportunities for vaccinations. In such settings communities can be engaged to bring dogs to temporary central vaccination points for inoculation. Mobilizing the general populous into a dog transportation workforce in this way has huge efficiency savings at the campaign level and was the basis for the vaccination of over 15 million dogs in Mexico during 1 week every year in the early 2000s (Velasco-Villa et al. 2017a). Similar CP approaches have been used to achieve high vaccination coverages in African cities of Chad and Malawi; however, these are yet to be expanded to the national level (Gibson et al. 2015; Lechenne et al. 2016; Mazeri et al. 2021; Mpolya et al. 2017; Zinsstag et al. 2017).

CP strategies fail to achieve sufficient vaccination coverage in areas where a high proportion of dogs are unowned or where dog owners are unable or unwilling to bring them to CP vaccination clinics during the campaign (Muthiani et al. 2015; Tohma et al. 2016). With awareness of the vaccination campaign being a major reason cited for lack of turn-out to CP clinics, mass SMS messages were recently demonstrated to be of benefit in broadcasting campaign dates and locations (Cleaton et al. 2018). In another recent study, the strategic adjustment of the distribution of CP clinics enabled vaccination coverage to be increased in an urban setting of Malawi, dramatically increasing campaign efficiency (Mazeri et al. 2021). Where the coverage of CP vaccination cannot be increased to the required level, more costly and resource-intensive methods of Door-to-Door vaccination (DDV), either in combination with CP or alone may be required and has been demonstrated to be effective in a number of African settings, where most dogs are owned and can be manually restrained for parenteral vaccination (Jibat et al. 2015).

In contrast to many areas of Africa, dog populations of Asia often comprise a higher proportion of unowned dogs (Gibson et al. 2015; Sánchez-Soriano et al. 2019; Sudarshan et al. 2001). As a result, CP and DDV methods, relying on the manual restraint of dogs by their owner, are ineffective at achieving sufficient vaccination coverage throughout the population (Belsare and Gompper 2013). CVR strategies using highly skilled teams to catch large numbers of dogs using nets has been shown to access 70% of dogs; however such resource-intensive approaches are unlikely to be feasible for national implementation in the short term (Gibson et al. 2020).

The ability to efficiently immunize dogs that are not amenable to handling has consequently become a central barrier to scaling dog vaccination efforts in these areas and has reignited discussion on the use of oral rabies vaccination (ORV) of dogs (Cliquet et al. 2018; Gibson et al. 2020; Wallace et al. 2020). Global health authorities have renewed their support for this well-established tool for rabies control to be used in conjunction with parenteral vaccination approaches in mass dog vaccination campaigns (Wallace et al. 2020). The efficacy of ORV in dogs has been demonstrated in a number of locations, both from immunological and operational perspectives (Chanachai et al. 2021; Gibson et al. 2019; Smith et al. 2019b), and the improved safety profile of modern third generation modified live ORVs would enable the investigation of ORV campaign strategies in urban settings (Head et al. 2019).

25.4.8 Dog Vaccination Campaign Evaluation

The vaccination coverage achieved by a particular vaccination method, or combination of methods, will be affected by the composition of the local dog population, community engagement, and sociocultural factors. During the initial phases of campaign development, it is beneficial to evaluate vaccination coverage to assess the efficacy of a particular vaccination approach in a specific locality (Sambo et al. 2017). This enables the strategy to be refined in the short term to reach a target vaccination coverage across much of the population and thus increase the chances of achieving the objective of rabies control. Ultimately the vaccination requirement to successfully control rabies at the community level will vary depending on local

epidemiological factors, with viral elimination occurring at lower coverages in some areas than others. Therefore, monitoring canine rabies case incidence through robust surveillance is essential for guiding vaccination strategy through the course of a control effort, determining regions where vaccination must persist or be intensified and areas that have achieved elimination.

Homogeneity of vaccination coverage across a region is thought to be of central importance to the successful local elimination of the canine RABV. A "Swiss-cheese" appearance to vaccination penetration across the dog population of a region (heterogeneous coverage) allows for sustained RABV transmission within unvaccinated pockets of dogs. This persistence of the virus through the vaccination campaign undermines prospects for elimination through rapid reintroduction into vaccinated areas as coverage wanes (Ferguson et al. 2015; Kitala et al. 2002; Townsend et al. 2013).

Mobile technologies and internet connectivity are transforming capabilities for communication and data sharing to benefit public heath interventions, with the term mHealth referring to systems in this space (World Health Organization 2011). Several tools have been developed to support the spatial coordination of dog vaccination workforces, with the specific focus of improving vaccination coverage homogeneity and robust post-vaccination evaluation (Gibson et al. 2018). These tools also improve the ability to capture high-resolution campaign data, at the individual dog, including time, date, GPS location, and demographic data offering deeper insights into campaign output, efficiency, and operational effectiveness (Athingo et al. 2021). The considerable resources required for the centralization and aggregation of campaign data using paper-based approaches are eliminated, as data are immediately available for remote review in a standardized digital format upon upload to a central server. The timely availability of granular operational data make it possible for campaign planners to redirect vaccination team movements during the course of a campaign (Gibson et al. 2015) as well as between campaigns (Mazeri et al. 2021) for maximum effect. This real-time oversight of field data was pivotal to the successful coordination of a multinational response to a rabies outbreak at the border of Haiti and the Dominican Republic in 2019 (Adrien et al. 2019; Mandra et al. 2019). The benefits of mHealth are also being applied to rabies surveillance systems to support the intersectoral sharing of information for efficient investigation and management of suspect rabid animals (Lushasi et al. 2020; Mtema et al. 2016).

25.4.9 Conclusion

The COVID-19 pandemic has shown the huge effort and expense that governments will expend and the disruption they will impose on their citizens to control a disease that has a broad impact on society. Rabies has been perceived by key decision makers not to be worth the investment in resources required to control it. However, with increased experience from implementing mass dog vaccination at scale enabling improvements in operational efficiency, while improving visibility of the significance of rabies control through improved surveillance, it may be possible to

bring rabies control into the realms of political feasibility. Ultimately a greater awareness of the global and individual human impact of canine rabies is needed and as global momentum builds through initiative such as ZeroBy30, the question of whether ours will be the generation to write history on global canine rabies control will need to be answered.

25.5 Road Blocks on the Way to Global Elimination of Dog-Mediated Rabies[4]

25.5.1 Introduction

Rabies is widely recognized as a public health threat in large parts of the globe, most markedly in Asia (Kole et al. 2014; Miranda and Miranda 2020) and Africa (Haselbeck et al. 2021; Sabeta and Ngoepe 2018). Human rabies, despite being preventable with existing tools, still claims an estimated 35 000–60 000 lives annually (Hampson et al. 2015). Even though these deaths could be prevented using post-exposure prophylaxis (PEP), the high costs for human vaccines as well as limited availability of these biologics are prohibitive to this solution. The elimination of rabies at its source – in over 95% of human cases, rabid dogs – is of a higher benefit both from an economical and a sustainability point of view. The elimination of canine rabies is considered a feasible objective (Lembo et al. 2010), although the RABV is characterized by multiple hosts and a range of variants (Rupprecht et al. 2008). The example of Latin America (Del Rio Vilas et al. 2017), as well as national and local efforts in other endemic regions have proven this principle (Le Roux et al. 2018; Mpolya et al. 2017; Lionel Harischandra et al. 2016). The international community, with more than 100 endemic countries, has set a global target of reaching zero human deaths from dog-transmitted rabies ("Zero by 30") worldwide by 2030 (Minghui et al. 2018). Through these efforts, health decision makers are increasingly aware that this fatal disease could be eliminated as a public health problem cost effectively in a relatively short time (World Health Organization 2015c; Shwiff et al. 2013; WHO and OIE 2016). As of today, however, rabies remains neglected in many places and progress remains slow on a global scale; the disease still being endemic in far more than 100 countries globally (Wallace et al. 2017; Fahrion et al. 2017).

This section describes some frequently cited main constraints to the prevention and elimination of dog transmitted rabies. It has to be recognized that most of the topics mentioned in this chapter are interconnected and fit under more than one subheading. For example, the presence of basic financial means is essential for any initiative, not to mention for running a program, and directly associated with the awareness and will of (political and financial) decision makers. Therefore, other

[4] Author: Anna Fahrion. Of note, this section contains extracts from last edition´s section, "What hampers global human rabies elimination?" by Tiziana Lembo, Lea Knopf, and Deborah J Briggs.

points mentioned here cannot be regarded in separation from the funding issue. The same is true, e.g., for the implementation of a One Health approach.

25.5.2 Awareness and Political Will

Rabies imposes huge burdens on individuals, families, societies, and economies (Hampson et al. 2015). Awareness of the disease is necessary to promote the ambitious but feasible goal of ending human rabies deaths, but is often lacking, on different levels and layers throughout society. Rabies needs to be on the agenda of politicians and decision makers globally, nationally, and locally, but this depends on and is intertwined with public perception and societal awareness. As communities become more aware of the threat and burden through dog rabies, political pressure to act will accumulate and raise the prestige of such activities. Champions at all hierarchy levels who directly advocate for the cause can make a significant difference (Balaram et al. 2016; World Health Organization 2015c). Building a proactive society, fully engaged in the dog rabies elimination efforts, through community involvement and education on rabies is therefore essential in mobilizing a country toward the elimination of rabies.

25.5.3 Public and Society

25.5.3.1 Low Awareness and Education in the Public/Community Engagement

Many unnecessary human rabies deaths occur because of insufficient awareness on effective post-exposure preventive measures at the community level, which is particularly marked in very remote areas. Increasing awareness about how to avoid and treat rabies exposures can therefore save lives. This has been successfully achieved by involving communities in the prevention and control process (Hampson et al. 2008). However, questions remain regarding the most effective avenues to reach communities at risk. For instance, while it is recognized that children are an important risk group (Kilic et al. 2006), uncertainties remain about the type of interventions that may be most effective for this audience. The emphasis so far has been on school-based interventions (Lapiz et al. 2012), despite the fact that in remote rabies-endemic areas non-schooled children still represent a large proportion of the community. Furthermore, interventions at the school level rarely involve parents, who are likely to play an important role in shaping children's health behavior, or key community members (e.g., community leaders), despite their potential role in encouraging community participation in rabies management initiatives. In many cases, efforts to raise public awareness mainly consist of promoting rabies information, but its translation into the desired behavioral changes remains unclear. Therefore, it is recommended to design communication strategies using the science of behavioral change, embracing the diversity of behavioral drivers, motivations, and larger sociocultural context of the targeted audience (Fahrion et al. 2017).

25.5.3.2 Companion Animal Health Issues Are Considered Low Status Activities in Rabies Endemic Countries

It has to be considered that in many rabies-endemic countries, the capacity within veterinary services is highly limited, especially when it comes to companion animals, given the greater focus on livestock health and agricultural problems. This problem could be overcome by providing training and support for veterinary services to handle dogs and dog diseases – resources and knowledge to run vaccination campaigns, however, can hardly be provided by veterinary services alone (see also sect. 25.5.10). Another challenging issue to address is that for most veterinary and medical professionals in Africa and Asia interventions involving domestic dogs are viewed as low status because they target an animal species of no economic value and an underrepresented segment of the human population, the rural poor. Yet, lessons can be learnt from areas of the world, such as Latin America, where national elimination programs led by an influential government sector (public health) have resulted in dramatic declines in human and canine rabies (Schneider et al. 2007).

25.5.4 Political Leadership

25.5.4.1 Lacking Prioritization at High Level Decision-Making

Community-level awareness alone is not sufficient to improve the rabies situation. There is also a need for commitment on the policy-making level to support the transition toward freedom from human rabies in a cohesive way (Fahrion et al. 2017). Too often, diseases of the rural poor slip the attention of political leadership and therefore face limitations in prioritization, leading to lacks of institutional and programmatic anchoring in the veterinary and public health systems, accompanied by lacking resource attribution. This issue is complicated by the necessary involvement of different sectors increasing the complexity of organization (see: intersectoral collaboration) and the lack of reliable numbers and data (see: surveillance). Increasing the awareness of political leaders and decision makers about the horrible fate of rabies victims, the high number of children affected, the well-known and proven principal interventions and their relatively rapid onset of measurable effects, as well as the potential benefit on other zoonotic diseases and on national, regional, and international recognition could make the case and raise the pressure for more engagement.

25.5.5 Surveillance, Data, and Diagnostics

One important key toward building awareness and investment of resources for a disease is recognition and prioritization by political leadership (Mangen et al. 2010). To this end, it is key to demonstrate the impact it has on public health and the economy and the potential benefit of targeting the disease. Very often, the true scale of the human disease problem on local communities and national economies (as stated by Knobel et al. 2005) is still unknown or ignored by high-level policy-making bodies. This might be due to insufficient science communication and simply

due to the fact that rabies is a neglected disease of the poor, with an animal reservoir often seen as "low status" and as a veterinary issue in the first place.

25.5.6 Underreporting

A lack of surveillance and diagnostic capacity is an enduring issue in regions of the world where the highest number of rabies deaths occurs. As a disease most of the times occurring in the remotest and poorest parts of the population that do not have a voice (Hampson et al. 2008), rabies deaths often go undiagnosed and unreported. It is estimated that human rabies deaths are commonly underreported 100-fold (Taylor et al. 2017a; Scott et al. 2017). This absence of solid evidence induces a cycle of neglect: because of the resulting underreporting and under-diagnosis, rabies is falsely perceived as an insignificant health issue leading to low priority, hence further neglect (Taylor and Nel 2015). Vice versa, a surveillance system that delivers better data, flowing from local authorities up to the international level is a precondition to increased awareness. In the presence of data, where quantitative assessments or prioritization exercises have been run, rabies consistently came up among the priority zoonotic diseases in different geographic locations (Yasobant et al. 2019; Sekamatte et al. 2018; Salyer et al. 2017). A way to formally establish reporting is declaring a disease notifiable (Taylor et al. 2015) as advised by OIE. For reasons of practicability and cost effectiveness, it seems advisable to integrate reporting on well-described rabies indicators solidly into the functionality of the general national surveillance system. Where the reporting happens in an isolated manner, without a clear data flow mechanism and dependent on individual's personal motivation, it is not likely that the data chain can work reliably. Often, a lack of such integrated surveillance systems, involving both the central- and local-level human and animal health sectors, remains a key gap in surveillance capacity in developing countries (Banyard et al. 2013; Molyneux et al. 2011).

25.5.7 Limited Diagnostic Capacity for Reporting According to International Standards

Testing animals to confirm rabies cases allows for better decision-making on the follow-up of human exposures and can generate data for program evaluation. In humans, diagnostics can yield results only by the time of disease manifestation, therefore limiting the purpose of testing to case confirmation either *intra vitam* or *postmortem*. Due to sophisticated laboratory requirements, the reference fluorescent antibody test is not an option in many rabies endemic settings. To this end, simplified techniques requiring less specialized equipment such as Direct Rapid Immunohistochemical Test (Dürr et al. 2008) and lateral flow devices have been developed, even though quality of these may be uncertain (Klein et al. 2020). However, international standards for rabies surveillance and evaluation of interventions are entirely reliant on laboratory confirmation of cases postmortem, which requires the establishment of rigorous systems for the collection and submission of

samples, and adequate laboratory infrastructure and capacity to perform rabies diagnostics (Meslin et al. 1999; Fooks et al. 2009; Banyard et al. 2013). On the other hand, the reality is that very few countries in rabies-endemic areas are likely to meet global surveillance and diagnostic standards in the foreseeable future and this should not further delay the implementation of large-scale rabies elimination programs. Approaches based on clinical case finding by local communities were the primary surveillance tool in the final stages of rinderpest eradication (Mariner et al. 2012; Mariner and Roeder 2003). Given the distinctive nature of rabies, recognition among local communities in affected areas is high (Hampson et al. 2009) and therefore participatory surveillance has the potential to play a key role in assessing the impacts of rabies interventions. Efforts should therefore be made to provide affected communities with user-friendly tools for rapid case reporting (using, for example, mobile phone technologies (Gibson et al. 2018)), and to shift the focus from laboratory-based surveillance as the sole means to evaluate intervention efforts.

25.5.8 Coordination and Implementation

25.5.8.1 Lack of Intersectoral Collaboration

The key justification why rabies is a prime example of a disease that needs to be resolved through applying a One Health approach lies in the realization that the one key strategy to avoid human deaths is interrupting transmission through the reservoir: by dog vaccination (Cleaveland and Hampson 2017). In other words: generating human public health benefits from a veterinary intervention. If a veterinary perspective is not included from the beginning, public health deciders too often ignore the economic savings from dog vaccination over just providing PEP to humans as they are used to focus solely on supplying the human population with medical treatment. However, providing PEP without interventions on dogs is expensive and purely symptomatic, lacking any perspective of sustainable improvement of the situation. Building the body of evidence by demonstrating the cost-beneficial impacts of dog rabies control due to reduced expenditure on costly human post-exposure vaccines is an important step toward the integration of budgets across ministries so to ensure sustained financial support (Hampson et al. 2011; González-Roldán et al. 2021). Beyond the veterinary and public health sectors, close involvement of social sciences, the education sector, and municipalities is now equally recognized (Srinivasan et al. 2019), for example, as a powerful method for preventing dog bites in children, increasing knowledge and awareness about rabies, and in sustainably managing dog populations in affected communities (Lapiz et al. 2012).

The effectiveness of intersectoral strategies for canine rabies elimination has been proven, e.g., in the Americas (Laing et al. 2020; Vigilato et al. 2013a) and elsewhere (Léchenne et al. 2021; Pudjiatmoko and Kadun 2013; Changalucha et al. 2019; Lushasi et al. 2020). While in general, the benefits of a One Health approach are more and more recognized at the highest international level (Seifman and Kaplan 2021), One Health operationalization at national or local levels remains a challenge

in many countries. The bottleneck frequently seems to be the question how to adjust existing systems and habits to realize working in an intersectoral manner (Belot et al. 2021). Given the nature of rabies, the responsibility for its control should be borne by a broad range of sectors. However, the range of "players" that ought to be involved makes operationalization problematic because of difficulties in harmonizing administrative and management structures and budget lines across sectors, and planning and implementing joint financing mechanisms involving different ministries (Jerolmack 2013). The creation of intersectoral zoonotic units or task forces, and integrating intersectoral processes in national rabies plans and guidance from the beginning, can help crossing these burdens. Integrated bite case management (IBCM) is an example of a locally implemented coordinated activity (Lushasi et al. 2020). Various international and national efforts exist to strengthen intersectoral, One Health collaboration. One example is the so-called IHR-PVS National Bridging Workshops (Belot et al. 2021). This methodology is currently being adapted specifically for rabies to enable countries to improve and strengthen the intersectoral network and collaboration at significant technical areas, providing them with a better organizational equipment to tackle the disease under a One Health approach.

25.5.8.2 Lack of Cohesive and Strategic Implementation Guidance

While challenges of One Health operationalization are often located at subnational levels (Munyua et al. 2016), it is the National authorities who prepare the ground for the general direction of rabies control and elimination efforts as well as the way the sectors will work together, by deciding on the national approach and its implementation. The authorities are mainly responsible for developing national strategies and implementing programs, but can easily be overwhelmed by multiple human and animal disease priorities and the challenges associated with programs stretched across sectors and administrative levels (Fahrion et al. 2017). A range of tools and materials that provide guidance are available but may lack cohesion, complicating the selection of a starting point and the most crucial measures to implement. For example, the Stepwise Approach Toward Rabies Elimination (SARE), which is embedded in the rabies blueprint (Global Alliance for Rabies Control), is a guidance and evaluation tool that has been used by countries across three continents, mostly at national or regional stakeholder consultations, to kick-start coordinated rabies control (Coetzer et al. 2016). More recently, additional tools and international mechanisms have been added and continue to evolve (United Against Rabies Collaboration 2019).

25.5.9 Challenges of Cross-Border Collaboration

Rabies, as other pathogens, does not stop at borders. For transboundary diseases, cross-border collaboration, cooperation, and transparency can only be of benefit for disease control. However, admittance of public health problems being perceived as "failures," authorities might tend not to share information with their neighbors (Fahrion et al. 2017). This represents a missed opportunity to address potential

transboundary issues and more general, bilateral or multilateral collaboration to jointly tackle infectious diseases, catalyzing more awareness and activity and an expanding, more sustainable approach. On a larger scale, regional approaches have been fundamental for the most successful rabies control and elimination efforts, e.g., in Western Europe (World Health Organization 2018b) and Latin America (Vigilato et al. 2013a. b). Regional efforts are also being undertaken in other parts of the world (Miranda and Miranda 2020; Pieracci et al. 2017; Taylor et al. 2021; Scott et al. 2015) and will continue to require strong commitment.

25.5.10 Issues Related to Dog Vaccination

Rabies is integrally linked to the proximity and the ways people and dogs live together. Most of the constraints around rabies elimination can be understood when taking a look at what is recognized as the single, most effective intervention to prevent and control transmission: dog vaccination. The constraints around this one key activity demonstrate the entire significance and difference that a true One Health approach could make if fully implemented: as a primarily veterinary intervention, dog vaccination is not firmly rooted in public health awareness, often underfunded and understaffed, linked with difficult logistics, research gaps and a need for high level of perseverance to make it sustainable.

Rabies control requires an adequate understanding of the dog ecology and dog-keeping practices in a country in locally differing sociocultural contexts (e.g., urban vs rural, among different economic, religious, or ethnic groups). Achieving the 70% dog vaccination coverage that is recognized to provide herd immunity (Conan et al. 2015) is often hampered by rapid population turn over, partly despite dog population management attempts (Taylor et al. 2017b). As rabies threatens communities, the fear of rabies also leads to unnecessary cruel treatment of dogs such as culling (Hiby et al. 2017). Especially free-roaming dog population act as drivers of rabies transmission cycles (Gamble et al. 2018), a population that has a growing tendency especially in connection to progressing urbanization (Krystosik et al. 2020). The problem is further aggravated by the associated increased solid waste production and mismanagement of waste disposal where dogs are attracted in high numbers and compete for food, increasing the risk for aggressive behavior (Wright et al. 2021). Proper management of poorly managed garbage disposal sites and landfills certainly benefits a broad range of public and veterinary health issues and should therefore be prioritized by the responsible authorities. When implementing dog vaccination campaigns, knowledge of the local dog population is essential (Gamble et al. 2018). In circumstances where dogs are privately owned or community owned, it might be the case that almost all dogs can be handled and vaccinated by the parenteral route (Morters et al. 2014) despite being free roaming. In other cases, inaccessible dogs may jeopardize vaccination coverage. Empirical work (e.g., on area-specific basic reproductive ratio (R_0)) suggests that vaccination strategies and coverages should be adapted to the local context to control rabies successfully. High vaccination coverage in high-risk areas may be more crucial than medium coverage across the whole country, but clear guidance on this is lacking. Better knowledge of area and country-

specific factors related to dog keeping practices, dog population turnover, and contact rates between dogs and wildlife can help in determining a more flexible, realistic required dog vaccination coverage (Sparkes et al. 2014). Research on oral rabies vaccination has recently delivered increasing evidence to be a well-suited complement to parenteral rabies vaccination activities in different contexts, especially with free roaming dogs (Gautam et al. 2020; Wallace et al. 2020).

25.5.11 Limited Availability of and Access to Rabies Biologicals

In addition to the problem that dog bite victims might not be aware of a potential rabies risk (see: awareness and political will), a common constraint following a potentially rabid dog bite is that of inaccessibility or unaffordability of rabies biologicals (Hampson et al. 2011). The fact that a course of rabies PEP, combined with potentially long travel routes to get the vaccine, just exceeds the household´s resources, remains a bitter truth for many. Newer developments to fill this gap including shortened PEP regimens (requiring fewer health facility visits), changed recommendations toward vaccine-saving intradermal administration and new technologies such as thermostable vaccines and monoclonal antibodies could contribute to alleviate the problem (see part on human prevention). However, a broad, general change toward better supply and distribution of these biologicals warrants systematic improvement of universal health coverage. A global push in these discussions and a decisive, facilitating shift for many countries seems possible through the recent uptake of human rabies vaccine on the portfolio of Gavi, the Vaccine Alliance (WHO Rabies Modelling Consortium 2019) (Mohammadi 2016). On the manufacturing level, an absence of accurate forecasting data on vaccine needs impairs production and procurement and can lead to shortages of stock and impaired long-term planning (Wallace et al. 2017). (Interrupted delivery chains in turn could cause countries to purchase at a higher cost or to turn to manufacturers not meeting international vaccine quality standards.) Vaccine banks or stockpiles at regional levels as managed by OIE or WHO have become a solid mechanism for countries to maintain the supply of quality-assured vaccines and allow manufacturers to forecast and stabilize their production over years with lowered pricing through bulk purchase (WHO and OIE 2015). Vaccine banks have contributed demonstrably to the scaling up and maintenance of local, national, or subregional programs in Asia and Africa (World Health Organization 2015a) and incentivized recipient countries to increase data collection, as reporting on vaccine use and results is required (Fahrion et al. 2017).

25.5.12 Funding and Sustainability

Like with all veterinary and public health issues, allocation of resources and the lack thereof is to be considered a root cause for the barriers to advance toward elimination of this horrifying disease that should be consigned to the history books (M. Chan, Director General of WHO (World Health Organization 2015b). It can be hoped that

the investment case will become more and more obvious to countries with the realization that investing, e.g., in dog vaccination is cost effective (Shwiff et al. 2013) (Anderson and Shwiff 2013). Setting up rabies programs and improving surveillance for rabies, integrated with other diseases and interventions, provides multiple public health benefits and strengthens the veterinary and public health systems in their entirety. Savings that can be made to reduce running costs, e.g., for PEP, such as intradermal vaccine administration, have been mentioned above. Private–public partnerships might provide ways to fund rabies control for countries (Taylor and Partners for Rabies Prevention 2013). Internationally, while there is no single pooled fund for rabies elimination and investments are small and fragmented, the United Against Rabies Forum has been set up as a platform, among other tasks, to secure funding resources from the international community (United Against Rabies 2021). However, it is up to countries to understand that investment in rabies is a long-term, but potentially highly cost-beneficial commitment, bringing along significant improvements to public health and to the life of people.

25.6 Cross-References

- ▶ Animal Bites and Zoonoses: From A to Z – Alligators to Zebras
- ▶ Bat-Related Zoonoses
- ▶ Cats – Revered and Reviled – and Associated Zoonoses
- ▶ Dogs and Transmission of Infection to Man, "Respected Member of the Family?"

References

Adrien J, Georges Y, Augustin PD, Monroe B, Gibson AD, Fenelon N et al (2019) Notes from the Field: A Multipartner Response to Prevent a Binational Rabies Outbreak - Anse-à-Pitre, Haiti, 2019. *MMWR Morb Mortal Wkly Rep* 68(32):707–709. https://doi.org/10.15585/mmwr.mm6832a6

Agnarsson I, Zambrana-Torrelio CM, Flores-Saldana NP, May-Collado LJ (2011) A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). PLoS Curr 3. https://doi.org/10.1371/currents.RRN1212

Aguilar-Setien A, Campos YL, Cruz ET, Kretschmer R, Brochier B, Pastoret PP (2002) Vaccination of vampire bats using recombinant vaccinia-rabies virus. *J Wildl Dis* 38(3):539–544

Allen, Toph; Murray, Kris A.; Zambrana-Torrelio, Carlos; Morse, Stephen S.; Rondinini, Carlo; Di Marco, Moreno et al. (2017): Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* 8 (1), 1124. https://doi.org/10.1038/s41467-017-00923-8.

Amarasinghe GK, Arechiga Ceballos NG, Banyard AC, Basler CF, Bavari S, Bennett AJ et al (2018) Taxonomy of the order Mononegavirales. update 2018. *Arch Virol* 163:2283–2294. https://doi.org/10.1007/s00705-018-3814-x

Anderson A, Shwiff SA (2013) The Cost of Canine Rabies on Four Continents. *Transbound Emerg Dis* 62(4):446–452. https://doi.org/10.1111/tbed.12168

Antunes KD, Matos JCC, Mol LP, Oliveira MA, Arcebispo TLM, Santos VG et al (2018) Descriptive analysis of rabies in wild animals in the state of Sergipe, Brazil. *Arq Bras Med Vet Zootec* 70:169–173

Appolinario CM, Jackson AC (2015) Antiviral therapy for human rabies. *Antivir Ther* 20(1):1–10. https://doi.org/10.3851/IMP2851

Arai YT, Kuzmin I, Kameoka Y, Botvinkin AD (2003) New Lyssavirus genotype from the lesser mouse-eared bat (Myotis blythi), Kyrghyzstan. *Emerg Infect Dis* 9(3):333–337

Aréchiga Ceballos N, Moron SV, Berciano JM, Nicolas O, Lopez CA, Juste J et al (2013) Novel lyssavirus in bat, Spain. *Emerg Infect Dis* 19(5):793–795. https://doi.org/10.3201/eid1905.121071

Athingo R, Tenzin T, Coetzer A, Hikufe EH, Peter J, Hango L et al (2021) Application of the GARC Data Logger—a custom-developed data collection device—to capture and monitor mass dog vaccination campaigns in Namibia. *PLoS Neglect Trop Dis* 14(12):e0008948. https://doi.org/10.1371/journal.pntd.0008948

Aubert, M. (1992): Epidemiology of fox rabies. In: K. Bögel, F. X. Meslin und M. Kaplan (Hg.): Wildlife Rabies Control. Kent: Wells Medical Ltd., S. 9–18.

Aubert M (1994) Control of Rabies in Foxes - What Are the Appropriate Measures. *Vet Rec* 134(3):55–59

Aubert MF (1999) Costs and benefits of rabies control in wildlife in France. *Rev Sci Tech* 18(2):533–543

Badrane H, Tordo N (2001) Host Switching in Lyssavirus History from the Chiroptera to the Carnivora Orders. *J Virol* 75(17):8096–8104

Badrane H, Bahloul C, Perrin P, Tordo N (2001) Evidence of Two Lyssavirus Phylogroups with Distinct Pathogenicity and Immunogenicity. *J Virol* 75(7):3268–3276

Baer GM, Abelseth MK, Debbie JG (1971) Oral vaccination of foxes against rabies. *Am J Epidemiol* 93(6):487–490

Bakker AB, Python C, Kissling CJ, Pandya P, Marissen WE, Brink MF et al (2008) First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine* 26(47):5922–5927

Bakker KM, Rocke TE, Osorio JE, Abbott RC, Tello C, Carrera JE et al (2019) Fluorescent biomarkers demonstrate prospects for spreadable vaccines to control disease transmission in wild bats. *Nat Ecol Evol* 3(12):1697–1704. https://doi.org/10.1038/s41559-019-1032-x

Balaram D, Taylor LH, Doyle KAS, Davidson E, Nel LH (2016) World Rabies Day - a decade of raising awareness. *Trop Dis Travel Med Vaccines* 2(1):S. 19. https://doi.org/10.1186/s40794-016-0035-8

Banyard AC, Horton D, Freuling C, Müller T, Fooks AR (2013) Control and prevention of canine rabies. the need for building laboratory-based surveillance capacity. *Antiviral Res* 98:357–364. https://doi.org/10.1016/j.antiviral.2013.04.004

Banyard AC, Selden D, Wu G, Thorne L, Jennings D, Marston D et al (2018) Isolation, antigenicity and immunogenicity of Lleida bat lyssavirus. *J Gen Virol* 12(99):1590–1599. https://doi.org/10.1099/jgv.0.001068

Banyard, Ashley C.; Davis, A.; Gilbert, Amy T.; Markotter, Wanda (2020a): Bat rabies. In: A. R. Fooks und A. C. Jackson (Hg.): Rabies - Scientific Basis of the Disease and its Management. 4 Aufl.: Elsevier Academic Press, S. 231–276.

Banyard, Ashley C.; Davis, April; Gilbert, Amy T.; Markotter, Wanda (2020b): Bat rabies. In: Anthony R. Fooks und Alan C. Jackson (Hg.): Rabies: Scientific Basis of the Disease and Its Management 4. Boston: Academic Press, S. 231–276.. Online verfügbar unter https://www.sciencedirect.com/science/article/pii/B9780128187050000078.

Bardosh K, Sambo M, Sikana L, Hampson K, Welburn SC (2014) Eliminating rabies in Tanzania? Local understandings and responses to mass dog vaccination in Kilombero and Ulanga districts. *PLoS Neglect Trop Dis* 8(6):e2935. https://doi.org/10.1371/journal.pntd.0002935

Bellan SE, Cizauskas CA, Miyen J, Ebersohn K, Kusters M, Prager KC et al (2012) Black-backed jackal exposure to rabies virus, canine distemper virus, and Bacillus anthracis in Etosha National Park, Namibia. *J Wildl Dis* 48(2):371–381. https://doi.org/10.7589/0090-3558-48.2.371

Belot G, Caya F, Errecaborde KM, Traore T, Lafia B, Skrypnyk A et al (2021) IHR-PVS National Bridging Workshops, a tool to operationalize the collaboration between human and animal health while advancing sector-specific goals in countries. *PloS one* 16(6):e0245312. https://doi.org/10.1371/journal.pone.0245312

Belotto AJ (2004) The Pan American Health Organization (PAHO) role in the control of rabies in Latin America. *Dev Biol (Basel)* 119:213–216

Belsare AV, Gompper ME (2013) Assessing demographic and epidemiologic parameters of rural dog populations in India during mass vaccination campaigns. *Prev Vet Med* 111(1-2):139–146. https://doi.org/10.1016/j.prevetmed.2013.04.003

Berentsen AR, Ellis CK, Johnson SR, Leinbach IL, Sugihara RT, Gilbert AT (2020) Immunogenicity of Ontario Rabies Vaccine for Small Indian Mongooses (Herpestes auropunctatus). *J Wildl Dis* 56(1):224–228. Epub 2019 Sep 30.

Berentsen A, Leinbach I, Rivera-Rodriguez M, Gilbert A (2021) Oral rabies vaccination of small Indian Mongooses (Urva auropunctata) with ONRAB via Ultralite Baits. *Viruses* 13(5):734. https://doi.org/10.3390/v13050734

Bernard KW, Fishbein DB, Miller KD, Parker RA, Waterman S, Sumner JW et al (1985) Pre-exposure rabies immunization with human diploid cell vaccine: decreased antibody responses in persons immunized in developing countries. *Am J Trop Med Hyg* 34(3):633–647. https://doi.org/10.4269/ajtmh.1985.34.633

Bernardi F, Nadin-Davis SA, Wandeler AI, Armstrong J, Gomes AA, Lima FS et al (2005) Antigenic and genetic characterization of rabies viruses isolated from domestic and wild animals of Brazil identifies the hoary fox as a rabies reservoir. *J Gen Virol* 86(11):3153–3162

Bharti OK, Tekta D, Shandil A, Sharma K, Kapila P (2019) Failure of postexposure prophylaxis in a girl child attacked by rabid dog severing her facial nerve causing possible direct entry of rabies virus into the facial nerve. *Hum Vaccin Immunother* 15(11):2612–2614. https://doi.org/10.1080/21645515.2019.1608131

Billah MA, Miah MM, Khan MN (2020) Reproductive number of coronavirus: A systematic review and meta-analysis based on global level evidence. *PloS one* 15(11):e0242128. https://doi.org/10.1371/journal.pone.0242128

Bingham J, Foggin CM, Wandeler AI, Hill FW (1999) The epidemiology of rabies in Zimbabwe. 2. Rabies in jackals (Canis adustus and Canis mesomelas). *Onderstepoort J Vet Res* 66(1):11–23

Blancou, J. (2004): Rabies in Europe and the Mediterranean Basin. From antiquity to the 19th century. In: A. A. King, A. R. Fooks, M. Aubert und A. I. Wandeler (Hg.): Historical perspective of rabies in Europe and the Mediterranean Basin. Paris: OIE, S. 15–23.

Blanton JD, Dyer J, McBrayer J, Rupprecht CE (2012) Rabies surveillance in the United States during 2011. *J Am Vet Med Assoc* 241(6):712–722

Both L, Banyard AC, van Dolleweerd C, Horton DL, Ma JKC, Fooks AR (2012) Passive immunity in the prevention of rabies. *Lancet Infect Dis* 12(5):397–407

Both L, Banyard AC, van Dolleweerd C, Wright E, Ma JK, Fooks AR (2013a) Monoclonal antibodies for prophylactic and therapeutic use against viral infections. *Vaccine* 31(12):1553–1559. https://doi.org/10.1016/j.vaccine.2013.01.025

Both L, van Dolleweerd C, Wright E, Banyard AC, Bulmer-Thomas B, Selden D et al (2013b) Production, characterization, and antigen specificity of recombinant 62-71-3, a candidate monoclonal antibody for rabies prophylaxis in humans. *FASEB J* 27(5):2055–2065

Botvinkin AD, Poleschuk EM, Kuzmin I, Borisova TI, Gazaryan SV, Yager P, Rupprecht CE (2003) Novel lyssaviruses isolated from bats in Russia. *Emerg Infect Dis* 12(9):1623–1625. https://doi.org/10.3201/eid0912.030374

Bourhy H, Nakoune E, Hall M, Nouvellet P, Lepelletier A, Talbi C et al (2016) Revealing the Micro-scale Signature of Endemic Zoonotic Disease Transmission in an African Urban Setting. *PLoS Pathog* 12(4):e1005525. https://doi.org/10.1371/journal.ppat.1005525

Brass DA (1994) Rabies-related viruses from bats in Europe and Asia. In: Rabies in Bats : Natural History and Public Health Implications. Livia Press, Ridgefield

Briggs DJ (2011) Rabies. The immunological basis for immunization. In: Series Module 17. World Health Organization, Geneva Switzerland, p 24

Briggs DJ (2012) The role of vaccination in rabies prevention. *Curr Opin Virol* 2(3):309–314. https://doi.org/10.1016/j.coviro.2012.03.007

Brookes SM, Healy DM, Fooks AR (2006) Ability of rabies vaccine strains to elicit cross-neutralising antibodies. *Dev Biol (Basel)* 125:185–193

Brunker K, Jaswant G, Thumbi SM, Lushasi K, Lugelo A, Czupryna AM et al (2020) Rapid in-country sequencing of whole virus genomes to inform rabies elimination programmes. *Wellcome Open Res.* 5:3. https://doi.org/10.12688/wellcomeopenres.15518.2

Buregyeya E, Atusingwize E, Nsamba P, Musoke D, Naigaga I, Kabasa JD et al (2020) Operationalizing the One Health Approach in Uganda: Challenges and Opportunities. *J Epidemiol Glob Health* 10(4):250–257. https://doi.org/10.2991/jegh.k.200825.001

Butler JR, Bingham J (2000) Demography and dog-human relationships of the dog population in Zimbabwean communal lands. *Vet Rec* 147(16):442–446. https://doi.org/10.1136/vr.147.16.442

Calisher CH, Child JE, Field H, Holmes KV, Schountz T (2006) Bats. important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19(3):531–545

Carter HE (1997) Britain Should Immediately Alter its Quarantine Rules for the Control of Rabies. *Rev Med Virol* 7(1):13–15. https://doi.org/10.1002/(sici)1099-1654(199704)7:1<13::aid-rmv186>3.0.co;2-t

Castillo-Neyra R, Zegarra E, Monroy Y, Bernedo R, Cornejo-Rosello I, Paz-Soldan V, Levy M (2017) Spatial Association of Canine Rabies Outbreak and Ecological Urban Corridors, Arequipa, Peru. *Trop Med Infect Dis* 2(3):38

Chanachai K, Wongphruksasoong V, Vos A, Leelahapongsathon K, Tangwangvivat R, Sagarasaeranee O et al (2021) Feasibility and Effectiveness Studies with Oral Vaccination of Free-Roaming Dogs against Rabies in Thailand. *Viruses* 13:S. 571. https://doi.org/10.3390/v13040571

Changalucha J, Steenson R, Grieve E, Cleaveland S, Lembo T, Lushasi K et al (2019) The need to improve access to rabies post-exposure vaccines. Lessons from Tanzania. *Vaccine* 37(Suppl. 1): A45–A35. https://doi.org/10.1016/j.vaccine.2018.08.086

Cirovic D, Rakovic M, Milenkovic M, Paunovic M (2011) Small Indian Mongoose Herpestes auropunctatus (Herpestidae, Carnivora). an invasive species in Montenegro. *Biol Invasions* 13 (2):393–399. https://doi.org/10.1007/s10530-010-9831-7

Cleaton JM, Wallace RM, Crowdis K, Gibson A, Monroe B, Ludder F et al (2018) Impact of community-delivered SMS alerts on dog-owner participation during a mass rabies vaccination campaign, Haiti 2017. *Vaccine* 36(17):2321–2325. https://doi.org/10.1016/j.vaccine.2018.03.017

Cleaveland S, Dye C (1995) Maintenance of a microparasite infecting several host species. rabies in the Serengeti. *Parasitology* 111(Suppl):S33–S47

Cleaveland S, Hampson K (2017) Rabies elimination research: juxtaposing optimism, pragmatism and realism. Proc R Soc B Biol Sci 284(1869):20171880. https://doi.org/10.1098/rspb.2017.1880

Cleaveland S, Kaare M, Tiringa P, Mlengeya T, Barrat J (2003) A dog rabies vaccination campaign in rural Africa. impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine* 21(17-18):1965–1973. https://doi.org/10.1016/s0264-410x(02)00778-8

Cliquet F, Guiot AL, Aubert M, Robardet E, Rupprecht CE, Meslin FX (2018) Oral vaccination of dogs: a well-studied and undervalued tool for achieving human and dog rabies elimination. *Vet Res* 49(1):S. 61. https://doi.org/10.1186/s13567-018-0554-6

Coertse J, Grobler C, Sabeta C, Seamark ECJ, Kearney T, Paweska J, Markotter W (eds) (2020) Lyssaviruses in Insectivorous Bats, South Africa, 2003–2018. *Emerg Infect Dis* 26(12):3056–3060. https://doi.org/10.3201/eid2612.203592

Coetzer A, Kidane AH, Bekele M, Hundera AD, Pieracci EG, Shiferaw ML et al (2016) The SARE tool for rabies control: Current experience in Ethiopia. *Antiviral Res* 135:74–80. https://doi.org/10.1016/j.antiviral.2016.09.011

Coleman PG, Dye C (1996) Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine* 14(3):185–186

Colombi D, Poletto C, Nakouné E, Bourhy H, Colizza V (2020) Long-range movements coupled with heterogeneous incubation period sustain dog rabies at the national scale in Africa. *PLoS Neglect Trop Dis* 14(5):e0008317. https://doi.org/10.1371/journal.pntd.0008317

Conan A, Akerele O, Simpson G, Reininghaus B, van Rooyen J, Knobel D (2015) Population Dynamics of Owned, Free-Roaming Dogs: Implications for Rabies Control. *PLoS Neglect Trop Dis* 9(11):e0004177. https://doi.org/10.1371/journal.pntd.0004177

Daoust PY, Wandeler AI, Casey GA (1996) Cluster of rabies cases of probable bat origin among red foxes in Prince Edward Island, Canada. *J Wildl Dis* 32(2):403–406

de Benedictis P, Minola A, Rota Nodari E, Aiello R, Zecchin B, Salomoni A et al (2016) Development of broad-spectrum human monoclonal antibodies for rabies post-exposure prophylaxis. *EMBO Mol Med* 8(4):407–421. https://doi.org/10.15252/emmm.201505986

de Melo GD, Sonthonnax F, Lepousez G, Jouvion G, Minola A, Zatta F et al (2020) A combination of two human monoclonal antibodies cures symptomatic rabies. EMBO Mol Med:e12628. https://doi.org/10.15252/emmm.202012628

Denis M, Knezevic I, Wilde H, Hemachudha T, Briggs D, Knopf L (2019) An overview of the immunogenicity and effectiveness of current human rabies vaccines administered by intradermal route. *Vaccine* 37(Suppl. 1):A99–A106. https://doi.org/10.1016/j.vaccine.2018.11.072

Dodet B, Goswami A, Gunasekera A, de Guzman F, Jamali S, Montalban C et al (2008) Rabies awareness in eight Asian countries. *Vaccine* 26(50):6344–6348

Du Pont V, Wirblich C, Yoon JJ, Cox RM, Schnell MJ, Plemper RK (2020) Identification and characterization of a Small-Molecule Rabies Virus Entry Inhibitor. *J Virol* 94(13):e00321–e00320. https://doi.org/10.1128/JVI.00321-20

Dunlop RH, Williams, DJ (1996) Veterinary medicine. An illustrated history: Mosby.

Dürr S, Naissengar S, Mindekem R, Diguimbye C, Niezgoda M, Kuzmin I et al (2008) Rabies Diagnosis for Developing Countries. *PLoS Neglect Trop Dis* 2(3):e206. https://doi.org/10.1371/journal.pntd.0000206

Eggerbauer E, de Benedictis P, Hoffmann B, Mettenleiter TC, Schlottau K, Ngoepe EC et al (2016) Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. *PLoS Neglect Trop Dis* 10(6):e0004776. https://doi.org/10.1371/journal.pntd.0004776

Eichner M, Dietz K (2003) Transmission potential of smallpox: estimates based on detailed data from an outbreak. *Am J Epidemiol* 158(2):110–117. https://doi.org/10.1093/aje/kwg103

Ertl HCJ (2019) New Rabies Vaccines for Use in Humans. *Vaccines* 7(2):54. https://doi.org/10.3390/vaccines7020054

Etheart MD, Kligerman M, Augustin PD, Blanton JD, Monroe B, Fleurinord L et al (2017) Effect of counselling on health-care-seeking behaviours and rabies vaccination adherence after dog bites in Haiti, 2014–15: a retrospective follow-up survey. *Lancet Glob Health* 5(10):e1017–e1025. https://doi.org/10.1016/S2214-109X(17)30321-2

Evans JS, Selden D, Wu G, Wright E, Horton DL, Fooks AR, Banyard AC (2018) Antigenic site changes in the rabies virus glycoprotein dictates functionality and neutralizing capability against divergent lyssaviruses. *J Gen Virol* 99(2):169–180. https://doi.org/10.1099/jgv.0.000998

Faber M, Pulmanausahakul R, Hodawadekar SS, Spitsin S, McGettigan JP, Schnell MJ, Dietzschold B (2002) Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. *J Virol* 76(7):3374–3381

Faber M, Li JW, Kean RB, Hooper DC, Alugupalli KR, Dietzschold B (2009) Effective preexposure and postexposure prophylaxis of rabies with a highly attenuated recombinant rabies virus. *Proc Natl Acad Sci U S A* 106(27):11300–11305. https://doi.org/10.1073/pnas.0905640106

Fahrion AS, Taylor LH, Torres G, Müller T, Durr S, Knopf L et al (2017) The Road to Dog Rabies Control and Elimination-What Keeps Us from Moving Faster? *Front Public Health* 5:103. https://doi.org/10.3389/fpubh.2017.00103

Favoretto SR, de Mattos CC, Morais NB, Araujo FA, de Mattos CA (2001) Rabies in Marmosets (Callithrix jacchus), Ceara, Brazil. *Emerg Infect Dis* 7(6):1062–1065

Feder HM Jr, Petersen BW, Robertson KL, Rupprecht CE (2012) Rabies: still a uniformly fatal disease? Historical occurrence, epidemiological trends, and paradigm shifts. *Curr Infect Dis Rep* 14(4):408–422. https://doi.org/10.1007/s11908-012-0268-2

Fehlner-Gardiner C (2018) Rabies control in North America - past, present and future. *Rev Sci Tech* 37(2):421–437. https://doi.org/10.20506/rst.37.2.2812

Fekadu M, Shaddock JH, Sanderlin DW, Smith JS (1988) Efficacy of rabies vaccines against Duvenhage virus isolated from European house bats (Eptesicus serotinus), classic rabies and rabies-related viruses. *Vaccine* 6(6):533–539

Ferguson EA, Hampson K, Cleaveland S, Consunji R, Deray R, Friar J et al (2015) Heterogeneity in the spread and control of infectious disease. consequences for the elimination of canine rabies. *Sci Rep* 5:S. 18232. https://doi.org/10.1038/srep18232

Filla C, Rajeev M, Randriana Z, Hanitriniana C, Rafaliarison RR, Edosoa GT et al (2021) Lessons Learned and Paths Forward for Rabies Dog Vaccination in Madagascar: A Case Study of Pilot

Vaccination Campaigns in Moramanga District. *Trop Med Infect Dis* 6:48. https://doi.org/10.3390/tropicalmed6020048

Fisher CR, Streicker DG, Schnell MJ (2018) The spread and evolution of rabies virus. conquering new frontiers. *Nat Rev Microbiol* 16(4):241–255. https://doi.org/10.1038/nrmicro.2018.11

Fisher CR, Lowe DE, Smith TG, Yang Y, Hutson CL, Wirblich C et al (2020) Lyssavirus Vaccine with a Chimeric Glycoprotein Protects across Phylogroups. *Cell reports* 32(3):S. 107920. https://doi.org/10.1016/j.celrep.2020.107920

Foggin CM (1988) Rabies and rabies-related viruses in Zimbabwe: historical, virological and ecological aspects. PhD Thesis in Medicine, University of Zimbabwe

Fooks AR, Johnson N, Brookes SM, Parsons G, McElhinney LM (2003) Risk factors associated with travel to rabies endemic countries. *J Appl Microbiol* 94 Suppl:31S–36S. https://doi.org/10.1046/j.1365-2672.94.s1.4.x.

Fooks AR, Johnson N, Freuling CM, Wakeley PR, Banyard AC, McElhinney LM et al (2009) Emerging technologies for the detection of rabies virus. challenges and hopes in the 21st century. *PLoS Neglect Trop Dis* 3(9):e530

Fooks AR, Banyard AC, Horton DL, Johnson N, McElhinney LM, Jackson AC (2014) Current status of rabies and prospects for elimination. *Lancet* 384(9951):1389–1399. https://doi.org/10.1016/S0140-6736(13)62707-5

Fooks AR, Cliquet F, Finke S, Freuling C, Hemachudha T, Mani RS et al (2017) Rabies. *Nat Rev Dis Primers* 3(17091):1–19. https://doi.org/10.1038/nrdp.2017.91

Fooks AR, Banyard AC, Ertl HCJ (2019) New human rabies vaccines in the pipeline. *Vaccine* 37 (Suppl):A140–A145. https://doi.org/10.1016/j.vaccine.2018.08.039

Fooks AR, Shipley R, Markotter W, Tordo N, Freuling CM, Müller T et al (2021) Renewed Public Health Threat from Emerging Lyssaviruses. *Viruses* 13:1769

Foord AJ, Heine HG, Pritchard LI, Lunt RA, Newberry KM, Rootes CL, Csiro DBB (2006) Molecular diagnosis of lyssaviruses and sequence comparison of Australian bat lyssavirus samples. *Aust Vet J* 84(7):225–230

Franka R, Rupprech CE (2011) Treatment of rabies in the 21st century. curing the incurable? *Future Microbiol* 6(10):1135–1140. https://doi.org/10.2217/fmb.11.92

Freuling CM, Selhorst T, Batza HJ, Müller T (2008) The financial challenge of keeping a large region rabies-free—the EU example. *Dev Biol (Basel)* 131:273–282

Freuling CM, Beer M, Conraths FJ, Finke S, Hoffmann B, Keller B et al (2011) Novel Lyssavirus in Natterer's Bat, Germany. *Emerg Infect Dis* 17(8):1519–1522. https://doi.org/10.3201/eid1708.110201

Freuling CM, Hampson K, Selhorst T, Schroder R, Meslin FX, Mettenleiter TC, Müller T (2013) The elimination of fox rabies from Europe. determinants of success and lessons for the future. Philos Trans R Soc Lond B Biol Sci 368(1623):20120142. https://doi.org/10.1098/rstb.2012.0142

Gamble L, Gibson AD, Shervell K, Lohr F, Otter I, Mellanby RJ (2018) The problem of stray dogs. *Rev Sci Tech* 37(2):543–550. https://doi.org/10.20506/rst.37.2.2822

Gautam, Vaishali; Bhardwaj, Pankaj; Saxena, Deepak; Kumar, Nitesh; S, Dileepan (2020): Multi-sectoral approach to achieve canine rabies controlled zone using Intervention Mapping: Preliminary results. *PloS one* 15 (12), e0242937. https://doi.org/10.1371/journal.pone.0242937.

Gavi - the Vaccine Alliance (2021) Vaccine investment strategy. Hg. v. https://www.gavi.org/our-alliance/strategy/vaccine-investment-strategy

Gibson AD, Ohal P, Shervell K, Handel IG, Bronsvoort BM, Mellanby RJ, Gamble L (2015) Vaccinate-assess-move method of mass canine rabies vaccination utilising mobile technology data collection in Ranchi, India. *BMC Infect Dis* 15(1):1–10. https://doi.org/10.1186/s12879-015-1320-2

Gibson AD, Mazeri S, Lohr F, Mayer D, Burdon Bailey JL, Wallace RM et al (2018) One million dog vaccinations recorded on mHealth innovation used to direct teams in numerous rabies control campaigns. *PloS one* 13(7):e0200942. https://doi.org/10.1371/journal.pone.0200942

Gibson AD, Yale G, Vos A, Corfmat J, Airikkala-Otter I, King A et al (2019) Oral bait handout as a method to access roaming dogs for rabies vaccination in Goa, India: A proof of principle study. *Vaccine X*(1):100015. https://doi.org/10.1016/j.jvacx.2019.100015

Gibson AD, Wallace RM, Rahman A, Bharti OK, Isloor S, Lohr F et al (2020) Reviewing Solutions of Scale for Canine Rabies Elimination in India. *Trop Med Infect Dis* 5:47. https://doi.org/10.3390/tropicalmed5010047

Gilbert AT, Petersen BW, Recuenco S, Niezgoda M, Gomez J, Alberto Laguna-Torres V, Rupprecht C (2012) Evidence of Rabies Virus Exposure among Humans in the Peruvian Amazon. *Am J Trop Med Hyg* 87(2):206–215. https://doi.org/10.4269/ajtmh.2012.11-0689

Global Alliance for Rabies Control (Hg.): Rabies Blueprint. Online verfügbar unter https://rabiesblueprint.org/, zuletzt geprüft am 30.07.2021

Gogtay N, Thatte U, Kshirsagar N, Leav B, Molrine D, Cheslock P et al (2012) Safety and pharmacokinetics of a human monoclonal antibody to rabies virus: a randomized, dose-escalation phase 1 study in adults. *Vaccine* 30(50):7315–7320. https://doi.org/10.1016/j.vaccine.2012.09.027

Gogtay NJ, Munshi R, Ashwath Narayana DH, Mahendra BJ, Kshirsagar V, Gunale B et al (2018) Comparison of a Novel Human Rabies Monoclonal Antibody to Human Rabies Immunoglobulin for Postexposure Prophylaxis: A Phase 2/3, Randomized, Single-Blind, Noninferiority, Controlled Study. *Clin Infect Dis* 66(3):387–395. https://doi.org/10.1093/cid/cix791

Gongal G, Sampath G (2019) Introduction of intradermal rabies vaccination - A paradigm shift in improving post-exposure prophylaxis in Asia. *Vaccine* 37(Suppl 1):A94–A98. https://doi.org/10.1016/j.vaccine.2018.08.034

González-Roldán JF, Undurraga EA, Meltzer MI, Atkins C, Vargas-Pino F, Gutiérrez-Cedillo V, Hernández-Pérez JR (2021) Cost-effectiveness of the national dog rabies prevention and control program in Mexico, 1990-2015. *PLoS Neglect Trop Dis* 15(3):e0009130. https://doi.org/10.1371/journal.pntd.0009130

Grange ZL, Goldstein T, Johnson CK, Anthony S, Gilardi K, Daszak P et al (2021) Ranking the risk of animal-to-human spillover for newly discovered viruses. *Proc Natl Acad Sci U S A* 118(15):e2002324118. https://doi.org/10.1073/pnas.2002324118

Gruzdev KN (2008) The rabies situation in Central Asia. *Dev Biol (Basel)* 131:37–42

Guerra FM, Bolotin S, Lim G, Heffernan J, Deeks SL, Li Y, Crowcroft NS (2017) The basic reproduction number (R 0) of measles: a systematic review. *Lancet Infect Dis* 17(12):e420–e428. https://doi.org/10.1016/S1473-3099(17)30307-9

Gunawardena PS, Marston DA, Ellis RJ, Wise EL, Karawita AC, Breed AC et al (2016) Lyssavirus in Indian Flying Foxes, Sri Lanka. *Emerg Infect Dis* 22(8):1456–1459. https://doi.org/10.3201/eid2208.151986

Hampson K, Dushoff J, Bingham J, Brückner G, Ali YH, Dobson A (2007) Synchronous cycles of domestic dog rabies in sub-Saharan Africa and the impact of control efforts. *Proc Natl Acad Sci U S A* 104(18):7717–7722. https://doi.org/10.1073/pnas.0609122104

Hampson K, Dobson A, Kaare M, Dushoff J, Magoto M, Sindoya E, Cleaveland S (2008) Rabies exposures, post-exposure prophylaxis and deaths in a region of endemic canine rabies. *PLoS Neglect Trop Dis* 2(11):e339. https://doi.org/10.1371/journal.pntd.0000339

Hampson K, Dushoff J, Cleaveland S, Haydon DT, Kaare M, Packer C, Dobson A (2009) Transmission dynamics and prospects for the elimination of canine rabies. *PLoS Biol* 7(3):e53. https://doi.org/10.1371/journal.pbio.1000053

Hampson K, Cleaveland S, Briggs D (2011) Evaluation of cost-effective strategies for rabies post-exposure vaccination in low-income countries. *PLoS Neglect Trop Dis* 5(3):e982. https://doi.org/10.1371/journal.pntd.0000982

Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M et al (2015) Estimating the global burden of endemic canine rabies. *PLoS Neglect Trop Dis* 9(4):e0003709. https://doi.org/10.1371/journal.pntd.0003709

Hampson K, Abela-Ridder B, Bharti O, Knopf L, Léchenne M, Mindekem R et al (2019) Modelling to inform prophylaxis regimens to prevent human rabies. *Vaccine* 37(Suppl 1):A166–A173. https://doi.org/10.1016/j.vaccine.2018.11.010

Hanke D, Freuling CM, Fischer S, Hueffer K, Hundertmark K, Nadin-Davis S et al (2016) Spatio-temporal Analysis of the Genetic Diversity of Arctic Rabies Viruses and Their Reservoir Hosts in Greenland. *PLoS Neglect Trop Dis* 10(7):e0004779. https://doi.org/10.1371/journal.pntd.0004779

Hanlon CA, Kuzmin IV, Blanton JD, Weldon WC, Manangan JS, Rupprecht CE (2005) Efficacy of rabies biologics against new lyssaviruses from Eurasia. *Virus Res* 111(1):44–54

Harmsen MM, de Haard HJ (2007) Properties, production, and applications of camelid single-domain antibody fragments. *Appl Microbiol Biotechnol* 77(1):13–22. https://doi.org/10.1007/s00253-007-1142-2

Haselbeck AH, Rietmann S, Tadesse BT, Kling K, Kaschubat-Dieudonné ME, Marks F et al (2021) Challenges to the Fight against Rabies-The Landscape of Policy and Prevention Strategies in Africa. Int J Environ Res Public Health 18:1736. https://doi.org/10.3390/ijerph18041736

Hassel R, Vos A, Clausen P, Moore S, van der Westhuizen J, Khaiseb S et al (2018) Experimental screening studies on rabies virus transmission and oral rabies vaccination of the Greater Kudu (Tragelaphus strepsiceros). Sci Rep 8(1):16599. https://doi.org/10.1038/s41598-018-34985-5

Hayman DT, McCrea R, Restif O, Suu-Ire R, Fooks AR, Wood JL et al (2012) Demography of straw-colored fruit bats in Ghana. *J Mammal* 93(5):1393–1404

Head JR, Vos A, Blanton J, Müller T, Chipman R, Pieracci EG et al (2019) Environmental distribution of certain modified live-virus vaccines with a high safety profile presents a low-risk, high-reward to control zoonotic diseases. Sci Rep 9(1):6783. https://doi.org/10.1038/s41598-019-42714-9

Hiby E, Atema KN, Brimley R, Hammond-Seaman A, Jones M, Rowan A et al (2017) Scoping review of indicators and methods of measurement used to evaluate the impact of dog population management interventions. *BMC Vet Res* 13(1):143. https://doi.org/10.1186/s12917-017-1051-2

Horie M, Akashi H, Kawata M, Tomonaga K (2020) Identification of a reptile lyssavirus in Anolis allogus provided novel insights into lyssavirus evolution. *Virus Genes* 57(1):40–49. https://doi.org/10.1007/s11262-020-01803-y

Horton DL, Banyard A, Marston DA, Wise E, Selden D, Nunez A et al (2014) Antigenic and genetic characterisation of a divergent African virus, Ikoma lyssavirus. *J Gen Virol* 95:1025–1032. https://doi.org/10.1099/vir.0.061952-0

Hossain M, Bulbul T, Ahmed K, Ahmed Z, Salimuzzaman M, Haque MS et al (2011) Five-year (January 2004-December 2008) surveillance on animal bite and rabies vaccine utilization in the Infectious Disease Hospital, Dhaka, Bangladesh. *Vaccine* 29(5):1036–1040. https://doi.org/10.1016/j.vaccine.2010.11.052

Hou Q, Jin Z, Ruan S (2012) Dynamics of rabies epidemics and the impact of control efforts in Guangdong Province, China. *J Theor Biol* 300:39–47. https://doi.org/10.1016/j.jtbi.2012.01.006

Hu WT, Willoughby RE Jr, Dhonau H, Mack KJ (2007) Long-term follow-up after treatment of rabies by induction of coma. *New Engl J Med* 357(9):945–946. https://doi.org/10.1056/NEJMc062479

Hu SC, Hsu CL, Lee MS, Tu YC, Chang JC, Wu CH et al (2018) Lyssavirus in Japanese Pipistrelle, Taiwan. *Emerg Infect Dis* 24(4):782–785. https://doi.org/10.3201/eid2404.171696

Huang F, Guan Z, Ahmad W, Qiao B, Song Y, Duan M, Zhang M (2014) Protective efficacy of live attenuated rabies virus against Lethal Challenged Virus Strain (CVS-11) infection in the Central Nervous System (CNS) of mice. *Rev Med Vet* 165(7-8):219–224

Huang F, Ahmad W, Duan M, Liu Z, Guan Z, Zhang M et al (2015) Efficiency of live attenuated and inactivated rabies viruses in prophylactic and post exposure vaccination against the street virus strain. *Acta Virol.* 59(2):117–124. https://doi.org/10.4149/av_2015_02_117

Hudson EG, Brookes VJ, Dürr S, Ward MP (2019) Targeted pre-emptive rabies vaccination strategies in a susceptible domestic dog population with heterogeneous roaming patterns. *Prev Vet Med* 172:104774. https://doi.org/10.1016/j.prevetmed.2019.104774

Hultberg A, Temperton NJ, Rosseels V, Koenders M, Gonzalez-Pajuelo M, Schepens B et al (2011) Llama-derived single domain antibodies to build multivalent, superpotent and broadened neutralizing anti-viral molecules. *PloS one* 6(4):e17665. https://doi.org/10.1371/journal.pone.0017665

Ichhpujani RL, Mala C, Veena M, Singh J, Bhardwaj M, Bhattacharya D et al (2008) Epidemiology of animal bites and rabies cases in India. A multicentric study. *J Commun Dis* 40(1):27–36

Iglesias R, Cox-Witton K, Field H, Skerratt LF, Barrett J (2021) Australian Bat Lyssavirus: Analysis of National Bat Surveillance Data from 2010 to 2016. Viruses 13:189. https://doi.org/10.3390/v13020189

Jackson AC (2008) Rabies. Neurol Clin 26(3):717–726
Jackson AC (2013) Current and future approaches to the therapy of human rabies. *Antiviral Res* 99 (1):61–67. https://doi.org/10.1016/j.antiviral.2013.01.003
Jackson AC, Warrell MJ, Rupprecht CE, Ertl HCJ, Dietzschold B, O'Reilly M et al (2003) Management of rabies in humans. *Clin Infect Dis* 36(1):60–63
Jentes ES, Blanton JD, Johnson KJ, Petersen BW, Lamias MJ, Robertson K et al (2013) The Global Availability of Rabies Immune Globulin and Rabies Vaccine in Clinics Providing Direct Care to Travelers. *J Travel Med* 20(3):148–158. https://doi.org/10.1111/jtm.12024
Jerolmack C (2013) Who's worried about turkeys? How 'organisational silos' impede zoonotic disease surveillance. *Sociol Health Illn* 35(2):200–212. https://doi.org/10.1111/j.1467-9566.2012.01501.x
Jibat T, Hogeveen H, Mourits MCM (2015) Review on dog rabies vaccination coverage in Africa: a question of dog accessibility or cost recovery? *PLoS Neglect Trop Dis* 9(2):e0003447. https://doi.org/10.1371/journal.pntd.0003447
Johnson N, Black C, Smith J, Un H, McElhinney LM, Aylan O, Fooks AR (2003) Rabies emergence among foxes in Turkey. *J Wildl Dis* 39(2):262–270. https://doi.org/10.7589/0090-3558-39.2.262
Johnson N, Un H, Vos A, Aylan O, Fooks AR (2006) Wildlife rabies in Western Turkey. the spread of rabies through the western provinces of Turkey. *Epidemiol Infect* 134(2):369–375. https://doi.org/10.1017/S0950268805005017
Johnson N, Dicker A, Mork T, Marston DA, Fooks AR, Tryland M et al (2007) Phylogenetic comparison of rabies viruses from disease outbreaks on the Svalbard Islands. Vector Borne Zoonotic Dis 7(3):457–460
Johnson N, Freuling C, Vos A, Un H, Valtchovski R, Turcitu M et al (2008) Epidemiology of rabies in Southeast Europe. *Dev Biol (Basel)* 131:189–198
Johnson N, Un H, Fooks AR, Freuling C, Müller T, Aylan O, Vos A (2010) Rabies epidemiology and control in Turkey. past and present. *Epidemiol Infect* 138(3):305–312. https://doi.org/10.1017/S0950268809990963
Johnson N, Arechiga-Ceballos N, Aguilar-Setien A (2014) Vampire bat rabies. ecology, epidemiology and control. *Viruses* 6(5):1911–1928. https://doi.org/10.3390/v6051911
Johnston, D. H.; Tinline, R. R. (2002): Rabies control in wildlife. In: A. C. Jackson und W. H. Wunner (Hg.): Rabies. 1 Band. San Diego: Academic Press Inc., S. 446–471.
Kansagra K, Parmar D, Mendiratta SK, Patel J, Joshi S, Sharma N et al (2020) A Phase 3, Randomised, Open-Label, Non-inferiority Trial Evaluating Anti-Rabies Monoclonal Antibody Cocktail (TwinrabTM) Against Human Rabies Immunoglobulin (HRIG). Clin Infect Dis: ciaa779. https://doi.org/10.1093/cid/ciaa779
Karunanayake D, Matsumoto T, Wimalaratne O, Nanayakkara S, Perera D, Nishizono A, Ahmed K (2014) Twelve years of rabies surveillance in Sri Lanka, 1999-2010. *PLoS Neglect Trop Dis* 8(10):e3205. https://doi.org/10.1371/journal.pntd.0003205
Kessels JA, Recuenco S, Navarro-Vela AM, Deray R, Vigilato M, Ertl H et al (2017) Pre-exposure rabies prophylaxis. a systematic review. *Bull World Health Organ* 95(3):210–219C. https://doi.org/10.2471/BLT.16.173039
Kessels J, Tarantola A, Salahuddin N, Blumberg L, Knopf L (2019) Rabies post-exposure prophylaxis: a systematic review on abridged vaccination schedules and the effect of changing administration routes during a single course. *Vaccine* 37(Suppl 1):A107–A117. https://doi.org/10.1016/j.vaccine.2019.01.041
Kgaladi J, Faber M, Dietzschold B, Nel LH, Markotter W (2017) Pathogenicity and Immunogenicity of Recombinant Rabies Viruses Expressing the Lagos Bat Virus Matrix and Glycoprotein: Perspectives for a Pan-Lyssavirus Vaccine. Trop Med Infect Dis 2(3):37. https://doi.org/10.3390/tropicalmed2030037
Khawplod P, Jaijaroensup W, Sawangvaree A, Prakongsri S, Wilde H (2012) One clinic visit for pre-exposure rabies vaccination (a preliminary one year study). *Vaccine* 30(19):2918–2920. https://doi.org/10.1016/j.vaccine.2011.12.028
Kilic B, Unal B, Semin S, Konakci SK (2006) An important public health problem. rabies suspected bites and post-exposure prophylaxis in a health district in Turkey. *Int J Infect Dis* 10(3):248–254

King AA, Meredith CD, Thomson GR (1993) Canid and viverrid rabies viruses in South Africa. *Onderstepoort J Vet Res* 60(4):295–299

Kitala P, McDermott J, Kyule M, Gathuma JM, Perry B, Wandeler A (2001) Dog ecology and demography information to support the planning of rabies control in Machakos District, Kenya. *Acta Trop* 78:217–230

Kitala PM, McDermott JJ, Coleman PG, Dye C (2002) Comparison of vaccination strategies for the control of dog rabies in Machakos District, Kenya. *Epidemiol Infect* 129(1):215–222

Klein A, Fahrion A, Finke S, Eyngor M, Novak S, Yakobson B et al (2020) Further Evidence of Inadequate Quality in Lateral Flow Devices Commercially Offered for the Diagnosis of Rabies. *Trop Med Infect Dis* 5(1). https://doi.org/10.3390/tropicalmed5010013

Knobel DL, Cleaveland S, Coleman PG, Fevre EM, Meltzer MI, Miranda ME et al (2005) Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 83(5):360–368

Kole AK, Roy R, Kole DC (2014) Human rabies in India: a problem needing more attention. Bull World Health Organ 92(4):230. https://doi.org/10.2471/BLT.14.136044

Krystosik A, Njoroge G, Odhiambo L, Forsyth JE, Mutuku F, LaBeaud AD (2020) Solid wastes provide breeding sites, burrows, and food for biological disease vectors, and urban zoonotic reservoirs: a call to action for solutions-based research. *Front Public Health* 7:405. https://doi.org/10.3389/fpubh.2019.00405

Kuhn JH, Adkins S, Alioto D, Alkhovsky SV, Amarasinghe GK, Anthony SJ et al (2020) 2020 taxonomic update for phylum Negarnaviricota (Riboviria: Orthornavirae), including the large orders Bunyavirales and Mononegavirales. *Arch Virol* 165(12):3023–3072. https://doi.org/10.1007/s00705-020-04731-2

Kularatne SA, Ralapanawa DM, Weerakoon K, Bokalamulla UK, Abagaspitiya N (2016) Pattern of animal bites and post exposure prophylaxis in rabies: A five year study in a tertiary care unit in Sri Lanka. *BMC Infect Dis* 16:62. https://doi.org/10.1186/s12879-016-1394-5

Kuzmin IV, Botvinkin AD, McElhinney LM, Smith JS, Orciari LA, Hughes GJ et al (2004) Molecular epidemiology of terrestrial rabies in the former Soviet Union. J Wildl Dis 40(4):617–631

Kuzmin IV, Hughes GJ, Botvinkin AD, Orciari LA, Rupprecht CE (2005) Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Res* 111(1):28–43

Kuzmin IV, Novella IS, Dietzgen RG, Padhi A, Rupprecht CE (2009) The rhabdoviruses: Biodiversity, phylogenetics, and evolution. *Infect Genet Evol*:9

Kuzmin IV, Shi M, Orciari LA, Yager PA, Velasco-Villa A, Kuzmina NA et al (2012) Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001-2009. *PLoS Pathog* 8(6):e1002786. https://doi.org/10.1371/journal.ppat.1002786

Laager M, Léchenne M, Naissengar K, Mindekem R, Oussiguere A, Zinsstag J, Chitnis N (2019) A metapopulation model of dog rabies transmission in N'Djamena, Chad. *J Theor Biol* 462:408–417. https://doi.org/10.1016/j.jtbi.2018.11.027

Laing G, Vigilato MAN, Cleaveland S, Thumbi SM, Blumberg L, Salahuddin N et al (2020) One Health for neglected tropical diseases. Trans R Soc Trop Med Hyg 115(2):182–184. https://doi.org/10.1093/trstmh/traa117

Lan YC, Wen TH, Chang CC, Liu HF, Lee PF, Huang CY et al (2017) Indigenous Wildlife Rabies in Taiwan. Ferret Badgers, a Long Term Terrestrial Reservoir. BioMed Res Int:5491640. https://doi.org/10.1155/2017/5491640

Lapiz SM, Miranda ME, Garcia RG, Daguro LI, Paman MD, Madrinan FP et al (2012) Implementation of an intersectoral program to eliminate human and canine rabies. the Bohol Rabies Prevention and Elimination Project. PLoS Neglect Trop Dis 6(12):e1891

Layan M, Dellicour S, Baele G, Cauchemez S, Bourhy H (2021) Mathematical modelling and phylodynamics for the study of dog rabies dynamics and control: A scoping review. *PLoS Neglect Trop Dis* 15(5):e0009449. https://doi.org/10.1371/journal.pntd.0009449

Le Roux K, Stewart D, Perrett KD, Nel LH, Kessels JA, Abela-Ridder B (2018) Rabies control in KwaZulu-Natal, South Africa. *Bull World Health Organ* 96(5):360–365. https://doi.org/10.2471/blt.17.194886

Lechenne M, Oussiguere A, Naissengar K, Mindekem R, Mosimann L, Rives G et al (2016) Operational performance and analysis of two rabies vaccination campaigns in N'Djamena, Chad. Vaccine 34(4):571–577. https://doi.org/10.1016/j.vaccine.2015.11.033

Léchenne M, Naïssengar K, Lepelletier A, Alfaroukh IO, Bourhy H, Zinsstag J, Dacheux L (2016) Validation of a Rapid Rabies Diagnostic Tool for Field Surveillance in Developing Countries. *PLoS Neglect Trop Dis* 10(10):e0005010. https://doi.org/10.1371/journal.pntd.0005010

Lechenne M, Mindekem R, Madjadinan S, Oussiguéré A, Moto DD, Naissengar K, Zinsstag J (2017) The Importance of a Participatory and Integrated One Health Approach for Rabies Control. The Case of N'Djaména, Chad. Trop Med Infect Dis 2(3):43

Léchenne M, Traore A, Hattendorf J, Kallo V, Oussiguere A, Tetchi M et al (2021) Increasing rabies data availability: The example of a One Health research project in Chad, Côte d'Ivoire and Mali. *Acta Trop* 215:105808. https://doi.org/10.1016/j.actatropica.2020.105808

Ledesma LA, Lemos ERS, Horta MA (2020) Comparing clinical protocols for the treatment of human rabies: the Milwaukee protocol and the Brazilian protocol (Recife). *Rev Soc Bras Med Trop* 53:e20200352. https://doi.org/10.1590/0037-8682-0352-2020

Lembo T, Hampson K, Kaare MT, Ernest E, Knobel D, Kazwala RR et al (2010) The feasibility of canine rabies elimination in Africa. dispelling doubts with data. PLoS Neglect Trop Dis 4(2): e626. https://doi.org/10.1371/journal.pntd.0000626

Leonova GN, Belikov SI, Kondratov IG, Krylova NV, Pavlenko EV, Romanova EV et al (2009) A fatal case of bat lyssavirus infection in Primorye Territory of the Russian Far East. *Rabies Bull Eur* 33(4):5–8

Leslie MJ, Messenger S, Rohde RE, Smith J, Cheshier R, Hanlon C, Rupprecht CE (2006) Bat-associated rabies virus in skunks. *Emerg Infect Dis* 12(8):1274–1277

Leung T, Davis SA (2017) Rabies Vaccination Targets for Stray Dog Populations. *Front Vet Sci* 4: 52. https://doi.org/10.3389/fvets.2017.00052

Li AJ, Sreenivasan N, Siddiqi UR, Tahmina S, Penjor K, Sovann L et al (2019) Descriptive assessment of rabies post-exposure prophylaxis procurement, distribution, monitoring, and reporting in four Asian countries: Bangladesh, Bhutan, Cambodia, and Sri Lanka, 2017-2018. *Vaccine* 37(Suppl 1):A14–A19. https://doi.org/10.1016/j.vaccine.2018.10.011

Lingappa UF, Wu X, Macieik A, Yu SF, Atuegbu A, Corpuz M et al (2013) Host-rabies virus protein-protein interactions as druggable antiviral targets. Proc Natl Acad Sci U S A 110(10): E861–E868. https://doi.org/10.1073/pnas.1210198110

Linhart SB, King R, Zamir S, Naveh U, Davidson M, Perl S (1997) Oral rabies vaccination of red foxes and golden jackals in Israel. preliminary bait evaluation. *Rev Sci Tech* 16(3):874–880

Lionel Harischandra PA, Gunesekera A, Janakan N, Gongal G, Abela-Ridder B (2016) Sri Lanka takes action towards a target of zero rabies death by 2020. WHO South East Asia J Public Health 5(2):113–116. https://doi.org/10.4103/2224-3151.206247

Liu Y, Chen Q, Zhang F, Zhang S, Li N, Lian H et al (2013a) Evaluation of rabies biologics against Irkut virus isolated in China. *J Clin Microbiol* 51(11):3499–3504. https://doi.org/10.1128/JCM.01565-13

Liu Y, Zhang SF, Zhao JH, Zhang F, Hu RL (2013b) Isolation of Irkut Virus from a Murina leucogaster Bat in China. PLoS Neglect Trop Dis 7(3):e2097. https://doi.org/10.1371/journal.pntd.0002097

Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, Pulliam JR et al (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses. are bats special? Proc R Soc B Biol Sci 280 (1756):20122753. https://doi.org/10.1098/rspb.2012.2753

Lushasi K, Steenson R, Bernard J, Changalucha JJ, Govella NJ, Haydon DT et al (2020) One Health in Practice: Using Integrated Bite Case Management to Increase Detection of Rabid Animals in Tanzania. *Front Public Health* 8:13. https://doi.org/10.3389/fpubh.2020.00013

Ly S, Buchy P, Heng NY, Ong S, Chhor N, Bourhy H, Vong S (2009) Rabies situation in Cambodia. PLoS Neglect Trop Dis 3(9):511

Ma X, Monroe BP, Cleaton JM, Orciari LA, Gigante CM, Kirby JD et al (2020) Public Veterinary Medicine: Public Health: Rabies surveillance in the United States during 2018. J Am Vet Med Assoc 256(2):195–208. https://doi.org/10.2460/javma.256.2.195

Ma X, Monroe BP, Wallace RM, Orciari LA, Gigante CM, Kirby JD et al (2021) Rabies surveillance in the United States during 2019. *J Am Vet Med Assoc* 258(11):1205–1220. https://doi.org/10.2460/javma.258.11.1205

MacInnes CD, Smith SM, Tinline RR, Ayers NR, Bachmann P, Ball DG, Calder LA, Crosgrey SJ, Fielding C, Hauschildt P, Honig JM, Johnston DH, Lawson KF, Nunan CP, Pedde MA, Pond B, Stewart RB, Voigt DR (2001) Elimination of rabies from red foxes in eastern Ontario. *J Wildl Dis* 37(1):119–132. https://doi.org/10.7589/0090-3558-37.1.119.

Madjadinan A, Hattendorf J, Mindekem R, Mbaipago N, Moyengar R, Gerber F et al (2020) Identification of risk factors for rabies exposure and access to post-exposure prophylaxis in Chad. *Acta Trop* 209:105484. https://doi.org/10.1016/j.actatropica.2020.105484

Maki J, Guiot AL, Aubert M, Brochier B, Cliquet F, Hanlon CA et al (2017) Oral vaccination of wildlife using a vaccinia-rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG(®)). a global review. *Vet Res* 48(1):57. https://doi.org/10.1186/s13567-017-0459-9

Malerczyk C, Selhorst T, Tordo N, Moore SA, Müller T (2009) Antibodies induced by vaccination with purified chick embryo cell culture vaccine (PCECV) cross-neutralize non-classical bat lyssavirus strains. *Vaccine* 27(39):5320–5325

Malerczyk C, Freuling C, Gniel D, Giesen A, Selhorst T, Müller T (2014) Cross-neutralization of antibodies induced by vaccination with Purified Chick Embryo Cell Vaccine (PCECV) against different Lyssavirus species. *Hum Vaccin Immunother* 10(10):2799–2804. https://doi.org/10.4161/21645515.2014.972741

Mandra A, Morán D, Santana PV, La de Marrero MC, Díaz E, Gil M et al (2019) Notes from the Field: Rabies Outbreak Investigation - Pedernales, Dominican Republic, 2019. MMWR Morb Mortal Wkly Rep 68(32):704–706. https://doi.org/10.15585/mmwr.mm6832a5

Mangen M-JJ, Batz MB, Käsbohrer A, Hald T, Morris JG, Taylor M, Havelaar AH (2010) Integrated approaches for the public health prioritization of foodborne and zoonotic pathogens. *Risk Anal* 30(5):782–797. https://doi.org/10.1111/j.1539-6924.2009.01291.x

Mansfield KL, McElhinney L, Hübschle O, Mettler F, Sabeta C, Nel LH, Fooks AR (2006a) A molecular epidemiological study of rabies epizooties in kudu (Tragelaphus strepsiceros) in Namibia. *BMC Vet Res* 2:2

Mansfield KL, Racloz V, McElhinney LM, Marston DA, Johnson N, Ronsholt L et al (2006b) Molecular epidemiological study of Arctic rabies virus isolates from Greenland and comparison with isolates from throughout the Arctic and Baltic regions. *Virus Res* 116(1-2):1–10. https://doi.org/10.1016/j.virusres.2005.08.007

Mariner JC, Roeder PL (2003) Use of participatory epidemiology in studies of the persistence of lineage 2 rinderpest virus in East Africa. *Vet Rec* 152(21):641–647

Mariner JC, House JA, Mebus CA, Sollod AE, Chibeu D, Jones BA et al (2012) Rinderpest eradication. appropriate technology and social innovations. Science (New York, N.Y.) 337 (6100):1309–1312

Markotter W, Coertse J, de Vries L, Geldenhuys M, Mortlock M (2020) Bat-borne viruses in Africa: a critical review. *J Zool* 311:77–98. https://doi.org/10.1111/jzo.12769

Marston DA, Horton DL, Ngeleja C, Hampson K, McElhinney LM, Banyard AC et al (2012) Ikoma Lyssavirus, Highly Divergent Novel Lyssavirus in an African Civet. *Emerg Infect Dis* 18(4): 664–667. https://doi.org/10.3201/eid1804.111553

Marston DA, Horton DL, Nunez J, Ellis RJ, Orton RJ, Johnson N et al (2017) Genetic analysis of a rabies virus host shift event reveals within-host viral dynamics in a new host. *Virus Evol* 3(2): vex038. https://doi.org/10.1093/ve/vex038

Marston DA, Banyard AC, McElhinney LM, Freuling CM, Finke S, de Lamballerie X et al (2018) The lyssavirus host-specificity conundrum — rabies virus — the exception not the rule. *Curr Opin Virol* 28:68–73. https://doi.org/10.1016/j.coviro.2017.11.007

Mauti S, Léchenne M, Naïssengar S, Traoré A, Kallo V, Kouakou C et al (2020) Field Postmortem Rabies Rapid Immunochromatographic Diagnostic Test for Resource-Limited Settings with Further Molecular Applications. *J Vis Exp* 160:e60008. https://doi.org/10.3791/60008

Mazeri S, Bailey B, Jordana L, Mayer D, Chikungwa P, Chulu J, Grossman PO et al (2021) Using data-driven approaches to improve delivery of animal health care interventions for public health. *Proc Natl Acad Sci U S A* 118(5):e2003722118. https://doi.org/10.1073/pnas.2003722118

Mazigo HD, Okumu FO, Kweka EJ, Mnyone LL (2010) Retrospective analysis of suspected rabies cases reported at bugando referral hospital, mwanza, Tanzania. *J Glob Infect Dis* 2(3):216–220

McElhinney LM, Marston DA, Wise EL, Freuling CM, Bourhy H, Zanoni R et al (2018) Molecular Epidemiology and Evolution of European Bat Lyssavirus 2. *Int J Mol Sci* 19(1):S. 156. https://doi.org/10.3390/ijms19010156

Medley A, Millien M, Blanton J, Ma X, Augustin P, Crowdis K, Wallace R (2017) Retrospective Cohort Study to Assess the Risk of Rabies in Biting Dogs, 2013–2015, Republic of Haiti. Trop Med Infect Dis 2(2):14

Meeting of the Strategic Advisory Group of Experts on immunization (2017) conclusions and recommendations. *Wkly Epidemiol Rec* 92(48):729–747

Meslin, F. X.; Kaplan, M. M.; Koprowski, H. (Hg.) (1999): Laboratory Techniques in Rabies. 4. Geneva: World Health Organization.

Mills DJ, Lau CL, Mills C, Furuya-Kanamori L (2021) Efficacy of one-dose intramuscular rabies vaccine as pre-exposure prophylaxis in travellers. *J Travel Med* 28(5):taab059. https://doi.org/10.1093/jtm/taab059

Minghui R, Stone M, Semedo MH, Nel L (2018) New global strategic plan to eliminate dog-mediated rabies by 2030. *Lancet Glob Health* 6(8):e828–e829. https://doi.org/10.1016/S2214-109X(18)30302-4

Miranda, Mary Elizabeth G.; Miranda, Noel Lee J. (2020): Rabies Prevention in Asia: Institutionalizing Implementation Capacities. In: Hildegund C. J. Ertl (Hg.): Rabies and rabies vaccines. Cham: Springer, S. 103–116.

Mohammadi D (2016) Moves to consign rabies to history. *Lancet Infect Dis* 16(10):1115–1116. https://doi.org/10.1016/S1473-3099(16)30342-5

Mollentze N, Biek R, Streicker DG (2014) The role of viral evolution in rabies host shifts and emergence. *Curr Opin Virol* 8:68–72. https://doi.org/10.1016/j.coviro.2014.07.004

Mollentze N, Streicker DG, Murcia PR, Hampson K, Biek R (2020) Virulence mismatches in index hosts shape the outcomes of cross-species transmission. *Proc Natl Acad Sci U S A* 117(46):28859–28866. https://doi.org/10.1073/pnas.2006778117

Molyneux D, Hallaj Z, Keusch GT, McManus DP, Ngowi H, Cleaveland S et al (2011) Zoonoses and marginalised infectious diseases of poverty. where do we stand? *Parasites Vectors* 4:106

Mork T, Prestrud P (2004) Arctic Rabies - A Review. *Acta Vet Scand* 45(1):1–9

Morters MK, McKinley TJ, Horton DL, Cleaveland S, Schoeman JP, Restif O et al (2014) Achieving population-level immunity to rabies in free-roaming dogs in Africa and Asia. *PLoS Neglect Trop Dis* 8(11):e3160. https://doi.org/10.1371/journal.pntd.0003160

Mpolya EA, Lembo T, Lushasi K, Mancy R, Mbunda EM, Makungu S et al (2017) Toward Elimination of Dog-Mediated Human Rabies. Experiences from Implementing a Large-scale Demonstration Project in Southern Tanzania. *Front Vet Sci* 4:S. 21. https://doi.org/10.3389/fvets.2017.00021

Mtema Z, Changalucha J, Cleaveland S, Elias M, Ferguson HM, Halliday JE et al (2016) Mobile Phones As Surveillance Tools. Implementing and Evaluating a Large-Scale Intersectoral Surveillance System for Rabies in Tanzania. *PLoS Med* 13(4):e1002002. https://doi.org/10.1371/journal.pmed.1002002

Müller T, Freuling CM (2018) Rabies control in Europe. an overview of past, current and future strategies. *Rev Sci Tech* 37(2):409–419. https://doi.org/10.20506/rst.37.2.2811

Müller, T.; Freuling, C. M. (2020a): Rabies in terrestrial animals. In: A. R. Fooks und A. C. Jackson (Hg.): Rabies - Scientific Basis of the Disease and its Management. 4. Aufl.: Elsevier Academic Press, S. 195–230.

Müller, T.; Freuling, C. M. (2020b): Rabies Vaccines for Wildlife. In: Hildegund C. J. Ertl (Hg.): Rabies and rabies vaccines. Cham: Springer, S. 45–70.

Müller T, Dietzschold B, Ertl H, Fooks AR, Freuling C, Fehlner-Gardiner C et al (2009) Development of a mouse monoclonal antibody cocktail for post-exposure rabies prophylaxis in humans. *PLoS Neglect Trop Dis* 3(11):e542

Müller, T.; Demetriou, P.; Moynagh, J.; Cliquet, F.; Fooks, A. R.; Conraths, F. J. et al. (2012): Rabies elimination in Europe – A success story. In: A. R. Fooks und T. Müller (Hg.): Rabies Control - Towards Sustainable Prevention at the Source, Compendium of the OIE Global Conference on Rabies Control, Incheon-Seoul, 7-9 September 2011, Republic of Korea. Paris: OIE, S. 31–44.

Müller T, Freuling CM, Wysocki P, Roumiantzeff M, Freney J, Mettenleiter TC, Vos A (2015) Terrestrial rabies control in the European Union. historical achievements and challenges ahead. *Vet J* 203(1):10–17. https://doi.org/10.1016/j.tvjl.2014.10.026

Munyua P, Bitek A, Osoro E, Pieracci EG, Muema J, Mwatondo A et al (2016) Prioritization of Zoonotic Diseases in Kenya, 2015. *PloS one* 11(8):e0161576. https://doi.org/10.1371/journal.pone.0161576

Muthiani Y, Traoré A, Mauti S, Zinsstag J, Hattendorf J (2015) Low coverage of central point vaccination against dog rabies in Bamako, Mali. *Prev Vet Med* 120(2):203–209. https://doi.org/10.1016/j.prevetmed.2015.04.007

Nadeem M, Panda PK (2020) Survival in human rabies but left against medical advice and death followed - Community education is the need of the hour. *J Family Med Prim Care* 9(3):1736–1740. https://doi.org/10.4103/jfmpc.jfmpc_1079_19

Nadin-Davis SA, Muldoon F, Wandeler AI (2006a) Persistence of genetic variants of the arctic fox strain of Rabies virus in southern Ontario. *Can J Vet Res* 70(1):11–19

Nadin-Davis SA, Torres G, Ribas ML, Guzman M, De La Paz RC, Morales M, Wandeler AI (2006b) A molecular epidemiological study of rabies in Cuba. *Epidemiol Infect* 134(6):1313–1324

Nel LH, Sabeta CT, von Teichman B, Jaftha JB, Rupprecht CE, Bingham J (2005) Mongoose rabies in southern Africa. a re-evaluation based on molecular epidemiology. *Virus Res* 109(2):165–173

Neville, J. (2004): Rabies in the ancient world. In: A. A. King, A. R. Fooks, M. Aubert und A. I. Wandeler (Hg.): Historical perspective of rabies in Europe and the Mediterranean Basin. Paris: OIE, S. 1–13.

Ngugi JN, Maza AK, Omolo OJ, Obonyo M (2018) Epidemiology and surveillance of human animal-bite injuries and rabies post-exposure prophylaxis, in selected counties in Kenya, 2011-2016. BMC Public Health 18(1):996. https://doi.org/10.1186/s12889-018-5888-5

Nokireki T, Tammiranta N, Kokkonen U-M, Kantala T, Gadd T (2018) Tentative novel lyssavirus in a bat in Finland. *Transbound Emerg Dis* 65:593–596. https://doi.org/10.1111/tbed.12833

O'Brien KL, Nolan T, Sage Wg on Rabies (2019) The WHO position on rabies immunization - 2018 updates. *Vaccine* 37(Suppl 1):A85–A87. https://doi.org/10.1016/j.vaccine.2018.10.014

O'Sullivan A, Willoughby RE, Mishchuk D, Alcarraz B, Cabezas-Sanchez C, Condori RE et al (2013) Metabolomics of Cerebrospinal Fluid from Humans Treated for Rabies. *J Proteome Res* 12(1):481–490. https://doi.org/10.1021/pr3009176

Office International des Epizooties (2021) Chapter 3.1.17, Rabies (Infection with Rabies virus and other Lyssaviruses). In: OIE (Hg.): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Unter Mitarbeit von OIE. 2 Bände. Paris. Online verfügbar unter https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.17_RABIES.pdf, zuletzt geprüft am 20.07.2021

Ortmann S, Vos A, Kretzschmar A, Walther N, Kaiser C, Freuling C, Lojkic I, Müller T (2018) Safety studies with the oral rabies virus vaccine strain SPBN GASGAS in the small Indian mongoose (Herpestes auropunctatus). *BMC Veterinary Research* 14(1):90 https://doi.org/10.1186/s12917-018-1417-0

Pancharoen C, Thisyakorn U, Lawtongkum W, Wilde H (2001a) Rabies exposures in thai children. *Wilderness Environ Med* 12(4):239–243

Pancharoen C, Thisyakorn U, Tantawichien T, Jaijaroensup W, Khawplod P, Wilde H (2001b) Failure of pre- and postexposure rabies vaccinations in a child infected with HIV. *Scand J Infect Dis* 33(5):390–391

Perry BD (1993) Dog ecology in eastern and southern Africa: implications for rabies control. *Onderstepoort J Vet Res* 60(4):429–436

Pieracci EG, Scott TP, Coetzer A, Athman M, Mutembei A, Kidane AH et al (2017) The Formation of the Eastern Africa Rabies Network: A Sub-Regional Approach to Rabies Elimination. *Trop Med Infect Dis* 2(3):29. https://doi.org/10.3390/tropicalmed2030029

Pudjiatmoko P, Kadun IN (2013) Integrated Bite Case Management (IBCM) in Bali: One Health in Action. Prince Mahidol Award Conference, Bangkok

Quiambao BP, Dimaano EM, Ambas C, Davis R, Banzhoff A, Malerczyk C (2005) Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals. *Vaccine* 23(14):1709–1714

Quiambao BP, Dy-Tioco HZ, Dizon RM, Crisostomo ME, Teuwen DE (2009) Rabies post-exposure prophylaxis with purified equine rabies immunoglobulin. One-year follow-up of patients with laboratory-confirmed category III rabies exposure in the Philippines. *Vaccine* 27 (51):7162–7166. https://doi.org/10.1016/j.vaccine.2009.09.036

Robardet E, Bosnjak D, Englund L, Demetriou P, Martín PR, Cliquet F (2019) Zero Endemic Cases of Wildlife Rabies (Classical Rabies Virus, RABV) in the European Union by 2020: An Achievable Goal. *Trop Med Infect Dis* 4:124. https://doi.org/10.3390/tropicalmed4040124

Robertson K, Recuenco S, Niezgoda M, Garcia EJ, Rupprecht CE (2010) Seroconversion following incomplete human rabies postexposure prophylaxis. *Vaccine* 28(39):6523–6526. https://doi.org/10.1016/j.vaccine.2010.06.102

Rohde RE, Rupprecht CE (2020) Update on lyssaviruses and rabies: will past progress play as prologue in the near term towards future elimination? *Fac Rev* 9:S. 9. https://doi.org/10.12703/b/9-9

Rosatte, R. C. (2013): Rabies Control in Wild Carnivores. In: A. C. Jackson und W. Wunner (Hg.): Rabies, Bd. 3. New York: Academic Press, S. 617–670.

Rosatte RC, Howard DR, Campbell JB, MacInnes CD (1990) Intramuscular vaccination of skunks and raccoons against rabies. *J Wildl Dis* 26(2):225–230

Rupprecht, C. E.; Plotkin, S. A. (2013): Rabies vaccines. In: S. Plotkin, W. Orenstein und P. Offit (Hg.): Vaccines. 6 Elsevier, S. 646–668.

Rupprecht CE, Salahuddin N (2019) Current status of human rabies prevention. remaining barriers to global biologics accessibility and disease elimination. *Expert Rev Vaccines* 18(6):629–640. https://doi.org/10.1080/14760584.2019.1627205

Rupprecht, C. E.; Stöhr, K.; Meredith, C. (2001): Rabies. In: E. S. Williams und K. Barker (Hg.): Infectious diseases of wild mammals. Ames: Iowa State University Press, S. 3–36.

Rupprecht CE, Willoughby R, Slate D (2006) Current and future trends in the prevention, treatment and control of rabies. *Expert Rev Anti Infect Ther* 4(6):1021–1038

Rupprecht CE, Barrett J, Briggs D, Cliquet F, Fooks AR, Lumlertdacha B et al (2008) Can rabies be eradicated? *Dev Biol (Basel)* 131:95–121

Rupprecht CE, Briggs D, Brown CM, Franka R, Katz SL, Kerr HD et al (2009) Evidence for a 4-dose vaccine schedule for human rabies post-exposure prophylaxis in previously non-vaccinated individuals. *Vaccine* 27(51):7141–7148. https://doi.org/10.1016/j.vaccine.2009.09.029

Rupprecht CE, Briggs D, Brown CM, Franka R, Katz SL, Kerr HD et al (2010) Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. *MMWR Recomm Rep* 59(RR-2): 1–9

Rupprecht C, Kuzmin I, Meslin F (2017) Lyssaviruses and rabies. current conundrums, concerns, contradictions and controversies. *F1000Research* 6:184. https://doi.org/10.12688/f1000research.10416.1

Rupprecht, Charles E.; Freuling, Conrad M.; Mani, Reeta S.; Palacios, Carlos; Sabeta, Claude T.; Ward, Michael (2020a): A history of rabies—The foundation for global canine rabies elimination. In: A. R. Fooks und A. C. Jackson (Hg.): Rabies - Scientific Basis of the Disease and its Management. 4. Aufl.: Elsevier Academic Press, S. 1–42.

Rupprecht, Charles E.; Yager, Mary L.; Newhouse, Richard H. (2020b): Passive Immunity in Rabies Prophylaxis. In: Hildegund C. J. Ertl (Hg.): Rabies and rabies vaccines. Cham: Springer, S. 117–139.

Sabeta C, Ngoepe EC (2018) Controlling dog rabies in Africa: successes, failures and prospects for the future. *Rev Sci Tech* 37(2):439–449. https://doi.org/10.20506/rst.37.2.2813

Sabeta C, Phahladira B (2013) Mokola virus (MOKV) in southern Africa. A review of genetic, epidemiologic and surveillance studies. iConcept Press

Sabeta CT, Bingham J, Nel LH (2003) Molecular epidemiology of canid rabies in Zimbabwe and South Africa. *Virus Res* 91(2):203–211

Salyer SJ, Silver R, Simone K, Behravesh B, Casey (2017) Prioritizing Zoonoses for Global Health Capacity Building-Themes from One Health Zoonotic Disease Workshops in 7 Countries, 2014-2016. *Emerg Infect Dis* 23(13):S57–S64. https://doi.org/10.3201/eid2313.170418

Sambo M, Johnson PC, Hotopp K, Changalucha J, Cleaveland S, Kazwala R et al (2017) Comparing Methods of Assessing Dog Rabies Vaccination Coverage in Rural and Urban Communities in Tanzania. *Front Vet Sci* 4:33. https://doi.org/10.3389/fvets.2017.00033

Sánchez-Soriano C, Gibson AD, Gamble L, Bailey B, Jordana L, Green S, Green M et al (2019) Development of a high number, high coverage dog rabies vaccination programme in Sri Lanka. *BMC Infect Dis* 19(1):977. https://doi.org/10.1186/s12879-019-4585-z

Saunders KO (2019) Conceptual approaches to modulating antibody effector functions and circulation half-life. *Front Immunol* 10:1296. https://doi.org/10.3389/fimmu.2019.01296

Schatz J, Fooks AR, McElhinney L, Horton D, Echevarria J, Vazquez-Moron S et al (2013) Bat Rabies Surveillance in Europe. *Zoonoses Public Health* 60(1):22–34. https://doi.org/10.1111/zph.12002

Schneider MC, Belotto A, Adé MP, Hendrickx S, Leanes LF, Rodrigues MJ et al (2007) Current status of human rabies transmitted by dogs in Latin America. *Cadernos de saude publica* 23(9):2049–2063

Scott TP, Hassel R, Nel L (2012) Rabies in kudu (*Tragelaphus strepsiceros*). *Berl Munch Tierarztl Wochenschr* 125(5-6):236–241

Scott TP, Coetzer A, de Balogh K, Wright N, Nel LH (2015) The Pan-African Rabies Control Network (PARACON). A unified approach to eliminating canine rabies in Africa. *Antiviral Res* 124:93–100. https://doi.org/10.1016/j.antiviral.2015.10.002

Scott TP, Coetzer A, Fahrion AS, Nel LH (2017) Addressing the Disconnect between the Estimated, Reported, and True Rabies Data. The Development of a Regional African Rabies Bulletin. *Front Vet Sci* 4:S. 18. https://doi.org/10.3389/fvets.2017.00018

Seetahal JF, Velasco-Villa A, Allicock OM, Adesiyun AA, Bissessar J, Amour K et al (2013) Evolutionary history and phylogeography of rabies viruses associated with outbreaks in Trinidad. *PLoS Neglect Trop Dis* 7(8):e2365. https://doi.org/10.1371/journal.pntd.0002365

Seetahal J, Vokaty A, Vigilato M, Carrington C, Pradel J, Louison B et al (2018) Rabies in the Caribbean. A Situational Analysis and Historic Review. Trop Med Infect Dis 3(3):89

Seifman R, Kaplan B (2021) Seismic shift by G7 in recognizing One Health as Critical to Everyone's Health. Online verfügbar unter https://impakter.com/g7-recognizing-one-health/, zuletzt geprüft am 30.07.2021

Sekamatte M, Krishnasamy V, Bulage L, Kihembo C, Nantima N, Monje F et al (2018) Multisectoral prioritization of zoonotic diseases in Uganda, 2017: A One Health perspective. *PloS one* 13(5):e0196799. https://doi.org/10.1371/journal.pone.0196799

Servat A, Wasniewski M, Cliquet F (2019) Cross-protection of inactivated rabies vaccines for veterinary use against bat lyssaviruses occurring in Europe. *Viruses* 11(10):936. https://doi.org/10.3390/v11100936

Setien AA, Brochier B, Tordo N, de Paz O, Desmettre P, Peharpre D, Pastoret PP (1998) Experimental rabies infection and oral vaccination in vampire bats (*Desmodus rotundus*). *Vaccine* 16(11-12):1122–1126

Shantavasinkul, P.; Wilde, H. (2011): Postexposure Prophylaxis for Rabies in Resource-Limited/Poor Countries. In: A. C. Jackson (Hg.): Adv Virus Res, Bd. 79. Amsterdam: Elsevier, S. 291–307.

Shantavasinkul P, Tantawichien T, Jaijaroensup W, Lertjarutorn S, Banjongkasaena A, Wilde H, Sitprija V (2010a) A 4-Site, Single-Visit Intradermal Postexposure Prophylaxis Regimen for Previously Vaccinated Patients. Experiences with > 5000 Patients. *Clin Infect Dis* 51(9):1070–1072. https://doi.org/10.1086/656585

Shantavasinkul P, Tantawichien T, Wacharapluesadee S, Jeamanukoolkit A, Udomchaisakul P, Chattranukulchai P et al (2010b) Failure of rabies postexposure prophylaxis in patients

presenting with unusual manifestations. *Clin Infect Dis* 50(1):77–79. https://doi.org/10.1086/649873

Shantavasinkul P, Tantawichien T, Wilde H, Sawangvaree A, Kumchat A, Ruksaket N et al (2010c) Postexposure Rabies Prophylaxis Completed in 1 Week. Preliminary Study. *Clin Infect Dis* 50 (1):56–60. https://doi.org/10.1086/649211

Shulpin MI, Nazarov NA, Chupin SA, Korennoy FI, Metlin AY, Mischenko AV (2018) Rabies surveillance in the Russian Federation. *Rev Sci Tech* 37(2):483–495. https://doi.org/10.20506/rst.37.2.2817

Shwiff S, Hampson K, Anderson A (2013) Potential economic benefits of eliminating canine rabies. *Antiviral Res* 98(2):352–356. https://doi.org/10.1016/j.antiviral.2013.03.004

Shwiff SA, Brown VR, Dao TT, Elser J, Trung HX, Tien NN et al (2018) Estimating the economic impact of canine rabies to Viet Nam 2005-2014. *PLoS Neglect Trop Dis* 12(10):e0006866. https://doi.org/10.1371/journal.pntd.0006866

Sidwa TJ, Wilson PJ, Moore GM, Oertli EH, Hicks BN, Rohde RE, Johnston DH (2005) Evaluation of oral rabies vaccination programs for control of rabies epizootics in coyotes and gray foxes: 1995–2003. *J American Veterinary Medical Association* 227(5):785–792 https://doi.org/10.2460/javma.2005.227.785

Silva ML, Rodrigues C, da Lima FS, de Gomes AAB, de Azevedo SS, Alves CJ, Bernardi F, Ito FH (2009) Isolation of rabies virus from the parotid salivary glands of foxes (Pseudalopex vetulus) from Paraíba State, Northeastern Brazil. *Braz J Microbiol* 40:446–449

Slate D, Algeo TP, Nelson KM, Chipman RB, Donovan D, Blanton JD et al (2009) Oral rabies vaccination in north america. opportunities, complexities, and challenges. *PLoS Neglect Trop Dis* 3(12):e549. https://doi.org/10.1371/journal.pntd.0000549

Sloan SE, Hanlon C, Weldon W, Niezgoda M, Blanton J, Self J et al (2007) Identification and characterization of a human monoclonal antibody that potently neutralizes a broad panel of rabies virus isolates. *Vaccine* 25(15):2800–2810

Smith TG, Wu X, Franka R, Rupprecht CE (2011) Design of future rabies biologics and antiviral drugs. *Adv Virus Res* 79:345–363

Smith SP, Wu G, Fooks AR, Ma J, Banyard AC (2019a) Trying to treat the untreatable: experimental approaches to clear rabies virus infection from the CNS. *J Gen Virol* 100(8):1171–1186. https://doi.org/10.1099/jgv.0.001269

Smith TG, Millien M, Vos A, Fracciterne FA, Crowdis K, Chirodea C et al (2019b) Evaluation of immune responses in dogs to oral rabies vaccine under field conditions. *Vaccine* 37(33):4743–4749. https://doi.org/10.1016/j.vaccine.2017.09.096

Smith TG, Jackson FR, Morgan CN, Carson WC, Martin BE, Gallardo-Romero N et al (2020) Antiviral Ranpirnase TMR-001 inhibits rabies virus release and cell-to-cell infection in vitro. *Viruses* 12(2):177. https://doi.org/10.3390/v12020177

Soentjens P, Andries P, Aerssens A, Tsoumanis A, Ravinetto R, Heuninckx W et al (2019) Preexposure intradermal rabies vaccination: a noninferiority trial in healthy adults on shortening the vaccination schedule from 28 to 7 days. *Clin Infect Dis* 68(4):607–614. https://doi.org/10.1093/cid/ciy513

Song M, Tang Q, Wang DM, Mo ZJ, Guo SH, Li H et al (2009) Epidemiological investigations of human rabies in China. *BMC Infect Dis* 9(210):1471–2334

Song M, Tang Q, Rayner S, Tao XY, Li H, Guo ZY et al (2014) Human rabies surveillance and control in China, 2005-2012. *BMC Infect Dis* 14:212. https://doi.org/10.1186/1471-2334-14-212

Sparkes J, Körtner G, Ballard G, Fleming PJS, Brown WY (2014) Effects of sex and reproductive state on interactions between free-roaming domestic dogs. *PloS one* 9(12):e116053. https://doi.org/10.1371/journal.pone.0116053

Sparrow E, Torvaldsen S, Newall AT, Wood JG, Sheikh M, Kieny MP, Abela-Ridder B (2019) Recent advances in the development of monoclonal antibodies for rabies post exposure prophylaxis. A review of the current status of the clinical development pipeline. *Vaccine* 37(Suppl 1):A132–A139. https://doi.org/10.1016/j.vaccine.2018.11.004

Sriaroon C, Sriaroon P, Daviratanasilpa S, Khawplod P, Wilde H (2006) Retrospective: animal attacks and rabies exposures in Thai children. *Travel Med Infect Dis* 4(5):270–274

Srinivasan K, Kurz T, Kuttuva P, Pearson C (2019) Reorienting rabies research and practice: Lessons from India. *Palgrave communications* 5:152. https://doi.org/10.1057/s41599-019-0358-y

Stading B, Ellison JA, Carson WC, Satheshkumar PS, Rocke TE, Osorio JE (2017) Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *PLoS Neglect Trop Dis* 11(10):e0005958. https://doi.org/10.1371/journal.pntd.0005958

Steck F, Wandeler AI, Bichsel P, Capt S, Schneider LG (1982) Oral Immunisation of Foxes against Rabies. *Zentralbl Veterinarmed B* 29:372–396

Sterner RT, Meltzer MI, Shwiff SA, Slate D (2009) Tactics and economics of wildlife oral rabies vaccination, Canada and the United States. *Emerg Infect Dis* 15(8):1176–1184. https://doi.org/10.3201/eid1508.081061

Stevenson B, Goltz J, Massé A (2016) Preparing for and responding to recent incursions of raccoon rabies variant into Canada. *Canada communicable disease report = Releve des maladies transmissibles au Canada* 42(6):125–129. https://doi.org/10.14745/ccdr.v42i06a03

Stokes W, McFarland R, Kulpa-Eddy J, Gatewood D, Levis R, Halder M et al (2012) Report on the international workshop on alternative methods for human and veterinary rabies vaccine testing. state of the science and planning the way forward. *Biologicals* 40(5):369–381

Streicker DG, Recuenco S, Valderrama W, Gomez Benavides J, Vargas I, Pacheco V et al (2012) Ecological and anthropogenic drivers of rabies exposure in vampire bats. implications for transmission and control. *Proc R Soc B Biol Sci* 279(1742):3384–3392. https://doi.org/10.1098/rspb.2012.0538

Sudarshan MK, Mahendra BJ, Narayan DH (2001) A community survey of dog bites, anti-rabies treatment, rabies and dog population management in Bangalore city. *J Commun Dis* 33(4):245–251

Sudarshan MK, Madhusudana SN, Mahendra BJ, Rao NS, Narayan DH, Rahman SA et al (2007) Assessing the burden of human rabies in India. results of a national multi-center epidemiological survey. *Int J Infect Dis* 11(1):29–35

Sudarshan MK, Narayana A, Hanumanthaiah D (2019) Appraisal of surveillance of human rabies and animal bites in seven states of India. *Indian J Public Health* 63(Supplement):S3–S8. https://doi.org/10.4103/ijph.IJPH_377_19

Sultanov AA, Abdrakhmanov SK, Abdybekova AM, Karatayev BS, Torgerson PR (2016) Rabies in Kazakhstan. *PLoS Neglect Trop Dis* 10(8):e0004889. https://doi.org/10.1371/journal.pntd.0004889

Taylor LH, Nel LH (2015) Global epidemiology of canine rabies: past, present, and future prospects. *Vet Med-Res Rep* 6:361–371. https://doi.org/10.2147/VMRR.S51147

Taylor L, Partners for Rabies Prevention (2013) Eliminating canine rabies. The role of public-private partnerships. *Antiviral Res* 98(2):314–318. https://doi.org/10.1016/j.antiviral.2013.03.002

Taylor LH, Knopf L, the Partners for Rabies, Prevention (2015) Surveillance of human rabies by national authorities - a global survey. *Zoonoses Public Health* 62(7):543–552. https://doi.org/10.1111/zph.12183

Taylor LH, Hampson K, Fahrion A, Abela-Ridder B, Nel LH (2017a) Difficulties in estimating the human burden of canine rabies. *Acta Trop* 165:133–140. https://doi.org/10.1016/j.actatropica.2015.12.007

Taylor LH, Wallace RM, Balaram D, Lindenmayer JM, Eckery DC, Mutonono-Watkiss B et al (2017b) The role of dog population management in rabies elimination-a review of current approaches and future opportunities. *Front Vet Sci* 4:109. https://doi.org/10.3389/fvets.2017.00109

Taylor E, Vilas DR, Victor, Scott T, Coetzer A, Prada JM, Alireza G et al (2021) Rabies in the Middle East, Eastern Europe, Central Asia and North Africa: Building evidence and delivering a

regional approach to rabies elimination. *J Infect Public Health* 14(6):787–794. https://doi.org/10.1016/j.jiph.2021.02.009

Te Kamp V, Freuling CM, Vos A, Schuster P, Kaiser C, Ortmann S et al (2020) Responsiveness of various reservoir species to oral rabies vaccination correlates with differences in vaccine uptake of mucosa associated lymphoid tissues. *Sci Rep* 10(1):S. 2919. https://doi.org/10.1038/s41598-020-59719-4

Tenzin, Wangdi K, Ward MP (2012) Human and animal rabies prevention and control cost in Bhutan, 2001-2008: the cost-benefit of dog rabies elimination. *Vaccine* 31(1):260–270. https://doi.org/10.1016/j.vaccine.2012.05.023

Tenzin T, Ahmed R, Debnath NC, Ahmed G, Yamage M (2015) Free-roaming dog population estimation and status of the dog population management and rabies control program in Dhaka City, Bangladesh. *PLoS Neglect Trop Dis* 9(5):e0003784. https://doi.org/10.1371/journal.pntd.0003784

Terryn S, Francart A, Lamoral S, Hultberg A, Rommelaere H, Wittelsberger A et al (2014) Protective effect of different anti-rabies virus VHH constructs against rabies disease in mice. *PloS one* 9(10):e109367. https://doi.org/10.1371/journal.pone.0109367

Terryn S, Francart A, Rommelaere H, Stortelers C, van Gucht S (2016) Post-exposure treatment with anti-rabies vhh and vaccine significantly improves protection of mice from Lethal rabies infection. *PLoS Neglect Trop Dis* 10(8):e0004902. https://doi.org/10.1371/journal.pntd.0004902

Tinsa F, Borgi A, Jahouat I, Boussetta K (2015) Rabies encephalitis in a child: a failure of rabies post exposure prophylaxis? BMJ Case Rep:bcr2014206191. https://doi.org/10.1136/bcr-2014-206191

Tohma K, Saito M, Demetria CS, Manalo DL, Quiambao BP, Kamigaki T, Oshitani H (2016) Molecular and mathematical modeling analyses of inter-island transmission of rabies into a previously rabies-free island in the Philippines. *Infect Genet Evol.* 38:22–28. https://doi.org/10.1016/j.meegid.2015.12.001

Townsend SE, Sumantra IP, Pudjiatmoko, Bagus GN, Brum E, Cleaveland S et al (2013) Designing programs for eliminating canine rabies from islands. Bali, Indonesia as a case study. *PLoS Neglect Trop Dis* 7(8):e2372. https://doi.org/10.1371/journal.pntd.0002372

Umeno S, Doi Y (1921) A study on the Antirabic inoculation of dogs, and the results of its practical application. *Kitasato Arch. Exp. Med* 4(2):89–108

Ün H, Eskiizmirliler S, Ünal N, Freuling C, Johnson N, Fooks AR et al (2012) Oral vaccination of foxes against rabies in Turkey between 2008 and 2010. *Berl Munch Tierarztl Wochenschr* 125(5-6):203–208

Undurraga EA, Meltzer MI, Tran CH, Atkins CY, Etheart MD, Millien MF et al (2017) Cost-effectiveness evaluation of a novel integrated bite case management program for the control of human rabies, Haiti 2014-2015. *Am J Trop Med Hyg* 96(6):1307–1317. https://doi.org/10.4269/ajtmh.16-0785

Undurraga EA, Millien MF, Allel K, Etheart MD, Cleaton J, Ross Y, Wallace RM (2020) Costs and effectiveness of alternative dog vaccination strategies to improve dog population coverage in rural and urban settings during a rabies outbreak. *Vaccine* 38(39):6162–6173. https://doi.org/10.1016/j.vaccine.2020.06.006

United Against Rabies (2021) United against rabies forum: zero by 30: One Health in action. Partnering for success. Eliminating rabies is an achievable goal, if we work together. Hg. v. B. Abela-Ridder. WHO and FAO and OIE. Online verfügbar unter https://www.who.int/publications/i/item/WHO-UCN-NTD-VVE-2021.1, zuletzt geprüft am 30.07.2021

United Against Rabies Collaboration (2019) Zero by 30: First Annual Progress Report. The Global Strategic Plan to end Human Deaths from Dog-mediated Rabies by 2030. Hg. v. United Against Rabies Collaboration, Geneva

Velasco-Villa A, Reeder SA, Orciari LA, Yager PA, Franka R, Blanton JD et al (2008) Enzootic rabies elimination from dogs and reemergence in wild terrestrial carnivores, United States. *Emerg Infect Dis* 14(12):1849–1854. https://doi.org/10.3201/eid1412.080876

Velasco-Villa A, Escobar LE, Sanchez A, Shi M, Streicker DG, Gallardo-Romero NF et al (2017a) Successful strategies implemented towards the elimination of canine rabies in the Western Hemisphere. *Antiviral Res* 143:1–12. https://doi.org/10.1016/j.antiviral.2017.03.023

Velasco-Villa A, Mauldin MR, Shi M, Escobar LE, Gallardo-Romero NF, Damon I et al (2017b) The history of rabies in the Western Hemisphere. *Antiviral Res* 146:221–232. https://doi.org/10.1016/j.antiviral.2017.03.013

Vigilato MA, Clavijo A, Knobl T, Silva HM, Cosivi O, Schneider MC et al (2013a) Progress towards eliminating canine rabies. policies and perspectives from Latin America and the Caribbean. Philos Trans R Soc Lond B Biol Sci 368(1623):20120143. https://doi.org/10.1098/rstb.2012.0143

Vigilato MAN, Cosivi O, Knöbl T, Clavijo A, Silva HMT (2013b) Rabies update for Latin America and the Caribbean. *Emerg Infect Dis* 19(4):678–679. https://doi.org/10.3201/eid1904.121482

Vilas DR, Victor J, de Carvalho F, Mary J, Vigilato MAN, Rocha F, Vokaty A, Pompei JA et al (2017) Tribulations of the Last Mile: Sides from a Regional Program. *Front Vet Sci* 4:4. https://doi.org/10.3389/fvets.2017.00004

Vos A, Freuling C, Eskiizmirliler S, Un H, Aylan O, Johnson N et al (2009) Rabies in foxes, Aegean region, Turkey. *Emerg Infect Dis* 15(10):1620–1622

Vos A, Nunan C, Bolles D, Müller T, Fooks AR, Tordo N, Baer GM (2011) The occurrence of rabies in pre-Columbian Central America. an historical search. *Epidemiol Infect* 139(10):1445–1452. https://doi.org/10.1017/S0950268811001440

Vos A, Ortmann S, Kretzschmar AS, Köhnemann B, Michler F (2012) The raccoon (Procyon lotor) as potential rabies reservoir species in Germany. a risk assessment. *Berl Munch Tierarztl Wochenschr* 125(5-6):228–235

Vos A, Kretzschmar A, Ortmann S, Lojkic I, Habla C, Müller T, Kaiser C, Hundt B, Schuster P (2013) Herpestes auropunctatus. *J Wildl Dis* 49(4) 1033–1036. https://doi.org/10.7589/2013-02-035

Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Dempsey DM et al (2020) Changes to virus taxonomy and the Statutes ratified by the International Committee on Taxonomy of Viruses (2020). *Arch Virol* 165(11):2737–2748. https://doi.org/10.1007/s00705-020-04752-x

Wallace RM, Reses H, Franka R, Dilius P, Fenelon N, Orciari L et al (2015) Establishment of a High Canine Rabies Burden in Haiti through the Implementation of a Novel Surveillance Program corrected. *PLoS Neglect Trop Dis* 9(11):e0004245. https://doi.org/10.1371/journal.pntd.0004245

Wallace RM, Undurraga EA, Blanton JD, Cleaton J, Franka R (2017) Elimination of dog-mediated human rabies deaths by 2030. Needs assessment and alternatives for progress based on dog vaccination. *Front Vet Sci* 4:9. https://doi.org/10.3389/fvets.2017.00009

Wallace RM, Undurraga EA, Gibson A, Boone J, Pieracci EG, Gamble L, Blanton JD (2019) Estimating the effectiveness of vaccine programs in dog populations. *Epidemiol Infect* 147:e247. https://doi.org/10.1017/S0950268819001158

Wallace RM, Cliquet F, Fehlner-Gardiner C, Fooks AR, Sabeta CT, Setién AA et al (2020) Role of oral rabies vaccines in the elimination of dog-mediated human rabies deaths. *Emerg Infect Dis* 26(12). https://doi.org/10.3201/eid2612.201266

Wandeler A (2000) Oral immunization against rabies. afterhoughts and foresight. *Schweiz Arch Tierheilkd* 142(8):455–462

Wandeler, A. (2004): Epidemiology and ecology of fox rabies in Europe. In: A. A. King, A. R. Fooks, M. Aubert und A. I. Wandeler (Hg.): Historical perspective of rabies in Europe and the Mediterranean Basin. Paris: OIE, S. 201–214.

Wang H, Zhang G, Wen Y, Yang S, Xia X, Fu ZF (2011a) Intracerebral administration of recombinant rabies virus expressing GM-CSF prevents the development of rabies after infection with street virus. *PloS one* 6(9):e25414. https://doi.org/10.1371/journal.pone.0025414

Wang Y, Rowley KJ, Booth BJ, Sloan SE, Ambrosino DM, Babcock GJ (2011b) G glycoprotein amino acid residues required for human monoclonal antibody RAB1 neutralization are

conserved in rabies virus street isolates. *Antiviral Res* 91(2):187–194. https://doi.org/10.1016/j.antiviral.2011.06.002

Warembourg C, Fournié G, Abakar MF, Alvarez D, Berger-González M, Odoch T et al (2021) Predictors of free-roaming domestic dogs' contact network centrality and their relevance for rabies control. Sci Rep 11(1):12898. https://doi.org/10.1038/s41598-021-92308-7

Warrell MJ (2012) Current rabies vaccines and prophylaxis schedules: preventing rabies before and after exposure. *Travel Med Infect Dis* 10(1):1–15

Warrell MJ (2019a) Rabies post-exposure vaccination in 2 visits within a week: a 4-site intradermal regimen. *Vaccine* 37(9):1131–1136. https://doi.org/10.1016/j.vaccine.2019.01.019

Warrell MJ (2019b) Simplification of Rabies Postexposure Prophylaxis: a New 2-Visit Intradermal Vaccine Regimen. *Am J Trop Med Hyg* 101(6):1199–1201. https://doi.org/10.4269/ajtmh.19-0252

Weyer J, Kuzmin IV, Rupprecht CE, Nel LH (2008) Cross-protective and cross-reactive immune responses to recombinant vaccinia viruses expressing full-length lyssavirus glycoprotein genes. *Epidemiol Infect* 136(5):670–678. https://doi.org/10.1017/S0950268807008965

Weyer J, Szmyd-Potapczuk AV, Blumberg LH, Leman PA, Markotter W, Swanepoel R et al (2011) Epidemiology of human rabies in South Africa, 1983-2007. *Virus Res* 155(1):283–290

WHO and OIE (Hg.) (2015) Human and Dog Rabies Vaccines and immunoglobulins. Report of a Meeting. Geneva, 12-13 October 2015. World Health Organization; World Organization for Animal Health, Geneva

WHO and OIE (Hg.) (2016) Global elimination of dog-mediated human rabies: the time is now! Report of the Rabies Global Conference 10-11 December 2015, Geneva. Switzerland. World Health Organization; World Organization for Animal Health, Paris

WHO Rabies Modelling Consortium (2019) The potential effect of improved provision of rabies post-exposure prophylaxis in Gavi-eligible countries. a modelling study. *Lancet Infect Dis* 19(1):P102–P111. https://doi.org/10.1016/S1473-3099(18)30512-7

Wilde H (2007) Failures of post-exposure rabies prophylaxis. *Vaccine* 25(44):7605–7609. https://doi.org/10.1016/j.vaccine.2007.08.054

Wilde H, Choomkasien P, Hemachudha T, Supich C, Chutivongse S (1989) Failure of rabies postexposure treatment in Thailand. *Vaccine* 7(1):49–52. https://doi.org/10.1016/0264-410x(89)90010-8

Wilde H, Sirikawin S, Sabcharoen A, Kingnate D, Tantawichien T, Harischandra PA et al (1996) Failure of postexposure treatment of rabies in children. *Clin Infect Dis* 22(2):228–232

Willoughby RE Jr, Tieves KS, Hoffman GM, Ghanayem NS, Amlie-Lefond CM, Schwabe MJ et al (2005) Survival after treatment of rabies with induction of coma. *New Engl J Med* 352(24):2508–2514

Willoughby RE Jr (2007) A cure for rabies? *Sci Am* 296(4):88–95

Willoughby RE, Roy-Burman A, Martin KW, Christensen JC, Westenkirschner DF, Fleck JD et al (2008) Generalised cranial artery spasm in human rabies. *Dev Biol (Basel)* 131:367–375

Willoughby RE, Opladen T, Maier T, Rhead W, Schmiedel S, Hoyer J et al (2009) Tetrahydrobiopterin deficiency in human rabies. *J Inherit Metab Dis* 32(1):65–72. https://doi.org/10.1007/s10545-008-0949-z

Windiyaningsih C, Wilde H, Meslin FX, Suroso T, Widarso HS (2004) The Rabies Epidemic on Flores Island, Indonesia (1998-2003). *J Med Assoc Thai* 84(11):1389–1393

World Health Organization (2011) mHealth. Second Global Survey on eHealth. World Health Organization, Geneva. Online verfügbar unter http://gbv.eblib.com/patron/FullRecord.aspx?p=851142

World Health Organization (2013) Expert consultation on rabies, second report. *WHO Tech Rep Ser* 982:1–150

World Health, Organization (2018) WHO expert consultation on rabies: third report, WHO technical report series;1012. World Health Organization, Geneva

World Health Organization (2018a) Rabies vaccines: WHO position paper, April 2018 - Recommendations. *Vaccine* 36(37):5500–5503. https://doi.org/10.1016/j.vaccine.2018.06.061

World Health Organization (2018b) WHO expert consultation on rabies, third report. *WHO Tech Rep Ser* 1012:195

World Health Organization (Hg.) (2015a) From Concept to Completion: The Rabies Control Pilot Project Supported by the Bill & Melinda Gates Foundation. World Health Organization. Online verfügbar unter https://www.who.int/activities/leading-and-coordinating-anti-rabies-efforts-with-One-Health-partners/control-and-elimination-strategies, zuletzt geprüft am 20.07.2021

World Health Organization (Hg.) (2015b) New global framework to eliminate rabies. News release. Online verfügbar unter https://www.who.int/news/item/10-12-2015-new-global-framework-to-eliminate-rabies, zuletzt geprüft am 30.07.2021

World Health Organization (Hg.) (2015c) Rationale for Investing in the Global Elimination of Dog-Mediated Human Rabies. World Health Organization, Geneva. Online verfügbar unter https://www.who.int/publications/i/item/9789241509558

Wright N, Subedi D, Pantha S, Acharya KP, Nel LH (2021) The role of waste management in control of rabies: a neglected issue. *Viruses* 13:225. https://doi.org/10.3390/v13020225

Wu XF, Smith TG, Rupprecht CE (2011) From brain passage to cell adaptation: the road of human rabies vaccine development. *Expert Rev Vaccines* 10(11):1597–1608. https://doi.org/10.1586/erv.11.140

Yale G, Gibson AD, Mani RS, Harsha PK, Costa NC, Corfmat J et al (2019) Evaluation of an Immunochromatographic Assay as a Canine Rabies Surveillance Tool in Goa, India. *Viruses* 11:649. https://doi.org/10.3390/v11070649

Yasobant S, Saxena D, Bruchhausen W, Memon FZ, Falkenberg T (2019) Multi-sectoral prioritization of zoonotic diseases: One health perspective from Ahmedabad, India. *PloS one* 14(7):e0220152. https://doi.org/10.1371/journal.pone.0220152

Zhang S, Tang Q, Wu X, Liu Y, Zhang F, Rupprecht CE, Hu R (2009) Rabies in ferret badgers, southeastern China. *Emerg Infect Dis* 15(6):946–949. https://doi.org/10.3201/eid1506.081485

Zieger U, Marston DA, Sharma R, Chikweto A, Tiwari K, Sayyid M et al (2014) The phylogeography of rabies in Grenada, West Indies, and implications for control. *PLoS Neglect Trop Dis* 8(10):e3251. https://doi.org/10.1371/journal.pntd.0003251

Zinsstag J (2013) Towards a science of rabies elimination. *Infect Dis Poverty* 2:S. 22. https://doi.org/10.1186/2049-9957-2-22

Zinsstag J, Durr S, Penny MA, Mindekem R, Roth F, Gonzalez SM et al (2009) Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proc Natl Acad Sci U S A* 106(35):14996–15001. https://doi.org/10.1073/pnas.0904740106

Zinsstag J, Lechenne M, Laager M, Mindekem R, Naissengar S, Oussiguere A et al (2017) Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure. *Sci Trans Med* 9:eaaf6984. https://doi.org/10.1126/scitranslmed.aaf6984

Zoonotic Transmission of *Chlamydia* spp.: Known for 140 Years, but Still Underestimated

26

Nicole Borel and Konrad Sachse

Contents

Abstract

Historically, the first documented cases of infections by chlamydiae involved humans with contact to psittacine birds. While birds have remained the main

N. Borel (✉)
Institute of Veterinary Pathology, Department of Pathobiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
e-mail: nicole.borel@uzh.ch

K. Sachse
Department of RNA Bioinformatics and High-Throughput Analysis, Faculty of Mathematics and Computer Science, Friedrich-Schiller-Universität, Jena, Germany

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_53

source of zoonotic transmission until now, the spectrum of chlamydial zoonoses has broadened in recent decades.

In the present chapter, we summarize current knowledge on etiology, pathology, epidemiology, and genomic markers of zoonotic chlamydial infections. In particular, *Chlamydia (C.) psittaci*, the agent of avian chlamydiosis, is continuing to affect human individuals in contact with birds. Clinical signs can range from flu-like to those of severe systemic illness. *C. abortus*, a pathogen causing enzootic abortion in small ruminants, was repeatedly shown to be responsible for cases of human abortion. *C. caviae*, an agent of ocular disease in guinea pigs, is known to have caused conjunctivitis in guinea pig owners. Also *C. felis*, which can cause acute and chronic conjunctivitis in cats, has a zoonotic potential and was associated with ocular disease in contact persons.

We outline the main characteristics of the agents' animal reservoirs, describe transmission routes, and summarize recent reports on outbreaks and individual cases of human infections by *Chlamydia* spp. The relatively low number of officially notified cases is probably due to underdiagnosis, since *C. psittaci* and other chlamydiae are usually not part of routine diagnosis in human medicine. As research in the past decades has led to the extension of the genus *Chlamydia* to 14 species and 4 taxa at *Candidatus* rank, the scope of zoonotic agents can be expected to rise in the future.

Keywords

Chlamydia psittaci · *Chlamydia abortus* · *Chlamydia caviae* · *Chlamydia felis* · Zoonosis · Transmission route · Human disease · Atypical pneumonia · Abortion · Conjunctivitis · Genome analysis

26.1 Introduction

Bacteria of the family *Chlamydiaceae* are defined as "coccoid, non-motile, obligate intracellular organisms of 0.2–1.5 μm diameter that reside in vacuole-like inclusions of eukaryotic cells, where they parasitize and multiply in a unique developmental cycle" (Sachse et al. 2015a). In the course of that cycle, chlamydiae appear in two different morphological forms, the small infectious elementary body (EB) and the larger intracellular reticulate body (RB). EBs enter the host cell using different routes involving various receptor molecules. Once within the cell, they initiate the formation of a vacuole-like inclusion, where they multiply in the RB form through binary fission. Finally, while RBs are transforming back into EBs, it comes to rupture of the inclusion with the release of newly infectious chlamydiae.

The fact that *Chlamydia* spp. rely on eukaryotic cells to survive and proliferate was always a great obstacle to culture and *in vitro* studies. While the first successful isolations of chlamydial strains were achieved in embryonated chicken eggs, nowadays cell culture is widely used in research and diagnostic laboratories.

Until very recently, all known species of the family *Chlamydiaceae* were taxonomically classified in a single genus *Chlamydia*, which currently comprises 18 species, among them four at *Candidatus* rank. Basic characteristics of these bacteria are given in Table 1.

Table 1 Basic characteristics of currently known *Chlamydia* spp.[a]

Species	Main hosts	Clinical manifestations	Zoonotic potential
Chlamydia abortus	Ruminants, swine	Abortion[b], vaginitis, endometritis, seminal vesiculitis, (latent) mastitis	Yes
Chlamydia avium	Pigeon	Enteritis and respiratory disease (link to pathology still uncertain)	Unclear
Chlamydia buteonis	Hawks	Conjunctivitis, respiratory disease (link to pathology still uncertain)	Unclear
Chlamydia caviae	Guinea pig	Conjunctivitis[b], keratitis, pneumonia[c]	Yes
Chlamydia felis	Cat	Conjunctivitis[b], rhinitis	Yes
Chlamydia gallinacea	Birds	No apparent pathology yet described	No
Chlamydia muridarum	Mouse, hamster	Pneumonitis, ileitis	No
Chlamydia pecorum	Ruminants Swine Koala	Encephalitis, polyarthritis, pneumonia, enteritis, vaginitis, endometritis Polyarthritis, serositis, enteritis, pneumonia Keratoconjunctivitis, vaginitis, ovarian cyst, infertility	No No No
Chlamydia pneumoniae	Koala, other marsupials, Horse	Rhinitis, pneumonia, conjunctivitis	No
	Reptiles, amphibians	Conjunctivitis, enteritis, granulomatous inflammation of internal organs	No
Chlamydia poikilotherma	Snakes	No apparent pathology yet described	No
Chlamydia psittaci	Birds Horse	Conjunctivitis, pneumonia, atypical pneumonia[b], enteritis, hepatitis Abortion, pneumonia[c]	Yes Yes
Chlamydia serpentis	Snakes	No apparent pathology yet described	No
Chlamydia suis	Swine	Conjunctivitis, pneumonia, enteritis, polyarthritis	Under debate
Chlamydia trachomatis	Human	Genital tract infections, ocular disease (trachoma)	No
Ca.[d] Chlamydia corallus	Snakes	No apparent pathology yet described	No
Ca. Chlamydia ibidis	Birds	No apparent pathology yet described	No
Ca. Chlamydia sanzinia	Snakes	No apparent pathology yet described	No
Ca. Chlamydia testudinis	Tortoise	Conjunctivitis, nasal discharge (link to pathology still uncertain)	No

[a]Adapted from reference (Sachse and Borel 2020)
[b]In both animal and human infection
[c]In cases of zoonotic transmission
[d]*Candidatus*

The most important members include the human pathogens *Chlamydia (C.) trachomatis*, *C. pneumoniae*, and the zoonotic agent *C. psittaci*, as well as *C. abortus, C. caviae*, and *C. felis*, which also have a zoonotic potential. Current knowledge on sources and reservoirs of zoonotic transmission of *Chlamydia* spp. is given in Fig. 1.

In 2021, new members of the family *Chlamydiaceae* were presented, when Vorimore et al. (Vorimore et al. 2021) defined the new genus *Chlamydiifrater*. These authors were able to show that their new chlamydial isolates from flamingos belonged to the species *Chlamydiifrater phoenicopteri* and *Chlamydiifrater volucris*, respectively. While the etiologic importance of these new taxa is still unknown, it seems certain that wild birds may harbor a large variety of yet unknown chlamydial organisms.

One of the main disease manifestations of zoonotic infections in humans is the so-called community-acquired pneumonia (CAP), defined as pneumonia acquired outside the hospital. CAP can be of zoonotic or non-zoonotic (*Chlamydia pneumoniae*, *Mycoplasma pneumoniae, Legionella pneumophila*) origin. Among atypical agents of CAP, the zoonotic pathogens *Chlamydia psittaci* (avian chlamydiosis, psittacosis, ornithosis), *Francisella tularensis* (tularemia), and *Coxiella burnetii* (Q fever) often remain undetected, because they are not included in routine procedures of human microbiology laboratories. *C. psittaci* is a rare cause of CAP (approximately 1%, Hogerwerf et al. 2017) but often remains undiagnosed because of the lack of rapid and accurate diagnostic methods in human microbiology laboratories. Underdiagnosis of this zoonosis is a serious problem as this may delay or prevent appropriate therapy. Moreover, the professional guidelines recommend beta-lactam antibiotic therapy for clinically diagnosed CAP, which is not effective against *C. psittaci*. Veterinarians and physicians are the key professionals to recognize and report zoonotic events; however, a lack of communication between these two healthcare fields often delays or even prevents the timely workup of such cases.

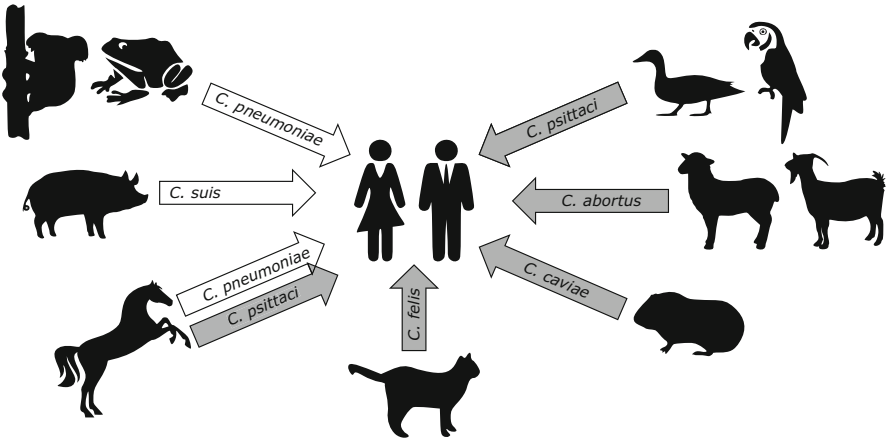

Fig. 1 Schematic presentation of zoonotic reservoirs and transmission routes of *Chlamydia* spp. Filled arrows, proven zoonotic transmission; empty arrows, zoonotic potential under debate

Current knowledge on chlamydial infections in animals and their zoonotic implications was compiled in a recent review (Sachse and Borel 2020). In the present chapter, we focus on those chlamydial species that have a proven zoonotic potential and discuss a few more whose status in terms of zoonosis is still uncertain.

26.2 Epidemiology of Zoonotic Infections in Animals

26.2.1 *Chlamydia psittaci*

C. psittaci, the causative agent of avian chlamydiosis and human psittacosis, is probably the most important veterinary chlamydial pathogen from economic and sanitary viewpoints.

This bacterium is disseminated worldwide, with birds representing its natural host. It was encountered in 9 domestic fowl species and at least 460 free-living or pet bird species of 30 different orders (Kaleta and Taday 2003).

Transmission of *C. psittaci* occurs through inhalation or ingestion of infected dust particles, as well as nasal and ocular discharges and droppings from infected birds. Many factors on both pathogen and host side can influence the course of infection, which is known to cover all stages from acute to chronic to subclinical. The acute form is more often observed in young birds, whereas adult birds tend to develop milder forms. Affected birds show signs of respiratory disease, conjunctivitis, coryza, mucopurulent discharge from nose and eyes, cough, dyspnea, or greenish to greyish feces, none of which can be regarded as specific. Generally speaking, subclinical or latent infection occurs more frequently than acute cases and outbreaks (Sachse et al. 2015b; Vanrompay 2013). Characteristic features of the various forms of avian chlamydiosis are summarized in Table 2.

Historically, chlamydial infections first gained public attention in the second half of the nineteenth century, when many cases of atypical pneumonia among the human population occurred in Europe and North America (Hegler 1930; Lepore 2009; Meyer and Eddie 1935; Ritter 1879). The larger outbreaks were associated with the arrival of parrot shipments from South America (see section below). Due to

Table 2 Characteristics of the disease course of avian chlamydiosis in birds[a]

Course	Incubation time (d)	Duration of disease	Symptoms
Acute (lethal systemic form)	3–7	8–14 d	Anorexia, apathy, dyspnea, diarrhea
Subacute to protracted	7–14	>3 weeks	Milder clinical signs of anorexia, apathy, dyspnea, diarrhea
Chronic	30–90	>2 months	Apathy, cachexia, diarrhea, dyspnea
Subclinical persisting	None	None	No clinical signs
Activated persisting	>3 months	>2 months	Clinical signs of chronic course (after activation by endogenous and exogenous factors)

[a]Modified from (Taise 2013)

crowding and poor housing conditions during the passage, the infectious agent (still unknown at that time) could spread unimpededly among the birds, so that many of them died while survivors transmitted the infection to humans. Meanwhile, fulminant and devastating outbreaks of avian chlamydiosis have become rare events. Nowadays, such infections lead to reduced feed intake and respiratory signs of affected birds. Due to the availability of antimicrobials, mortality can be kept low.

C. psittaci was reported to be nearly endemic in turkey flocks, where coinfection with *Ornithobacterium rhinotracheale* could exacerbate the course and sequelae (Van Loock et al. 2005). This finding may be exemplary for chlamydial coinfections with other microbial agents, but epidemiological or experimental evidence is still lacking.

Infections in flocks of domestic ducks have been reported from Europe (Haas et al. 2007; Laroucau et al. 2009) and Asia (Yin et al. 2015). The agent is also frequently encountered in pigeons. In a number of epidemiological studies in urban or feral pigeon populations, *C. psittaci* prevalence values from 12 to nearly 100% were reported (Magnino et al. 2009). However, these findings need to be rechecked in the light of the recent discovery of *C. avium*, another chlamydial species typically found in *Columba livia* (Sachse et al. 2014). Initial studies also suggest that coinfections involving *C. psittaci* and *C. avium* are quite common (Burt et al. 2018; Krautwald-Junghanns et al. 2013).

Until a few years ago, *C. psittaci* was believed to be endemic in domestic chicken flocks. However, following the discovery of *C. gallinacea* (Sachse et al. 2014), more recent studies suggest that this is the predominant chlamydial species in chickens, whereas *C. psittaci* is rare (Hulin et al. 2015). The risk assessment study by Dickx et al. (Dickx et al. 2010), which found 85% of chicken flocks *C. psittaci*-positive at slaughtering, is in contrast to studies from 2017 and later. Li et al. (Li et al. 2017) in the USA, Donati et al. (Donati et al. 2018) in Italy, and Heijne et al. (Heijne et al. 2018) in the Netherlands presented findings underpinning the predominant presence of *C. gallinacea* in chicken flocks. Finally, a large epidemiological survey in commercial and backyard poultry flocks in Mexico revealed *C. gallinacea* as the only chlamydial agent present, while no *C. psittaci* was detected (Ornelas-Eusebio et al. 2020).

The effects of *C. psittaci* outbreaks in the poultry industry are measurable as the health of affected birds is deteriorated, thus reducing production output. Unfortunately, systematic studies are missing. Therefore, the consequences on bird health and economic parameters of prolonged subclinical carriage are still poorly understood. Based on studies in cattle (Reinhold et al. 2011), it is anticipated that carriage of *C. psittaci* over a long period could result in chronicity and, consequently, retarded development and reduced weight gain of infected birds.

The main reason why these issues have not been systematically studied is probably the easy access to antibiotic therapy. To prevent zoonotic transmission, tetracycline was added to feedstuff of imported parrots as early as in the 1950s. Later on, this practice was also introduced in poultry farms as a measure of prophylaxis or metaphylaxis (Page and Grimes 1978; Wachendorfer and Luthgen 1974). More recently, legislation in Europe and elsewhere has been encouraging abandonment or at least substantial reduction of the use of antimicrobials in the poultry industry.

In horses, *C. psittaci* infection has been linked to conjunctivitis, respiratory disease, polyarthritis, and abortion (Borel et al. 2018). *C. psittaci*-induced equine abortion cases have been observed throughout Europe for decades, but associated zoonotic events were not reported (Henning et al. 2000; Szeredi et al. 2005). More recently, a cluster of human respiratory illness in a veterinary school in Australia was linked to equine reproductive failure (Chan et al. 2017). Limited epidemiological studies of *C. psittaci*-related equine abortion cases are available reporting prevalences ranging from only 0.6% in Switzerland (Baumann et al. 2020) to up to 14% in Hungary (Szeredi et al. 2005) and 20% in Australia (Taylor et al. 2018), the latter at a sentinel site in Australia where the initial outbreak was reported. A retrospective study in Australia (1994–2019) detected *C. psittaci* in 6.5% of equine abortion cases indicating that *C. psittaci* is not an emerging cause of equine abortion but was rather underdiagnosed in the past (Akter et al. 2021). In mares, the disease manifests as late-term abortion and neonatal losses; the pathogen can be detected in fetal membranes or fetal organs (lung, liver). Coinfections with *C. psittaci* and equine Herpesvirus type 1 (EHV-1) have been reported (Anstey et al. 2021; Baumann et al. 2020). In most countries, detection of chlamydial pathogens is not part of routine veterinary diagnostic procedures for equine abortion cases; therefore, *C. psittaci*-induced cases might be missed.

26.2.2 *Chlamydia abortus*

C. abortus is the cause of enzootic abortion in ewes (EAE), also known as ovine enzootic abortion (OEA) or ovine chlamydiosis, which is present worldwide except in Australia and New Zealand. *C. abortus* is causing economic losses and is relevant for public health as well. It is the most common cause of infectious abortion in sheep and goats in Europe and can also occur in cattle, pigs, horses, wild ruminants, and yaks, but to a lesser extent. *C. abortus* infection causes late-term abortion (last 2–3 weeks of pregnancy), stillbirth, and newborn lambs or kids that are weak and often die within 48 hours (Longbottom and Coulter 2003).

When the pathogen is first introduced in a naïve sheep or goat flock, small numbers of abortions occur in the first year, followed by an abortion storm of 30% or more animals (more than 60% in goats) in the second and third years. In endemically infected flocks, only 1–5% of abortions are observed, mostly affecting primiparous females or newly introduced animals.

Horizontal transmission is the main route, with the pathogen spreading from one animal to another (Longbottom and Coulter 2003). The oronasal route of exposure is considered the primary way of transmission, and this can occur through direct contact between animals, their abortion premises (placental membranes, dead fetuses, coat of live/dead lambs/kids born to infected mothers), or the contaminated environment (pasture, bedding). Very little evidence supports a role for sexual transmission. After abortion, ewes can shed the pathogen during the following periovulation period and subsequent lambing, but evidence from molecular analysis of vaginal swabs taken at these points suggests that such shedding poses a minimal

risk of transmission to naïve animals (Livingstone et al. 2009). Following *C. abortus* infection via the oronasal route, a complex host-pathogen interaction is established with a latent phase in nonpregnant sheep, followed by an active disease phase in the placenta during pregnancy (Longbottom et al. 2013). Latency can be achieved by experimental infection with relatively low doses of the organisms inoculated intranasally (Longbottom et al. 2013). It has been suggested that the primary infection is first established in the tonsils, from where it is disseminated via blood and lymph to other organs. During pregnancy, *C. abortus* travels to the placenta, initiating placental inflammation and insufficiency resulting in abortion or stillbirth. In the placenta, chlamydial growth and pathology are not observed until around day 85–90 of gestation (Buxton et al. 1990; Maley et al. 2009). From then on, rapid chlamydial replication and pathological changes occur in the placenta, including necrosis, inflammation, and arteritis (Buxton et al. 2002; Sammin et al. 2009). An experimental infection model reproduced latency of *C. abortus* infection in nonpregnant sheep but failed to identify a correlation between disease outcome and humoral immune responses (Longbottom et al. 2013). Cellular immunity seems to be important for the control of *C. abortus* infection, but several cytokines have been shown to be elevated during the active and the latent phase in protected but also in aborting sheep. This means that protection cannot be correlated to specific cellular responses (Wattegedera et al. 2020).

Infectious elementary bodies massively shed during abortion are the source of environmental contamination and can remain viable in the environment up to months, depending on climate conditions (Longbottom and Coulter 2003).

Prevention of chlamydial abortion is possible using inactivated or attenuated vaccines. However, the live *C. abortus* vaccine strain 1B is not attenuated and has the potential to cause disease in sheep and is hazardous to pregnant women (Longbottom et al. 2018). Protective immunity is most likely caused by the administration of high doses of *C. abortus* elementary bodies contained in the vaccine, which induces similar placental pathology as the experimental infection with a wild-type strain (Caspe et al. 2021).

26.2.3 *Chlamydia caviae*

The guinea pig is the main host of *C. caviae*, although this chlamydial species has also been reported in rabbits, a dog, a cat, and in horses (Gaede et al. 2010; Lutz-Wohlgroth et al. 2006; Pantchev et al. 2010). In research settings, *C. caviae* is commonly used as an ocular and genital experimental guinea pig model to study human chlamydial infections (Zhang et al. 2020). Initially, *C. caviae* was isolated from the conjunctiva of an infected young laboratory guinea pig and originally named the guinea pig inclusion conjunctivitis (GPIC) virus (Murray 1964). *C. caviae*-induced GPIC implies clinical signs ranging from mild to severe keratoconjunctivitis with serous to purulent ocular discharge, conjunctival chemosis, follicular hypertrophy, and pannus formation. This keratoconjunctivitis is usually self-limiting and clears within 3–4 weeks. Apart from ocular disease, *C. caviae* can

also induce rhinitis, pneumonia, as well as genital tract infection and abortion, but the infection can also remain asymptomatic (Borel et al. 2018). Transmission can occur through close contact between animals or sexually, spreading fast within one husbandry (Mount et al. 1973). Outbreaks in guinea pig farms with high *C. caviae* prevalence (48%) associated with clinical signs, such as conjunctivitis, ocular discharge, pneumonia, and abortion, have been reported in the literature (Lutz-Wohlgroth et al. 2006). It can be assumed that the prevalence is much lower in clinically healthy guinea pig husbandries. Moreover, juvenile guinea pigs might have a higher risk to get *C. caviae* infected than adults or older animals (Lutz-Wohlgroth et al. 2006).

26.2.4 *Chlamydia felis*

C. felis has a predilection for conjunctival epithelial cells in cats and is an important cause of feline acute and chronic conjunctivitis. The conjunctivitis usually starts unilaterally but frequently extends to the other eye and is characterized by conjunctival chemosis, blepharospasm, ocular discharge, and hyperemia of the nictitating membrane (Sykes 2005). The discharge is initially serous but can then become more mucoid to mucopurulent. Some cats may show additional clinical signs, such as fever, lethargy, inappetence, sneezing, as well as nasal discharge (Sykes 2005). Submandibular lymph node enlargement, lameness, and reduced weight gain can also be present, mostly in kittens (Sykes 2005). Moreover, *C. felis* has also occasionally been detected in the reproductive tract of experimentally and naturally infected cats (Sykes 2005).

Usually, the clinical signs appear after an incubation period of 2–7 days (Gruffydd-Jones et al. 2009) and last for a few weeks to months (Sykes 2005). Spontaneous recovery is possible, but most untreated cats develop chronic conjunctivitis with ocular signs persisting for 22–45 days. The duration of ocular shedding can last up to 60 days, but intermittent shedding up to 8 months has also been observed in experimental cats, suggesting an asymptomatic carrier state (Sykes 2005). Experimental infections were successful using the ocular or intranasal application route (Baker 1944; Shewen et al. 1978; Sykes et al. 1999b; TerWee et al. 1998) and not only resulted in conjunctivitis and mild respiratory symptoms as expected but also led to vaginal and rectal excretion of the bacteria in 50% and 40% of the infected animals, respectively (Wills et al. 1987). *C. felis* has also been isolated from internal organs of cats, such as the lung, peritoneum, liver, and spleen, but it remains unclear so far whether those findings are clinically relevant (Sykes 2005). The bacterium is shed in ocular secretions and requires close contact between cats for transmission. Natural transmission of *C. felis* occurs most likely by aerosols when either symptomatically or asymptomatically infected cats are in close contact to healthy cats (Sykes 2005).

C. felis was first isolated from a cat with respiratory disease in 1942 and originally called the feline pneumonitis agent (Baker 1942, 1944). However, only experimental intranasal infection caused pneumonia (Baker 1944; Hoover et al. 1978). Moreover,

the term feline pneumonitis agent is misleading as proof is missing that *C. felis* has ever been involved in natural cases of lower respiratory tract disease (Bart et al. 2000; Schmal-Filius et al. 2020).

Studies from different countries show that *C. felis* is more frequently detected in cats suffering from conjunctivitis than in healthy animals (Low et al. 2007; McDonald et al. 1998; Rampazzo et al. 2003) and that stray cats are more commonly affected compared to pet cats (Halánová et al. 2011; Yan et al. 2000). In pet cats, the chlamydial prevalence in different countries assessed by PCR, isolation, or immunofluorescence assays ranges from 0% to 10% in healthy animals and 5.6% to 30.9% in cats with conjunctivitis. In stray cat populations, the prevalence usually reaches positivity rates from 24.4% to 35.7% up to 65.8% in subgroups with conjunctivitis. *C. felis* is more common in younger cats with a prevalence significantly higher in cats aged 5 weeks to 9 months (Sykes et al. 1999a; Wills et al. 1987). Sex predisposition is not observed, although one study reported that male cats had a significantly higher prevalence of chlamydial infections than females (Wills et al. 1987).

The gold standard for diagnosing a *C. felis*-induced conjunctivitis is made by using flocked swab samples or cytobrushes from the conjunctiva and performing PCR on these samples (Bressan et al. 2021). In positive cases, treatment with doxycycline is indicated (Sykes 2005). Live and inactivated vaccines for *C. felis* are available, and combination vaccines for common viral diseases exist.

26.3 Epidemiology of Chlamydial Zoonoses in Humans

26.3.1 *Chlamydia psittaci*

The zoonotic properties of *C. psittaci* are well-documented in the literature (see reviews in (Beeckman and Vanrompay 2009; Knittler and Sachse 2014; Sachse et al. 2015b).

The first case of human psittacosis reported in a scientific journal dates back to 1879, when Ritter (Ritter 1879) described an outbreak of "typhoid pneumonia" involving seven members of a family, with three deaths. The author associated this endemic infection with sick parrots living in that household. In 1892, the first major epidemic occurred in Paris. Two merchants sold more than 100 parrots that had become infected during their shipment from South America. As a result, 49 individuals fell ill, of which 16 died (Dujardin-Beaumetz 1893).

The term "psittacosis" was first used by Morange in a paper describing the clinical course of this zoonosis and its association with imported psittacine birds (Morange 1895). The first world war and the years of crisis following it put a temporary end to the import of exotic birds. Psittacosis became a temporarily forgotten disease, while its causative agent still remained unknown.

This changed at the end of the 1920s, when local fairs with large-scale parrot sales in the Argentinian towns of Córdoba and Tucumán triggered numerous human infections and a number of casualties (Barros 1929). Later, a number of larger

outbreaks of human psittacosis occurred in Europe and North America, and all of them could be traced back to parrot shipments from South America (Lepore 2009; Meyer and Eddie 1935; Winkle 2000). As more and more veterinarians and laboratory workers fell ill with psittacosis while trying to isolate the causative agent, this gave rise to the assumption that the infection could be acquired through inhalation. The hypothesis of airborne transmission also provided an explanation of the annually occurring respiratory disease on the Faroe Islands in the first half of the twentieth century. It affected those inhabitants who took part in the capture or processing of young fulmars *(Fulmarus glacialis)*, which were part of their diet at that time (Haagen and Mauer 1938).

In the late twentieth and early twenty-first centuries, mass outbreaks seem to have disappeared altogether. A typical episode of psittacosis now affects individuals or small groups with previous contact to birds, while fulminant manifestations in humans usually occur only when antimicrobials are not administered in time.

The course of the human disease ranges from asymptomatic to flu-like to severe systemic illness, with the latter manifesting as pneumonia, myocarditis, encephalitis, or sepsis. Most frequently, mild symptoms are seen in affected individuals, whereas immunocompromised persons are more likely to develop clinical signs. But, occasionally, also apparently healthy individuals can be severely affected (Arenas-Valls et al. 2017; Gaede et al. 2008).

Typical sources of human *C. psittaci* infections nowadays include psittacine birds (Ferreira et al. 2017), as well as ducks (Hinton et al. 1993; Vorimore et al. 2015), turkeys (Van Droogenbroeck et al. 2009), and mixed domestic poultry (Gaede et al. 2008). A major outbreak leading to hospitalization of eight individuals, who had worked at a mixed poultry farm in France, could be attributed to *C. psittaci ompA* genotype E/B, which was present in the duck flocks of the farm. Interestingly, those workers were also exposed to *C. gallinacea* when handling chickens carrying this agent. While not detected in any of the patients, the question remains whether previous *C. gallinacea* infection could have contributed to the outbreak (Laroucau et al. 2015). Generally speaking, zoonotic cases ascribed to contact with chickens have become rare lately (Lagae et al. 2014), as it is known that *C. psittaci* is not the predominant chlamydial agent in chicken flocks. *C. psittaci* genotype E/B, which is typically encountered in ducks and chickens, was reported to have occasionally caused mild human infections (Vanrompay et al. 2007).

Wild birds are another known reservoir of the pathogen, as was documented in a number of cases (Haagen and Mauer 1938; Herrmann et al. 2006; Rehn et al. 2013; Wang et al. 2020). Although direct contact to humans is rare, zoonotic transmission can occur in specific circumstances, e.g., at avian refuge centers. Thus, *C. psittaci* genotype B found in three birds of such a center was later detected in three workers, who developed more or less pronounced clinical signs (Kalmar et al. 2014). In this context, bird handlers at avian refuge centers are also at risk.

More recently, *C. psittaci* infections from non-avian sources were associated with psittacosis as well. Thus, *C. psittaci*-induced equine abortion cases and ensuing zoonotic infections in Australia have raised public awareness (Jelocnik et al. 2017; Polkinghorne and Greub 2017). A 2014 outbreak of human psittacosis in New South

Wales, Australia, was linked to contact with *C. psittaci*-infected placental material from a horse (Polkinghorne and Greub 2017), representing the first report of mammal-to-mammal *C. psittaci* transmission and a novel zoonotic risk for this well-known avian pathogen (Polkinghorne et al. 2019). Human patients showed clinical signs of pneumonia after direct exposure to the equine fetal membranes of a mare who delivered a foal that subsequently died (Chan et al. 2017). Staff from an equine stud farm, veterinary staff and veterinary students, were affected as they got infected either during the delivery of the foal or the handling of abnormal placental membranes. Genetic typing revealed *C. psittaci* strains belonging to the 6 BC clade, originating from native Australian parrots (*ompA* genotype A; Jelocnik et al. 2017; Branley et al. 2016) or a pigeon-type *C. psittaci* strain (*ompA* genotype B′; Jelocnik et al. 2017). It is hypothesized that these virulent *C. psittaci* strains can be transmitted through indirect contact, presumably via fecal environmental contamination from *C. psittaci*-infected birds shedding the pathogen in their feces. Free-roaming parrots might be the most likely reservoir in Australia, whereas free-roaming pigeons could play a role in Europe. *C. psittaci* should be considered in the differential diagnosis of acute febrile illness in humans occurring after exposure to horses, in particular equine abortion (Polkinghorne and Greub 2017).

The main transmission routes of *C. psittaci* to humans involve inhalation of infectious aerosol or dust and direct contact with contaminated feces or feathers. Human-to-human transmission was long thought to be irrelevant, but recent reports of such cases suggest that it should be taken into consideration as well. For instance, a psittacosis outbreak in Scotland originated from a pneumonia patient and subsequently affected four family members and one healthcare worker. Four of these developed severe clinical signs, with two even requiring intensive care unit admission (McGuigan et al. 2012). A Swedish group reported transmission from a severely ill psittacosis patient to ten contact persons, i.e., two family members, one hospital roommate, and seven hospital caregivers (Wallensten et al. 2014). This study is remarkable because, unlike in most others, the diagnostic testing used was specific for *C. psittaci*. Nosocomial transmission had already been suggested in 1997, when a cluster of seven pneumonia cases was attributed to a pet shop worker hospitalized with psittacosis (Hughes et al. 1997). Single cases of possible person-to-person transmission were also reported from Japan (Ito et al. 2002) and Germany (Fischer et al. 2014).

Professions at elevated risk include veterinarians, poultry workers, birdkeepers, and pet shop employees (Arenas-Valls et al. 2017; Deschuyffeleer et al. 2012).

The current epidemiological situation is difficult to assess, because comprehensive studies are rare. In a recent meta-analysis, it was estimated that 1% of the cases of CAP were caused by *C. psittaci* (Hogerwerf et al. 2017). Examination of pharyngeal swabs from 780 CAP patients in Germany revealed a *C. psittaci* detection rate of 2.2% (Dumke et al. 2015). In most, if not all, countries of Europe and North America, a steady decline of notified cases of human psittacosis has been observed over the last two decades. However, as mentioned above, *C. psittaci* is usually not part of the routine laboratory diagnosis, so that many cases may remain unreported and official numbers may be an underestimation of the true incidence.

26.3.2 *Chlamydia abortus*

Human abortion due to *C. abortus* has been reported in several European countries, such as France, Switzerland, and the Netherlands and also in the USA (Longbottom and Coulter 2003; Rodolakis and Mohamad 2010). These human abortion cases, some with severe complications, have been associated with exposure to *C. abortus*-infected placentas of ewes and does but not of bovine origin (Buxton 1986; Essig and Longbottom 2015; Hyde and Benirschke 1997; Meijer et al. 2004; Pospischil et al. 2002; Roberts et al. 1967; Walder et al. 2003, 2005). The isolation of the bacteria from the placenta and fetus of a young woman who had assisted with lambing on her husband's farm, on which ewes had aborted, corroborated the zoonotic risk of *C. abortus* (Buxton 1986). The worldwide prevalence of these abortions is not known and might be underestimated, as the causes of infectious abortion are often not investigated in humans. The infection via ingestion or inhalation is usually acquired from infected abortion products or at parturition. In nonpregnant women and in men, *C. abortus* can cause subclinical infection to acute influenza-like illness, but this seems to be rare (Rodolakis and Mohamad 2010). In contrast, the consequences for pregnant women after close contact with infected sheep and goats are severe. *C. abortus* colonizes the human placenta causing abortion, stillbirth, and maternal illness (Essig and Longbottom 2015). In pregnant women, fever, headache, malaise, nausea, and vomiting are usually the first symptoms associated with lower abdominal pain followed by abortion. If left untreated, severe complications, such as acute renal failure, disseminated intravascular coagulation, or respiratory distress necessitating mechanical ventilation, might ensue. Transmission is mostly associated with exposure to infected sheep and goats, but indirect transmission from contaminated clothing, food, and other sources as well as through inhalation is also possible (Longbottom and Coulter 2003). Pregnant women should avoid exposure to small ruminants, particularly during the lambing and kidding periods. Ewes should be immediately separated; all dead fetuses, placental membranes, contaminated material, and bedding should be carefully disposed. Lambing pens must be cleaned and disinfected to limit the spread of infection and the possibility of zoonotic transmission. In general, chlamydial infections must be considered as an occupational hazard for pregnant women who come into contact with domestic ruminants (Essig and Longbottom 2015).

A zoonotic risk is not only relevant for pregnant women but also for laboratory workers when handling infectious material as they could subsequently develop atypical pneumonia (Ortega et al. 2015). Diagnosis of such an infection is best accomplished using PCR-based detection of *C. abortus* in bronchoalveolar lavage. This implies that *C. abortus* should be included as differential diagnosis for atypical pathogens in CAP in humans. In particular, farmers, veterinarians, laboratory personnel, and public health officials are at risk. The zoonotic potential of novel avian *C. abortus* strains (Longbottom et al. 2021; Szymanska-Czerwinska et al. 2017), which seem to be distributed worldwide in diverse bird families, is unknown and needs further investigations.

26.3.3 *Chlamydia caviae*

C. caviae can cause conjunctivitis in guinea pig owners (Lutz-Wohlgroth et al. 2006). The owner of the latter study reported mild serous ocular discharge, while *C. caviae* was detected in the conjunctival swab by PCR, suggesting a zoonotic infection. Between 2013 and 2018, severe CAP cases in human patients due to *C. caviae* after contact to guinea pigs emerged in the Netherlands (Ramakers et al. 2017; van Grootveld et al. 2018). Clinical signs in human patients included fever, malaise, coughing, headache, and myalgia, resulting in diagnosis of pneumonia and severe respiratory insufficiency with intensive care unit admission and mechanical ventilation. Sequencing of the *ompA* gene confirmed transmission between the guinea pigs and their owners. While contact to ill guinea pigs (respiratory signs, conjunctivitis, rhinitis) were confirmed in all three cases of the first outbreak (Ramakers et al. 2017), an additional case of severe CAP in an older patient remained unexplained, as no previous contact to guinea pigs was reported (van Grootveld et al. 2018). Diagnosis is best made on bronchoalveolar lavage samples using species-specific PCR protocols, including *ompA* genotyping based on variable domain 4, or whole-genome sequencing. Treatment of human patients with doxycycline is usually successful. *C. caviae* bears a zoonotic potential that should not be underestimated, especially since guinea pigs are beloved pets, in particular for children. Veterinarians should be aware of the potential zoonotic risk of infection when handling ill guinea pigs. Moreover, researchers handling experimentally *C. caviae*-infected guinea pigs might be at risk too. A single paper (Gaede et al. 2010) reported *C. caviae* in horses on a farm suffering from conjunctivitis and mucopurulent rhinitis but without contact to guinea pigs, thus illustrating that this chlamydial species is able to cross host barriers.

26.3.4 *Chlamydia felis*

In 1969, *C. felis* was isolated from conjunctival scrapings of a 25-year-old man with follicular keratoconjunctivitis (Schachter et al. 1969). Apparently, he got infected through close contact to his cat that had previously suffered from rhinitis and conjunctivitis. This case report was the first documentation of probable zoonotic transmission of *C. felis* from a cat. Chronic conjunctivitis due to *C. felis* acquired from a cat was also reported in a HIV-positive patient (Hartley et al. 2001), and isolation of the agent could confirm the link between the owner and the kitten. Apart from this latter confirmed case, no serious systemic disease or pneumonia have been reported (Browning 2004). More reports of zoonotic infections originate from the pre-PCR era, but evidence remains circumstantial (Browning 2004). In summary, a zoonotic potential due to *C. felis* in cats could be real, but the risk appears to be low. Still, cat owners and professionals working with cats might be at risk due to their exposure to potentially infected animals (Di Francesco et al. 2006; Wons et al. 2017), and precaution is warranted when handling diseased cats (Sykes 2005). Moreover, uncontrolled, non-vaccinated stray cat populations might also pose a zoonotic risk.

Table 3 Epidemiological and clinical features of *Chlamydia* spp. with confirmed zoonotic potential

	Animal infection		Human infection	
Species	Natural host (occasional hosts)	Disease(s) and clinical signs in the main host	Transmission	Disease(s) and clinical signs
C. psittaci	Birds (cattle, horse, swine)	Respiratory disease, conjunctivitis, coryza, mucopurulent discharge from nose and eyes, cough, dyspnea, greenish to grayish feces	Inhalation	From flu-like symptoms to severe systemic illness; atypical pneumonia
C. abortus	Sheep, goat (cattle, deer, horse, swine)	Enzootic abortion	Inhalation, ingestion	Abortion in pregnant woman, atypical pneumonia
C. caviae	Guinea pig (horse)	Conjunctivitis, pneumonia, abortion	Direct contact	Conjunctivitis, severe atypical pneumonia
C. felis	Cat	Conjunctivitis	Direct contact	(Kerato-) conjunctivitis

A summary of the epidemiological and clinical features of *Chlamydia* spp. with confirmed zoonotic potential is provided in Table 3.

26.3.5 Other Chlamydial Species

C. pneumoniae is considered a primary human respiratory pathogen (Hahn 1999) but has also been found in a variety of animal species including horses, marsupials, amphibians, and reptiles (Borel et al. 2018). Molecular data suggests that human *C. pneumoniae* originated from animals and cross-transmission to humans occurred earlier (Myers et al. 2009). However, no transmission events of *C. pneumoniae* from animals to humans have been observed to date.

C. suis is a ubiquitous pathogen in domestic pigs and has been associated with conjunctivitis, diarrhea, failure to gain weight, and other clinical signs (Schautteet and Vanrompay 2011). Though the zoonotic transmission of *C. suis* from pigs to humans has not yet been demonstrated, its DNA has been found in conjunctival swab samples of Nepalese trachoma patients (Dean et al. 2013). *C. suis* has also been detected in mucosal swab samples (rectal, conjunctival, pharyngeal) collected from farmers and slaughterhouse workers in Belgium and the Netherlands (De Puysseleyr et al. 2014a, b, 2017), including the detection of species-specific antibodies (Kieckens et al. 2018). These individuals did not have any clinical signs, but the concern is more related to the tetracycline resistance present in *C. suis* and its potential of transfer to the closely related human pathogen *C. trachomatis* (Suchland

et al. 2009). *C. suis* is the only chlamydial species harboring a stable tetracycline resistance (Sandoz and Rockey 2010). So far, all *C. suis* strains found in humans were tetracycline-susceptible.

Other veterinary chlamydial species, such as *C. pecorum* and *C. muridarum*; the new avian chlamydial species *C. avium* and *C. gallinacea* (Sachse et al. 2014), *C. buteonis* (Laroucau et al. 2019), and *Candidatus* Chlamydia ibidis (Vorimore et al. 2013); as well as the newly emerging chlamydial strains retrieved from snakes (*C. serpentis, C. poikilotherma*, Candidatus C. sanzinia, Candidatus C. corallus) (Staub et al. 2018; Taylor-Brown et al. 2016; Taylor-Brown and Polkinghorne 2017) or tortoises (Candidatus Chlamydia testudinis) (Laroucau et al. 2020) are not considered of zoonotic importance based on today's knowledge.

26.4 Genome Analysis of Zoonotic Agents

Recent advances in sequencing technology have enabled whole-genome sequencing of many chlamydial strains. Currently, sequences of the human pathogen *C. trachomatis* still make up the majority of database entries, but the main zoonotic agents *C. psittaci* and *C. abortus* are also well represented. Comparative studies of these data were conducted with the aim of understanding specific properties of *C. psittaci* and *C. abortus* (Holzer et al. 2020; Joseph et al. 2015; Read et al. 2013; Seth-Smith et al. 2017).

26.4.1 Common Genomic Elements

Sized approximately 1000 kbp, *Chlamydia* spp. have a small genome compared to most other bacteria. When it comes to comparison among *Chlamydia* spp. genome sequences, the high proportion of common coding sequences (CDS) is the most striking feature. A recent study involving 33 strains of 12 chlamydial species revealed that chlamydiae share about four-fifths of their genome (Holzer et al. 2020). The core genome, i.e., the number of CDS shared by all 33 strains, comprised 784 genes. This part of the genome is assumed to be indispensable and responsible for the typical properties of chlamydiae, such as the main stages of the obligate intracellular lifestyle. However, despite the highly conserved gene content of *Chlamydia* spp. genomes, the species belonging to this genus show significant diversity in terms of tissue tropism, host preference, immune and stress response patterns, as well as pathogenicity. Therefore, it seems straightforward to assume that the more variable genomic regions should account for species-specific features.

In this context, the following genomic regions are of major interest:

The plasticity zone (PZ), i.e., the hypervariable region near the predicted replication termination region, the families of polymorphic membrane proteins (Pmps) and inclusion membrane proteins (Incs), and a few others. As a general conclusion

from recent literature, the expectations on genomic studies should be realistic, since analysis of genome sequences can provide many hints and suggestions, but conclusive answers often will require additional functional studies.

In the following, we will summarize the data on characteristic genomic features of zoonotic chlamydiae.

26.4.2 *Chlamydia psittaci*

This species is rather heterogeneous from the genetic point of view. A serotype classification scheme for *C. psittaci* strains had been introduced more than three decades ago, which was later transformed into a genotyping system based on nucleotide sequences of the outer membrane protein A (*ompA*) (Vanrompay et al. 1997). Fifteen *ompA* genotypes are currently used, i.e., A to F, E/B, M56, WC, 1 V, 6 N, Mat116, R54, YP84, and CPX0308 (Sachse et al. 2008; Sachse and Ruettger 2015).

About 70 genome sequences of *C. psittaci* strains have been deposited to date in public databases. Genome size varies between 1.14 and 1.17 Mbp, and the number of CDS is approximately 1000 (Holzer et al. 2020).

The PZ of *C. psittaci* is sized in the range of 29,929 (strain MN) to 24,603 nt (strain WS-RT-E30), which is relatively small compared to *C. suis* (82,805 nt, strain 1–25a), *C. muridarum* (82,115 nt, strain Nigg), and *C. trachomatis* (55,445 nt, strain D-UW-3-CX). Among the major PZ constituents are genes encoding biotin modification (*accB, accC*), purine synthesis (*guaA, guaB* and ADA), a MAC/perforin, and an additional MAC/perforin domain-containing protein. Moreover, the large cytotoxin gene *toxB* was found in all strains examined so far, with type strain 6 BC harboring the largest version (10,074 nt). A typical characteristic of the *C. psittaci* genome is the absence of a tryptophan operon.

C. psittaci strains possess 21 different *pmp* genes, numbered *pmp1–17* and *pmp19–22*, which are classified into subtypes A, B, D, E G, and H. While 14 of these family members belong to subtype G and 3 to E, the other subtypes are represented by a single *pmp* gene.

An exhaustive description of the Inc. protein family in *C. psittaci* has not been achieved to date. So far, six different subtypes have been identified: A, B, C, V, X, and Y. The latter two were only found in *C. psittaci* and *C. abortus*, while subtype V is characteristic for the "psittaci cluster," which also includes *C. caviae* and *C. felis*.

26.4.3 *Chlamydia abortus*

This chlamydial species is distinguished by its remarkable genetic homogeneity. A recent genomic study involving 57 *C. abortus* isolates demonstrated unusual stability and lack of interspecies diversity (Seth-Smith et al. 2017).

The genome size of *C. abortus* strains sequenced to date ranges from 1.13 to 1.17 Mbp. The PZ is smaller than that of *C. psittaci*, but, at the same time,

characteristic differences between the typical ruminant strains and the recently discovered avian strains of *C. abortus* need to be taken into account. Ruminant strains, such as type strain B577 and strain S26–3, contain a PZ sized 11.7 kbp (ca. 17,770 nt), while the same region is 22,240 bp in the avian strain 16 DC122. Only the latter was found to have a *toxB* gene of 9312 nt reminiscent of *C. psittaci*. Furthermore, the PZ of *C. abortus* lacks a tryptophan operon and a CDS for MAC/perforin. Purine synthesis genes as present in *C. psittaci* have either evolved to pseudogenes or are absent altogether.

Strains of this species harbor 18 different *pmp* genes numbered 1–18 and belonging to subtypes A, B, D, E G, and H. Also here, subtype G is the most extensive one comprising 11 members, and E comprises 3. As mentioned above, Inc subtypes A, B, C, V, X, and Y are encountered.

26.4.4 *Chlamydia caviae*

The only genome sequence available is from the type strain GPIC. Its size is 1,173,390 bp.

Although the PZs of *C. caviae* and *C. felis* are not the most extensive ones (those of *C. suis* and *C. muridarum* are about twice as large), they possess the most complete set of genes and operons in this genomic region. Thus, the 34,753 nt PZ of *C. caviae* contains a *toxB* gene of 10,041 nt, the biotin modification genes (*accB, accC*), the purine synthesis genes (*guaA, guaB*, ADA), as well as a complete tryptophan operon (*trpA, trpB, trpD, trpF, trpR, kynU*).

Only the MAC/perforin seems to be missing.

Eighteen *pmp* genes were identified in the genome of this species. Subtype E/F, which was introduced here instead of E, has 5 representatives and G has 9. *Inc* genes were assigned to subtypes A, B, C, and V.

26.4.5 *Chlamydia felis*

The genome of strain Fe/C-56 is sized 1,166,239 bp. The PZ sized 39,924 nt contains a 9897 nt *toxB* gene, a CDS encoding MAC/perforin and, as *C. caviae*, the biotin modification genes, the purine synthesis genes and a complete tryptophan operon.

Furthermore, the *C. felis* genome harbors 20 *pmp* genes distributed among subtypes A, B, D, E G, and H, with 11 classified as subtype G and 4 as E. The *inc* genes of this species belong to subtypes A, B, C, and V (Holzer et al. 2020).

26.5 Unresolved Issues and Outlook

Animal chlamydiae are not a part of the traditional microbiological diagnostic procedure in human laboratories. Therefore, human chlamydial infections of zoonotic origin often remain underreported, misdiagnosed, or undiagnosed. Chlamydial

infections can be identified as a diagnosis of exclusion if routine diagnostic tests on blood, sputum, BAL fluid, and cultures remain negative. For example, *C. psittaci* is widely underdiagnosed as a cause of CAP, as pan-chlamydial PCRs do not identify the pathogen and species-specific diagnostics are not included in the routine evaluation of human cases. This omission can result in severe disease and empiric CAP treatment, which is not effective against chlamydiae.

Concerning laboratory diagnosis, successful approaches include the combined use of highly sensitive *Chlamydiaceae*-specific qPCR with species-specific qPCR to be supplemented by melting curve analysis, or sequencing, or microarray detection. Serological methods are not very sensitive and usually not species-specific. In older studies, *C. psittaci* was detected by complement fixation test, which is known to be neither sensitive nor specific. Moreover, serology requires the testing of paired sera, which delays the final result for 2 or more weeks. Using DNA-based methods, human samples can be further genotyped and matched with animal or environmental samples to search for the infection source.

New typing techniques, such as multilocus sequence typing (MLST) or culture-independent genome sequencing technologies, are helpful to interrogate strains of the same chlamydial species from different hosts to gain insight into their relationship, origin, and the potential for cross-host transmission.

Another important point to highlight is the choice of the appropriate sample type, which depends on the organ affected and chlamydial species involved. For the diagnosis of a *C. psittaci* infection, lower respiratory specimens are more suitable than nasopharyngeal swabs, albeit the latter are easier to collect (McGovern et al. 2021).

More recently, the use of metagenomic next-generation sequencing (mNGS) was suggested as a diagnostic tool for psittacosis. In a retrospective study by Chen et al. (2020), blood and BALF samples of nine psittacosis patients were subjected to mNGS to explore the suitability of this technology for diagnostic purposes. The presence of *C. psittaci* was revealed within 48–72 h.

However, although mNGS showed a satisfying diagnostic performance and had an overall superior detection rate to culture in initial studies (Zhang et al. 2020), it does not seem to offer advantages over established qPCR assays in terms of time and, more important, costs. While mNGS may provide a greater amount of information in the form of sequences, the question remains whether this is required in a clinical setting, where rapid pathogen identification enabling efficient treatment of the patient at the earliest time point is crucial.

Chlamydial infections in wildlife and spillover infections to domestic animals and humans (Burnard and Polkinghorne 2016) have to be carefully monitored, with wildlife hosts including birds, mammals, marsupials, amphibians, and reptiles. So far, mainly wild birds are considered to play a role as reservoir hosts for *C. psittaci*, but they might only represent the "tip of the iceberg." *C. abortus* has been detected in free-living frogs, marine reptiles and crocodiles, as well as wild ungulates (Burnard and Polkinghorne 2016). Chlamydial species in new and/or uncommon hosts, as exemplified by the zoonotic transmission of *C. psittaci* from equine abortion cases, require surveillance of transmission events and spreading. Recently, a case of a fatal

C. psittaci infection in a domestic kitten suffering of hepatitis and pneumonia was reported (Sanderson et al. 2021), but the source of infection remained unclear.

Novel and uncommon hosts or reservoirs for zoonotic chlamydiae might play an increasing role in the future. For example, ticks (*Ixodes ricinus*) removed from pet cats in Italy were PCR positive for *C. abortus* and *C. psittaci* (Chisu et al. 2020). Ticks are vectors for a wide variety of pathogens, including chlamydiae. In addition, chlamydia-like organisms (CLOs) and members of the family *Chlamydiaceae* have been found in bats, thus raising the question of the importance of bats as reservoir hosts or transmission source as known for other pathogens, especially viruses. More recently, ectoparasites of bats have been identified as potential vector candidates for chlamydial bacteria. These findings illustrate that our understanding of the ecology, diversity, and epidemiology of *Chlamydiaceae* and CLOs is still limited and further research is required.

References

Akter R, Sansom FM, El-Hage CM, Gilkerson JR, Legione AR, Devlin JM (2021) A 25-year retrospective study of *Chlamydia psittaci* in association with equine reproductive loss in Australia. J Med Microbiol 70(2):001284

Anstey S, Lizárraga D, Nyari S, Chalmers G, Carrick J, Chicken C, Jenkins C, Perkins N, Timms P, Jelocnik M (2021) Epidemiology of *Chlamydia psittaci* infections in pregnant Thoroughbred mares and foals. Vet J. 273:105683

Arenas-Valls N, Chacon S, Perez A, Del Pozo R (2017) Atypical *Chlamydia psittaci* pneumonia. Four related cases. Arch Bronconeumol 53:277–279

Baker JA (1942) A virus obtained from a pneumonia of cats and its possible relation to the cause of atypical pneumonia in man. Science 96:475–476

Baker JA (1944) A virus causing pneumonia in cats and producing elementary bodies. J Exp Med 79:159–172

Barros E (1929) Epidemia de psittacosis. Diario médico 152

Bart M, Guscetti F, Zurbriggen A, Pospischil A, Schiller I (2000) Feline infectious pneumonia: a short literature review and a retrospective immunohistological study on the involvement of Chlamydia spp. and distemper virus. Vet J 159:220–230

Baumann S, Gurtner C, Marti H, Borel N (2020) Detection of *Chlamydia* species in 2 cases of equine abortion in Switzerland: a retrospective study from 2000 to 2018. J Vet Diagn Invest 32:542–548

Beeckman DS, Vanrompay DC (2009) Zoonotic Chlamydophila psittaci infections from a clinical perspective. Clin Microbiol Infect 15:11–17

Borel N, Polkinghorne A, Pospischil A (2018) A review on chlamydial diseases in animals: still a challenge for pathologists? Vet Pathol 55:374–390

Branley J, Bachmann NL, Jelocnik M, Myers GS, Polkinghorne A (2016) Australian human and parrot *Chlamydia psittaci* strains cluster within the highly virulent 6BC clade of this important zoonotic pathogen. Sci Rep 6:30019

Bressan M, Rampazzo A, Kuratli J, Marti H, Pesch T, Borel N (2021) Occurrence of *Chlamydiaceae* and *Chlamydia felis* pmp9 typing in conjunctival and rectal samples of Swiss stray and pet cats. Pathogens 10

Browning GF (2004) Is *Chlamydophila felis* a significant zoonotic pathogen? Aust Vet J 82:695–696

Burnard D, Polkinghorne A (2016) Chlamydial infections in wildlife-conservation threats and/or reservoirs of 'spill-over' infections? Vet Microbiol 196:78–84

Burt SA, Roring RE, Heijne M (2018) *Chlamydia psittaci* and *C. avium* in feral pigeon *(Columba livia domestica)* droppings in two cities in the Netherlands. Vet Q 38:63–66

Buxton D (1986) Potential danger to pregnant women of *Chlamydia psittaci* from sheep. Vet. Rec 510–511

Buxton D, Barlow RM, Finlayson J, Anderson IE, Mackellar A (1990) Observations on the pathogenesis of *Chlamydia psittaci* infection of pregnant sheep. J Comp Pathol 102:221–237

Buxton D, Anderson IE, Longbottom D, Livingstone M, Wattegedera S, Entrican G (2002) Ovine chlamydial abortion: characterization of the inflammatory immune response in placental tissues. J Comp Pathol 127:133–141

Caspe SG, Palarea-Albaladejo J, Underwood C, Livingstone M, Wattegedera SR, Milne E, Sargison ND, Chianini F, Longbottom D (2021) Distribution and severity of placental lesions caused by the *Chlamydia abortus* 1B vaccine strain in vaccinated ewes. Pathogens 10

Chan J, Doyle B, Branley J, Sheppeard V, Gabor M, Viney K, Quinn H, Janover O, McCready M, Heller J (2017) An outbreak of psittacosis at a veterinary school demonstrating a novel source of infection. One Health 3:29–33

Chen X, Cao K, Wei Y, Qian Y, Liang J, Dong D, Tang J, Zhu Z, Gu Q, Yu W (2020) Metagenomic next-generation sequencing in the diagnosis of severe pneumonias caused by *Chlamydia psittaci*. Infection 48:535–542

Chisu V, Foxi C, Masu G, B DA, Masala G (2020) Detection of potentially pathogenic bacteria from *Ixodes ricinus* carried by pets in Tuscany, Italy. Vet Rec Open 7:e000395

De Puysseleyr K, De Puysseleyr L, Dhondt H, Geens T, Braeckman L, Morre SA, Cox E, Vanrompay D (2014a) Evaluation of the presence and zoonotic transmission of *Chlamydia suis* in a pig slaughterhouse. BMC Infect Dis 14:560

De Puysseleyr K, De Puysseleyr L, Geldhof J, Cox E, Vanrompay D (2014b) Development and validation of a real-time PCR for *Chlamydia suis* diagnosis in swine and humans. PLoS One 9: e96704

De Puysseleyr L, De Puysseleyr K, Braeckman L, Morre SA, Cox E, Vanrompay D (2017) Assessment of *Chlamydia suis* infection in pig farmers. Transbound Emerg Dis 64:826–833

Dean D, Rothschild J, Ruettger A, Kandel RP, Sachse K (2013) Zoonotic Chlamydiaceae species associated with trachoma, Nepal. Emerg Infect Dis 19:1948–1955

Deschuyffeleer TP, Tyberghien LF, Dickx VL, Geens T, Saelen JM, Vanrompay DC, Braeckman LA (2012) Risk assessment and management of *Chlamydia psittaci* in poultry processing plants. Ann Occup Hyg 56:340–349

Di Francesco A, Donati M, Mazzeo C, Battelli G, Piva S, Cevenini R, Baldelli R (2006) Feline chlamydiosis: a seroepidemiological investigation of human beings with and without contact with cats. Vet Rec 159:778–779

Dickx V, Geens T, Deschuyffeleer T, Tyberghien L, Harkinezhad T, Beeckman DS, Braeckman L, Vanrompay D (2010) *Chlamydophila psittaci* zoonotic risk assessment in a chicken and Turkey slaughterhouse. J Clin Microbiol 48:3244–3250

Donati M, Laroucau K, Guerrini A, Balboni A, Salvatore D, Catelli E, Lupini C, Levi A, Di Francesco A (2018) Chlamydiosis in backyard chickens (*Gallus gallus*) in Italy. Vector Borne Zoonotic Dis 18:222–225

Dujardin-Beaumetz G (1893) Enquete sur des cri de pneumonie infectieuse paraissant avoir été occassionés par des perruches. Bull. Conseil. Hyg. publ. salubrité Départ. Seine April 1893

Dumke R, Schnee C, Pletz MW, Rupp J, Jacobs E, Sachse K, Rohde G, Capnetz Study G (2015) *Mycoplasma pneumoniae* and *Chlamydia* spp. infection in community-acquired pneumonia, Germany, 2011–2012. Emerg Infect Dis 21:426–434

Essig A, Longbottom D (2015) *Chlamydia abortus*: new aspects of infectious abortion in sheep and potential risk for pregnant women. Curr Clin Microbiol Rep 2:22–34

Ferreira VL, Silva MV, Bassetti BR, Pellini ACG, Raso TF (2017) Intersectoral action for health: preventing psittacosis spread after one reported case. Epidemiol Infect 145:2263–2268

Fischer N, Rohde H, Indenbirken D, Gunther T, Reumann K, Lutgehetmann M, Meyer T, Kluge S, Aepfelbacher M, Alawi M, Grundhoff A (2014) Rapid metagenomic diagnostics for suspected outbreak of severe pneumonia. Emerg Infect Dis 20:1072–1075

Gaede W, Reckling KF, Dresenkamp B, Kenklies S, Schubert E, Noack U, Irmscher HM, Ludwig C, Hotzel H, Sachse K (2008) *Chlamydophila psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. Zoonoses Public Health 55:184–188

Gaede W, Reckling KF, Schliephake A, Missal D, Hotzel H, Sachse K (2010) Detection of *Chlamydophila caviae* and *Streptococcus equi* subsp. *zooepidemicus* in horses with signs of rhinitis and conjunctivitis. Vet Microbiol 142:440–444

Gruffydd-Jones T, Addie D, Belak S, Boucraut-Baralon C, Egberink H, Frymus T, Hartmann K, Hosie MJ, Lloret A, Lutz H, Marsilio F, Pennisi MG, Radford AD, Thiry E, Truyen U, Horzinek MC (2009) *Chlamydophila felis* infection. ABCD guidelines on prevention and management. J Feline Med Surg 11:605–609

Haagen E, Mauer G (1938) Über eine auf den Menschen übertragbare Viruskrankheit bei Sturmmöwen und ihre Beziehung zur Psittakose. Zbl Bakteriologie, Parasitenkunde und Infektionskrankheiten 143, I. Abt., Originale, 81–88

Haas WH, Swaan CM, Meijer A, Neve G, Buchholz U, Beer M, van Steenbergen JE, Krause G (2007) A Dutch case of atypical pneumonia after culling of H5N1 positive ducks in Bavaria was found infected with *Chlamydophila psittaci*. Euro Surveill 12(E071129):071123

Hahn DL (1999) *Chlamydia pneumoniae*, asthma, and COPD: what is the evidence? Ann Allergy Asthma Immunol 83:271–288, 291; quiz 291–272

Halanova M, Sulinova Z, Cislakova L, Trbolova A, Palenik L, Weissova T, Halan M, Kalinova Z, Holickova M (2011) *Chlamydophila felis* in cats–are the stray cats dangerous source of infection? Zoonoses Public Health 58:519–522

Hartley JC, Stevenson S, Robinson AJ, Littlewood JD, Carder C, Cartledge J, Clark C, Ridgway GL (2001) Conjunctivitis due to *Chlamydophila felis* (*Chlamydia psittaci* feline pneumonitis agent) acquired from a cat: Case report with molecular characterization of isolates from the patient and cat. J Infection 43:7–11

Hegler C (1930) Psittacosis – clinical experience with humans [in German]. Dt Tierärztl Wschr 38: 677–681

Heijne M, van der Goot JA, Fijten H, van der Giessen JW, Kuijt E, Maassen CBM, van Roon A, Wit B, Koets AP, Roest HIJ (2018) A cross sectional study on Dutch layer farms to investigate the prevalence and potential risk factors for different *Chlamydia* species. PLoS One 13: e0190774

Henning K, Sachse K, Sting R (2000) Nachweis von Chlamydien bei einem Stutenabort [Demonstration of Chlamydia from an equine abortion]. In Dtsch Tierarztl Wochenschr pp 49–52

Herrmann B, Persson H, Jensen JK, Joensen HD, Klint M, Olsen B (2006) *Chlamydophila psittaci* in fulmars, the Faroe Islands. Emerg Infect Dis 12:330–332

Hinton DG, Shipley A, Galvin JW, Harkin JT, Brunton RA (1993) Chlamydiosis in workers at a duck farm and processing plant. Aust Vet J 70:174–176

Hogerwerf L, DE Gier B, Baan B, Hoek WVANDER (2017) *Chlamydia psittaci* (psittacosis) as a cause of community-acquired pneumonia: a systematic review and meta-analysis. Epidemiol Infect 145:3096–3105

Holzer M, Barf LM, Lamkiewicz K, Vorimore F, Lataretu M, Favaroni A, Schnee C, Laroucau K, Marz M, Sachse K (2020) Comparative genome analysis of 33 *Chlamydia* strains reveals characteristic features of *Chlamydia psittaci* and closely related species. Pathogens 9

Hoover EA, Kahn DE, Langloss JM (1978) Experimentally induced feline chlamydial infection (feline pneumonitis). Am J Vet Res 39:541–547

Hughes C, Maharg P, Rosario P, Herrell M, Bratt D, Salgado J, Howard D (1997) Possible nosocomial transmission of psittacosis. Infect Control Hosp Epidemiol 18:165–168

Hulin V, Oger S, Vorimore F, Aaziz R, de Barbeyrac B, Berruchon J, Sachse K, Laroucau K (2015) Host preference and zoonotic potential of *Chlamydia psittaci* and *C. gallinacea* in poultry. Pathog Dis 73:1–11

Hyde SR, Benirschke K (1997) Gestational psittacosis: case report and literature review. Mod Pathol 10:602–607

Ito I, Ishida T, Mishima M, Osawa M, Arita M, Hashimoto T, Kishimoto T (2002) Familial cases of psittacosis: possible person-to-person transmission. Intern Med 41:580–583

Jelocnik M, Branley J, Heller J, Raidal S, Alderson S, Galea F, Gabor M, Polkinghorne A (2017) Multilocus sequence typing identifies an avian-like *Chlamydia psittaci* strain involved in equine placentitis and associated with subsequent human psittacosis. Emerg Microbes Infect 6:e7

Joseph SJ, Marti H, Didelot X, Castillo-Ramirez S, Read TD, Dean D (2015) *Chlamydiaceae* genomics reveals interspecies admixture and the recent evolution of *Chlamydia abortus* infecting lower mammalian species and humans. Genome Biol Evol 7:3070–3084

Kaleta EF, Taday EM (2003) Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. Avian Pathol 32:435–461

Kalmar ID, Dicxk V, Dossche L, Vanrompay D (2014) Zoonotic infection with *chlamydia psittaci* at an avian refuge Centre. Vet J 199:300–302

Kieckens E, Van den Broeck L, Van Gils M, Morre S, Vanrompay D (2018) Co-occurrence of *Chlamydia suis* DNA and *Chlamydia suis*-specific antibodies in the human eye. Vector Borne Zoonotic Dis 18:677–682

Knittler MR, Sachse K (2014) *Chlamydia psittaci*: update on an underestimated zoonotic agent (Minireview). FEMS Pathog Dis 73. https://doi.org/10.1093/femspd/ftu1007

Krautwald-Junghanns ME, Stolze J, Schmidt V, Bohme J, Sachse K, Cramer K (2013) Efficacy of doxycycline for treatment of chlamydiosis in flocks of racing and fancy pigeons. Tierarztliche Praxis. Ausgabe K, Kleintiere/Heimtiere 41:392–398

Lagae S, Kalmar I, Laroucau K, Vorimore F, Vanrompay D (2014) Emerging *Chlamydia psittaci* infections in chickens and examination of transmission to humans. J Med Microbiol 63:399–407

Laroucau K, Aaziz R, Meurice L, Servas V, Chossat I, Royer H, de Barbeyrac B, Vaillant V, Moyen JL, Meziani F, Sachse K, Rolland P (2015) Outbreak of psittacosis in a group of women exposed to *Chlamydia psittaci*-infected chickens. Euro Surveill 20:pii: 21155

Laroucau K, de Barbeyrac B, Vorimore F, Clerc M, Bertin C, Harkinezhad T, Verminnen K, Obeniche F, Capek I, Bebear C, Durand B, Zanella G, Vanrompay D, Garin-Bastuji B, Sachse K (2009) Chlamydial infections in duck farms associated with human cases of psittacosis in France. Vet Microbiol 135:82–89

Laroucau K, Vorimore F, Aaziz R, Solmonson L, Hsia RC, Bavoil PM, Fach P, Holzer M, Wuenschmann A, Sachse K (2019) *Chlamydia buteonis*, a new *Chlamydia* species isolated from a red-shouldered hawk. Syst Appl Microbiol 42:125997

Laroucau K, Ortega N, Vorimore F, Aaziz R, Mitura A, Szymanska-Czerwinska M, Cicerol M, Salinas J, Sachse K, Caro MR (2020) Detection of a novel *Chlamydia* species in captive spur-thighed tortoises (*Testudo graeca*) in southeastern Spain and proposal of *Candidatus* Chlamydia testudinis. Syst Appl Microbiol 43:126071

Lepore J (2009) It's spreading – outbreaks, media scares, and the parrot panic of 1930. The New Yorker American Chronicles June 1

Li L, Luther M, Macklin K, Pugh D, Li J, Zhang J, Roberts J, Kaltenboeck B, Wang C (2017) *Chlamydia gallinacea:* a widespread emerging *Chlamydia* agent with zoonotic potential in backyard poultry. Epidemiol Infect 145:2701–2703

Livingstone M, Wheelhouse N, Maley SW, Longbottom D (2009) Molecular detection of *Chlamydophila abortus* in post-abortion sheep at oestrus and subsequent lambing. Vet Microbiol 135:134–141

Longbottom D, Coulter LJ (2003) Animal chlamydioses and zoonotic implications. J Comp Pathol 128:217–244

Longbottom D, Livingstone M, Maley S, van der Zon A, Rocchi M, Wilson K, Wheelhouse N, Dagleish M, Aitchison K, Wattegedera S, Nath M, Entrican G, Buxton D (2013) Intranasal infection with *Chlamydia abortus* induces dose-dependent latency and abortion in sheep. PLoS One 8:e57950

Longbottom D, Sait M, Livingstone M, Laroucau K, Sachse K, Harris SR, Thomson NR, Seth-Smith HMB (2018) Genomic evidence that the live *Chlamydia abortus* vaccine strain 1B is not attenuated and has the potential to cause disease. Vaccine 36:3593–3598

Longbottom D, Livingstone M, Ribeca P, Beeckman DSA, van der Ende A, Pannekoek Y, Vanrompay D (2021) Whole genome de novo sequencing and comparative genomic analyses

suggests that *Chlamydia psittaci* strain 84/2334 should be reclassified as *Chlamydia abortus* species. BMC Genomics 22:159

Low HC, Powell CC, Veir JK, Hawley JR, Lappin MR (2007) Prevalence of feline herpesvirus 1, *Chlamydophila felis*, and *Mycoplasma* spp DNA in conjunctival cells collected from cats with and without conjunctivitis. Am J Vet Res 68:643–648

Lutz-Wohlgroth L, Becker A, Brugnera E, Huat ZL, Zimmermann D, Grimm F, Haessig M, Greub G, Kaps S, Spiess B, Pospischil A, Vaughan L (2006) *Chlamydiales* in guinea-pigs and their zoonotic potential. J Vet Med A Physiol Pathol Clin Med 53:185–193

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovc A, Prukner-Radovcic E, Residbegovic E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF (2009) Chlamydial infections in feral pigeons in Europe: review of data and focus on public health implications. Vet Microbiol 135:54–67

Maley SW, Livingstone M, Rodger SM, Longbottom D, Buxton D (2009) Identification of *Chlamydophila abortus* and the development of lesions in placental tissues of experimentally infected sheep. Vet Microbiol 135:122–127

McDonald M, Willett BJ, Jarrett O, Addie DD (1998) A comparison of DNA amplification, isolation and serology for the detection of *Chlamydia psittaci* infection in cats. Vet Rec 143:97–101

McGovern OL, Kobayashi M, Shaw KA, Szablewski C, Gabel J, Holsinger C, Drenzek C, Brennan S, Milucky J, Farrar JL, Wolff BJ, Benitez AJ, Thurman KA, Diaz MH, Winchell JM, Schrag S (2021) Use of real-time PCR for *Chlamydia psittaci* detection in human specimens during an outbreak of psittacosis – Georgia and Virginia, 2018. MMWR Morb Mortal Wkly Rep 70:505–509

McGuigan CC, McIntyre PG, Templeton K (2012) Psittacosis outbreak in Tayside, Scotland, December 2011 to February 2012. Eur Secur 17:20186

Meijer A, Brandenburg A, de Vries J, Beentjes J, Roholl P, Dercksen D (2004) *Chlamydophila abortus* infection in a pregnant woman associated with indirect contact with infected goats. Eur J Clin Microbiol Infect Dis 23:487–490

Meyer KF, Eddie B (1935) Avian psittacosis. J Bacteriol 29:67

Morange A (1895) De la psittacose, ou infection spéciale déterminée par des perruches. Thesis, Fac. de Mèdecine, Paris

Mount DT, Bigazzi PE, Barron AL (1973) Experimental genital infection of male guinea pigs with the agent of guinea pig inclusion conjunctivitis and transmission to females. Infect Immun 8:925–930

Murray ES (1964) Guinea pig inclusion conjunctivitis virus. I. Isolation and identification as a member of the psittacosis-lymphogranuloma-trachoma group. J Infect Dis 114:1–12

Myers GS, Mathews SA, Eppinger M, Mitchell C, O'Brien KK, White OR, Benahmed F, Brunham RC, Read TD, Ravel J, Bavoil PM, Timms P (2009) Evidence that human *Chlamydia pneumoniae* was zoonotically acquired. J Bacteriol 191:7225–7233

Ornelas-Eusebio E, Garcia-Espinosa G, Vorimore F, Aaziz R, Durand B, Laroucau K, Zanella G (2020) Cross-sectional study on *Chlamydiaceae* prevalence and associated risk factors on commercial and backyard poultry farms in Mexico. Prev Vet Med 176:104922

Ortega N, Caro MR, Gallego MC, Murcia-Belmonte A, Alvarez D, Del Rio L, Cuello F, Buendia AJ, Salinas J (2015) Isolation of *Chlamydia abortus* from a laboratory worker diagnosed with atypical pneumonia. Ir Vet J 69:8

Page LA, Grimes JE (1978) Avian chlamydiosis (ornithosis). In: Hofstad MS, Barnes HJ, Calnek BW, Yoder WM, Yoder HW Jr (eds) Diseases of poultry, 8th edn. Iowa State University Press, Ames, IA, pp 283–308

Pantchev A, Sting R, Bauerfeind R, Tyczka J, Sachse K (2010) Detection of all *Chlamydophila* and *Chlamydia* spp. of veterinary interest using species-specific real-time PCR assays. Comp Immunol Microbiol Infect Dis 33:473–484

Polkinghorne A, Greub G (2017) A new equine and zoonotic threat emerges from an old avian pathogen, *Chlamydia psittaci*. Clin Microbiol Infect 23:693–694

Polkinghorne A, Borel N, Heijne M, Pannekoek Y (2019) New evidence for domesticated animals as reservoirs of *Chlamydia*-associated community-acquired pneumonia. Clin Microbiol Infect 25:131–132

Pospischil A, Thoma R, Hilbe M, Grest P, Zimmermann D, Gebbers JO (2002) Abortion in humans by *Chlamydophila abortus* (*Chlamydia psittaci* serovar 1). In Schweiz. Arch. Tierheilk pp 463–466

Ramakers BP, Heijne M, Lie N, Le TN, van Vliet M, Claessen VPJ, Tolsma PJP, De Rosa M, Roest HIJ, Vanrompay D, Heddema ER, Schneeberger P, Hermans MHA (2017) Zoonotic *Chlamydia caviae* presenting as community-acquired pneumonia. N Engl J Med 377:992–994

Rampazzo A, Appino S, Pregel P, Tarducci A, Zini E, Biolatti B (2003) Prevalence of *Chlamydophila felis* and feline herpesvirus 1 in cats with conjunctivitis in northern Italy. J Vet Intern Med 17:799–807

Read TD, Joseph SJ, Didelot X, Liang B, Patel L, Dean D (2013) Comparative analysis of *Chlamydia psittaci* genomes reveals the recent emergence of a pathogenic lineage with a broad host range. MBio 4

Rehn M, Ringberg H, Runehagen A, Herrmann B, Olsen B, Petersson AC, Hjertqvist M, Kuhlmann-Berenzon S, Wallensten A (2013) Unusual increase of psittacosis in southern Sweden linked to wild bird exposure, January to April 2013. Euro Surveill 18:20478

Reinhold P, Sachse K, Kaltenboeck B (2011) *Chlamydiaceae* in cattle: commensals, trigger organisms, or pathogens? Vet J 189:257–267

Ritter J (1879) Über Pneumotyphus, eine Hausepidemie in Uster. Dtsch Arch Klin Med 25:53

Roberts W, Grist NR, Giroud P (1967) Human abortion associated with infection by ovine abortion agent. Br Med J 4:37

Rodolakis A, Yousef Mohamad K (2010) Zoonotic potential of *Chlamydophila*. Vet Microbiol 140:382–391

Sachse K, Bavoil PM, Kaltenboeck B, Stephens RS, Kuo CC, Rossello-Mora R, Horn M (2015a) Emendation of the family *Chlamydiaceae*: proposal of a single genus, *Chlamydia*, to include all currently recognized species. Syst Appl Microbiol 38:99–103

Sachse K, Borel N (2020) Recent advances in epidemiology, pathology and immunology of veterinary chlamydiae (Chapter 17). Caister Academic Press, pp 403–428. https://doi.org/10.21775/9781912530281

Sachse K, Laroucau K, Hotzel H, Schubert E, Ehricht R, Slickers P (2008) Genotyping of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of ompA genes. BMC Microbiol 8:63

Sachse K, Laroucau K, Riege K, Wehner S, Dilcher M, Creasy HH, Weidmann M, Myers G, Vorimore F, Vicari N, Magnino S, Liebler-Tenorio E, Ruettger A, Bavoil PM, Hufert FT, Rossello-Mora R, Marz M (2014) Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. Syst Appl Microbiol 37:79–88

Sachse K, Laroucau K, Vanrompay D (2015b) Avian Chlamydiosis. Curr Clin Microbiol Rep 2:10–21

Sachse K, Ruettger A (2015) Rapid microarray-based genotyping of *Chlamydia* spp. strains from clinical tissue samples. Methods Mol Biol 1247:391–400

Sammin D, Markey B, Bassett H, Buxton D (2009) The ovine placenta and placentitis-A review. Vet Microbiol 135:90–97

Sanderson H, Vasquez M, Killion H, Vance M, Sondgeroth K, Fox J (2021) Fatal *Chlamydia psittaci* infection in a domestic kitten. J Vet Diagn Invest 33:101–103

Sandoz KM, Rockey DD (2010) Antibiotic resistance in *Chlamydiae*. Future Microbiol 5:1427–1442

Schachter J, Ostler HB, Meyer KF (1969) Human infection with the agent of feline pneumonitis. Lancet 1:1063–1065

Schautteet K, Vanrompay D (2011) *Chlamydiaceae* infections in pig. Vet Res 42:29

Schmal-Filius E, Nedorost N, Weissenbacher-Lang C, Weissenbock H (2020) A retrospective study on the presence of selected infectious agents in lung samples of cats with pneumonia. Acta Vet Hung 68:275–284

Seth-Smith HMB, Buso LS, Livingstone M, Sait M, Harris SR, Aitchison KD, Vretou E, Siarkou VI, Laroucau K, Sachse K, Longbottom D, Thomson NR (2017) European *Chlamydia abortus* livestock isolate genomes reveal unusual stability and limited diversity, reflected in geographical signatures. BMC Genomics 18:344

Shewen PE, Povey RC, Wilson MR (1978) Case report. Feline chlamydial infection. Can Vet J 19:289–292

Staub E, Marti H, Biondi R, Levi A, Donati M, Leonard CA, Ley SD, Pillonel T, Greub G, Seth-Smith HMB, Borel N (2018) Novel *Chlamydia* species isolated from snakes are temperature-sensitive and exhibit decreased susceptibility to azithromycin. Sci Rep 8:5660

Suchland RJ, Sandoz KM, Jeffrey BM, Stamm WE, Rockey DD (2009) Horizontal transfer of tetracycline resistance among *Chlamydia* spp. *in vitro*. Antimicrob Agents Chemother 53:4604–4611

Sykes JE (2005) Feline chlamydiosis. Clin Tech Small Anim Pract 20:129–134

Sykes JE, Anderson A, Studdert VP, Browning GF (1999a) Prevalence of feline *Chlamydia psittaci* and feline Herpesvirus 1 in cat with upper respiratory tract disease. In J Vet Intern Med 153–162

Sykes JE, Studdert VP, Browning GF (1999b) Comparison of the polymerase chain reaction and culture for the detection of feline Chlamydia psittaci in untreated and doxycycline-treated experimentally infected cats. J Vet Intern Med 13:146–152

Szeredi L, Hotzel H, Sachse K (2005) High prevalence of chlamydial (*Chlamydophila psittaci*) infection in fetal membranes of aborted equine fetuses. Vet Res Commun 29(Suppl 1):37–49

Szymanska-Czerwinska M, Mitura A, Niemczuk K, Zareba K, Jodelko A, Pluta A, Scharf S, Vitek B, Aaziz R, Vorimore F, Laroucau K, Schnee C (2017) Dissemination and genetic diversity of chlamydial agents in Polish wildfowl: isolation and molecular characterisation of avian *Chlamydia abortus* strains. PLoS One 12:e0174599

Taise SM (2013) Avian Chlamydiosis – retrospective analysis of an epidemic with zoonotic potential, from its first description to the present [in German]. Thesis, University of Giessen, Germany. http://geb.unigiessen.de/geb/volltexte/2014/10664/pdf/TaiseSabine_12013_10612_10609.pdf

Taylor-Brown A, Bachmann NL, Borel N, Polkinghorne A (2016) Culture-independent genomic characterisation of *Candidatus* Chlamydia sanzinia, a novel uncultivated bacterium infecting snakes. BMC Genomics 17:710

Taylor-Brown A, Polkinghorne A (2017) New and emerging chlamydial infections of creatures great and small. New Microbes New Infect 18:28–33

Taylor KA, Durrheim D, Heller J, O'Rourke B, Hope K, Merritt T, Freeman P, Chicken C, Carrick J, Branley J, Massey P (2018) Equine chlamydiosis-An emerging infectious disease requiring a one health surveillance approach. Zoonoses Public Health 65:218–221

TerWee J, Sabara M, Kokjohn K, Sandbulte J, Frenchick P, Dreier KJ (1998) Characterization of the systemic disease and ocular signs induced by experimental infection with *Chlamydia psittaci* in cats. Vet Microbiol 59:259–281

Van Droogenbroeck C, Beeckman DS, Verminnen K, Marien M, Nauwynck H, Boesinghe Lde T, Vanrompay D (2009) Simultaneous zoonotic transmission of *Chlamydophila psittaci* genotypes D, F and E/B to a veterinary scientist. Vet Microbiol 135:78–81

Van Grootveld R, Bilsen MP, Boelsums TL, Heddema ER, Groeneveld GH, Gooskens J, de Boer MGJ (2018) *Chlamydia caviae* causing community-acquired pneumonia: An emerging zoonosis. Vector Borne Zoonotic Dis

Van Loock M, Geens T, De Smit L, Nauwynck H, Van Empel P, Naylor C, Hafez HM, Goddeeris BM, Vanrompay D (2005) Key role of Chlamydophila psittaci on Belgian Turkey farms in association with other respiratory pathogens. Vet Microbiol 107:91–101

Vanrompay D (2013) Avian Chlamydiosis. In: Diseases of poultry, Bacterial diseases, vol Section III, 13th edn. Wiley- Blackwell Publishing

Vanrompay D, Butaye P, Sayada C, Ducatelle R, Haesebrouck F (1997) Characterization of avian *Chlamydia psittaci* strains using omp1 restriction mapping and serovar-specific monoclonal antibodies. Res Microbiol 148:327–333

Vanrompay D, Harkinezhad T, van de Walle M, Beeckman D, van Droogenbroeck C, Verminnen K, Leten R, Martel A, Cauwerts K (2007) *Chlamydophila psittaci* transmission from pet birds to humans. Emerg Infect Dis 13:1108–1110

Vorimore F, Holzer M, Liebler-Tenorio EM, Barf LM, Delannoy S, Vittecoq M, Wedlarski R, Lecu A, Scharf S, Blanchard Y, Fach P, Hsia RC, Bavoil PM, Rossello-Mora R, Laroucau K, Sachse K (2021) Evidence for the existence of a new genus *Chlamydiifrater* gen. nov. inside the

family *Chlamydiaceae* with two new species isolated from flamingo (*Phoenicopterus roseus*): *Chlamydiifrater phoenicopteri* sp. nov. and *Chlamydiifrater volucris* sp. nov. Syst Appl Microbiol 44:126200

Vorimore F, Hsia RC, Huot-Creasy H, Bastian S, Deruyter L, Passet A, Sachse K, Bavoil P, Myers G, Laroucau K (2013) Isolation of a new *Chlamydia* species from the Feral Sacred Ibis (*Threskiornis aethiopicus*): *Chlamydia ibidis*. PLoS One 8:e74823

Vorimore F, Thebault A, Poisson S, Cleva D, Robineau J, de Barbeyrac B, Durand B, Laroucau K (2015) *Chlamydia psittaci* in ducks: a hidden health risk for poultry workers. Pathog Dis 73:1–9

Wachendorfer G, Luthgen W (1974) Chlortetracycline impregnated food-pellets for the prophylaxis and therapy of psittacosis/ornithosis in psittacines and pigeons. Avian Pathol 3:105–114

Walder G, Meusburger H, Hotzel H, Oehme A, Neunteufel W, Dierich MP, Wurzner R (2003) *Chlamydophila abortus* pelvic inflammatory disease. In Emerg Infect Dis 1642–1644

Walder G, Hotzel H, Brezinka C, Gritsch W, Tauber R, Wurzner R, Ploner F (2005) An unusual cause of sepsis during pregnancy: recognizing infection with *Chlamydophila abortus*. Obstet Gynecol 106:1215–1217

Wallensten A, Fredlund H, Runehagen A (2014) Multiple human-to-human transmission from a severe case of psittacosis, Sweden, January-February 2013. Euro Surveill 19

Wang H, Jensen JK, Olsson A, Vorimore F, Aaziz R, Guy L, Ellstrom P, Laroucau K, Herrmann B (2020) *Chlamydia psittaci* in fulmars on the Faroe Islands: a causative link to south American psittacines eight decades after a severe epidemic. Microbes Infect 22:356–359

Wattegedera SR, Livingstone M, Maley S, Rocchi M, Lee S, Pang Y, Wheelhouse NM, Aitchison K, Palarea-Albaladejo J, Buxton D, Longbottom D, Entrican G (2020) Defining immune correlates during latent and active chlamydial infection in sheep. Vet Res 51:75

Wills JM, Grufydd-Jones TJ, Richmond SJ, Gaskell RM, Bourne FJ (1987) Effect of vaccination on feline *Chlamydia psittaci* infection. Infect Immun 55:2653–2657

Winkle S (2000) The history of the parrot fever epidemic [in German: Die Seuchengeschichte der Papageienkrankheit]. Hamb Arztebl 2:50–58

Wons J, Meiller R, Bergua A, Bogdan C, Geissdorfer W (2017) Follicular conjunctivitis due to *Chlamydia felis*-Case report, review of the literature and improved molecular diagnostics. Front Med (Lausanne) 4:105

Yan C, Fukushi H, Matsudate H, Ishihara K, Yasuda K, Kitagawa H, Yamaguchi T, Hirai K (2000) Seroepidemiological investigation of feline chlamydiosis in cats and humans in Japan. Microbiol Immunol 44:155–160

Yin L, Kalmar ID, Boden J, Vanrompay D (2015) *Chlamydia psittaci* infections in Chinese poultry: a literature review. Worlds Poult Sci J 71:473–482

Zhang Y, Cui P, Zhang HC, Wu HL, Ye MZ, Zhu YM, Ai JW, Zhang WH (2020) Clinical application and evaluation of metagenomic next-generation sequencing in suspected adult central nervous system infection. J Transl Med 18:199

Part V

Zoonoses in Domestic Animals

Dogs and Transmission of Infection to Man, "Respected Member of the Family?" 27

Paul Overgaauw and Frans van Knapen

Contents

Abstract

Many reviews on dog zoonoses address long-lasting lists of zoonotic infections, either observed worldwide or very specifically only in certain regions. In this chapter, the average pet dog in the western hemisphere will be described. It is assumed that the dog is owned by an average family with limited knowledge of the potential hazards their pet might be carrying, and possibly transmitting to family members.

It is based on semiquantitative risk analysis in order to rank potential health risks transmitted from pet dogs to humans. Surprisingly, everyday risk is different from the expected general potential risk according to the traditional ranking of hazards (zoonoses) in dogs. Attention will be given to human behavior regarding

P. Overgaauw (✉) · F. van Knapen
Faculty of Veterinary Medicine, Institute of Risk Assessment Sciences, Veterinary Public Health, Utrecht University, Utrecht, The Netherlands
e-mail: p.a.m.overgaauw@uu.nl

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_22

pet dogs and to responsible dog ownership. Modern trends include pet importation from endemic to non-endemic areas, without sufficient knowledge among pet owners or public health institutes. In Europe, the advice provided by ESCCAP (www.esccap.org) is of great value to veterinarians and pet-owners alike and includes information about prevalence and the prevention of parasitic infection in dogs and cats in the major European languages.

Other new trends include feeding bones and raw meat to dogs, which may have serious consequences for the spread of zoonoses like *Salmonella* and parasitic infections not only between dogs, but also to family members.

Finally, yet importantly, is the prevention of attracting wildlife zoonoses, via dogs, to family members (e.g., *Echinococcus multilocularis* and *Baylisascaris* spp.). Public health authorities should be encouraged to pay more attention to this, not only by increased regulation, but primarily by enforcing existing rules and encouraging responsible pet ownership. Companion animal veterinarians and (local) public health authorities, including physicians, should contribute equally in zoonosis prevention programs ("One Health" approach).

Keywords

Zoonoses · Emerging zoonoses · Dogs · Risk analysis · Human behavior · Trends

27.1 Introduction

Until around the turn of the twenty-first century, zoonoses were regarded by the medical profession as "just a number" of well-known infectious diseases in the modern ("western") world. As infectious diseases in that part of the world were abated by increased public hygiene, food safety, vaccinations, and proper antibiotic treatment, there seemed no need for further attention. However, a number of events causing human casualties have alarmed public health authorities in many countries and have sparked public and political awareness for new and reemerging zoonoses. Such events include the rapid spread of severe acute respiratory syndrome SARS in 2002 (Peiris et al. 2003), the outbreaks of avian influenza in South East Asia, but also in Europe (Shortridge et al. 2003) with varying types (H5N1, H7N7), West Nile virus throughout the United States (Shephard et al. 2006), Middle East Respiratory Syndrome-CoV coronavirus MERS (Zaki et al. 2012), the Zika-virus in 2013 (Cao-Lormeau et al. 2014), and the COVID-19 coronavirus in 2020 (WHO 2020).

In 2004, a joint WHO/FAO/OIE consultation on emerging zoonoses was held in Geneva (WHO/FHO/OIE 2004). Other incidents fostered this change such as antibiotic resistance believed to be related to animal treatments (MRSA, ESBL) (Weese 2010; Friese et al. 2013), and the significant spread of ordinary gray ticks (*Ixodus ricinus*) and consequently Lyme disease in man (Giessen et al. 2010). A central question to be answered is what role do veterinarians play in public health in the twenty-first century? (WHO 2002).

As a result of emerging and reemerging diseases, the One Health concept became important as a worldwide initiative recognizing that public health relates to animal

health and the environment. The multidisciplinary collaboration between physicians, veterinarians, environmental scientists, public health professionals, and other experts enhances the knowledge of how zoonotic diseases can be shared between animals and people with the goal of achieving optimal health outcomes (Overgaauw et al. 2020). The World Small Animal Veterinary Association (WSAVA) One Health Committee identified three key areas regarding companion animals: the human–companion animal bond, comparative and translational medicine, and zoonotic infectious disease (WSAVA 2011).

A traditional approach to dealing with zoonoses is to use a model, which includes the biology of the germ, epidemiology, disease in man and animals, diagnosis and prevention or control of the disease. Often it involves a simple enumeration of zoonoses found in dogs, without exploring the relative health risks for dog owners or the population at large through environmental pollution (Macpherson et al. 2012; Baneth et al. 2016). Depending on endemicity in certain areas of the world, more attention could be paid to zoonoses such as echinococcosis and toxocarosis (Carmena and Cardona 2013; Deplazes et al. 2011).

In this chapter, we will concentrate on relevant dog zoonoses and the role of the pet owner in an average (north) western European country without endemicity for diseases such as leishmaniosis, rabies, or heartworm disease. We will focus on an average family dog, within an average household and will assume limited awareness of the potential health risks of enjoying a pet at home.

27.2 Risk Analysis

The relative risks to human health or the attribution of a huge variety of dog zoonoses to having dogs, are either largely unknown or studies remain inconclusive. This is due to failure in examining both pet owners and their pets simultaneously, and by not comparing isolates by genotyping, serotyping, or other identification methods to suggest a one-way cross infection or a common source of infection elsewhere. There are, however, also indications that some infections may be transferred from humans to their dogs, which is called reverse zoonotic disease transmission or zooanthroponosis. For companion animals, MRSA infection was especially reported but *M. tuberculosis*, influenza A, *Candida albicans*, *Microsporum* spp., and *Trichophyton* spp. were also discussed (Lefebvre et al. 2009; Messenger et al. 2014). The zoonotic potential of *G. duodenalis* is considered evident, based on findings of assemblages A and B in humans, as well as in dogs. Authors automatically concluded therefore a one-way transmission route to the human (Marangi et al. 2010; Dado et al. 2012). The risk of transmission from dogs and cats to humans is, however, considered very low. Dog- and cat-specific *Giardia* assemblages are very rarely found in humans, but human assemblages A and B may circulate within dog and cat populations and therefore it could be that humans are a source of infection to a dog or cat, which may then in turn represent a zoonotic risk. Evidence on the contribution and frequency of the zoonotic potential is (still) lacking (Sprong et al. 2009).

Each risk analysis begins with an assessment of the potential dog zoonoses in an area, depending on the endemicity (the hazard, H). Hazard characterization also

includes prevalence in animals (the reservoir), virulence to man, transmission routes, and survival of the agent in the environment. These criteria are then weighed, mostly based on expert opinion.

The second step is exposure assessment (E). Who is exposed to the potential hazard and for how long or how often? How much of the potential pathogen is needed to become a health risk? This is inevitably directly related to human behavior in relation to their dog.

The third step is to assess the impact of getting infected. How serious is the disease, what is the chance of complications, and what economic consequences may be expected (e.g., labor hours lost)? For this purpose, the disease burden can be expressed in disability adjusted life years (DALYs). This quantifies health loss based on two components: life years lost due to premature death and the proportional loss of quality of life as a result of the disease.

Each of the parameters can be ranked in order from negligible (1) to the most serious possibility (5). Ranking is based on literature data, own observations (measuring), or expert opinion, thus arbitrarily.

The final risk assessment can be achieved by multiplying the outcome of hazard characterization, exposure assessment, and impact ($H \times E \times I = a$ number). The outcome is nothing more than a ranking of the potential health hazards and as such, can be compared with other zoonotic agents.

An example of such a risk assessment was carried out in a large companion animal referral clinic in the Netherlands (Berends 2006). Table 1 shows the top five dog zoonoses from this study, according to the hazard characterization ranking order. Table 2 shows the ranking order of exposure to these zoonoses. In Table 3, the top five most important dog zoonoses in an average small animal clinic are listed, based on the multiplication of hazard, exposure, and impact. This can be used as the point of departure because the intensive contact that clinicians have with dogs will certainly be comparable to that of an owner, with only one important exception: the duration of exposure. Since this is also subject to the owner's behavior, this will be discussed in sect. 27.22.

Table 1 Ranking order of biological hazard characterization in dogs

1.	Rabies
2.	*Capnocytophaga canimorsus*
3.	*Leptospira* spp.
4.	*Salmonella* spp.
5.	*Campylobacter* spp.

Table 2 Ranking order of exposure assessment

1.	Dermatophytes
2.	*Pasteurella multocida, P. canis*
3.	*Staphylococcus aureus/intermedius/pseudointermedius*
4.	*Campylobacter*
5.	*Salmonella*

Table 3 Ranking order of potential human health risks due to dog zoonoses

1.	*Campylobacter*
2.	*Pasteurella* spp.
3.	*Salmonella* spp.
4.	*Staphylococcus* spp.
5.	*Listeria* spp.

27.2.1 Dog Ownership

Companion animals have an important emotional value and promote socialization among the lonely elderly because they facilitate additional contact with people. Pets create purpose in life, reduce stress, and encourage physical activity. Most research, addressing the health benefits of pet ownership, shows a reduction in distress and anxiety, a decrease in loneliness and depression, and an increase in physical condition (Friedmann and Son 2009).

Dogs also play an increasing role as co-therapist or supporter for people with psychological or physical disabilities. The benefits of these animal-assisted activities are improved mood and decreased physiological distress, depression, dementia, and loneliness (Olsen et al. 2016).

Animal-assisted therapies can be used alongside other methods to facilitate psychotherapy or to provide specific types of therapeutic interventions such as improving motor skills or behavior. Such interventions were effective in improving the health status of children or adults with, or at risk of developing, mental disorders (Friedmann and Son 2009).

Dogs play an important role in the development and treatment of behavioral problems in children, the well-being of the elderly, and in decreasing absenteeism due to illness and visits to the doctor (Purewal et al. 2017; Hoagwood et al. 2017). Pet ownership has certainly been associated with health benefits, although not all (social) studies have been based on correct methodologies (Koivusilta and Ojanlatva 2006; Wells 2019).

On the other hand, more than six out of ten known infectious diseases in people can be spread by animals, and three out of four new or emerging infectious diseases in people are derived from animals (CDC). The recent pandemic of the COVID-19 coronavirus (SARS-CoV-2) that may originate from bats, is a good example of a recent emerging zoonotic infectious disease. A few dogs have tested positive but are not considered a source of infection for humans.

Here we deal with potential biological hazards (zoonoses) that may have negative health consequences for the owner. The starting point is Table 3, which ranks the zoonoses by potential health risks for individuals with short but intensive contact, such as veterinarians or breeders. It may be assumed that exposure to the potential hazards mentioned will be much higher and longer lasting when the owner (family) is involved. The actual top five zoonoses will, however, remain similar and concern the fecal-oral route of transmission (*Campylobacter, Salmonella, Giardia*), direct contact (*Staphylococcus* spp.), and injuries as result of bite incidents, licking, and scratching (*Pasteurella multocida, P. canis*).

27.2.2 Human Behavior

In 80–90% of households, pets are considered members of the family, therefore physical contact is very common. Cuddling, stroking, and playing with animals is normal among dog owners, and especially their children. It is all part of enjoying pet animals, but pets have increasingly become substitutes for childbearing and childcare, sometimes leading to excessive pet care and intensive contact (Chomel and Sun 2011). This kind of behavior is the result of attributing human cognitive processes and emotional states to animals, such as feelings of happiness, love, or guilt. This perception that animals have awareness, thoughts, and feelings is called anthropomorphism (Szánthó et al. 2017).

It is remarkable that some owners allow their dogs to approach the table, beg for snacks, are often stroked or even worse are allowed to join the table at mealtimes. Therefore, washing hands before a meal would demonstrate an awareness of hygienic practice. The number of potential pathogens such as enterobacteriaceae (Westgarth et al. 2008) or parasite eggs (Keegan and Holland 2010) from the fur of most animals, including dogs, is easily detectable and can be washed off by using water and ordinary household soap. It is self-evident behavior to do so before sitting down for dinner or after visiting the toilet and is all part of upbringing and education.

Licking the face or sharing an ice cream is a sign of mutual affection for some and is allowed by as much as 50% of households (Westgarth et al. 2008; Overgaauw et al. 2009). The notion that a dog's tongue is clean and may even be used to cleanse wounds is widespread among the general public and sometimes even among first aid health professionals (Verrier 1970; Overgaauw et al. 2020). Dogs regularly lick their anus and it has been found that over half of all dog and cat owners allow their pets to lick their hands or faces. By doing this, bacteria may be spread. Literature increasingly indicates that dogs licking humans may lead to infections (Booij-Vrieling et al. 2010; Haesebrouck et al. 2009); or serious health consequences in individual cases (Shewring and Rushforth 1990; Wade et al. 1999; Overgaauw and van Knapen 2012).

Allowing dogs to sleep in the bedroom (33–56%) or to even sleep in the owner's bed (18–50%) is certainly contributing to the transmission of zoonoses, including parasites (Overgaauw et al. 2020; Chomel and Sun 2011). Intensive contact with the skin and nose, (*Staphylococcus* spp.) even when the dog is healthy and without skin lesions, may lead to contamination with antimicrobial resistant strains (MRSA) (Manian 2003; Cain 2013).

Having one or more dogs in the household means that soil from outdoors is regularly spread throughout the house. Dogs were regularly reported to have soil-transmitted parasitic infections in their fur (Keegan and Holland 2010) with, as yet, unknown consequences for the owner. However, it should be kept in mind that even *Toxocara* eggs may easily be found in household dust taken from the houses of dog owners (Overgaauw and Boersema 1998; Panova and Khrustalev 2018).

Among soil-transmitted diseases, serious consideration must be given to toxoplasmosis because dogs might act as active distributors of oocysts from the environment. Dogs regularly and actively roll in the feces of other animals or eat them (Frenkel et al. 1996; Nijsse et al. 2014).

An important role of the veterinarian is to make owners aware of the potential risks and to emphasize the importance of personal hygiene and thorough cleaning of the house/kitchen, without causing alarm (Stull et al. 2015). Awareness and responsible pet ownership are the key issues in achieving a healthy relationship between owner and dog.

27.2.3 Responsible Pet Ownership

The benefits of pet ownership come with certain obligations. This concept is called responsible pet ownership and includes, among other things, providing the preventive (e.g., vaccinations, parasite control) and therapeutic healthcare needs for the duration of the pet's life (AVMA 2021; Overgaauw et al. 2020) to prevent the transmission of pathogens to humans. Responsible pet ownership also means recognizing that the other half of the population does not own a dog or may not even like dogs. Annoyance over dog feces in the streets or noisiness is common. Dog owners can contribute to public discussions within communities to demonstrate their positive attitude toward regular deworming or parasite control, health certification, and cleaning up their dog's feces while out walking. It is surprising to see the difference in dog owners' attitudes between the various countries of Europe. While British dog owners are used to cleaning up dog feces, Dutch citizens are less likely to do so. Only 39% of Dutch dog owners report to regularly clean up their dog's feces (Overgaauw et al. 2009).

27.3 New Trends

27.3.1 Emerging Zoonoses

"Emerging zoonoses are zoonoses that are newly recognized or newly evolved or that have occurred previously but show an increase in incidence or expansion in the geographic host, or vector range" (WHO/FAO/OIE 2004). There is an increasing trend to rescue and import dogs from endemic, predominantly Mediterranean regions, with stray animal problems. Another trend is the increasing puppy trade from Eastern Europe. In the EU without borders, such transportation only requires a minimum age, mandatory health certificate (signed by an official veterinarian), and proper vaccinations (e.g., rabies). Rabies, *Echinococcus granulosus* and *Leishmania infantum* are therefore "imported" infections in many countries (Menn et al. 2010; Otranto et al. 2017). The import and settlement of canine babesiosis in Western Europe, including its vector (*Dermacentor reticulatus*), is a clear sign of transport and spreading of (zoonotic) infections (Daugschies 2001). The harmonization of parasite control in Europe has taken place by the independent organization ESCCAP (www.esccap.org) to enable veterinarians to inform clients about the differing endemic diseases in the various European countries and how to prevent contracting or importing infectious diseases when traveling with

dogs (holidays, assistance dogs) or purchasing dogs from abroad. Obviously, this is not only of value regarding parasitic infections, but also for bacterial or viral zoonoses (e.g., brucellosis, rabies).

27.3.2 Feeding Raw Meat to Dogs

Although many dog owners in the Western world feed their pets industrially processed food, an upward trend is emerging toward feeding homemade food instead of pre-prepared food, which may consist of leftovers, homemade and prepared meals, bones, raw meat, and offal. Publications were found reporting the presence of *Escherichia coli* O157, *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., and antibiotic-resistant bacteria in the feed (Van Bree et al. 2018). Outbreaks of *Salmonella* have been described, which could be linked to contaminated animal food. Caregivers and family members can become infected with the *Salmonella* bacteria, not only when preparing food that contains raw meat, but also after being in contact with infected animals that secrete the bacteria (Finley et al. 2006; Lefebvre et al. 2008; Schlesinger 2002; Davies et al. 2019) and after being in contact with infected food bowls, for example, when cleaning them (Weese and Rousseau 2006).

We traditionally know that young children (younger than 5 years), the elderly (older than 65 years), patients with an impaired immunity, and pregnant women carrying a fragile fetus are at more than average risk of becoming ill after an infection. Moreover, they may have more severe disease, have symptoms for a longer duration, or develop more severe complications compared to other patients (Stull et al. 2015). Therefore, it is recommended that animals living with these risk groups or those being used therapeutically to care for people, are not allowed to eat raw meat. Health authorities warn, therefore, that raw pet food diets can be dangerous to the owner and their pets (FDA, Nemser et al. 2014). If dogs are fed with commercially (complete) canned or dry food, which are free from pathogens, there will be no danger in becoming infected through the food chain.

27.3.3 Contact with Wildlife Zoonoses

Hunting dogs that are allowed to feed on the carcasses of wild animals or free-ranging dogs hunting or scavenging for their own food are at an increased risk of picking up zoonoses that may have serious consequences for their owner's health. In *Echinococcus multilocularis* (fox tapeworm) endemic areas (see www.esccap.org), there is a real risk that (hunting) dogs become infected with this tapeworm. Because *E. multilocularis* easily grows in dogs, the threat of it spreading to their owners is also realistic. Monthly deworming in endemic areas is strongly recommended (Hegglin and Deplazes 2013). Moreover, in large areas of Europe home to wild raccoons and raccoon dogs, another emerging zoonosis is the appearance of *Baylisascaris* spp. in dogs. This may cause severe larva migrans infection in animals and humans, but infection in children particularly needs further attention (Okulewicz and Bunkowska 2009; Lee et al. 2010).

Table 4 Additional potential health risks for dog owners depending on behavior, traveling, or the importation of dogs from areas endemic for zoonoses not covered by Table 3

1.	*Echinococcus granulosus*
2.	*Echinococcus multilocularis*
3.	*Toxocara* spp.
4.	*Baylisascaris* spp.
5.	*Rhipicephalus sanguineus*
6.	(Influenza/norovirus)
7.	(*Toxoplasma*)

27.3.4 Dogs and Transmission of Human Viruses

The increasing role of the influenza A virus and human disease and the threat of new pandemics of new types (hemagglutinin (H) and neuraminidase (N)) also affects dogs and cats. So far, canine influenza (H3N8) has not caused harm to humans, but pathogenic avian influenza (H3N2) and human influenza (H1N1) isolates are able to infect dogs and cats. These animals may therefore play a role in interspecies transmission and the spread of the influenza virus (Song et al. 2008; Tangwangvivat et al. 2019). Small animal practitioners may play an important role in early warning systems for influenza in humans and dogs (Beeler 2009).

Recently, it was shown that human noroviruses can survive in dogs' gastrointestinal tracts. It is suggested that this major source of human diarrheal disease worldwide is transmitted from man to dog and consequently, may be transferred to others (Summa et al. 2012; Caddy et al. 2015).

Additional potential health risks for dog owners (depending on behavior, traveling, or importation of dogs from areas endemic for zoonoses) not covered by Table 3 are presented in Table 4.

27.4 Authorities' Involvement

Emerging zoonoses, early warning, and surveillance are all important issues (van der Giessen et al. 2010). Although active surveillance systems exist, particularly for livestock and wildlife, no such system exists for pet animals. Notifiable diseases from pet animals in the Netherlands include brucellosis, campylobacteriosis, echinococcosis, leptospirosis, rabies, salmonellosis, toxoplasmosis, tuberculosis, and yersiniosis. Data are scarce however and underreporting undoubtedly occurs. This may be due to improper diagnoses or ignorance. Enforcement of existing legislation should be the first goal for authorities. Community administration however is regularly confronted with complaints from citizens about dogs and their behavior and indirectly about dog owner behavior. This was previously mentioned and requires more attention from local authorities. Responsible pet ownership should get more attention and could be encouraged locally. Dogs undergoing health certification by a veterinarian at regular intervals, including proper parasite control, vaccinations, and general health checks, could be rewarded with a recognizable medal for their collar. By doing so, the dog owner would demonstrate his or her public responsibility (social control). Moreover, the obligation to clean up dog feces

should be encouraged by the national governments in those countries where this is not yet commonly practiced.

27.5 Conclusions

The role of the companion animal veterinarian is not only to care for animals with diseases but is increasingly becoming important in the field of veterinary public health.

Livestock veterinarians and official veterinarians have long since taken up this responsibility. Disease detection, reporting, and prevention are important issues. Companion animals, including dogs, may act as important sentinels for public health. Veterinarians should advise pet (dog) owners more about health education with regard to husbandry, dog behavior, and responsible pet ownership. Cooperation with community health services and local government should be part of the contribution of small animal practitioners in the twenty-first century "One Health" approach (Trevejo 2009; Gyles 2016).

27.6 Cross-References

- ▶ Animal Bites and Zoonoses: From A to Z – Alligators to Zebras
- ▶ *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control
- ▶ Cystic and Alveolar Echinococcosis: Fraternal Twins both in Search of Optimal Treatment
- ▶ Elimination of Rabies: A Missed Opportunity
- ▶ The Zoonotic Agent Salmonella

References

AVMA (Am. Vet. Med. Ass.). Guidelines for responsible pet ownership. Pet ownership brochure, Available online: www.avma.org/resources-tools/avma-policies/guidelines-responsible-pet-ownership. Accessed on 25 June 2021

Baneth G, Thamsborg SM, Otranto D, Guillot J, Blaga R, Deplazes P et al (2016) Major parasitic zoonoses associated with dogs and cats in Europe. Comp Pathol 155:S54–S74

Beeler E (2009) Influenza in dogs and cats. Vet Clin North Am: Small Anim Pract 39:251–264

Berends BR (2006) Risk inventory and evaluation of non-targeted work with microbiological agents in the University Clinic for Companion Animals in Utrecht (in Dutch). Internal report Institute for Risk Assessment Sciences (IRAS) division Veterinary Public Health. Utrecht

Booij-Vrieling HE, van der Reijden WA, Houwers DJ, de Wit WEAJ, Bosch-Tijhof CJ, Penning LC et al (2010) Comparison of periodontal pathogens between cats and their owner. Vet Microbiol 144:147–152

Cain CL (2013) Antimicrobial resistance in staphylococci in small animals. Vet Clin North Am Small Anim Pract 43(1):19–40

Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP et al (2014) Zika virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis 20:1085–1086

Caddy SL, de Rougemont A, Emmott E, El-Attar L, Mitchell JA, Hollinshead M et al (2015) Evidence for human norovirus infection of dogs in the UK. J Clin Microbiol 53(6):1873–1883
Carmena C, Cardona GA (2013) Canine echinococcosis: global epidemiology and genotypic diversity. Acta Trop 128(3):441–460
CDC, Centers for Disease Control and Prevention (CDC). Zoonotic Diseases. Available online: www.cdc.gov/onehealth/basics/zoonotic-diseases.html. Accessed on 25 June 2021
Chomel BB, Sun B (2011) Zoonoses in the bedroom. Emerg Infect Dis 17:167–172
Dado D, Montoya A, Blanco MA, Miró G, Saugar JM, Bailo B et al (2012) Prevalence and genotypes of *giardia duodenalis* from dogs in Spain: possible zoonotic transmission and public health importance. Parasitol Res 111:2419–2422
Daugschies A (2001) Importation of parasites by tourism and animal trading (in German). Dtsch Tierartzl Wochenschr 108(8):348–352
Davies RH, Lawes JR, Wales AD (2019) Raw diets for dogs and cats: a review, with particular reference to microbiological hazards. J Small Anim Pract 60:329–339
Deplazes P, van Knapen F, Schweiger A, Overgaauw PAM (2011) Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. Vet Par 182:41–53
FDA, U.S. Food and drug administration. Available online: https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-raw-pet-food-diets-can-be-dangerous-you-and-your-pet. Accessed 25 June 2021
Finley R, Reid-Smith R, Weese JS (2006) Human health implications of *salmonella*-contaminated natural pet treats and raw pet food. Clin Infect Dis 42:686–691
Frenkel JK, Dobesh M, Parker BB, Lindsay DS (1996) Xenophilia of dogs: a habit favoring the mechanical transmission of *Toxoplasma gondii* and other fecal microbes. Programme guide and abstracts for the joint meeting of the American Society of Parasitologists and the Society of Protozoölogists, p 110
Friedmann E, Son H (2009) The human-companion animal bond: how humans benefit. Vet Clin North Am Small Anim Pract 39:293–326
Friese A, Schulz J, Laube H, von Salviati C, Hartung J, Roesler U (2013) Faecal occurrence and emissions of livestock-associated methicillin-resistant *Staphylococcus aureus* (laMRSA) and ESbl/AmpC-producing *E. coli* from animal farms in Germany. Berl Munch Tierarztl Wochenschr 126:175–180
Giessen JWB van der, Giessen AW van de, Braks MAH. Emerging zoonoses: early warning and surveillance in the Netherlands. 2010; RIVM report 330214002. Available online: www.rivm.nl/bibliotheek/rapporten/330214002.pdf. Accessed on 25 June 2021
Gyles C (2016) One medicine, one health. One World Can Vet J 57:345–346
Haesebrouck F, Pasmans F, Flahou B, Chiers K, Baele M, Meyns T et al (2009) Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clin Microbiol Rev 22:202–223
Hegglin D, Deplazes P (2013) Control of *Echinococcus multilocularis*: strategies, feasibility and cost-benefit analysis. Int J Parasitol 43(5):327–337
Hoagwood KE, Acri M, Morrissey M, Peth-Pierce R (2017) Animal-assisted therapies for youth with or at risk for mental health problems: a systematic review. Appl Dev Sci 21:1–13
Keegan JD, Holland CV (2010) Contamination of the hair of owned dogs with the eggs of *Toxocara* spp. Vet Parasitol 173:161–164
Koivusilta LK, Ojanlatva A (2006) To have or not to have a pet for better health. PLoS One 1(1): e 109
Lee ACY, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD (2010) Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol 26:155–161
Lefebvre SL, Reid-Smith R, Boerlin P, Weese JS (2008) Evaluation of the risks of shedding salmonellae and other potential pathogens by therapy dogs fed raw diets in Ontario and Alberta. Zoonoses Public Health 55:470–480
Lefebvre SL, Reid-Smith RJ, Waltner-Toews D, Weese JS (2009) Incidence of acquisition of methicillin-resistant *Staphylococcus aureus, Clostridium difficile*, and other health-care–

associated pathogens by dogs that participate in animal-assisted interventions. J Am Vet Med Ass 234:1404–1417
Macpherson CNL, Meslin FX, Wandeler AI (eds) (2012) Dogs, Zoonoses and public health, 2nd edn. Cabi Publishing, New York
Manian FA (2003) Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis 36(2):e26–e28
Marangi M, Berrilli F, Otranto D, Giangaspero A (2010) Genotyping of *Giardia duodenalis* among children and dogs in a closed socially deprived community from Italy. Zoonoses Public Health 57:e54–e58
Menn B, Lorentz S, Naucke TJ (2010) Imported and travelling dogs as carriers of canine vector-borne pathogens in Germany. Parasit Vectors 3:34
Messenger AM, Barnes AN, Gray GC (2014) Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. PLoS One 9:e89055
Nemser SM, Doran T, Grabenstein M, McConnell T, McGrath T, Pamboukian R et al (2014) Investigation of *Listeria, Salmonella*, and toxigenic *Escherichia coli* in various pet foods. Foodborne Pathog Dis 11:706–709
Nijsse ER, Mughini-Gras L, Wagenaar J, Ploeger HW (2014) Coprophagy in dogs interferes in the diagnosis of parasitic infections by faecal examination. Vet Parasitol 204:304–309
Okulewicz A, Bunkowska K (2009) *Baylisascaris* – a new dangerous zoonosis (in polish). Wiad Parazytol 55(4):329–334
Olsen C, Pedersen I, Bergland A, Enders-Slegers MJ, Ihlebæk C (2016) Effect of animal-assisted activity on balance and quality of life in home-dwelling persons with dementia. Geriatr Nurs 37: 284–291
Otranto D, Dantas-Torres F, Mihalca AD, Traub RJ, Lappin M, Baneth G (2017) Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis. Trends Parasitol 33:813–825
Overgaauw PAM, Boersema JM (1998) Nematode infections in dog breeding kennels in the Netherlands, with special reference to *Toxocara*. Vet Quart 20:12–15
Overgaauw PAM, van Knapen F (2012) Is being licked by dogs not dirty? (in Dutch). Tijdschr Diergeneeskd 137:594–596
Overgaauw PAM, van Zutphen L, Hoek D, Yaya FO, Roelfsema J, Pinelli E, van Knapen F, Kortbeek L (2009) Zoonotic parasites in fecal samples and fur from dogs and cats in the Netherlands. Vet Par 163:115–122
Overgaauw PAM, Vinke CM, van Hagen MAE, Lipman LJA (2020) A one health perspective on the human–companion animal relationship with emphasis on zoonotic aspects. Int J Environ Res Public Health 17:3789
Panova OA, Khrustalev AV (2018) Dog walking brings *Toxocara* eggs to people's homes. Vet Parasitol 262:16–19
Peiris JSM, Yuen KY, Osterhaus ADME, Stöhr K (2003) The severe acute respiratory syndrome. N Engl J Med 349:2431–2441
Purewal R, Christley R, Kordas K, Joinson C, Meints K, Gee N, Westgarth C (2017) Companion animals and child/adolescent development: a systematic review of the evidence. Int J Environ Res Public Health 14:234
Schlesinger JDJ (2002) Preliminary assessment of the risk of *Salmonella* infection in dogs fed raw chicken diets. Can Vet J 43(6):441–442
Shephard R, Aryel RM, Shaffer L (2006) Animal health. In: Wagner MM, Moore AW, Aryel RM (eds) Handbook of biosurveillance. Elsevier Academic Press, Boston, pp 111–127
Shewring DJ, Rushforth GF (1990) A bizarre postoperative wound infection. Br Med J 300:1557
Shortridge KF, Peiris JS, Guan Y (2003) The next influenza pandemic: lessons from Hong Kong. J Appl Microbiol 94:70S–79S
Song D, Kang B, Lee C, Jung K, Ha G, Kang D et al (2008) Transmission of avian influenza virus (H3N2) to dogs. Emerg Inf Dis 14:741–746

Sprong H, Cacciò SM, van der Giessen JW (2009) ZOOPNET network and partners. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis 3:e558

Stull JW, Brophy J, Weese JS (2015) Reducing the risk of pet-associated zoonotic infections. Can Med Assoc J 187:736–743

Summa M, von Bonsdorff CH, Maunula L (2012) Pet dogs – a transmission route for human noroviruses? J Clin Vir 53:244–247

Szánthó F, Miklósi A, Kubinyi E (2017) Is your dog empathic? Developing a dog emotional reactivity survey. PLoS One 12:e0170397

Tangwangvivat R, Chanvatik S, Charoenkul K, Chaiyawong S, Janethanakit T, Tuanudom R et al (2019) Evidence of pandemic H1N1 influenza exposure in dogs and cats, Thailand: a serological survey. Zoonoses Public Health 66:349–353

Trevejo RT (2009) Public health for the twenty first century: what role do veterinarians in clinical practice play? Vet Clin N Am Small Anim Pract 39:215–224

Van Bree FPJ, Bokken GCAM, Mineur R, Franssen F, Opsteegh M, van der Giessen JWB, Lipman LJA, Overgaauw PAM (2018) Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. Vet Rec 182(2):50

Verrier L (1970) Dog licks man. Lancet 295:615

Wade T, Booy R, Teare EL, Kroll S (1999) *Pasteurella multocida* meningitis in infancy (a lick may be as bad as a bite). Eur J Pediatr 159:875–878

Weese JS, Rousseau J (2006) Survival of *Salmonella* Copenhagen in food bowls following contamination with experimentally inoculated raw meat: effects of time, cleaning, and disinfection. Can Vet J 47:887–889

Weese JS (2010) Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J 51:233–244

Wells DL (2019) The state of research on human–animal relations: implications for human health. Anthrozoös 32:169–181

Westgarth C, Pinckbeck GL, Bradshaw JWS, Dawson S, Gaskell RM, Christley RM (2008) Dog-human and dog-dog interactions of 260 dog owning households in a community in Cheshire. Vet Rec 162:436–442

WHO, World Health Organization (2002) Future trends in veterinary public health. World Health Organ Tech Rep Ser 907(i-vii):1–85

WHO, World Health Organization. Statement on novel coronavirus in Thailand. Available online: www.who.int/news/item/13-01-2020-who-statement-on-novel-coronavirus-in-thailand. Accessed on 22 June 2020

WHO/FAO/OIE. Report of the joint consultation on emerging zoonotic diseases. (2004). Available online: https://apps.who.int/iris/handle/10665/68899. Accessed on 25 June 2021

WSAVA, World Small Animal Veterinary Association. One Health Initiative (2011) Eur J Companion Anim Pract 21:11

Zaki AM, Boheemen S van, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367: 1814–1820

Cats – Revered and Reviled – and Associated Zoonoses

28

Killing You Softly with Feces and Fleas

Andreas Sing and Anja Berger

Contents

A. Sing (✉)
Dept. of Public Health Microbiology, Bavarian Health and Food Safety Authority (LGL), National Consiliary Laboratory on Diphtheria and National Reference Center for Borrelia, Oberschleißheim, Germany
e-mail: andreas.sing@lgl.bayern.de

A. Berger
Bavarian Health and Food Safety Authority (LGL), National Consiliary Laboratory on Diphtheria, Oberschleißheim, Germany
e-mail: anja.berger@lgl.bayern.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_23

Abstract

In many countries worldwide, cats – revered and reviled – have become "man's really best friend." In the following chapter the public health relevance of cats will be highlighted by introducing its most relevant zoonotic pathogens including *Toxoplasma gondii, Bartonella henselae*, *Toxocara cati*, *Rickettsia felis*, enteropathogenic bacteria and parasites, as well as the emerging cat-related pathogen *Corynebacterium ulcerans.* Moreover, cats and their role in the SARS-CoV-2 pandemic will also be discussed.

Keywords

Cat · Lion · Tiger · Puma · Cougar · *Toxplasma gondii* · *Toxocara cati* · *Giardia* spp. · *Campylobacter* spp. · *Cryptosporidium felis* · *Bartonella* spp. · *Chlamydia felis* · *Opisthorchis felineus* · *Rickettsia felis* · Cat scratch disease · SARS-CoV-2 · Feline Infectious Peritonitis (FIP) · *Salmonella* spp. · *Enterohemorrhagic E. coli* (EHEC) · *Carnivora* · *Corynebacterium ulcerans* · Diphtheria

28.1 Introduction

The family *Felidiae* of the Order *Carnivora* is of comparatively recent evolutionary origin with a proposed common ancestor living <11 million years ago (Agnarsson et al. 2010; Johnson et al. 2006). Based on morphological and molecular data at least 38 extant and two extinct species can be divided into eight major lineages: the two "big cat" clades of the pantherine lineage with four roaring and one non-roaring species and the Asian leopard cat group, the ocelot lineage, the caracal group, the puma group, the baycat group, the *lynx* genus, and the domestic cat lineage (Agnarsson et al. 2010; Serpell 2000; Johnson and O'Brien 1997; Johnson et al. 2006; Kitchener et al. 2017).

The latter lineage originated in the Mediterranean region about 6–7 million years ago (Johnson et al. 2006; Driscoll et al. 2007; Crowley et al. 2020) and consists of six small cat species from which four, i.e., the domestic cat (*Felis catus*), the European wildcat (*F. silvestris*), the African wildcat (*F. libyca*), and the sand cat (*F. margarita*), diverged later on. Based mainly on morphological, archeological, and behavioral findings, *F. libyca* is hypothesized to be the most likely ancestor of the domestic cat. Even etymological findings support this idea, since the word for cat in many languages (e.g., English *cat*, fourth century Latin *cattus*, the ancient Greek

καττος, the medieval Byzantine Greek *κατης* [Nicholas 1999], Spanish *gato*, French *chat*, German *Katze*, Lithuanian *katė*, the modern Arabic *quttah*) is thought to be derived from the Nubian word for cat, *kadiz*.

Domestication of cats started presumably 10,000–25,000 years later than the domestication of dogs (Germonpré et al. 2009) and probably began in Egypt about 4000 years ago, where cats even enjoyed a sacred status and were associated with the cult of the cat-shaped deity Bastet. One strain of etymological speculation links the English diminutive *pussy* and the Romanian word for cat *pissica* to this Egyptian goddess (Serpell 2000). Several reasons for cat domestication have been discussed in the literature based on its hunting properties: (i) protection of grain storages by killing small rodents (indirect benefit from hunting); (ii) use as a companion animal for bird hunting (direct benefit from hunting); (iii) use for religious reasons or as status symbol due to its resemblance of a lion, the king of the carnivorous hunters (Serpell 2000; Jores 2004).

Interestingly and in a pronounced contrast to dogs, the domestication process of cats did not induce major changes either in the physical shape or in the behavior of domestic cats when compared to wildcats. This process is sometimes referred to as "self-domestication" probably driven by a fortuitous combination of ecological and sociocultural circumstances (Crowley et al. 2020). Modern cats still remain capable of sustaining themselves without human assistance; this independence from human support is reflected by the increasing number of the so-called "feral cats" raising major problems in many human societies and ecological settings, e.g., due to their carnivore and predator lifestyle contributing even to the extinction of other animals especially in islands or certain geographic areas (Crowley et al. 2020; Parliament of the Commonwealth of Australia 2020).

From its sacred origins in Ancient Egypt, the domestic cat spread – supported by its remarkable "sea sickness resistance" – often by ships across the Mediterranean – first throughout Europe and finally worldwide (Engels 1999; Jores 2004). While during the first millennia of the human–cat relationship the animal enjoyed valuable respect nearly in all human cultures, the esteem of cats dramatically changed in the Middle Ages at least in Christian Europe and Japan when demonic features were attributed to them; the respective iconic pictures commonly propagated in fairy tails are those of a cat sitting on the hunchback of a witch or even being a companion of the devil. Probably the previously as benevolent cat attributes described features such as (female) fertility and sexuality were now disregarded as demonic for cultural or religious reasons and consequently the cat was transformed into a symbol of these now as vicious connotated features. These associations were also based on facets of the natural behavior of cats, e.g., their obvious independence, affectionate nature, search for physical caresses, sexual behavior, and promiscuity, which all were now interpreted in the light of a sexualized, often misogynic perspective (Serpell 2000). After this emotional setback in the history of the human–cat relationship things clearly improved in the last few centuries making the cat now in many countries "man's really best friend" with the numbers of household cats outnumbering those of domestic dogs. However, the ambiguous nature of cat's image as revered and reviled still prevails in many human societies and subgroups (Crowley et al. 2020; Vocelle 2017).

28.1.1 Cat Demography

The worldwide domestic cat population is estimated to exceed 500 million of which about 58% are thought to be be "stray" or "feral" (International Companion Animal Management Coalition 2011; Hiby et al. 2013). The largest pet cat population lives in the USA with about 74 million cats present in 32–38% of US households (Rowan 2018, Humane Society of the USA 2022), followed by China (53 million), Russia (17.8 million), and Brazil (12.5 million) (www.mappr.co/thematic-maps/world-pet-ownership/). In Europe about 110 million cats are living in about 25% of European households; Russia (22.7 million), Germany (15.7 million; 2012: 8.2 million), France (15.1 million; 2012: 11.4 million), UK (7.9 million; 2012: 8.5 million), and Italy (7.9 million; 2012: 7.5 million) ranked as the five countries with the highest pet cat populations in Europe in 2020 (FEDIAF 2014, 2021). Interestingly and similarly to 2012, the highest rates of households with cat ownership in Europe are found in the East, e.g., in Romania and Poland (42% of households), the Czech Republic (41%), Lithuania (37%), Hungary (34%), and Slovenia (31%) with Portugal being the only exception from this geographical trend (38%) (FEDIAF 2021). The Parliament of the Commonwealth of Australia reported 3.77 million pet cats in 27% of Australian households and 2.8. million feral cats, respectively (Parliament of the Commonwealth of Australia 2020). Cat ownership is relatively low in African countries besides South Africa (2 million) and Spanish-speaking Southern America besides Argentina (3.0 million).

In a random sample of 2,980 UK households in 2007 pet cat ownership was significantly correlated with female sex of the owner (OR 1.63), having a garden (OR 3.66), living in a semi-urban or rural environment (OR 1.30), and having a higher education degree (OR 1.36) (Murray et al. 2010). In an Irish study, pet cat ownership was significantly associated with house type (apartment compared with detached housing, OR: 0.13), female sex (OR: 1.48), age (55–64 years compared with 18–24 years, OR: 2.25) and the presence of a pet dog in the house (compared with no pet dog, OR: 1.85) (Downes et al. 2009). Similarly, in a British study, the presence of an adult female in the household or of people in the age groups 20–29 years and 30–59 years were associated with increased odds of owning a cat (Westgarth et al. 2010). Female sex, age class between 40–59 years, separated marital status, and living in a rural area were associated with cat ownership in a study from Central Italy (Carvelli et al. 2006). In the USA more than 75% of the persons taking care of a cat within a household are women; this rate is highest among 19–29 year-old women (AVMA 2012). Indirect evidence for a higher proportion of women caring for cats might be drawn from a US multicenter study on 110 patients with pet-afflicted bite wounds showing that 72% of people bitten by cats were women, while only 38% of dog bites documented in the study population affected women (Talan et al. 1999). In a 2003 population-based study from California, female single respondents had a higher odds of owning a cat; smaller household sizes, home ownership, living in a home, full time employment of the household, and more rural location were associated with higher odds of cat ownership (Saunders et al. 2017).

28.1.2 Public Health Impact of Cats

Cats may kill humans directly by attacking, violating or – seldomly and then reaching literally fame (Anderson 1955) – eating them. About 14,009 deadly attacks by large carnivorous cats on humans have been recorded worldwide in the twentieth century, the vast majority caused by tigers – probably a gross underestimation (Löe and Röskaft 2004). In the nineteenth and early twentieth centuries, tigers killed approximately 34,075 persons in the Indian subcontinent, in the twentieth century another 12,600 persons resulting in 600 to 800 human deaths per year from tiger attacks in Asia (Shepherd et al. 2014). The utmost unfriendly behavior, i.e., man-eating, was mainly reported for tigers in Singapore, India, Russia, and South China. For other big feline carnivores available twentieth century data allow the conservative estimate that lions and leopards have killed 552 and 840 people, respectively, in their natural African and Asian habitats (Shepherd et al. 2014), with more scaring man-eating events reported than for tigers giving rise to eternal fame, for instance, for the Tsavo brothers, two lion man-eaters (Peterhans et al. 2001), and the Panar man-eating leopard (Corbett 1954).

Not only wildlife felines or captive carnivores such as Siegfried and Roy's Montecore, but also pet cats may attack humans: about 400,000 cat bites occur every year in the USA (WHO 2018), in Bologna/Italy the incidence of cat-bite-related injuries was estimated to be 17.9/100,000 (Ostanello et al. 2005), in Brazil a national health survey calculated 76,512 cat bites/year corresponding with an annual incidence of 41 cat bites/100,000 (Benavides et al. 2020).

While these literally crude data show quite obviously a direct impact of cats on human health, the zoonotic health risks of cats to humans are much less visible and indirect.

Starting from a comprehensive risk assessment review on companion animal-associated zoonoses by the Robert Koch-Institut, Germany (Weber and Schwarzkopf 2003), and based on an intensive literature review as well as expert interviews of 14 scientists from the fields of veterinary medicine, human medicine and microbiology, a list of eight, three, and six pathogens with a public human health risk scored as "high," "low," and "unsure," respectively, for the German situation was compiled at the Veterinary University Hannover (Möbius 2013). *Bartonella henselae*, *Capnocytophaga* spp., *Pasteurella multocida*, Orthopoxvirus, *Cryptosporidium parvum*, *Toxoplasma gondii*, *Microsporum canis*, and *Trichophyton* spp. were ranked among the "high risk" zoonotic pathogens, *Coxiella burnetii*, *Dipylidium caninum*, and *Echinococcus* spp.) among the "low risk" group and *Campylobacter jejuni*, *Escherichia coli*, *Leptospira* spp., *Salmonella* spp., *Toxocara* spp., and *Giardia* spp. among the ambiguous zoonotic agents. Pathogens for which only a theoretical transmission risk exists (e.g., Lyssa viruses in a rabies-free country) or for which only anecdotal case reports could be found in the literature were excluded. A similar, but not risk-oriented list on zoonotic infectious diseases of companion animals including cats was published by Day et al. as an electronic document for the *Emerging Infectious Diseases* journal of the CDC, Atlanta, USA (Day et al. 2012), while in a more recent document the US CDC list on their homepage cat

scratch disease, roundworms, toxoplasmosis, rabies, campylobacteriosis, salmonellosis, cryptosporidiosis, giardiasis, hookworm, MRSA, plague, ringworm, different tick-borne diseases, and sporotrichosis as diseases associated with cats (CDC 2021). The American Association of Feline Practitioners (AAFP) mentions in their 2019 Feline Zoonoses Guidelines 14 enteric, seven scratch-, bite-, or exudate-associated, six ocular or respiratory, eight flea- and tick-borne zoonotic agents of cats, respectively, without further weighting (Lappin et al. 2019). Table 1 comprises the most important cat-associated zoonotic pathogens.

In the following we will mainly concentrate on pathogens with significant public health importance or a quite unique association with cats. Mainly bite-transmitted pathogens (e.g., *Capnocytophaga* spp. and *Pasteurella* spp.) and dermatophytes (e.g., *Microsporum* spp. and *Trichophyton* spp.) will not be dealt due to their quite special transmission pathways. Antibiotic resistant bacteria with the potential of zoonotic transmission, especially Methicillin-resistant *Staphylococcus aureus* will also not be addressed in this chapter.

28.2 The Usual Suspects

28.2.1 *Toxoplasma gondii*

28.2.1.1 The Pathogen – Life Cycle and Transmission

Three remarkable, if not ironical, events mark the first descriptions of *T. gondii* in 1908 by the French Nobel laureates Charles Nicolle and Louis Herbert Manceaux and the Calabria-born microbiologist and physician Alfonso Splendore (Meira 2010): (i) although spanning perhaps the widest host range of any parasite *T. gondii* was discovered independently not in wildlife, but in two laboratory animals, i.e., a North African comb rat named gundi in the Pasteur Institute in Tunis/Tunisia and a rabbit in the Hospital da Real Sociedade de Beneficência Portuguesa in São Paulo/Brazil, respectively (Dubey 2008, 2020). (ii) the two French microbiologists misspelled the Maghreb Arabian name of the rodent gundi as "gondi" resulting in the parasite's proposed species name as *gondii*; (iii) funnily, both the French and the Italian researchers misidentified the parasite discovered in their laboratory animals as a *Leishmania* species (Dubey 2008).

The parasitic life cycle of this coccidian protozoan of the Phylum Apicomplexa depends on the carnivorous lifestyle of felids and was finally resolved as late as the 1970s by the work of Frenkel and Dubey (Dubey 2008). Despite the parasite's extremely wide host range in air (birds [Dubey et al. 2021a]), on earth (nearly all warm-blooded animals), and in sea (maritime mammals) (see an extended list of animal species in Hill et al. 2005 and of rodents alone in Dubey et al. 2021b, respectively), the only known definitive hosts for *T. gondii* are members of the family Felidae. The important role of cats in the natural transmission of *T. gondii* is underlined by seroepidemiological studies on isolated Pacific, Australian, or US islands showing very low seroprevalence for *T. gondii* in the absence of cats (Wallace 1969; Munday 1972; Dubey et al. 1997); higher *T. gondii* seroprevalences on basically cat-free Arctic islands have been linked to migratory birds (Prestrud

et al. 2007). The obligate intracellular parasite can only sexually reproduce and, thereby, complete its life cyle in felids, the only mammals lacking delta-6-desaturase activity in their intestines resulting in a systemic excess of linoleic acid needed for *Toxoplasma*'s sexual reproduction (Martorelli Di Genova et al. 2019; English and Striepen 2019) manifesting in the subsequent production of the massive numbers of unsporulated oocysts. These oocysts are shed in the cat's feces and need to sporulate for 1–5 days in the environment to become infective. Intermediate hosts, e.g., birds and rodents, but principally any warm-blooded species including humans, get infected by ingesting oocysts-contaminated soil, water, or plant material. Shortly after ingestion, oocysts transform within the intermediate host into tachyzoites, which travel mainly within macrophages primarily into neural or muscular tissue (but principally in any tissue) where they develop into bradyzoites. Several thousands of bradyzoites form tissue cysts, which are ingested by cats when feeding on their prey, e.g., the "regular" intermediate hosts. Moreover, cats may also become infected directly by ingestion of sporulated oocysts. The infectious dose for cats is estimated to be around 100 oocysts or one bradyzoite, respectively (Dubey 2020). Infection of cats via tissue cysts is probably more effective than via oocysts resulting in a higher percentage of oocyst shedding (Zhu et al. 2021).

Humans may become infected (i) by slowly dividing bradyzoites, e.g., when eating undercooked meat of animals harboring tissue cysts[1], (ii) by sporozoites-containing oocysts, e.g., when consuming food or water contaminated with cat feces or when ingesting environmental items – either directly contaminated by a cat (e.g., via fecally contaminated soil or pet cat litter boxes) or indirectly by another animal (e.g., by dogs carrying oocysts in their fur after having rolled in cat feces due to their behavioral trait of the so-called xenosmophilia [Frenkel and Parker 1996]), (iii) by rapidly dividing tachyzoites, e.g., via blood transfusion or transplacentally, and (iv) by bradyzoites, e.g., via organ transplantation when transplanting tissue cysts-containing transplant material (Hill and Dubey 2002; Flegr et al. 2014).

As derived from toxoplasmosis outbreaks, neither disease severity nor clinical symptoms are probably related to the parasitic stage, i.e., oocysts vs. bradyzoites, ingested (Dubey 2021; Pinto-Ferreira et al. 2019).

28.2.1.2 Epidemiology in Cats and Humans

T. gondii strains are highly diverse, but only a few lineages are widely spread. In Europe, North America, and Africa there are three dominant clonal lineages, i.e., the more mouse-virulent I and the less mouse-virulent II and III; in South America the variety of genotypes is much more diverse and comprises more atypical strains, possibly due to more frequent sexual replication of *Toxoplasma* in this part of the world and associated with higher pathogenicity (Flegr et al. 2014; Su et al. 2012;

[1] Human transmission by different stages of undercooked meat (esp. lamb chops) or raw meat juice was shown in a French study with hospitalized children, supplemented by a detailed diet survey on beef, lamb, and horse meat preparing habits („*très cuit*," „*á point*," „*saignant*," „*très saignant*," and „*cru*"); while the culinary procedures are described in a quite sophisticated manner, no information is given regarding an ethical approvement of the performed diet studies (Desmonts et al. 1965).

Amouei et al. 2020; Bertranpetit et al. 2017; Galal et al. 2019; Lehmann et al. 2006; Khan et al. 2007).

Cats have been implicated to be involved in shaping the global phylogeny of *T. gondii* by two major spreading events: (i) after the reconnection of the Panamian land bridge 1-2 Mya comigration of feline definite hosts with the parasite might have resulted in the genetic divergence of both felids and *Toxoplasma* in South America (Khan et al. 2007); (ii) sixteenth century transatlantic slave trade by ships populated with rodents and domestic cats might have enforced the spread of *Toxoplasma* and its clonal formation in Europe, North America, and Africa (Lehmann et al. 2006).

Globally, seroprevalence in cats ranges from around 5% to more than 90% and is higher in feral and stray cats than in domestic pets (Dabritz and Conrad 2010; Dubey and Jones 2008; Nutter et al. 2004; Dubey et al. 2020; Zhu et al. 2021) with an estimated global pooled seroprevalence of 35% and 59% in domestic and wild felis, respectively (Montazeri et al. 2020). Seroprevalence rises with cat age (Vollaire et al. 2005); e.g., in a Belgian study on 567 health domestic cats seroprevalence increased from 2% in kittens below 12 months of age to 44% in 7-year old cats (De Craeye et al. 2008). Probably less than 1% of mainly young cats – often being infected for the first time during their first outdoor hunting experiences – are shedding oocysts once in their lifetime for an average period of 1 week and up to 3 weeks, although data from experimentally infected cats suggest that re-excretion of oocysts is possible (Dubey et al. 2020) questioning the previous assumption of a single oocyst shedding period (Zhu et al. 2021). Reported rates of shedding domestic cats range from 0% to 34% in microscopic (Dabritz and Conrad 2010; Barutzki and Schaper 2011; Epe et al. 2004) and up to 20% in DNA studies (Dubey et al. 2020). A single cat may excrete more than 50 million oocysts per day (Dabritz and Conrad 2010: data are based on 73 experimentally or naturally infected cats from five different studies) with 3 to 810 million oocysts per cat infection. With an estimated 40 g of daily feces production per cat, the amount of annual fecal production by feral cats was calculated to reach up to 2.4×10^6 tons in the USA matching up to 2.4×10^{15} oocysts deposited in the US environment (Dabritz and Conrad 2010; Torrey and Yolken 2013). It should be noted, however, that laboratory methods for oocyst detection especially in environmental samples are not very sensitive; direct measurements and source detection in outbreak situations are therefore often not possible.

Toxoplamosis is probably the most prevalent infection in humans affecting 30–50% of the world population thus outnumbering even latent tuberculosis (Flegr et al. 2014). It ranks among the three most important food-borne diseases at least in industrialized countries (Aguirre et al. 2019; WHO 2015) with the highest disability-adjusted life years (DALY) among food-borne infections (Flegr et al. 2014).

Seroprevalence in humans shows a similar range as in cats, i.e., from about 3% to around 90% in different human populations and geographical regions (Dabritz and Conrad 2010; Bigna et al. 2020). In most European, Central/South American, and North American countries, about one-fourth to two thirds – from a public health point of view – of the most relevant human population, i.e., pregnant women or women of child-bearing age, show anti-*Toxoplasma* IgG antibodies (Dabritz and Conrad 2010; Nogareda et al. 2014; Galvan-Ramirez et al. 2012), with the Americas

as the WHO region with the highest (around 45%) and the Western Pacific with the lowest (around 11%) IgG prevalence, respectively (Bigna et al. 2020). In some countries, including France, the prevalence has fallen in the last two decades, probably due to hygienic and awareness building measures (Petersen et al. 2010; Nogareda et al. 2014).

The global annual incidence of the most severe form of toxoplasmosis, i.e., congenital toxoplasmosis, was estimated in a WHO review to be 190,100 cases matching an incidence of 1.5 cases of congenital toxoplasmosis per 1,000 live births (Torgerson and Mastroiacovo 2013). A recent review found the highest incidences for congenital toxoplasmosis in Brazil, France, and Austria, respectively (Dubey et al. 2021c).

28.2.1.3 Disease in Cats

Infection in cats is usually asymptomatic or subclinical with only minor symptoms such as short-term diarrhea, lymphadenopathy, or fever. Overt disease is more likely in cats with immunosuppression, including young kittens and cats with, e.g., feline leukemia virus (FELV) or feline immunodeficiency virus (FIV) infection. During the parasite's extraintestinal tachyzoite phase and depending on the affected organ system, pneumonia with cough and breathing problems, longer episodes of diarrhea, uveitis, iritis, chorioretinitis, myocarditis, or encephalitis may develop. Very rarely, sudden death may occur especially in very young kittens (Dubey et al. 2020).

28.2.1.4 Disease in Humans

Most infections affecting humans are asymptomatic. If symptomatic, mainly immunosuppressed people are concerned. Lymphadenopathy, sometimes associated with fever, fatigue, muscle pain, sore throat, and headache, is the most frequently observed acute clinical form of postnatally acquired toxoplasmosis in humans.

In striking contrast, multivisceral symptomatic infection, sometimes referred to as "Amazonian toxoplasmosis," acute and severe ocular forms (e.g., retinochoroiditis), and even deaths among immunocompetent patients have been reported mainly in connection with both oocyst- and bradyzoites-associated outbreaks in South America and the Carribean, sometimes linked to atypical and more pathogenic *Toxoplasma* strains or genetic host susceptibility (Galal et al. 2019; Carme et al. 2009; Dubey 2021).

Most important clinical findings in immunosuppressed patients include encephalitis, chorioretinitis, or pneumonitis due to reactivation of bradyzoites, which are otherwise immunologically well controlled in tissue cysts and might develop into tachyzoites during phases of immunosuppression. Recently, evidence is rising that latent *Toxoplasma* infection might be linked to behavioral or mental changes and psychiatric disease (Flegr and Horáček 2020), e.g., an association with self-directed violence (Pedersen et al. 2012), suicidal behavior (Groër et al. 2011; Postolache et al. 2021), or automotive accidents (Flegr et al. 2002); however, if and which *Toxoplasma*-specific underlying causes might be involved is not yet understood (Flegr 2013). These findings are paralleled by behavioral changes observed in experimentally infected mice, which have been linked to *Toxoplama*'s life cycle postulating that the parasite manipulates its intermediate hosts to lose their innate fear of

predators including cats or to feel attracted to cat urine thus increasing the probability to be devoured by their definite hosts (Vyas et al. 2007; Boillat et al. 2020; Aguirre et al. 2019; Tong et al. 2021). Congenital toxoplasmosis is caused by transplacental infection when a non-immune mother infected for the first time during pregnancy transmits tachyzoites to her fetus. The clinical spectrum of congenital toxoplasmosis ranges from asymptomatic infection to severe syndromes including hydrocephalus, microcephaly, or intracranial calcifications – leading to mental and/or psychomotoric retardation – and chorioretinitis causing vision impairment or even blindness. Stillbirth or death in the neonatal period may also occur.

28.2.1.5 Public Health Importance

A WHO review estimates the global burden of congenital toxoplasmosis to be as high as 1.20 million DALYs (Torgerson and Mastroiacovo 2013). The highest burdens were seen in South America and in some Middle Eastern – especially in low-income – countries. For their calculations, the WHO review authors took into account different disease patterns, manifestations, and severities possibly due to different pathogenic strains and their respective geographical distribution.

In the 1990s, about 750 deaths per year were attributed to toxoplasmosis in the USA (Mead et al. 1999), while more recent estimates report 327 deaths per year making *Toxoplasma* the food-borne pathogen with the second most annual deaths after *Salmonella* spp. (Batz et al. 2021). These estimates of lethal outcomes might mainly refer to infections acquired by immunosuppressed people via ingestion of tissue cysts. However, due to the larger amount of people affected, oocyst-transmitted infections may be both clinically and from a public health point of view more severe than tissue cyst-induced infections as mainly concluded from outbreak situations (Beneson et al. 1982; Burnett et al. 1998; Teutsch et al. 1979; Bowie et al. 1997).

On a global scale, the relative contribution of human infection via oocysts from food or drinking water contaminated with cat feces on the one hand and from tissue cysts by eating undercooked meat on the other hand is very difficult to establish and probably differs in different geographical locations and human populations due to different environmental conditions, sociocultural customs, and also the pathogenicity of the prevalent *Toxoplasma* strain (VanWormer et al. 2013; Milne et al. 2020). Interestingly, case-control studies failed to explain up to 40% of human infections due to any known risk factor (Petersen et al. 2010). In a European multicenter study from France, Italy, Belgium, Denmark, and Sweden, between one and two thirds of infections could be attributed to consumption of undercooked meat products and 6–17% to soil contact (Cook et al. 2000). However, considerable seroprevalence data in vegetarian human populations from India (Rawal 1959: 21%), among Seventh Day Adventists in the USA (Roghmann et al. 1999: 18%) and in Amerindian aborigines from Venezuela (Chacin-Bonilla et al. 2001: 43.5% to 62.4%) as well as symptomatic outbreaks of human toxoplasmosis mainly linked to probably contaminated drinking water sources (Balasundaram et al. 2010; Petersen et al. 2010; Dabritz and Conrad 2010; Dubey 2021; Pinto-Ferreira et al. 2019), e.g., the most prominent and best analyzed 1995 Vancouver outbreak

presumably caused by cougars shedding oocysts (Aramini et al. 1998, 1999; Bowie et al. 1997), or to contaminated vegetables (Ekman et al. 2012) suggest a significant proportion of oocyst-transmitted toxoplasmosis at least in some circumstances (Peterson et al. 2010). Moreover, when finding with a novel sporozoite-(and therefore oocyst-)specific serological test that 78% of 76 mothers of congenitally infected infants in a US-wide survey had a primary infection with oocysts, Boyer et al. speculated that a major number of congenital toxoplasmosis cases and at least four North American epidemics are due to infection via oocysts (Boyer et al. 2011). Similarly, based on sporozoite-specific seroprevalence studies (Hill et al. 2011), *T. gondii* transmission has been largely attributed to oocysts for regions of Brazil with poor socioeconomic and hygienic conditions and oocyst-favorable warm, humid climate (Milne et al. 2020). In contrast, in Poland with approximately 80% of livestock reported as *T. gondii* seropositive, bradyzoites from undercooked meat have been implicated to be the most significant source of infection (Flegr et al. 2014).

Data from 11 epidemiological studies performed from 1990 to 2006 in different European, African, and American countries analyzing soil contact associated with seropositivity found odds ratios (OR) from 1.4 to 10.3 suggesting a possible risk of oocyst-transmitted infections via cat feces (Dabritz and Conrad 2010). However, direct daily contact with cats or cat-ownership was not associated with an increased risk of *T. gondii* infection in one USA and two European case-control studies (Cook et al. 2000; Kapperud et al. 1996; Jones et al. 2009) or in studies from South Korea (Jung et al. 2017) or the UK (Flatt and Shetty 2013), respectively. In contrast, the OR for owing a cat was 1.25 in a study from the Czech Republic (Kolbekova et al. 2007). Seroprevalence studies in pregnant women from Iran (Foroutan-Rad et al. 2016) and France (with an OR of 4.5 for cat ownership [Baril et al. 1999]), in hemodialysis patients (OR 3.73) and a healthy control group (OR 1.80) in Iran (Soltani et al. 2020), in a population-based control study from Taiwan (OR of 2.9 [Chiang et al. 2014]) and in school children in central China (Wang et al. 2020) identified cat ownership as an independent risk factor for *Toxoplasma* infection. Interestingly, owing three or more kittens was associated with an adjusted OR (aOR) of nearly 28 in the very same US study which showed a "protective" effect of owing one or two cats (aOR 0.6) (Jones et al. 2009).

In conclusion, there is considerable indirect evidence for a direct role of oocyst-shedding cats in *Toxoplasma* transmission to humans. Unfortunately, although oocysts have been detected both in environmental and in water samples by different methods (Dabritz and Conrad 2010), often the implicated sources suggested by epidemiological considerations cannot be corroborated or certified by direct detection of oocysts due to the lack of laboratory facilities or methodologically based sensitivity problems. Therefore, the public health impact of directly cat-associated, i.e., oocyst-transmitted, toxoplasmosis cannot be clearly calculated.

28.2.1.6 Public Health Measures

When assessing the risk of directly cat-associated transmission, it should be noted that probably one oocyst might be enough to cause infection as data from

experimentally infected swine suggest (Dubey et al. 1996). Considering the mean of 50 million oocysts daily excreted by a single freshly infected cat methods preventing oocyst contact, ingestion or inhalation are obviously reasonable. Care should especially be taken when dealing with sandboxes where cats that do usually not defecate randomly, but rather select places for defecation, often deposit and subsequently cover their feces resulting in the accumulation of more than 1 million oocysts per square foot in certain areas of sandboxes (Torrey and Yolken 2013).

Interestingly, in a 19 months lasting German study on more than 18,000 feline fecal samples the proportion of *T. gondii*-positive samples collected between January and June was significantly lower than between July and December (Herrmann et al. 2010).

Cat-related public health measures for reducing the risk of toxoplasmosis might therefore include (i) hygienic measures such as wearing gloves when and washing hands after gardening, working with soil (especially in places where domestic and above all feral or stray cats might defecate) or having contact with cat feces; (ii) thoroughly washing of vegetables and fruits; (iii) restricting pet cats', especially kittens', access to wild rodents (although estimated bradyzoite rates for rodents are quite low, e.g., 0.1–0.4% in Germany); (iv) avoiding disposal of cat feces into drinking water sources (e.g., the respective Californian legislation [Dabritz and Conrad 2010]); (v) adopting a stray cat policy reducing roaming of unowned and not sufficiently supervised cats; (vi) preventing contact of cats and their feces to highly susceptible intermediate host animals such as swine, lambs, or chicken (Dubey 2010); (vii) development of a feasible and robust vaccine for cats (Dabritz and Conrad 2010; Petersen et al. 2010; Aguirre et al. 2019). Recently, a CRISPR/Cas9-based genetically attenuated live vaccine generating defective oocysts failing to produce sporozoites was succesfully shown to completely prevent oocyst excretion thus blocking *T. plasma* transmission (Ramakrishnan et al. 2019).

28.2.2 Bartonellosis

28.2.2.1 The Pathogen

The genus *Bartonella* comprises a group of Gram-negative facultative intracellular bacteria with a unique life cycle involving one or few closely related mammals as reservoir hosts and different bloodsucking arthropods as vectors. Currently, 37 *Bartonella* species/subspecies and additional candidates, e.g., not fully characterized and named species, are listed (www.bacterio.net), which have been identified in a wide range of domestic and wild animals, including at least 16 causing disease in humans and 10 zoonotic species from cats and/or dogs (Cicuttin et al. 2014; Chomel et al. 2012; Harms and Dehio 2012; Zangwill 2013; Okaro et al. 2017). Following transmission by an arthropod vector, bartonellae colonize a not yet definitely identified primary niche, which probably involves migratory cells as well as additional cell types and are transported to the vascular endothelium where they persist intracellularly (see Harms and Dehio 2012 for an intense review of the molecular pathogenesis of *Bartonella* spp.). *Bartonella* is known to infect a range of host cells,

but the endothelial cell is thought to be the primary niche location (Okaro et al. 2017). From the primary niche the bartonellae invade erythrocytes where they finally persist to be again taken up by bloodsucking arthropods for transmission to another host. Both vector (including its ecology) and host (including the primary niche as first step in the intrahost replication cycle) factors are responsible for the reservoir host specificity of the respective *Bartonella* species (Harms and Dehio 2012). Their lifestyle both in the primary and the intraerythrocytic niches allow the bartonellae to escape the immune system, to replicate within their host, to relapse from time to time and to evade antibiotic treatment.

The majority of human infections is caused by three *Bartonella* species (*B. henselae*, *B. bacilliformis*, and *B. quintana*) of which one is zoonotic and has cats as reservoir hosts, i.e., *Bartonella henselae*. Cats are also the main reservoir for *B. clarridgeiae* and *B. koehleae* that may be both causative agents of cat-scratch disease and endocarditis in humans (Cheslock and Embers 2019; Alvarez-Fernandez et al. 2018). The natural and most important arthropod vector both for direct intra- (i.e., cat-to-cat) and probably also for indirect interspecies (i.e., cat-to-human) transmission is the cat flea *Ctenocephalides felis* (Breitschwerdt and Kordick 2000; Mosbacher et al. 2011; Harms and Dehio 2012). Transmission to humans is thought to occur mainly via *B. henselae*-contaminated cat flea feces after inoculation by a cat scratch or a cat bite; interestingly, *B. henselae* DNA could be isolated from gingiva and claw beds of domestic cats in the USA (Lappin and Hawley 2009).

Besides, in cat fleas, *B. henselae* has also been identified in hard ticks from Europe and North America (i.e., *Ixodes ricinus* and *Ixodes pacificus*) suggesting a possible role of *Ixodes* spp. as vectors as it is proven for another *Bartonella* species (Mosbacher et al. 2011; Reis et al. 2011; Regier et al. 2016). Furthermore ticks have been clinically implicated in the transmission of *Bartonella* infection to humans in the absence of other known transmission modes (Maggi et al. 2013).

While cats are the natural reservoir host for *B. henselae*, infection has also been shown serologically or by DNA detection in a variety of accidental hosts including dogs, coyotes, cattle, horses, and deers (Mosbacher et al. 2011; Regier et al. 2019). In addition, bats and wild rodents were found to be novel hosts for potentially zoonotic *Bartonella* spp. (Stuckey et al. 2017; Rozental et al. 2017).

28.2.2.2 Epidemiology in Cats and Humans

Bartonellosis has a worldwide distribution. In cats, seroprevalence usually ranging from 40% to 70% is much higher in warm, humid regions in which high flea infestation is expected (Breitschwerdt and Kordick 2000; Mosbacher et al. 2011; Alvarez-Fernandez et al. 2018). In Europe seroprevalence for pet cats was determined to be, for instance, 23% in Austria (Skerget et al. 2003), 24.7–71.4% in Spain (Aylo et al. 2012; Solano-Gallegro et al. 2006), 41.1% in France (Gurfiel et al. 2001), 18.8–68.7% in Germany (Mietze et al. 2011; Morgenthal et al. 2012), 10.9–57.1% in Italy (Zobba et al. 2009; Mansueto et al. 2012), and 41.2 in the UK (Barnes et al. 2000). An absolute exception is the seroprevalence of 0% in 100 domestic and feral cats from Norway (Bergh et al. 2002).

In Africa 21% and 59.6% of the investigated cats in South Africa and North Africa, respectively, had antibodies against *Bartonella* spp. (Kelly et al. 1996; Al-Kappany et al. 2011). On the Asian continent the seroprevalence of cats ranged from 1.5 to 68% in the Middle East and the Philippines, respectively (Alvarez-Fernandez et al. 2018) and the main clinico-epidemiological studies from America report on 3.7 –75% (Jameson et al. 1995; Case et al. 2006; Nutter et al. 2004) and 5.6–75% (Levy et al. 2008; Müller et al. 2017) serogical positive cats in North and South America, respectively.

Hunting for prey, having access to outdoor environments, living previously as a stray cat or living with other pet cats in the same household were found to be associated with a higher risk of seropositivity (Al-Majali 2004; Gurfield et al. 2001). When compared with pet cats from the same geographical region, feral cats show usually significantly higher seroprevalence rates, e.g., in Sicily (35.4% vs. 68.3%; Mansueto et al. 2012), in North Carolina, USA (75% vs. 93%; Nutter et al. 2004), in Taiwan (26.7% vs. 40%; Chang et al. 2006) or in Turkey (22.9% vs. 31.7%; Guzel et al. 2011). In contrast, seroprevalence in shelter cats seems to be bimodal, i.e., either most cats or only very few show a positive serology (Breitschwerdt and Kordick 2000) with flea infestation as the most important risk factor. Prevalence of bacteremia in both domestic (DC) and stray cats (SC) is usually lower than the respective seroprevalence with 0% in DC in Madrid, Spain (Gil et al. 2013), 4% in Catalonia and northeastern Spain (Pons et al. 2005; Solano-Gallego et al. 2006), 3% in Brazil (DC; Braga et al. 2012), 5.8% (DC) and 18.6% (SC), in China (Yuan et al. 2011), 16% (DC) and 40% (SC), in Australia (Branley et al. 1996), 14.9% in Argentina (Cicuttin et al. 2014), 15.7% (DC) and 35.5% (SC) in La Rioja, Spain (Gil et al. 2013), 16.5% (DC; Gurfield et al. 2001) and 37.2% (SC; Heller et al. 1997) in different parts of France, 16.7% (DC) and 31.3% (SC) in Taiwan (Chang et al. 2006), 17% in Algeria (SC; Azzag et al. 2012) and New Zealand (DC; Joseph et al. 1997), 25% (DC) and 26% (SC) in the Netherlands (Bergmans et al. 1997) or 39.5% in California, USA (Chomel et al. 1995). Risk factors for *B. henselae* bacteremia are grossly those associated with seroprevalence.

In free-ranging or captured big cats such as lions, panthers, and cougars *B. henselae* infection could also be documented serologically or directly by PCR and/or bacterial culture. Seroprevalence rates ranged from 0% in 44 Amur tigers (Goodrich et al. 2012), 18% for panthers in Florida, USA and 28% for cougars from Texas, USA (Rotstein et al. 2000), 17% for lions and 31% for cheetahs in Africa collected between 1982 and 2002 (Molia et al. 2004), 29% in African lions (Pretorius et al. 2004), 37% for Californian mountain lions (Girard et al. 2012), 30% in captive wild felids, 35% for mountain lions, to 53% in bobcats from California, USA, respectively (Yamamoto et al. 1998). Bacteremia with *B. henselae* detected either by culture and/or PCR was found in 1.5% in 65 African lions (Pretorius et al. 2004), in 3.4% of 58 lions (Molia et al. 2004), in 6% of in Iriomote wildcats (*Prionailurus iriomotensis*) from Japan (Tateno et al. 2013), in 15% of neotropical felids mostly of the genus *Leopardus* from a Brazilian shelter (Guimaraes et al. 2010), and in 35% in feral cats (*Felis catus*) from St. Simons Island, Georgia, USA (Hwang and Gottdenker 2013).

In humans, the annual number of infections has been estimated to range between 22,000 and 24,000 in the USA, with about 2,000 cases requiring hospitalization (Jackson et al. 1993; Nelson et al. 2016; Okaro et al. 2017). Thousands of cases may occur yearly in Europe (Chomel et al. 2006; Blanco et al. 1998; Müller 2016). In the USA, there seems to be a seasonal distribution of human cat-scratch disease (CSD) incidence, with the majority of cases occurring between July and January (Carithers 1985; Reynolds et al. 2005; Jackson et al. 1993). This pattern might be due to the seasonal breeding patterns of domestic cats, the acquisition of kittens as family pets, and the peak temporal presence of the cat flea among cats (Anderson and Neuman 1997).

Screening of healthy human blood donors in different industrialized countries, e.g., Sweden, France, Austria, and the USA, found a seroprevalence with anti-*B. henselae* antibodies of 2–22% (Mosbacher et al. 2011; Müller et al. 2016; Breitschwerdt and Kordick 2000). However, studies in certain human populations with a presumably higher risk of attracting infection, e.g., veterinarians, cat owners, hunters, or farm workers, detected higher seroprevalence rates. Seropositivity was 9.6% in Chinese farm workers (Zhang et al. 2008), 15% in Japanese veterinarians (Kumasaka et al. 2001), 45% and 53.3% in Polish veterinarians and cat-owners, respectively (Chmielewski et al. 2007), and 51.1% in veterinarians from Austria (Nowotny et al. 1997). In Austrian hunters, seropositivity was found in 23% (Müller et al. 2016). In contrast, a Taiwanese study among veterinary clinic staff found only a seropositivity of 1.7% (Chang et al. 2006). Also, a seroprevalence rate of 7.1%, which is in the range of that found in the normal population, was detected in attendees of a veterinary conference in Ohio, USA (Noah et al. 1997). Moreover, no association of anti-*B. henselae* antibody positivity with cat ownership was seen in two studies from Austria (Skerget et al. 2003) and Germany (Rath et al. 1997), respectively. Taken together, these data suggest that frequent or even close contact with cats per se does not necessarily lead to *B. henselae* infection and other factors, e.g., flea infestation of cats, have also to be considered.

Only very few studies analyzing anti-*B. henselae* antibody seroprevalence in HIV-positive CSD- or bacillary angiomatosis (BA)-asymptomatic patients have been published; most of them do not differentiate between *B. henselae* and other *Bartonella* spp., have only very small study population sizes and/or lack control groups. Despite these biases and shortcomings, most of the study authors, however, postulate a higher seroprevalence in HIV-patients than in the HIV-negative population (Blanco et al. 1998; Frean et al. 2002; Pons et al. 2008; Trataris et al. 2012), while other studies found no significant differences between both groups (Tsukahara et al. 1999; Pawelczyk et al. 2019).

28.2.2.3 Disease in Cats

Naturally infected cats are usually asymptomatic, although *B. henselae* might cause chronic, relapsing bacteremia for months to years (Alvarez-Fernandez et al. 2018). In experimentally infected cats, in cats infected with non-reservoir-adapted *Bartonella* species (i.e. non-*B.henselae* species) or in immunosuppressed cats, e.g., with a coinfection with Feline Immunodeficiency Virus (FIV), symptoms such as lymphadenopathy, fever, mild transient anemia, or cardiac and renal lesions might be present (Breitschwerdt and Kordick 2000).

28.2.2.4 Disease in Humans

Cat scratch disease (CSD) in immunocompetent people and bacillary angiomatosis (BA) in mainly immunosuppressed patients are the prominent clinical syndromes caused by *B. henselae* (Zangwill 2013). Typically, CSD is a benign and self-limiting disease in humans, presenting with lymphadenopathy (> 90% of patients) mainly of an upper extremity or the neck and often showing suppuration (15–30%), low-grade fever (26–60%), a primary cutaneous lesion at the inoculation site (25–90%), malaise and weight loss (10–45%) (Zangwill 2013). Rare symptoms include erythema nodosum, figurate erythemas, thrombocytopenic purpura, Perinaud's oculoglandular syndrome, encephalopathy, hepatic granulomas, osteomyelitis, pulmonary disease, optic neuritis, and endocarditis. In most cases, the clinical findings resolve spontaneously after 6–12 weeks, while lymphopathy may persist for weeks to months. BA is an uncommon, but severe and potentially fatal disease especially seen in AIDS patients and caused by the pathogen's direct and indirect pro-angiogenic features (Harms and Dehio 2012). Hallmarks of cutaneous manifestations are often multiple (up to hundreds) erythematous, highly vasculated exophytic lesions, or subcutaneous nodules. Basically, any organ might be involved, but besides the skin mainly bones, liver (i.e., peliosis hepatitis), and spleen are affected. Due to their intracellular lifestyle as "intruders below the radar," bartonellae in their niches are difficult to reach by antibiotics (Harms and Dehio 2012). So far, no randomized clinical trials showing an effective antibiotic treatment for CSD exist (Prutsky et al. 2013), although guidelines based on expert consensus have been published (Rolain et al. 2004).

Angioproliferative dieseases such as BA and peliosis hepatitis can be fatal and are usually treated with macrolides or tetracyclines (Zangwill 2013; Rolain et al. 2004; Regier et al. 2016). *Bartonella* spp. have been reported to be the emerging cause of culture-negative endocarditis or fever of unknown origin with high anti-Bartonella-IgG antibody-titers (Regier et al. 2016). Especially, *B. henselae* and *B. quintana* harbor special surface adhesins, which may be an important factor for auto-aggregation, biofilm formation, and, potentially, persistence in vegetative masses in endocarditis. Thus, current recommendations for treatment of *Bartonella* endocarditis include a two drug regimen including aminoglycosides (Okaro et al. 2017).

28.2.2.5 Public Health Importance

Data obtained by multi-locus sequence typing (MLST) of feline and human *B. henselae* isolates indicate that certain sequence types (ST), e.g., ST1, ST2, ST5, and ST8 might be associated with zoonotic transmission and human disease, while others, e.g., ST6 and ST7, are more restricted or even exclusively found in cats (Arvand et al. 2007; Iredell et al. 2003). However, due to different geographic distributions, e.g., in Asia, which differs significantly from that in other parts of the world, a bias regarding an overemphasized association of certain STs with human disease cannot be excluded (Arvand et al. 2007; Bouchouicha et al. 2009; Li et al. 2006; Gil et al. 2013). An overrepresentation of ST1 among human isolates was reported by Arvand analyzing 182 strains from Europe, North America, and Australia by Iredell on 37 strains from France, Germany, the USA, and Australia

and – together with ST8 – by Gil et al. from northeastern Spain (Arvand et al. 2007; Iredell et al. 2003; Gil et al. 2013). Moreover, among 26 *B. henselae* strains isolated from stray cats in China comprising 18 different STs nearly two thirds belonged to ST1, which is associated with human disease (Yuan et al. 2011). ST1 was also predominant in 9 *B. henselae* strains obtained from cats in Buenos Aires, Argentina, followed by ST8, ST5 and ST6 (Cicuttin et al. 2014). In contrast, ST1 does probably not present the greatest public health risk in the UK: in an England-based MLST study on 118 *B. henselae* strains isolated from humans and cats, the vast majority (85%) of zoonosis-associated strains belonged to ST2, ST5, and ST8, respectively, while 74% of the feline isolates belonged to ST4, ST6, and ST7 also indicating that a few, uncommon STs were responsible for the majority of symptomatic human infections in the UK (Chaloner et al. 2011). Interestingly, ST1 and ST5 were found to be significantly more common in countries outside Europe than in England, ST5 and ST7 more common in continental Europe than in England, and ST4 and ST6 more common in England than in the rest of the world (Chaloner et al. 2011). ST1 was also found to be only rare or even absent in North West European countries, but dominating in the Mediterranean region (Arvand et al. 2007). In a Spanish study analyzing both 35 feline strains and the to date largest number of human isolates (n=46), ST5 was by far the most frequent ST among both feline and human isolates comprising more than half of all STs in the respective group (humans: 54.3%; cats: 61.5%) (Gil et al. 2013). Moreover, ST5 – besides ST7 – was also the most often identified ST in 42 German pet cat isolates (Mietze et al. 2011). Both in England (Chaloner et al. 2011) and Spain (Gil et al. 2013), ST5 and ST8 were among the three STs most frequently associated with symptomatic human infection.

However, in contrast to most studies using MLST or MLVA as molecular epidemiological tools, two complementary French studies (Li et al. 2006, 2007) and one Japanese study (Yanagihara et al. 2010) performing the more discriminatory multispacer typing (MST) did not find statistically significant differences in genotypic diversity between human and feline isolates. The reason for this discrepancy is not yet clear, but may be explained by the predominance of different zoonosis-associated strains in different geographic regions.

Not only an association between certain *B. henselae* strains and zoonotic transmission, but also with clinical presentation in humans, either with CSD or endocarditis, has been shown in different studies suggesting the existence of strains with a higher pathogenic potential for humans (Bergmans et al. 1996; Gil et al. 2013). Interestingly Boulouis et al. found specific MVLA profiles in free-ranging and captive wild felids suggesting that these *B. henselae* strains are highly adapted to a specific feline reservoir (Boulouis et al. 2020).

28.2.2.6 Public Health Measures

Except for recommendations to avoid cat contact, cat owners should be advised to screen their cats for fleas and ticks and should be protected year-round from infestation by the regular use of acaricides in spot-on or oral formulations thus prohibiting exposure in- and outside of human habitats (Alvarez-Fernandez et al. 2018; Mosbacher et al. 2011; Breitschwerdt 2008). Immunosuppressed people

should be advised not to adopt stray or flea-infested cats (Stützer and Hartmann 2012). Antibiotic treatment of individual cats has not been proven to be effective in eliminating the carrier status (Mosbacher et al. 2011) and is useless in cats older than 2 years of age due to the self-limiting character of the disease (Breitschwerdt 2008; Stützer and Hartmann 2012). However, it has been proposed to consider doxycycline treatment of both symptomatic and asymptomatic cats younger than 2 years of age when living in the same household with an immunosuppressed human to reduce the bacterial load (Stützer and Hartmann 2012). On a public health scale the development of vaccines to protect pets against *Bartonella* infections and thus reduction of the zoonotic risks would be eligible (Alvarez-Fernandez et al. 2018).

28.2.3 *Toxocara cati*

28.2.3.1 The Pathogen – Life Cycle and Transmission

Toxocara cati, the cat roundworm, first described as *Ascaris cati* in 1788 by the Bavarian Jesuit priest, botanist, and entomologist Franz von Paula Schrank (Schrank 1788), is the most common endoparasite in cats. As its close relative, *Toxocara canis*, it belongs to the ascarid nematodes in the order *Ascaridida*, superfamily Ascaridiodea, family Toxocaridae. Its definitive hosts are cats, in which they live as adults within the lumen of the small intestine. Cats might get infected by ingesting viable, embryonated eggs from contaminated sources (e.g., soil or paratenic[2] hosts such as mice, other rodents, earthworms, ants, or soil-dwelling invertebrates). After ingestion within 2–4 h, the eggs hatch in the duodenum to release juvenile larvae, which penetrate the small intestine, enter the circulation, migrate via the bloodstream or the lymph vessels throughout the body and may invade any organ, especially the liver (after 24 h), the heart, or the lungs (after another 12–24 h). Similarly to the human roundworm *Ascaris lumbricoides*, the larvae migrate especially in young kittens through the lungs, penetrate the trachea, enter the esophagus, get swallowed, and finally reach the lumen of the small intestine where they mature and mate. A single female worm produces about 200,000 non-embryonated eggs/day. After a prepatent period of around 8 weeks the eggs are excreted *per vias naturales* together with the cat feces into the environment. Embryonation occurs in the soil within 2–6 weeks after deposition depending on the temperature and environmental conditions. In adult animals with some degree of acquired immunity the larvae may also remain as dormant larvae in any tissue without reaching the intestine. Besides infection by ingestion of eggs, transmammary or lactogenic transmission is also possible and probably the major route of infection in kittens; larvae migrated to the mammary

[2] The phrase „paratenic host" was introduced by the Swiss parasitologist Jean Georges Baer in 1951, It is derived from the Greek παρατειvω meaning „to prolong, to draw out, to expand, to pass from one to another" or – in Baer's words – „to complicate the life cycle." A paratenic host is „an optional intermediate host (. . .) which the larvae usually enters passively, (. . .) such larvae will invariably remain at the same stage of development as when first swallowed by this host" (Baer 1951; Bowman 2020).

glands of lactating queens when infected late in pregnancy may infect nursing kittens throughout the entire lactation period (Coati et al. 2004; Traversa 2012; Swerczek et al. 1971; Baneth et al. 2016; Morelli et al. 2021). Vertical transmission and infection by the ingestion of paratenic hosts (preferably mice, but also earthworms, cockroaches, or other invertebrates), which harbor encapsulated larvae may also happen. The latter is supposed to be particularly important in cats due to their predatory lifestyle, while dogs might be more prone to infection via the fecal-oral route ingesting *Toxocara* eggs when sniffing or licking at feces-contaminated surfaces (Morelli et al. 2021). Encapsulated larvae may stay infective within paratenic hosts for up to 10 years allowing the parasite to continue their life cycle at prolonged periods waiting to be eaten by the final feline host (Strube et al. 2013; Beaver 1969). In contrast to *T. canis* in dogs, where transplacental transmission is of major importance for the completion of the parasite's life cycle, this route of transmission has not been described for *T. cati* in cats (Macpherson 2013; Strube et al. 2013; Rubinsky-Elefant et al. 2010; Carlin and Tyungu 2020; Rostami et al. 2019; Morelli et al. 2021).

Humans may become infected by accidental ingestion of embryonated infective third-stage larvae (L3) containing eggs present in cat-feces contaminated soil (therefore its classification as a primarily telluric zoonosis or saprozoonosis by the WHO) via geophagy (a certain type of pica), contaminated hands or onychophagy or – less frequently – food or water (Fillaux and Magnaval 2013; Deshayes et al. 2016; Rostami et al. 2019). The infectious dose of 100–200 eggs has been determined for *T. canis* in a single human volunteer (Chaudhuri and Saha 1959) and two "mentally defective children" (Smith and Beaver 1953), respectively[3]. Probably, more rarely, transmission can be achieved through consumption of encysted larvae in raw or undercooked paratenic hosts (e.g., chicken, ducks, sheep, cattle, especially raw liver) (Bowman 2020; for a review of cases see: Hoffmeister et al. 2007). Mainly children may also get infected by eating invertebrate paratenic hosts, e.g. earthworms (Macpherson 2013). Transmission by direct animal contact, e.g. via eggs in fur, might also be possible, although only a low percentage of detectable eggs sticking to cat fur were found to be embryonated and therefore infective in studies from Iran and Turkey (Bakshani et al. 2019; Öge et al. 2014).

In contrast to cats, humans are aberrant or dead-end hosts with respect to the completion of the *Toxocara* life cycle. Infective larvae may hatch after ingestion of eggs, but – in contrast to the human-adapted *A. lumbricoides* larvae – the juvenile stages fail to develop to mature adult worms. Instead, they may migrate throughout the human body and finally get encysted for months or years in basically any organ, causing inflammatory or immunologically driven damage to the respective tissue they happen to reach (Macpherson 2013; Overgaauw 1997; Despommier 2003). Since larvae do not develop to adult worms in humans, reports on finding adult *T. cati* worms in the human gut or in human feces (Rodan and Buckley 1969; Bisseru

[3] Unfortunately and sadly, no information on the circumstances of these *in vivo* experiments, esp. on an ethic approval of these studies, is given in the two publications.

et al. 1966) can only be explained by ingestion of advance-staged larvae in or from feline feces (Beaver 1969); the same holds true for the unique report on – most likely – *T. cati* eggs in the intestinal region of an eighteenth century Franciscan from a Portuguese monastery (Sianto et al. 2017).

28.2.3.2 Epidemiology in Cats and Humans

The epidemiology of toxocarisasis in cats and humans is difficult to assess for several reasons: (i) Most infections in humans, but also in cats are asymptomatic. (ii) *T. canis* and *T. cati* eggs are morphologically very similar and basically only distinguishable by slight size differences (74.8 x 86.0 µm vs. 62.3 x 72.7 µm, respectively [Fahrion et al. 2011]) making microscopical species differentiation probably impossible in a routine laboratory setting (Macpherson 2013; Uga et al. 2000), while molecular diagnostic tools for differentiating *T. canis* from *T. cati* eggs or larvae have been developed only recently (Jarosz et al. 2021; Durant et al. 2012). (iii) Serological techniques to differentiate reliably between *T. cati*- and *T. canis*-specific antibodies in either definite host species or humans do not yet exist (Macpherson 2013; Fillaux and Magnaval 2013; Poulsen et al. 2015; Holland 2017).

Therefore, most epidemiological data on *Toxocara* spp. or toxocariasis, esp. from the pre-molecular era, are not *T. cati*-specific, but will reflect more likely the situation for both *Toxocara* species. It is likely that for convenience reasons most studies deduct the respective *Toxocara* species from the name-giving animal in which they were detected. However, both *T. cati* and *T. canis* can be found in the respective name-giving mammal species. Interestingly, in a study applying species-specific ITS-2-based PCR only about two third of *Toxocara* eggs were *T. canis* and about one third *T. cati* in dog feces, while all eggs from cats were *T. cati* (Fahrion et al. 2011); the finding was explained by the canine behavior of coprophagy causing *T. cati* to be present also in dog feces. Moreover, also *T. malaysiensis*, a potentially zoonotic species first described in 2001 (Gibbons et al. 2001), which is genetically more closely related to *T. cati* than *T. canis* (Fava et al. 2020), shows a similar egg morphology as the two other species and has been found so far only in cats in Vietnam, China, and Malaysia (Gasser 2013; Le et al. 2016).

A recent systematic review and meta-analysis of cross-sectional studies covering more than 2 million cats from 51 countries estimates the global pooled prevalence of *Toxocara* infection in cats at 17.0%, being highest in African (43.3%) and lowest in South American (12.6%) countries (Rostami et al. 2020). For other WHO regions, the respective prevalence rates of *Toxocara* in cats were calculated as follows: Eastern Mediterranean (21.6%), North America (18.3%), Europe (17.8%), Western Pacific (17.3%), and South-East Asia (14.9%). In studies from different European countries, infection rates for *T. cati* in cats are reported to range from 6.2% to 76% (Strube et al. 2013; Epe et al. 2004; Barutzki and Schaper 2003; Chen et al. 2018 [Table S3]).

While prevalence studies of *Toxocara* spp. in cats from 26 European countries performed over the past 25 years found a prevalence rate of 24.5% for *T. cati* (Overgaauw and Nijsse 2020), a multicenter study from seven European countries (Austria, Belgium, France, Hungary, Italy, Romania, and Spain) found an overall

T. cati prevalence in cats of 19.7% with a range from 7.2% (Liège/Belgium) to 25.2% (Bari and Naples/Italy) (Beugnet et al. 2014). Systematic reviews on *Toxocara* prevalence in cats revealed prevalences of 22% in China (Zhang et al. 2020), 16.7% (range: 0.3–43.1%) in Brazil (Dantas-Torres 2020), 5.0–78% in South America (excluding Brazil) (López-Osorio et al. 2020), 3.5–5.1% in the USA on a national level (Ketzis and Lucio-Forster 2020; De Santis et al. 2006, Lucio-Forster et al. 2016), 11% in Canada (Jenkins 2020), 20.7–50.3% in Mexico (Ketzis and Lucio-Forster 2020), 6–52% in Russia (Lukashev et al. 2020), 9% in Sub-Saharan Africa (Omonijo et al. 2019), 9–55.9% in different African countries including Egypt, Ethiopia, Kenya, Nigeria (Chidumayo 2020), and 32.6% resp. 37% in Iran (Eslahi et al. 2020; Abbaszadeh Afshar et al. 2020), respectively. A national survey for *T. cati* in cats in Australia found a prevalence of only 3.2% (Palmer et al. 2008).

In a global perspective, the prevalence of *Toxocara* is higher in low-income tropical countries and also in stray (28.6%) and young ($\leq$12 months of age) (27.7%) cats than in pet (11.6%) and older cats (>12 months of age) (23.8%) (Rostami et al. 2020). A decline in *T. cati* prevalence in cats over the last 20 years is observed in countries with wider use of anthelmintics in the pet population, more stringent fecal hygiene practices in the pet environment, and increased feeding of commercial pet foods, e.g. in Canada and the USA (Jenkins 2020).

Toxocariasis is one of the most prevalent parasitic zoonoses worldwide occurring from the sub-arctic to the tropics (Rubinsky-Elefant et al. 2010; Macpherson 2013). About 1.4 billion humans (one fifth of the world population) and 118–150 million cats (a fourth to a third of the world cat population) are estimated to be affected by toxocariasis (Rostami et al. 2019, 2020). Thus, toxocariasis is probably the most common zoonotic helminthiasis at least in temperate climates (Deshayes et al. 2016).

Estimates of the *Toxocara* and esp. *T. cati* prevalence in humans has several important immanent methodological limitations: (i) due to the *Toxcara* life cycle with humans serving only as paratenic non-definite hosts, fecal examination for eggs is not possible in contrast to the situation in cats; therefore only indirect laboratory methods, i.e., serology, have to be used; (ii) indirect serological methods have immanent sensitivity and specificity problems; especially specificity might be affected by cross-reactivity with antigens from other nematodal helminths, e.g., *Ascaris* spp. or *Toxascaris* spp. (iii) serological methods to differentiate between *T. canis* and *T. cati*, if available at all, are not widely distributed.

Mainly for theoretical considerations the majority of human toxocariasis infections have been considered to be caused by *T. canis*. These arguments are mainly based on data from Iceland (Overgaauw 1997; Rubinsky-Elefant et al. 2010), where the importation of foreign dogs was banned for *Echinococcus* control reasons from 1909 to 1991, dog ownership was prohibited in the capital Reykjavík since 1924, and a serological study failed to detect anti-*Toxocara* antibodies in 307 adult Icelanders (Woodruff et al. 1982). This study, however, did not give any epidemiological information on the study population, e.g., on any exposure risks such as cat contact, on *T. cati* prevalence in cats, cat population density, environmental load of *T. cati* eggs, or their survival in soil under Iceland's climate conditions. Moreover,

neither was *Toxocara* infection completely absent in dogs from Iceland during this time period or shortly thereafter (Richter 1981) nor was the percentage of *Toxocara* eggs shedding cats (12.5%) very high when compared to the situation in cats from other countries (Ágústsson and Richter 1993; Sandholt 2004) making it an over-simplification to claim that a lack of *Toxocara* seropositivity in the study population proves a much lesser transmission risk of *T. cati* to humans when compared to *T. canis*. Besides that, this belief might also be fostered by experimental data showing that *T. canis* larvae migrate faster than *T. cati* through the body of infected mice (Overgaauw and van Knapen 2013; Strube et al. 2013).

Very recently, the global human seroprevalence of anti-*Toxocara* spp. antibodies was estimated in a systematic review and meta-analysis comprising the results of 253 databases on more than 250,000 participants in 71 countries (Rostami et al. 2019) to be 19.0% (Rostami et al. 2019). Seroprevalence was highest in the African region (37.7%), followed by the WHO regions of South-East Asia (34.1%), the Western Pacific (24.2%), the Americas (22.8%), Europe (10.5%), and lowest in the Eastern Mediterranean region (8.2%). More detailed subregional meta-analyses show often wide ranges of human *Toxocara* seroprevalence, e.g., from 3.9% to 84.6% in Southeast Asia (Chou and Fan 2020), from 1% to 56% in Turkey (Taylan-Ozkan 2020), from 4.7% to 13.8% in Mexico (Ponce-Macotela and Martínez-Gordillo 2020), from 6.6% to 9.3% and 11% in Iran (Shafiei et al. 2020; Eslahi et al. 2020; Abbaszadeh Afshar et al. 2020), from 12.1% to 44.8% in China (Kong and Peng 2020), and 16% in Russia (Akhmadishina et al. 2020). In Europe, the overall seroprevalence was estimated at 6.2% (Strube et al. 2020), while the most recent national *Toxocara* survey in the USA revealed a seroprevalence of 5.1% (Farmer et al. 2017; Liu et al. 2018), considerably lower than two decades ago (Bradbury and Hobbs 2020). The highest prevalences with more than 50% in basically healthy people were found in subpopulations, mostly from rural locations or other remote areas: for instance in Africa (Lötsch and Grobusch 2020) in La Réunion (92.8%) (Magnaval et al. 1994), Nigeria (92.5%; Ikotun et al. 2020 or 86.1%, Gyang et al. 2015), Gabon (59.9%) (Lötsch et al. 2016), Ghana (53.5%) (Kyei et al. 2015), and Burundi (50.8%) (Nicoletti et al. 2007); in South-East Asia in Indonesia (84.6%) (Hayashi et al. 2005), Nepal (81.0%) (Rai et al. 1996), Taiwan (76.6%) (Fan et al. 2004), Bali/Indonesia (63.2%) (Chomel et al. 1993), and Thailand (58.2%) (Phasuk and Punsawad 2020); in the Western Pacific region in the Marshall Islands (68.7%) (Fu et al. 2014) and in the Philippines (66.7%) (Rostami et al. 2019); in the Americas in St. Lucia (86%) (Thompson et al. 1986), Colombia (79.3%) (Waindok et al. 2021), Brazil (71.8%) (Araújo et al. 2018), and in Trinidad and Tobago (62.3%) (Rostami et al. 2019); and in Europe in Romania (40.8%) (Cojocariu et al. 2012).

The global prevalence in humans is influenced by a variety of environmental, geographic, cultural, and socioeconomic factors as well as individual components, e.g., susceptibility to *Toxocara* infection due to immunity, coinfection, genetics, age, gender, nutrition, and the behavior of both the human and the definitive hosts (Congdon and Lloyd 2011; Macpherson 2013). Poverty as measured by lower income levels and a lower human development index, lack of education, a high

percentage of untreated or uncontrolled definitive hosts, poor hygienic conditions both for humans and the definitive hosts, lower geographical latitude, a climate showing higher humidity, higher temperature, and higher precipitation thus allowing embryonation of *Toxocara* eggs to infective stages (optimum temperatures: 20–30 °C, detrimental effects at <10 °C or > 37 °C [Gamboa 2005, Azam et al. 2012]) or longer survival of embryonated eggs in the environment are associated with higher prevalences (Macpherson 2013; Rostami et al. 2019). Human seroprevalence in industrialized countries ranges from 0.7% to 44% with the majority of studies indicating prevalences far below 20% (Macpherson 2013); in children who are most at risk prevalences between 1.0% and 17.1% in European countries with a pooled prevalence of 7.8% for children and young adults up to 20 years of age as compared to 4.6% in the 21–50 years old population (Strube et al. 2020), 2.5% in Germany (Overgaauw and van Knapen 2013), and 12.0% in the USA (Bradbury and Hobbs 2020) are reported. In tropical and/or less industrialized countries seroprevalences are much higher ranging from 30% to 92,8% (Strube et al. 2013; Overgaauw and van Knapen 2013; Macpherson 2013; for an intensive review of human seroprevalence surveys published since 1990, see Rubinsky-Elefant et al. 2010; and for the period 1980 to 2019 Rostami et al. 2019, respectively).

28.2.3.3 Disease in Cats

Usually, adult *Toxocara* living in the lumen of the small intestine of their definite hosts do not cause pathological changes. Therefore, mild infections are mostly subclinical. Clinical signs are more often evident during larval migration causing respiratory symptoms (e.g., pneumonia, cough, nasal discharge) or intestinal infections with a moderate or high parasitic load of adult worms (Morelli et al. 2021). Infected kittens may show a catarrhal enteritis with loss of appetite, vomiting, diarrhea alternated with constipation, developmental disturbances, anemia or anorexia, esp. after heavy infections. In adult cats *T. cati* may cause vomiting (e.g., of adult worms), abdominal enlargement, anorexia, or intestinal obstruction with subsequent rupture of the intestine (Traversa 2012; Morelli et al. 2021).

28.2.3.4 Disease in Humans

T. cati larvae may reach basically any organ where they finally get encysted. Therefore, there is a considerable variability in clinical presentation depending on the systemic migration pattern of the active larvae or the final destination of the encysted larvae. Experimental data with mice suggest that *T. cati* migrate more slowly and/or less often into the CNS or the eye when compared to *T. canis* (Overgaauw and van Knapen 2013; Strube et al. 2013). Most authors differentiate between four (or five) different syndromes of toxocariasis: (i) visceral larva migrans (VLM), which might be characterized by fever, hepatosplenomegaly, abdominal pain, pulmonary disease, asthma-like symptoms, anorexia, weight loss, fatigue, occasionally urticaria, and eosinophilia; (ii)ocular larva migrans (OLM; also known as ocular toxocariasis, OT), characterized by a usually unilateral granulomatous retinitis causing visual impairment and possibly blindness; (iii) neurotoxocariasis (NT), characterized by progressive neurologic disease (Deshayes et al.

2016); (iv) and covert or common toxocariasis (CT; occasionally also as two separate, but overlapping entities named covT and comT [Auer and Walochnik 2020]), in which chronic abdominal pain or other nonspecific symptoms can develop (Despommier 2003; Macpherson 2013; Rubinsky-Elefant et al. 2010; Chen et al. 2018; Ma et al. 2018). Both VLM and OLM most frequently affect children, VLM more often those aged less than 5 years of age, OLM usually those 5–10 years old. OLM is considered to be much rarer than VLM and probably caused by a lower level of infection. Human toxocariasis is usually diagnosed serologically and can be treated with albendazole.

28.2.3.5 Public Health Importance

Toxocara eggs need 2–6 weeks outside the definitive host to finally contain infective larvae.

In a recent meta-analysis of 42,797 soil samples from 40 countries, the pooled global prevalence of *Toxocara* eggs in public places was 21%, ranging from 13% to 35% (in descending order: Western Pacific: 35%; Africa: 27%; South America: 25%; South-East Asia: 21%; Middle East and North Africa: 18%; Europe: 18%; North and Central Americas: 13%) (Fakhri et al. 2018). Some recent surveys from Poland (Mizgajska-Wiktor et al. 2017), Portugal (Otero et al. 2018), and the USA (Tyungu et al. 2020) indicate that *Toxocara* eggs are found more commonly in sandpits rather than in the soil of parks and in household-near backyards than in public places, respectively, with sandy, silty, and loamy soil textures more prone for *Toxocara* contamination than clay grounds (Paller and Chavez 2014) and *T. cati* being more abundant than *T. canis* in public places (Mizgajska-Wiktor et al. 2017). In contrast, using a mathematic modeling approach a UK-based study estimated the *T. canis*-linked burden in the Bristol area to be much higher than the *T.cati*-caused environmental contamination and as a consequence also the relative contribution of dogs to *Toxocara* contamination when compared to cats (Morgan et al. 2013). Similar data using a stochastic model were obtained for the Netherlands (Nijsse et al. 2015).

Since the most important *Toxocara* transmission pathway for humans is ingestion of soil resulting in "contact with soil" as the factor with the highest odds ratio (OR: 2.1) for seropositivity in a global meta-analysis (Rostami et al. 2019), children are more at risk than adults due to their less strict hygienic behavior and their tendency to put possibly egg-contaminated objects and soil or larvae-containing paratenic hosts such as earthworms into their mouth.

Direct cat contact is obviously of minor importance, since studies have shown conflicting results on a possible association between seropositivity and cat ownership or close contact with cats (Rostami et al. 2019: OR 1.61 in a global meta-analysis of 16 studies; Paludo et al. 2007: OR 1.957 in Brazil; Negri et al. 2013: OR 1.647; Jarosz et al. 2010: OR 2.0; Won et al. 2008: OR 1.2 in the USA; Rubinsky-Elefant et al. 2008: OR 0.57 in Brazil; Woodruff et al. 1982). A possible infection route by *Toxocara* eggs sticking on the cat's fur seems unlikely, since eggs have to be embryonated to cause infection, which would need time. Moreover, cats – when compared to dogs – show less often xenosmophilic behavior, which might lead to get contaminated with soil- or feces-derived embryonated *Toxocara* eggs.

28.2.3.6 Public Health Measures

To date, no specific national control programs against *Toxocara* spp. have ever been attempted.

Of the four main reservoirs of parasite infection (intestinal infections in the definitive host, eggs in the environment, larvae in paratenic hosts, larvae in the definitive host), the easiest to control are the definitive hosts, e.g., cats and dogs (Macpherson 2013). Therefore, prevention of the initial contamination of the environment is the most obvious approach, for instance, by regularly deworming cats (and dogs) [see recommendations from CAPC or ESCCAP], preventing defecation of pet animals in public areas (especially in playgrounds), reduction of free-roaming cats and dogs, fostering pet hygiene, and educating the public (Overgaauw and van Knapen 2013). However, the paradoxical finding known for *T. canis* eggs that low levels of egg exposure are more successful in establishing patent infections in dogs than larger egg amounts might hamper public health measures, if this finding is also valid for *T. cati* and cats (Macpherson 2013).

When addressing hygienic measures for animals, the differences in canine and feline defecation patterns is important: in contrast to dogs, cats tend to defecate in more thoroughly selected and less open places, e.g., in corners, often covering and hiding their feces thereafter. Therefore, playgrounds and especially sandpits are probably much more important for cat-transmitted *Toxocara* eggs than for those excreted by dogs.

For exposure prophylaxis with respect to *Toxocara* eggs, especially children should be educated about hand hygiene and taught to avoid geophagy.

28.3 Eponymous, But Probably Seldom (or Not Relevant): *Rickettsia felis*, *Chlamydia felis*, *Cryptosporidium felis*, and *Opisthorchis felineus*

28.3.1 The Enigmatic *Rickettsia felis*

28.3.1.1 The Pathogen

Rickettsia felis is an obligate intracellular Gram-negative bacterium, which is commonly attributed to the spotted fever group (SFG) – more recently to the transitional group (TG) – of *Rickettsia* (Caravedo Martinez et al. 2021; Blanton and Walker 2017; Shpynov et al. 2018; Parola 2011; Reif and Macaluso 2009; Pérez-Osorio et al. 2008; Abdad et al. 2011). It has been definitely described in 2002 (La Scola et al. 2002). The cat flea (*Ctenocephalides felis*) is the primary and so far only known natural vector and the reservoir of *R. felis*, although *R. felis* has been found in more than 40 other arthropods including ticks, fleas, chigger mites (Reif and Macaluso 2009; Legendre and Macaluso 2017; Brown and Macaluso 2016), and even non-hematophagous organisms such as booklice (*Liposcelis bostrychophila*) (Behar et al. 2010) and moths (*Phereoeca* spp) (Araújo et al. 2021). *R. felis* was shown to be prevalent in cat fleas from more than 20 countries on 5 continents with positivity rates ranging from 2% to 70% (Parola 2011). It has been detected in several

peri-domestic species including cats, dogs, raccoons, rodents, and opossums, but the definitive mammalian host(s) has not yet been identified. However, very recently, a possible role for the domestic dog (*Canis familiaris*) as a vertebrate reservoir of *R. felis* has been shown (Ng-Nguyen et al. 2020). Humans might possibly get infected via flea saliva and flea bites (Brown et al. 2015; Blanton and Walker 2017) or – as it is the case with *R. typhi* and *R. prowazekii* – via flea feces through dermal micro-traumas (Legendre and Macaluso 2017).

28.3.1.2 Epidemiology in Cats and Humans

Due to antibodies cross-reacting against antigens from different *Rickettsia* spp., species-specific seroprevalence data are often difficult to obtain. In seroprevalence studies, *R. felis*-specific antibodies have been found in 4–15% of mainly client-owned cats from several parts of the USA (Bayliss et al. 2009; Case et al. 2006) and Italy (Ebani et al. 2021; Morganti et al. 2019; Morelli et al. 2019). So far, the highest antibody positivity rate with up to 70% was found in 22 cats from a single household in Chile (Labruna et al. 2007). In contrast, outside from experimental infection settings, *R. felis* DNA has only extremely rarely been detected in blood of cats making it very unlikely that cats serve as an important reservoir for *R. felis* (Hoque et al. 2020).

In humans, serological studies have shown an antibody prevalence of 2.7% in German forest workers (Wölfel et al. 2017), of 4.4% in HIV-positive patients from Spain (Nogueras et al. 2014), of 3.2% and 6.5% in healthy individuals from Catalonia (Nogueras et al. 2006) and from southern Spain (Bernabeu-Wittel et al. 2006), respectively, of 16.1% and 22.5% in healthy farm workers and indigenous Orang Asli people from Malaysia (Kho et al. 2017), and of 17.8% and 24% in volunteers (Hidalgo et al. 2013) and symptomatic patients (Pérez et al. 2021), respectively, from the Caldas province, Colombia.

So far, more than 100 human clinical infections have been described either in case reports or in diagnostic studies from more than 20 countries of 5 continents including Brazil, Mexico (Zavala-Castro et al. 2009), the USA, Sweden (Lindblom et al. 2010), France, Germany, Spain, Thailand, Sri Lanka (Angelakis et al. 2012), Taiwan (Yang et al. 2021), South Korea, Laos, Israel, Tunesia (Kaabia and Letaief 2009), Algeria (Taleb et al. 2013; Mokrani et al. 2012), Egypt, Kenya (Maina et al. 2012; Richards et al. 2010), Senegal (Socolovschi et al. 2010), St. Kitts from the West Indies (Kelly et al. 2010), Australia (Williams et al. 2011), New Zealand (Lim et al. 2012), Indonesia (Mawuntu et al. 2020), and China (Zeng et al. 2021; Ye et al. 2021) (countries not specified by an own reference were cited in Renvoisé et al. 2009, Parola 2011 and Pérez-Osorio et al. 2008).

28.3.1.3 Disease in Cats

The majority of cats are probably asymptomatic (McElroy et al. 2010).

28.3.1.4 Disease in Humans

Clinical manifestations of human *R. felis* infection, sometimes referred to as flea-borne spotted fever, may include fever (>90%), fatigue, headache, dermatological

symptoms such as maculopapular rash (>70%) and – less frequently – an eschar (>10%) (Legendre and Macaluso 2017), less frequently neurological signs (15%) including meningitis-like symptoms (Lindholm et al. 2010, Ye et al. 2021, Zeng et al. 2021, Mawuntu et al. 2020), hepatosplenomegaly (Zavala-Castro et al. 2009), intestinal disease (<10%), or pneumonia (<10%) (Parola 2011; Renvoisé et al. 2009; Caravedo Martinez et al. 2021; Legendre and Macaluso 2017). The disease is usually self-limiting and is most often treated with doxycycline. So far, there have been no reports of flea-borne spotted fever causing death (Abdad et al. 2011) except for two fatal cases presenting with acute meningoencephalitis in whom alternate diagnoses could not be definitely ruled out (Mawuntu et al. 2020).

Importantly, so far laboratory diagnosis of human *R. felis* infections basically relies on serology or PCR (and subsequent sequencing) from blood, CSF, or skin lesions, while cultivation of the organism from clinical samples has not yet been achieved (Blanton and Walker 2017; Caravedo Martinez et al. 2021). Therefore, pathological and clinical findings are at least in some cases difficult to interpret. This holds especially true for two clinical syndromes suspected to be linked to *R. felis*: (i) increasing reports from Sub-Saharan Africa and also Asia finding *R. felis* DNA in the blood of a considerable proportion of febrile patients suggested the pathogen to be an important cause of uneruptive "fever of unknown origin" (FUO) esp. in Africa (Socolovschi et al. 2010; Mediannikov et al. 2013b; Maina et al. 2012). However, in recent years, *R. felis* DNA has been also detected in nonfebrile controls as well as in febrile patients with simultaneous *Plasmodium* parasitemia complicating the previously suggested interpretation of the pathological role of *R. felis* in FUO (Caravedo Martinez et al. 2021). (ii) Similarly enigmatic is the finding of *R. felis* DNA in two cutaneous manifestations, a vesicular fever disease known among the local population in Senegal as "yaaf" (Mediannikov et al. 2013a) and in maculopapular rash reported in three patients from Yucatan, Mexico (Zavala-Velázquez et al. 2000), when considering that *R. felis* DNA has also been found on the skin of healthy afebrile people in Africa (Mediannikov et al. 2013a; Caravedo Martinez et al. 2021).

28.3.1.5 Public Health Importance

The geographic distribution of *R. felis* in the cosmopolitan cat flea reinforces the hypothesis that *R. felis* might be found in most, if not all, human populations where cats and other domestic animals are kept as pets (Abdad et al. 2011). Unfortunately, most human case reports do not comment on possible animal contact. One report from Australia indicates that direct contact with cat fleas harboring cats might be a risk for transmission, since three siblings, their grandmother and a neighbor showed serological and clinical signs of *R. felis* infection, all of them having had contact to a cat family, while the children's parents without cat contact remained asymptomatic (Williams et al. 2011). However, in several studies *R. felis* DNA has been detected in cat fleas (*Ctenocephalides felis*) harvested from pet cats (Abdullah et al. 2020; Kamrani et al. 2008; Noden et al. 2017); in contrast, the finding of *R. felis* DNA in naturally infected pet cats seems to be rare and was reported only very recently (Muz et al. 2021; Hoque et al. 2020).

28.3.1.6 Public Health Measures

The individual and public health measures recommended for prevention of bartonellosis might also be useful for avoiding *R. felis* infection due to the supposed similar transmission via *Ctenocephalides felis*.

28.3.2 Chlamydia felis

28.3.2.1 The Pathogen

Chlamydia felis (previously named as *Chlamydophila felis and earlier Chlamydia psittaci* var. *felis*) is an obligate intracellular bacterium, which grows in the cytoplasm of epithelial cells where it produces inclusion bodies. Other species in the genus include the epidemiologically by far more important *Chlamydia pneumoniae* and *Chlamydia psittaci* as well as *Chlamydia pecorum, Chlamydia abortus*, and *Chlamydia caviae* (Sachse et al. 2015). Transmission occurs via aerosols or secretions from the eyes or noses of infected cats (Sykes 2005).

28.3.2.2 Epidemiology in Cats and Humans

Feline chlamydiosis is the most common cause of acute or chronic conjunctivitis, particularly in kittens, but occasionally also in adult cats (TerWee et al. 1998; Yan et al. 2000; Sykes 2005). Moreover, *C. felis* may also cause upper respiratory tract infection in cats. Seroprevalence data range from 2.5% to 58% in studies from China (Wu et al. 2013; Kang et al. 2016), Slovenia (Dovč et al. 2008), Spain (Millán and Rodríguez 2009; Ravicini et al. 2016), Sweden (Holst et al. 2006), Japan (Yan et al. 2000), Slovakia (Halánová et al. 2011), Italy (Di Francesco et al. 2004), and the USA (Nasisse et al. 1993). Stray cats, cats from catteries or animal shelters, and cats younger than 1 year of age seem to be more affected by *C. felis* infection in different studies including PCR surveys (Bressan et al. 2021; Halánová et al. 2019). PCR studies showed only a low *C. felis* prevalence in asymptomatic cats of less than 5–11% (Sykes 2005; Bressan et al. 2021; Barimani et al. 2019). Not many studies were done in human populations; seroprevalence in the normal population and in veterinarians was 1.7% and 8.8%, respectively, in a study from Japan (Yan et al. 2000). In all serological studies, however, cross-reacting antibodies might affect the positivity rate.

28.3.2.3 Disease in Cats

Clinical signs of *C. felis* infection are conjunctivitis, sneezing, transient fever, inappetence, weight lost, nasal discharge, rarely lameness or lethargy, and possibly, but not yet proven, reproductive tract disease including stillbirth or abortion (Sykes 2005). Chlamydial disease may be complicated by coinfection with other microorganisms. Cats are usually treated with doxycycline for several weeks (Sykes 2005).

28.3.2.4 Disease in Humans

C. felis infection may be associated occasionally with conjunctivitis and/or very rarely respiratory tract disease (Browning 2004). In a few serological studies using

antibodies reported to be relatively specific for *C. felis*, *C. felis* was found to be at best an uncommon cause of community-acquired pneumonia (CAP) in less than 0.5% of CAP patients from Canada (2/539; Marrie et al. 2003) or Japan (1/506; Miyashita et al. 2005).

28.3.2.5 Public Health Importance

Exposure to *C. felis* is possible when handling infected cats via contact with their aerosols or contaminated body fluids, e.g., tear fluid, but maybe also by feces, since *C. felis* DNA was recently detected in rectal samples (Bressan et al. 2021). The zoonotic potential of *C. felis*, however, appears to be very low. Only sporadic cases with possible cat-to-human transmission have been reported (Browning 2004). So far, only two PCR-proven cases have been published showing identity of a human and a feline isolate in an HIV-positive patient with chronic conjunctivitis and his recently acquired cat (Hartley et al. 2001; Wons et al. 2017). Moreover, in one Japanese patient presenting with CAP and serological evidence of possible *C. felis* infection cat contact was documented (Miyashita et al. 2005).

28.3.2.6 Public Health Measures

Care should be taken especially by immunosuppressed people when handling cats with conjunctivitis and/or upper respiratory tract infection. A vaccine for cats is available.

28.3.3 Cryptosporidium felis

28.3.3.1 The Pathogen

Cryptosporidium spp. are coccidian obligate intracellular parasites mainly infecting epithelial cells of the gastrointestinal tract. They have been reported from a large variety of different hosts including humans. The most important species for humans infections are the zoonotic *C. parvum* and the host-adapted *C. hominis* (Ryan et al. 2014, 2021), while *C. felis* is one of several species identified in different mammals and the most commonly species found in cats (Li et al. 2021; Meng et al. 2021; Taghipour et al. 2021). Infection occurs via ingestion of viable oocysts, often by drinking contaminated water, eating contaminated food and/or fecal-orally.

28.3.3.2 Epidemiology in Cats and Humans

Fecal oocyst shedding by cats ranges from 0% to around 30% according to data from Spain (Gracenea et al. 2009; Gil et al. 2017), the Netherlands (Overgaauw et al. 2009), Denmark (Enemark et al. 2020), Greece (Kostopoulou et al. 2017), Australia (Palmer et al. 2008; Yang et al. 2015), Canada (Shukla et al. 2006), Colombia (Santin et al. 2006), Brazil (Coelho et al. 2009; Alves et al. 2018; de Oliveira et al. 2021), the UK (Gow et al. 2009; Tzannes et al. 2008), Italy (Rambozzi et al. 2007), Thailand (Tangtongstrup et al. 2020), Japan (Ito et al. 2017; Ito et al. 2016; Yoshiuchi et al. 2010), China (Li et al. 2015, 2019a, b, 2021; Xu et al. 2016), Iran (Homayouni et al. 2019), and the USA (Ballweber et al. 2009; Mekaru et al. 2007; Nutter et al. 2004;

Lucio-Forster et al. 2010) with most of the studies reporting prevalences below 10% or even lower (Li et al. 2021). In most cases, younger age of the cats and living outside a household were risk factors for *C. felis* infection in cats.

Worldwide, far more than 100 findings of *C. felis* from humans have been reported to date (for a review see Lucio-Forster et al. 2010; Raccurt 2007; Ryan et al. 2014, 2021; Jiang et al. 2020; Das et al. 2006; Cieloszyk et al. 2012; Llorente et al. 2006; Cama et al. 2008; Elwin et al. 2012; Matos et al. 2004). In the majority of cases immunosuppressed people were affected, previously mainly HIV-positive adults (about two thirds of all *C. felis*-positive individuals for whom clinical information regarding their immunostatus is known), but in recent years also obviously immunocompetent children mainly from developing countries and patients presenting with diarrhea (Lucio-Forster et al. 2010; Jiang et al. 2020; Liu et al. 2020). For instance, *C. felis* was the underlying *Cryptosporidium* species in 0.4% of 14,469 human stool samples obtained in England and Wales between 2000 and 2008 (Elwin et al. 2012), in 1% of 108, 98, and 175 samples from Spain (Llorente et al. 2007), from the Netherlands (Wielinga et al. 2008) and from children in Kenya (Gatei et al. 2006), respectively, in 2.5% from 40 samples from India (Das et al. 2006), in 0.3% of 394 samples from Nigeria (Ukwah et al. 2017), and in 4.3% from 109 *Cryptosporidium*-infected children from Peru (Cama et al. 2008).

Very recently, the development of a *gp60* gene subtyping tool for *C. felis* (Rojas-Lopez et al. 2020) dividing the species in so far five different subtype families called XIXa to XIXe allows a clearer picture of the molecular epidemiology of the cat-adapted species *C. felis* due to its highly polymorphic composition (Li et al. 2021). Since XIXa and XIXb have been found both in humans and cats, while XIXc-e has nearly exclusively been detected in humans, it has been suggested that the latter subtypes might be (more) human-adapted (Li et al. 2021; Jiang et al. 2020).

28.3.3.3 Disease in Cats

Oocyst shedding cats are most often asymptomatic. Occasionally, infection may be associated with persistent diarrhea, especially in younger and/or immunocompromised cats (Lucio-Forster et al. 2010).

28.3.3.4 Disease in Humans

In humans, *Cryptosporidia* may cause an enteric disease with watery diarrhea, abdominal pain, and nausea, occasionally accompanied by low grade fever or headache. Immunocompromised individuals and children are especially at risk of developing prolonged or profuse diarrhea. Deaths due to exsiccation may occur mainly in severely immunosuppressed patients or malnourished children in developing countries. In rare cases, mainly in immunocompromised patients, also other organ systems might be infected, e.g., the respiratory tract, the pancreas, or the bile ducts. Asymptomatic infections have also been reported.

28.3.3.5 Public Health Importance

Case-control studies from several countries were not able to show that contact with companion animals is associated with an increased risk of acquiring cryptosporidiosis (for a review see Xiao and Feng 2008). While in some of the few case reports on human *C. felis* infections the patients had previous cat contact (Matos et al. 2004), no anamnestic cat contact was reported in others (Pedraza-Díaz et al. 2001; Cacciò et al. 2002; Llorente et al. 2006; de Lucio et al. 2017). Similarly, in a Swiss-US American study by Morgan et al. three HIV-positive *C. felis*-infected patients were reported to have had contact with a cat, while two others denied the presence of cats in their household (Morgan et al. 2000). So far, only one case of possible cat-to-human transmission has been documented based on molecular subtyping showing an identical *C. felis* strain in a pet cat and its owner, both suffering from diarrhea (Beser et al. 2015). Taken together, it may be concluded that pet cats impose only a minimal risk for zoonotic transmission of cryptosporidiosis in general and for *C. felis* in peculiar (Lucio-Forster et al. 2010; Raccurt 2007).

28.3.3.6 Public Health Measures

Despite an only minimal risk of zoonotic transmission, especially immunocompromised persons should be advised to minimize contact to cat feces and to observe basic hand hygienic measures.

28.3.4 Opisthorchis felineus

Opisthorchis felineus and its close relatives *O. viverrini* and *Clonorchis sinensis* are the three most common liver flukes involved in human health. *O. felineus* was named after one of the definite host species in which it was first described, e.g., cats from Pisa, Italy (Rivolta 1884). Although the species name suggests a zoonotic risk due to cat contact, humans – as other fish-eating mammals including cats – acquire an infection by eating raw fish harboring the trematode's metacercariae (Pozio et al. 2013; Petney et al. 2013; Fedorova et al. 2020). Therefore, cats do not pose a direct zoonotic risk for human infections, although in some regions they might be required for maintaining the parasite's life cycle (Scaramozzino et al. 2018).

28.4 The (un)usual Suspects from the Gut

Cats may excrete a plethora of bacteria, viruses, and parasites via their feces (Barutzki and Schaper 2003, 2011; Gow et al. 2009; Waap et al. 2014; Weber et al. 1995; Weese 2011; Spain et al. 2001; Philbey et al. 2009; Paris et al. 2014; Hill et al. 2000; Morato et al. 2009; Dantas-Torres and Otranto 2014; Ballweber et al. 2010; Hoelzer et al. 2011; Esch and Petersen 2013; Oh et al. 2021). However, for most of the respective pathogens the presumed zoonotic potential is mainly based on isolation of identical species, subtypes, serotypes, or – in better circumstances – of similar genotypic strains from within the pet and human population. However,

clearly defined outbreak or case reports with molecular proof of cat-to-human transmission are still lacking for most enteric pathogens. Moreover, the relative contribution of cat-associated transmission to the overall zoonotic disease load for most of these pathogens is not clear and probably very low for most of them when compared to other zoonotic sources, as it is the case with campylobacteriosis or salmonellosis in which chicken predominate the picture when compared to pet animals.

Recent reviews and studies on the zoonotic potential of *Giardia* spp., of which different genotypic assemblages are relatively host-specific with assemblage F found almost exclusively among cats and assemblages A and B in a wide range of species including cats and humans (Ballweber et al. 2010; Morelli et al. 2021; Lecová et al. 2020; de Lucio et al. 2017; Ramírez-Ocampo et al. 2017), and non-typhoidal salmonellosis (Hoelzer et al. 2011) suggest only a minimal risk of cats as source for human infection with the respective pathogens. However, indirect epidemiological data suggest at least a potential zoonotic risk, since a multivariable analysis matched on age group, which was conducted in Michigan, USA revealed that children with *Salmonella* infections had reported more commonly than controls contact with cats (matched OR = 2.53) (Younus et al. 2010). Interestingly, seasonal outbreaks of *Salmonella* Typhimurium among wild birds, domestic cats, and humans have been linked to cats preying on salmonellosis-diseased singing birds or passerines subsequently transmitting the bacteria to their respective owners (Tauni and Österlund 2000; Söderlund et al. 2019).

Importantly, also antibiotic-resistant *Salmonella* strains have been isolated from cats and partly linked to human outbreaks (Low et al. 1996; Van Immerseel et al. 2004; Wright et al. 2005).

Carriage of thermotolerant *Campylobacter* spp. by cats ranges between 9.9% and 41.9% (Thépault et al. 2020) suggesting a potential source of pet cats as source for human exposure. However, data on zoonotic transmission between cats and humans are scarce. An analysis of *Campylobacter* strains isolated from humans and animals in Switzerland using different genotyping methods showed that only very few genotypes among human and cat isolates were similar (Keller et al. 2007). Similarly, a recent study from France showed only very minor cluster prevalence similarities between *Campylobacter* spp. from cats and humans (Thépault et al. 2020). In an extensive study from the Netherlands comparing *Campylobacter* genotypes of strains obtained from pet cats, pet dogs, pet-owners, and non-pet owners by MLST, an increased risk for human *C. jejuni* or *C. coli* infection was associated with dog-ownership (OR 2.5 in an overall model, 9.2 in a model for campylobacteriosis of probable pet origin), but not with cat-ownership (Mughini Gras et al. 2013). In contrast, another Dutch study found an OR of 1.7 and 2.0 of cat-ownership for human infection with *C. jejuni* and *C. coli*, respectively (Doorduyn et al. 2010), but interestingly no association with visiting other cat-owning households. Although pet ownership was estimated in both studies to contribute in 10–25% to human *Campylobacter* infections, the route of transmission – e.g., pet-to-human or human-to-pet or a common infection source such as food items – cannot be inferred from these modeled attributions (Mughini Gras

et al. 2013; Doorduyn et al. 2010). In general, the risk of human infection via pet cat contact is low (Thépault et al. 2020).

With respect to pathogenic *Escherichia coli*, only a few genetic association studies and/or case reports exist indicating a minor zoonotic potential for cat-related human infections.

A Brazilian MLST analysis of Enteropathogenic *Escherichia coli* (EPEC) strains has shown close genetic similarity between O111:H25 and O125:H6 strains of human and cat origin (Morato et al. 2009). Two case reports on human and cat infections caused by a molecularly identical strain of *Enterohemorrhagic E. coli* (EHEC) have been published from Germany (Busch et al. 2007) and Argentina (Rumi et al. 2012), respectively. However, infection of pet cats with the classical *E. coli* O157:H7 has not yet been described (Kataoka et al. 2010; Persad and Lejeune 2014).

Since most of the cat infections due to the above mentioned enteric bacteria are asymptomatic and remain unnoticed, it might be reasonable to advise immunosuppressed people, especially people with HIV-infection, not only to get their pets tested for *Salmonella*, *Campylobacter*, or *Cryptosporidium* when these develop diarrhea, but also to educate about proper hygienic measures when handling animals in general or to avoid exposure to them even if they are asymptomatic (Spain et al. 2001).

28.5 An Emerging Cat-Related Pathogen: Toxigenic *Corynebacterium ulcerans*

28.5.1 The Pathogen

C. ulcerans are facultative anaerobic, nonmotile, non-sporulating, unencapsulated, pleomorphic, partially acid-fast Gram-positive rods. Similar to *Corynebacterium diphtheriae*, the classical diphtheria agent of humans, and *Corynebacterium pseudotuberculosis*, the agent of caseous lymphadenitis in sheep and goats and a very rare zoonotic pathogen, *C. ulcerans* may harbor a lysogenic beta-corynephage bearing the *tox* gene. Toxigenic strains may produce the *tox*-encoded diphtheria toxin (DT), which is responsible for the systemic symptoms of diphtheria. Additionally, pathogenicity of *C. ulcerans* arises from the production of phospholipase D (Hacker et al. 2016). Domestic animals such as cats, dogs, and pigs serve as reservoirs for possible zoonotic *C. ulcerans* infection (Meinel et al. 2014; Schuhegger et al. 2009; Berger et al. 2011a, b). Moreover, it has been found in many non-domestic animals, such as macaques, ferrets, red foxes, and hedgehogs (Hirai-Yuki et al. 2013; Marini et al. 2014; Sting et al. 2015; Berger et al. 2019). Recently a toxigenic *C. ulcerans* diphtheria like infection in a horse was reported (Zendri et al. 2021).

C. silvaticum, formerly called *C. ulcerans* wild boar cluster, was previously described as a novel potentially zoonotic NTTB (non-toxigenic tox gene-bearing) species in wild boars and deers (Dangel et al. 2020; Möller et al. 2020). Since the

final taxonomic description of *C. ulcerans* is only from 1995 (Riegel et al. 1995) and the differentiation between *C. ulcerans*, *C. pseudotuberculosis* and *C. silvaticum* by classical biochemical tools might be difficult or is not possible at all in case of *C. silvaticum* (Torres et al. 2013; Berger et al. 2014), prevalence data on, or case reports of, *C. ulcerans* infections, which were published before 1995 or which do not explain their differentiation methods used have to be handled with care. Today MALDI-TOF mass spectrometry and Fourier-transform infrared (FT-IR) spectroscopy are suitable tools to identify these isolates (Rau et al. 2019; Berger et al. 2019; Dangel et al. 2020).

28.5.2 Epidemiology in Cats and Humans

Epidemiological data on the prevalence of diphtheria-causing *Corynebacterium* spp. in cats are sparse. Since the first description of *C. ulcerans* in two cats from Scotland in 2002 (PHLS 2002; Taylor et al. 2002), only sporadic and often asymptomatic infections have been identified in cats mostly as part of public health measures during the search for an infectious source in case of a human index patient. A recent study report carriage rates of *C. ulcerans* in healthy animals were 0.42% (2/479) in dogs and 0.00% (0/72) in cats, whereas in animals with signs of upper respiratory tract infection prevalence rates were 0.53% (1/189) in dogs and 6.25% (4/64) in cats (Abbott et al. 2020).

Human diphtheria is a WHO-notifiable disease. In 2020, there were 22,916 cases reported globally (WHO 2020), most of them in Nigeria, India, and Ethopia. On a global scale, no WHO data exist on the relative contribution of the three potentially toxigenic *Corynebacterium* species. In 24 member countries of the European Diphtheria Surveillance Network (DIPNET) except the high diphtheria endemic country Latvia, *C. diphtheriae* and *C. ulcerans* accounted for 53 reported human infections each between the period 2000 and 2009 (Wagner et al. 2012). In many industrialized countries, cases of diphtheria-like infection caused by toxigenic *C. ulcerans* have recently outnumbered those caused by toxigenic *C. diphtheriae* (Wagner et al. 2010; Bonmarin et al. 2009; Zakikhany and Efstratiou 2012; Torres et al. 2013; Gower et al. 2020). In Germany, we observe between 2 and 20 cases of *tox+C. ulcerans* infections (usually skin located) in humans per year since 2007 (https://survstat.rki.de).

28.5.3 Disease in Cats

In most cases in which human *C. ulcerans* infections were associated with pet cat contact, the respective cats were either asymptomatic or presented with bilateral nasal discharge probably due to an underlying Feline Calicivirus (FCV) infection (Hatanaka et al. 2003; De Zoysa et al. 2005). In analogy to dogs (Lartigue et al. 2005), also skin ulcers and bronchopneumonia might be expected (Abbott et al. 2020).

28.5.4 Disease in Humans

Toxigenic *C. ulcerans* may cause classical respiratory diphtheria or diphtheria-like syndromes as well as cutaneous diphtheria (Berger et al. 2011a; Schuhegger et al. 2009). Classical diphtheria is an upper respiratory tract illness characterized by sore throat, low fever, and an adherent eponymous pseudomembrane (Greek: διφθέρα "pair of leather scrolls") on the tonsils, pharynx, and/or nasal cavity (Bonnet and Begg 1999). Systemic sequelae may appear after several days including myocarditis and peripheral neuropathy (Haywood et al. 2017; Schuhegger et al. 2009). Fatal cases of zoonotic *C. ulcerans* infections have also been reported (Hatanaka et al. 2003; Wellinghausen et al. 2002; Hogg et al. 2009; Mattos-Guaraldi et al. 2008; Tiwari et al. 2008; Gower et al. 2020). Treatment of respiratory diphtheria is by immediate antitoxin application and subsequent antibiosis with usually penicillin or erythromycin (Bonnet and Begg 1999; Marosevic et al. 2020).

Cutaneous diphtheria usually presents as skin ulcer and is often caused by a minor skin trauma; frequently, toxigenic *C. ulcerans* are isolated from an infected wound together with *Staphylococcus aureus* and *Streptococcus pyogenes* as additional pathogens.

Non-toxigenic strains may very rarely be associated with human disease, e.g., as cause of bacteremia or skin ulceration (Corti et al. 2012; Gower et al. 2020).

28.5.5 Public Health Importance

C. ulcerans infection was originally associated with consumption of raw milk and dairy products or contact with cattle, but since the first isolation of toxigenic *C. ulcerans* from two domestic cats from the same household in Scotland (PHLS 2002; Taylor et al. 2002), *C. ulcerans* has increasingly been isolated from domestic animals such as pet dogs (Lartigue et al. 2005; Hogg et al. 2009) and cats (De Zoysa et al. 2005). Although most human cases reported in the last decade in the UK (Wagner et al. 2010), France (Bonmarin et al. 2009), and Germany (Sing and Heesemann 2008; Berger et al. 2011a; Meinel et al. 2014) were associated with pet animal contact – this sums up to 94% of case-patients for which the respective information was available within Europe from 2000 to 2009 (Wagner et al. 2012) – epidemiological links of human infections by toxigenic *C. ulcerans* exclusively restricted to cat contact have only been reported in two patients from Japan with six and nearly twenty pet cats, respectively (Urakawa et al. 2013; Hatanaka et al. 2003), one patient with four cats from Belgium (Detemmerman et al. 2013) and in two patients from France (Bonmarin et al. 2009). A recent study in the UK reported on 15 toxigenic *C. ulcerans* from 2009 to 2017 with exposure to domestic animals as the major risk factor. Exposure to either dogs or cats was documented for 12/15 and 7/15 *C. ulcerans* cases, respectively (Gower et al. 2020).

In the last years molecular strain typing methods have improved for the epidemiologic research of *C. ulcerans* infections (Both et al. 2015; König et al. 2014) in order to enable the confirmation of strain transmissions between animals and

humans. Isolation of an identical toxigenic *C. ulcerans* strain from a cat and its owner has been documented in a few cases, for example, of an asymptomatic pet cat and a person with pharyngeal diphtheria-like illness (Berger et al. 2011a), in an HIV-patient with an axillary lymph node abscess (Yoshimura et al. 2014) and a patient with flu-like symptoms, dyspnea, and pseudomembrane formation (Wake et al. 2021). Meinel et al. demonstrated the superior resolution of next generation sequencing compared to multi-locus sequence typing for epidemiologic research of *C. ulcerans* zoonotic transmissions including three cat-associated cases (Meinel et al. 2014). Indirect evidence for zoonotic transmission comes from a case of cat-bite-transmitted cutaneous diphtheria in which toxigenic *C. ulcerans* was isolated from the infected wound; however, no *C. ulcerans* could be detected in the biting cat (Berger et al. 2011b). Additionally, toxigenic *C. ulcerans* was isolated from a pharyngeal pseudomembrane of a Japanese woman with refractory pharyngitis and discharge material from her cat's eyes suggesting zoonotic transmission (Kamada et al. 2012). Interestingly, when ribotyping 50 human and 7 feline *C. ulcerans* isolates from the UK, all ribotypes generated by the cat isolates were found among the human isolates; moreover, six out of seven of the cat strains belonged to one of the predominant ribotypes seen among the clinical strains, i.e., U1, U2, and U4 (De Zoysa et al. 2005).

Besides toxigenic *C. ulcerans*, also non-toxigenic *tox*-bearing (NTTB) *C. diphtheriae* strains have been isolated during contact tracing due to a human diphtheria index patient from the ears of two pet cats with otitis in the USA (Hall et al. 2010) and from an asymptomatic cat in Belgium (Detemmerman et al. 2013). Interestingly, all feline isolates from both continents showed a 1-bp deletion at nucleotide 55 in the *tox*-gene explaining the non-production of a functional DT; moreover, the *rpoB* sequence of the US strains showed less than 98% identity when compared to other *C. diphtheriae* suggesting a novel subspecies. The authors of both studies speculate that cats might also serve as reservoirs for – a possibly cat-specific subspecies of – *C. diphtheriae*.

28.5.6 Public Health Measures

Although diphtheria is rare in humans in industrialized countries due to vaccination programs, it is a notifiable disease that has to be reported to the relevant health authorities. In contrast to *C. diphtheriae*, *C. ulcerans* was for a long time thought to be exclusively transmitted from animal to human. However, in one instance from 1996 person-to-person transmission has been suspected due to isolation of *C. ulcerans* from two siblings (Bonnet and Begg 1999 citing a personal communication). Another report on a possible human-to-human transmission of toxigenic *C. ulcerans* between a 13-year-old girl suffering from tonsillitis and her asymptomatic grandmother harboring the identical sequence type ST 332 is documented (Konrad et al. 2015). The authors suggested an initial zoonotic transmission and a subsequent human-to-human transmission event because the family lived on a farm with many domestic animals. Gower et al. documented the transmission of a

toxigenic strain from a fully vaccinated individual to an unvaccinated contact in the UK (Gower et al. 2020).

Although probably only a very minor risk of human-to-human spread might exist, the English public health authorities have recommended that the public health response to human *C. ulcerans* infection should be the same as that for *C. diphtheriae*, e.g., isolation and treatment of the index case, tracing and taking nose and throat swabs from close contacts, as well as providing prophylactic antibiotics and booster vaccination for close contacts (Bonnet and Begg 1999; Public Health England 2015). Additionally, effective management of a *C. ulcerans* case also requires coordination between human and animal health agencies, especially because of several ethical and practical issues, including the lack of legal compulsion for owners to treat non-symptomatic companion animals harboring a toxigenic *C. ulcerans* strain (Hogg et al. 2009). In some instances dogs or cats as zoonotic sources of a human index case have successfully been treated using antibiotics, sometimes over a prolonged period (Berger et al. 2011a, b; Hogg et al. 2009; Abbott et al. 2020). There are also no proven vaccines specifically directed against *C. ulcerans*. Scanty data from clinical case reports showing attenuated clinical symptoms in some patients as well as from cytotoxicity assays using a very limited number of clinical isolates indicate that despite differences in the aminoacid sequence of DT from *C. diphtheriae* and *C. ulcerans* the currently used diphtheria toxoid vaccine might also protect from diphtheria due to infection with toxigenic *C. ulcerans* (Schuhegger et al. 2008; Gower et al. 2020).

28.6 SARS-CoV-2: A Reason for Cats (or Us) to Pan(dem)ic?

28.6.1 The Pathogen

Coronaviruses (CoVs) belonging to the order Nidovirales, suborder Cornidovirineae, family Coronaviridae, and subfamily Orthocoronavirinae, are pleomorphic, enveloped, positive-sense, single-stranded RNA viruses containing the largest viral RNA genomes known so far of around 30 kb. The Orthocoronavirinae subfamily comprises four genera, i.e., alpha-, beta-, gamma-, and delta-CoVs (Maurin et al. 2021; Ghai et al. 2021; Parkhe and Verma 2021). Common human coronaviruses (HCoVs) usually cause mild disease, mainly common cold, and include two α-CoV (HCoV-229E and HCoV-NL63) and two β-CoV (HCoV-OC43 and HCoV-HKU1) strains. Besides these seasonal, non-zoonotic and endemic human coronaviruses, three β-CoVs of animal origin have led to severe epi- or pandemics in the twenty-first century, i.e., the successfully eradicated SARS-CoV-1 causing Severe Acute Respiratory Syndrome (SARS) in 2002/3, the Middle-Eastern Respiratory Syndrome virus (MERS-CoV) enzootic in dromedary camels and having emerged in 2012, and the COVID-19 agent SARS-CoV-2 (genus: Betacoronavirus, subgenus: Sarbecovirus) first identified in Wuhan, China, at the end of 2019.

Host susceptibility and organotropism are dependent on the presence and cellular distribution of the SARS-CoV-2 receptor angiotensin-converting enzyme 2 (ACE2) interacting with the viral spike (S) protein and its receptor-binding domain (RBD) (Hoffmann et al. 2020; Fischhoff et al. 2021). Human and domestic cat ACE2 sequences show a high degree of protein homology > 85% (Ekstrand et al. 2021; de Morais et al. 2020) esp. in the predicted RBD key binding sites (Wei et al. 2021; Piplani et al. 2021; Mathavarajah and Dellaire 2020). Additionally, SARS-CoV-2 cell entry is also supported by the transmembrane serine protease 2 (TMPRSS2) (Hoffmann et al. 2020), which shows a 79.7% identity between human and domestic cat (Huang et al. 2021). Not to be confused with the three β-CoVs SARS-CoV-1 and -2 as well as MERS-CoV, respectively, are feline coronaviruses (FCoV) belonging to the α-CoVs. In contrast to SARS-CoV-2 with several thousands of sublineages, there are only two FCoV, i.e., type I and type II. FCoV may cause mild enteric infections to fatal Feline Infectious Peritonitis (FIP) and differ in many aspects from SARS-CoV-2, e.g., cell receptor (feline aminopeptidase, fAPN, at least for type II FCoV), cellular tropism (enterocytes, but also monocytes and macrophages), and route of infection (fecal-oral) (Paltrinieri et al. 2021).

28.6.2 Epidemiology in Cats and Humans

SARS-CoV-2 is responsible for one of the most severe pandemics in human history causing more than 300 million human infections and more than 5 million deaths worldwide by the beginning of 2022 as updated routinely on the Johns Hopkins University Coronavirus Resource Center (https://coronavirus.jhu.edu/map.html) as well as the WHO dashboard (https://covid19.who.int/). Molecular database platforms (https://www.gisaid.org; https://cov-lineages.org/) allow an intense and unprecendented real-time surveillance of the pandemic helping to identify new SARS-CoV-2 variants of concern (VOCs) with the potential to supersede previous prevalent virus lines due to higher transmissibility or immune escape. In most countries and regions, different waves of the pandemic were caused or at least intensely influenced by certain rapidly spreading VOCs, e.g., the third wave by Alpha (B.1.1.7), the fourth wave by Delta (B.1.617.2) including its numerous sublines and the fifth wave by Omicron (B.1.1.529).

Although felines belong to the most SARS-CoV-2-susceptible animal species as known from experimental (Shi et al. 2020; Halfmann et al. 2020; Bosco-Lauth et al. 2020; Gaudreault et al. 2020) as well as sequence- or structure-based modeling data (Fischhoff et al. 2021; for a review see Ekstrand et al. 2021; Dróżdż et al. 2021; Giraldo-Ramirez et al. 2021), only 124 SARS-CoV-2-infections in cats have been documented globally until 15 March 2021 (Giraldo-Ramirez et al. 2021, similar: Dróżdż et al. 2021 and OIE 2021), when already more than 120 million humans had been infected. Most recently, Islam et al. reported 156 domestic cats infections worldwide (Islam et al. 2021), while as of 15 January 2021 for the USA alone only 132 (of 3625 tested) SARS-CoV-infected animals and more than 27 million human cases were counted (Davis and Innes 2021).

The first case of SARS-CoV-2 infection in a domestic cat was reported from Belgium approximately 6 weeks after the first human case in this country in March 2020 (Garigliany et al. 2020). Subsequent domestic cat infections during the first (or second) wave have been found mostly within 3 months after the onset of the COVID-19 pandemic in Hong Kong (Barrs et al. 2020), Germany (Schulz et al. 2021a), the USA (Newman et al. 2020; UDSA 2020), Spain (Segalés et al. 2020), Chile (Neira et al. 2021), France (Sailleau et al. 2020), and the UK (Hosie et al. 2021a). Infected cats were identified 4–6 months after the first documented human case in Italy (Klaus et al. 2021a; Musso et al. 2020), Russia (OIE 2021), and Thailand (Jairak et al. 2021), while in some countries it took even longer until the first feline infections were reported (6 months in Japan [OIE 2021], more than 8 months in Switzerland [Chan et al. 2021] and more than 11 months in Canada [OIE 2021]) and South Korea (Han et al. 2021).

Similarly, in later pandemic waves it took at least 3 months until the respective VOC was detected in domestic cats after the first human case in the respective country, e.g., Alpha (B.1.1.7) in the USA (Hamer et al. 2021a), Italy (Zoccola et al. 2021), the UK (Ferasin et al. 2021), France (Krafft et al. 2021), and Germany (Keller et al. 2021) or Delta (B.1.617.2) in Harbin, China (Kang et al. 2021). Alpha and Delta genome sequences were also reported from Thailand and the USA as well as from Belgium, respectively (Islam et al. 2021).

In conclusion, the comparatively very low number and delayed detection of SARS-CoV-2 infections in domestic cats suggest that cats are not of major importance for the ongoing COVID-19 pandemic. Interestingly, so far all described pet cat infections detected by RT-PCR were linked to a human index case including reports showing the same viral strain in human and pet cat (Garigliany et al. 2020; Zoccola et al. 2021; Barrs et al. 2020; Han et al. 2021); up to now, cat-to-human "re-spillback" transmission of SARS-CoV-2 is extremely rare and has not been reported prior to July 2022 (Dróżdż et al. 2021; Giraldo-Ramirez et al. 2021; Maurin et al. 2021), when a suspected (and NGS-corroborated) cat-to-human Alpha SARS-CoV-2 transmission from a sneezing pet cat to a veterinarian was published having taken place in Thailand in August 2021 (Sila et al. 2022).

Interestingly, during a country-wide SARS-CoV-2 outbreak in Dutch mink farms, mink-to-(stray) cat transmission has been shown resulting in an estimated 12% chance of cats being infected by minks (van Aart et al. 2021) and indicating that cross-species animal-to-animal transmission might be relevant not only in experimental settings, but also in natural situations. Similarly, one of 24 farm cats (4.2%) from two COVID-2 affected Dutch mink farms were SARS-CoV-2 PCR positive, while seven of them seroconverted (29.2%) (Oreshkova et al. 2020). Moreover, in Danish mink farms SARS-CoV-2 was detected in a single domestic farm cat, but not in 30 stray cats (Boklund et al. 2021), indicating the possibility of mink- or- human-to-cat transmission in a similar setting.

The first documented natural SARS-CoV-2 infections in nondomestic animals (being also the first animal infection in the USA) happened in two Malayan (*Panthera tigris jacksoni*) and Amur (*Panthera tigris altaica*) tigers, respectively, and in three lions (*Panthera leo krugeri*) in March 2020 in the Bronx Zoo, New York

(McAloose et al. 2020), about 2 months after the first human case had been detected in the USA. All except one big cat of this outbreak were symptomatic. Whole genome sequence data suggested at least two independent SARS-CoV-2 introductions from zoo staff to the animals. Since then, more than 45 SARS-CoV-2 infections in zoo-kept *Panthera, Puma*, and *Prionailurus* spp. were described (Giraldo-Ramirez et al. 2021; OIE 2021; Islam et al. 2021), e.g., in pumas in South Africa and the USA, in a lynx in Croatia, in a fishing cat (*Prionailurus viverrinus*) in the USA (www.aphis.usda.gov), in three snow leopards (*Panthera uncia*) in the USA, in lions in Croatia, Colombia, Estonia, Singapore, South Africa, Spain (Fernández-Bellon et al. 2021), Sweden, and the USA, in tigers in Indonesia, Sweden, the UK, and the USA (for all: OIE 2021; Islam et al. 2021). Infections by Delta (B.1.617.2) variants including possible lion-to-lion transmission events were recently described in 12 *Panthera leo persica* from zoos and safari parks in Tamil Nadu (Mishra et al. 2021), Uttar Pradesh, and Rajasthan/India (Karikalan et al. 2021). In nearly all reported SARS-CoV-2 infections of big cats a human-to-animal transmission was proven or at least suspected; most of the animals were symptomatic, primarily with respiratory symptoms, but also more systemic signs, e.g., loss of appetite and lethargy (Giraldo-Ramirez et al. 2021). Alpha genome sequences were also documented from a lion, a leopard, and three tigers from the Czech Republic and the USA, respectively (Islam et al. 2021). Until December 2021, no SARS-CoV-2 infections affecting free-ranging wildlife big cats have been reported. Very recently, a systemic SARS-CoV-2 Delta variant infection affecting brain, spleen, lymph nodes, and lungs of a free-ranging non-captive leopard (*Panthera pardus fusca*) probably killed by another carnivore has been reported from Uttar Pradesh/India (Mahajan et al. 2022).

Several seroprevalence studies have been carried out to evaluate the burden of SARS-CoV-2 infections in pet cats. Three of the first studies were performed in China including the city of Wuhan from where the COVID-19 pandemic started. Deng et al. found no SARS-CoV-2 antibodies in 66 pet and 21 stray cats sampled between November 2019 and March 2020 (Deng et al. 2020a) confirmed by a subsequent study from the same group finding no SARS-CoV-2 antibodies in 423 cats from 20 Chinese cities (including 48 from Wuhan and 42 from the province of Hubei) sampled between February and April 2020 during the first wave (Deng et al. 2020b); a strictly Wuhan-based study performed between January and March 2020 yielded a seroprevalence of 14.2% in 102 cats; 3 of 15 (20%) pet cats from COVID-19 families were seropositive, six of 46 (13%) cats from shelters and six of 41 (14.5%) from animal hospitals, respectively (Zhang et al. 2020). The three cats with the highest titers were patient-owned suggesting potential direct human-to-cat transmission rather than cat-to-cat transmission.

Serological studies in cats were also conducted in other hotspots during the first pandemic wave, esp. in Northern Italy, yielding similar or even lower seroprevalence rates, e.g., 0% among 24 cats in Lombardy (Klaus et al. 2021a); 0.95% in 105 stray and shelter cats from Lombardy (Spada et al. 2021) 5.8% among 191 cats mostly from Lombardy (Patterson et al. 2020) with a positivity rate of 4.5% (1/22) and 2.6% (1/38) in COVID-19 positive and negative households, respectively; 16.2% among

68 cats from all over Italy (with all positive-tested pet cats living with COVID-19 positive owners; seropositivity rate in these animals was 20.4%) (Colitti et al. 2021); 0.4% in 500 cats from the Netherlands (Zhao et al. 2021); 0.69% in 920 cats from Germany (Michelitsch et al. 2020); 0.76% in 131 cats from Croatia using micro-neutralization assays (Stevanovic et al. 2021); 3.3.%, 4.2%, 4.2%, and 6.4% in domestic cats in 331, 333, 1136, and 360 domestic cats from the UK, Italy, Germany, and Spain (Schulz et al. 2021b); 3.5% in 114 stray cats from Zaragoza/Spain (Villanueva-Saz et al. 2021); 8% in 239 cats from Minnesota/USA (Dileepan et al. 2021).

Studies covering a longer period of the pandemic or later waves found similarly low or even lower seroprevalence rates, e.g., 0% in 99 stray cats from Lodi/Italy sampled throughout the first year of the pandemic until end of 2020 (Stranieri et al. 2021), 0% in 80 domestic cats during the second wave in Thailand (Jairak et al. 2022); 0.42% (4/956) in cats from 48 US states sampled between March and November 2020 (Barua et al. 2021); 0.8% (2/240) in shelter cats in the Netherlands during the second wave (van der Leij et al. 2021); 1.36% in 1173 cats in second wave in Germany (Michelitsch et al. 2021), double as high as the seropositivity rate detected by the same group in the first wave (Michelitsch et al. 2020); and 1.79% in 279 domestic cats in Poland during the second and third wave (Pomorska-Mól et al. 2021). The constantly low seroprevalence rates indicate that neither domestic pet nor stray cats are heavily affected by the SARS-CoV-2 pandemic or by "re-spillback" effects.

In contrast, in studies performed in households with confirmed human COVID-19 cases, the seroprevalence was usually higher than in random or convenient sample studies, e.g., 23.5% (8/34) in Eastern France being significantly higher than in pet cats from households with unknown COVID-19 status (6.3%; 1/16) (Fritz et al. 2021); 17.1% (7/41) to 31.7% (13/41) in Peru (Jara et al. 2021); 30.1% (4/11) in Utah and Wisconsin/USA (Goryoka et al. 2021); 40% (4/10) in Brazil (Calvet et al. 2021); and 43.8% (7/16) in Texas/USA (Hamer et al. 2021b). The only exception from this trend is a study from France among a cluster of COVID-19 patients from a veterinary campus living in close proximity with their pets, where no SARS-CoV-2 antibodies could be detected in nine cats (Temmam et al. 2020). Most interestingly, seroprevalence in 44 stray cats from Dutch SARS-CoV-2-affected mink farms (22.7%) was significantly higher than in a convenience sample of cat sera from the Netherlands (0.4%) (Zhao et al. 2021).

As expected, SARS-CoV-2 viral prevalence in cats as detected by RT-PCR was usually even lower than seroprevalence, e.g., 0% in 99 stray cats from Lodi/Italy sampled during the first pandemic year (Stranieri et al. 2021); 0% in 93 cats from five different subdistricts of Thailand during the second wave (Jairak et al. 2022); 0.17% in 569 randomly selected and 1.63% in 184 COVID-19 and/or COVID-19 symptomatic cats, respectively, from different regions of Spain sampled between July 2020 and April 2021 (Barroso-Arévalo et al. 2021); 0.38% in 260 cats mainly from first wave hotspot areas in Munich/Germany and in Lombardy or Piedmont/Italy with the only positive cat living in a COVID-19 household (Klaus et al. 2021a); 0.8% in domestic cats from northwestern Iran (Mohebali et al. 2022); 2.4% in

asymptomatic cats from France sampled from April 2020 to April 2021 (Krafft et al. 2021).

SARS-CoV-2 viral prevalence was higher in some outbreak investigations from cats living in COVID-19 positive households, e.g. 11.1% (1/9), 12% (6/50), 12.5% (1/8) and 17.6% (3/17) in studies from Thailand (Jairak et al. 2021), Hong Kong (Barrs et al. 2020), Spain (Ruiz-Arrondo et al. 2021), and Texas/USA (Hamer et al. 2021b), respectively. However, also instances of zero SARS-CoV-2 RT-PCR positivity in outbreak settings have been reported, e.g., in 19 cats from Utah and Wisconsin (Goryoka et al. 2021) and in 9 cats from France (Temmam et al. 2020).

One of the key parameters in epidemiology indicating the transmissibility of a pathogen and therefore the risk of epidemic transmission is the basic reproduction number R_0. A value for $R_0 > 1$ suggests sustained transmission, while $R_0 < 1$ indicates a likely tapering off of an ongoing epi- or pandemic. Using experimental and household observational data the SARS-CoV-2 reproduction number R_0 in cats was calculated to be 2.3–3.3 (Gonzales et al. 2021), while R_0 of the ancestral SARS-CoV-2 strain in humans was estimated to be 2.5–3.8 (Liu and Rocklöv 2021; Anderson et al. 2021). For subsequent variants dominating the human pandemic, e.g., Alpha, Delta, and Omicron, significantly higher R_0 values have been estimated (Davies et al. 2021; Liu and Rocklöv 2021; Nishiura et al. 2021).

28.6.3 Disease in Cats

After experimental (mostly intranasal and/or oral) infection cats, esp. when older than 4 months, remained basically asymptomatic exhibiting generally mild-to-moderate pathological changes mainly in the respiratory tract in several studies (Gaudreault et al. 2020; Shi et al. 2020; Bosco-Lauth et al. 2020; Bao et al. 2021; Patania et al. 2021). Juvenile cats might be more susceptible and prone to more severe overt disease as indicated in one study (Shi et al. 2020). Viral replication was found predominantly in the upper respiratory tract, while SARS-CoV-2 RNA could be detected in a wide range of tissues corresponding with the wide distribution of ACE2 in many organs (e.g., lymph nodes, liver, heart, kidney) (Gaudreault et al. 2020; Meekins et al. 2021).

Regarding natural infections observed in animals, a selection bias is to be expected with a focus on testing symptomatic animals or animals with close contact to COVID-19 human patients therefore probably overrepresenting the tip of the iceberg in the clinical spectrum of SARS-CoV-2 infection. However, even in a review of the first 124 SARS-CoV-2 reports in cats, the majority of cats with available clinical information were completely asymptomatic, i.e., 54% (corresponding to 38/70) (Giraldo-Ramirez et al. 2021). The wider clinical spectrum of symptomatic pet cats in this review comprised mainly respiratory signs (sneezing [41%], dyspnea [13%], nasal or ocular discharge [both 9%]), but also digestive (diarrhea [6%], emesis [3%]), systemic (lethargy [38%], loss of appetite [16%], fever [6%]), and cardiovascular (congestive heart failure [25%], ventricular arrhythmia [13%], hypertrophic cardiomyopathy [9%]) symptoms (Giraldo-Ramirez et al.

2021). Some of the more severe symptoms in naturally vs. experimentally infected cats might be due to preexisting comorbidities (Meekins et al. 2021) including very few fatal outcomes complicated by underlying coinfections or severe systemic disease (Segalés et al. 2020; Giraldo-Ramirez et al. 2021). Moreover, myocarditis has been described in SARS-CoV-2 infected pet cats, most of them associated with the Alpha variant (Ferasin et al. 2021; Chetboul et al. 2021). Usually, the disease is self-limiting in naturally infected domestic cats.

In big cats the data situation is different. For obvious reasons no experimental data are available. In contrast to the published evidence involving naturally infected domestic cats, the infections reported in captive big cats were usually symptomatic, with coughing (97%), sneezing (79%), and loss of appetite (51%) being the most prevalent clinical signs (for a review Giraldo-Ramirez et al. 2021; Maurin et al. 2021; see also case and outbreak reports in McAloose et al. 2020; Mishra et al. 2021; Fernández-Bellon et al. 2021). However, these findings might be biased due to the symptom-driven diagnostic work-up of the diseased zoo animals. Disease was usually self-limiting, however, sometimes with a prolonged course of infection; deaths due to COVID-19 were also reported for at least two lions (Mishra et al. 2021).

28.6.4 Disease in Humans

SARS-CoV-2-caused COVID-19 is a systemic disease affecting many different organ systems due to the abundance of the SARS-CoV-2 receptor ACE2 in the respective tissues (for a review see Synowiec et al. 2021). Accordingly, many different short-term symptoms and long-term effects of the disease have been described.

The major organ system affected is the respiratory system, but direct viral as well as indirect host immune response effects may cause cardiovascular, gastrointestinal, renal, neurological, ocular, cutaneous, musculoskeletal, hematological, and endocrine symptoms (Mehta et al. 2021). In general, COVID-19 tends to be a mild to moderate disease in 80–85% of infected humans (including basically asymptomatic people); severe disease is often associated with elder age over 65 years, comorbidities including diabetes, hypertension, cardiovascular, or respiratory disorders, or other chronic conditions, e.g., obesity. However, severe and lethal infections may also be observed in previously healthy and young patients. The global gross case fatality rate (CFR) may be calculated as about 1.7% using data from WHO (January 18, 2022), the infection fatality rate (IFR) was estimated to be 0.68% in 2020 (Meyerowitz-Katz and Merone 2020); IFRs and CFRs, however, largely depend on many different parameters including test policies and differ between countries, populations, and over time (Anderson et al. 2021; Levin et al. 2020).

During the first wave of the pandemic caused basically by the ancestral SARS-CoV-2 Wuhan strain, the most common initial clinical manifestations of COVID-19 included fever (82%), cough (61%), myalgia and/or fatigue (36%), shortness of breath (26%), headache (12%), sore throat (10%), and gastrointestinal symptoms

(9%) (Yazdanpanah et al. 2021). According to the report from WHO-China-Joint Mission on COVID-19, the most prevalent respiratory and systemic symptoms during the first Corona wave in China comprised fever (87.9%), dry cough (67.7%), fatigue (38.1%), sputum production (33.4%), difficulty breathing (18.6%), sore throat (13.9%), chills (11.4%), nasal congestion (4.8%), and hemoptysis (0.9%) (WHO-China Joint Mission 2019). Of special interest in the course of and at different time-points during the pandemic became – among a plethora of other symptoms – mostly self-limiting hyp- or anosmia (in nearly half of the patients) (Karamali et al. 2022), cardiac arrhythmias (in about 5–10% of hospitalized patients) (Pandat et al. 2021), thromboembolic events (in 5–17% of patients) (Gorog et al. 2022). Depending on the human population (e.g., vaccination status, age distribution, underlying diseases) and the predominant variant (e.g., Alpha, Delta, Omicron) the distribution of clinical symptoms and entities may differ. Additional concerns arise from the Pediatric Multisystem Inflammatory Syndrome temporally associated with COVID-19 (PIMS-TS or PIMS) or Multisystem Inflammatory Syndrome in Children (MIS[-C]) (Hoste et al. 2021; Case and Son 2021) as well as from long COVID-19 entities including post-COVID-19 (Jennings et al. 2021).

28.6.5 Public Health Importance

As known from experimental data, domestic cats can transmit SARS-CoV-2 to co-housed cats via close contact and to a lesser degree via aerosols (Bao et al. 2021; Shi et al. 2020; Halfmann et al. 2020; Gaudreault et al. 2020). Interestingly, with respect to transmission by close contact, SARS-CoV-2 RNA has been detected on fur and/or bedding swabs from cats in studies from Switzerland (Klaus et al. 2021b), Spain (Barroso-Arévalo et al. 2021), and the USA (Hamer et al. 2021a) indicating environmental contamination as a possible source for cat-to-cat transmission. Importantly, in experimental settings, previously SARS-CoV-2 infected cats could be successfully reinfected, but did not transmit SARS-CoV-2 to other co-housed naïve sentinels (Gaudreault et al. 2021).

In contrast to intraspecies transmission, situations with cats as inter- or cross-species SARS-CoV-2 spreaders have been described so far only in a single case of cat-to-human transmission involving a sneezing pet cat and its veterinarian during a medical procedure in Thailand (Sila et al. 2022), suggesting only a very low risk for cat owners to attract COVID-19 from their pet. Importantly, also in the large SARS-CoV-2 outbreaks on mink farms in Denmark and the Netherlands, no cases of cat-to-mink transmission were suspected, while all SARS-CoV-2 infected domestic pet and stray cats with farm contact were probably infected by minks as indicated at least in some cases by whole genome-based viral typing (van Aart et al. 2021).

In contrast to the sustained human-to-human transmission observed over time, cat-to-cat transmissibility is reduced after serial passaging of the virus in co-housing experiments (Bao et al. 2021) suggesting limited transmission in the cat population after viral entry, e.g., from humans. More disturbing, however, is the observation that

despite a narrow bottleneck of 2–5 virions expected to help slowing down the pace of viral adaptation and shown to leave SARS-CoV-2 consensus sequences largely unchanged over time SARS-CoV-2 (Braun et al. 2021; Bao et al. 2021) variants involving amino acid mutations such as D614G, D138Y, or H655Y also found in VOCs known from human infections may develop very rapidly and even get fixed in infected cats (Bashor et al. 2021; Braun et al. 2021). These findings illustrate that reverse zoonosis can result in the emergence of new SARS-CoV-2 variants. Therefore, monitoring of SARS-CoV-2 evolution in animals is important, even in cases where transmissibility to humans seems to be very low (Bashor et al. 2021; Davis and Innes 2021).

28.6.6 Public Health Measures

According to the high susceptibility of cats for SARS-CoV-2 and their very low potential as SARS-CoV-2 transmitters to humans (Ekstrand et al. 2021), public health recommendations primarily target humans, e.g., infected cat owners or zoo staff, to prevent human-to-cat transmission. In comparison, "One Health/One Medicine" aspects to avoid (i) cat-to-cat transmission (ii) cross-species "spill-over" to other animals; or (iii) cat-to-human "spill-back" effects are only in second line of current recommendations.

Several public health and veterinary public health institutions as well as animal or pet welfare organizations have issued guidelines and recommendations, e.g., the US Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html), the World Organization for Animal Health OIE (https://www.oie.int), the American Veterinary Medical Association (AVMA) (https://www.avma.org/resources-tools/animal-health-and-welfare/covid-19), or the European Advisory Board on Cat Diseases (ABCD) (http://www.abcdcatsvets.org/sars-coronavirus-2-and-cats/).

Most documents recommend that (i) SARS-CoV-2 infected people should restrict contact with mammalian animals, including pet cats, and apply good hygiene practices when having to care for their pets (e.g., wearing a mask, handwashing, avoiding kissing their pets, or sharing food, towels, or the bed with them); (ii) cats from SARS-CoV-2-infected households should be kept indoors; (iii) animals with suspected or confirmed SARS-CoV-2 infection should remain separated from other animals and humans; (iv) pet owners should monitor their pets to detect any health problems suggestive for SARS-CoV-2 infection; (v) pet owners should not abandon their animals or compromise their welfare during the COVID-19 pandemic (Huang et al. 2020; Hosie et al. 2021b).

On a more general "One Health/One Medicine" scale, it is of utmost importance to establish both national and international surveillance systems to closely monitor the development and occurrence of SARS-CoV-2 and other agents of public health relevance in animals with respect to possible "spill over" and "spill-back" effects thus contributing substantially to the urgently needed global pandemic preparedness efforts (Giraldo-Ramirez et al. 2021).

28.7 Cross-References

- ▶ Animal Bites and Zoonoses: From A to Z – Alligators to Zebras
- ▶ Severe Acute Respiratory Syndrome Coronaviruses-2 (SARS-CoV-2)
- ▶ Toxoplasmosis: A Widespread Zoonosis Diversely Affecting Humans and Animals

References

Abbaszadeh Afshar MJ, Zahabiun F, Heydarian P, Mozafar Saadati H, Mohtasebi S, Khodamoradi F, Raissi V (2020) A Systematic Review and Meta-analysis of Toxocariasis in Iran: Is it Time to Take it Seriously? Acta Parasitol 65:569–584

Abbott Y, Efstratiou A, Brennan G, Hallanan S, Leggett B, Leonard FC, Markey BK, Tuite C, Fry NK (2020 Sep) Toxigenic *Corynebacterium ulcerans* associated with upper respiratory infections in cats and dogs. J Small Anim Pract 61(9):554–560

Abdad MY, Stenos J, Graves S (2011) *Rickettsia felis*, an emerging flea-transmitted human pathogen. Emerg Health Threats J 4:7168

Abdullah S, Lait P, Helps C, Newbury H, Wall R (2020) The prevalence of *Rickettsia felis* DNA in fleas collected from cats and dogs in the UK. Vet Parasitol 282:109143

Agnarsson I, Kuntner M, May-Collado LJ (2010) Dogs, cats, and kin: A molecular species-level phylogeny of Carnivora. Mol Phylogenet Evol 54:726–745

Aguirre AA, Longcore T, Barbieri M, Dabritz H, Hill D, Klein PN, Lepczyk C, Lilly EL, McLeod R, Milcarsky J, Murphy CE, Su C, VanWormer E, Yolken R, Sizemore GC (2019) The One Health approach to toxoplasmosis: epidemiology, control, and prevention strategies. Ecohealth 16:378–390

Ágústsson T, Richter SH (1993) Sníkjudýr í og á köttum í Reykjavík og nágrenni. (Parasites of cats in South-West Iceland). Dýralæknaritið 10:24–29. (in Icelandic)

Akhmadishina LV, Ruzina MN, Lukasheva MA, Kyuregyan KK, Mikhailov MI, Lukashev AN (2020) Seroprevalence and incidence of human toxocarosis in Russia. Adv Parasitol 109:419–432

Al-Kappany YM, Lappin MR, Kwok OCH, Abu-Elwafa SA, Hilali M, Dubey JP (2011 Apr) Seroprevalence of *Toxoplasma gondii* and concurrent *Bartonella* spp., feline immunodeficiency virus, feline leukemia virus, and *Dirofilaria immitis* infections in Egyptian cats. J Parasitol 97 (2):256–258

Al-Majali AM (2004) Seroprevalence of and risk factors for *Bartonella henselae* and *Bartonella quintana* infections among pet cats in Jordan. Prev Vet Med 64:63–71

Alvarez-Fernandez A, Breitschwerd EB, Solano-Gallego L (2018) *Bartonella* infections in cats and dogs including zoonotic aspects. Parasit Vectors 11(1):624

Alves MEM, Martins FDC, Bräunig P, Pivoto FL, Sangioni LA, Vogel FSF (2018) Molecular detection of *Cryptosporidium* spp. and the occurrence of intestinal parasites in fecal samples of naturally infected dogs and cats. Parasitol Res 117(9):3033–3038

Amouei A, Sarvi S, Sharif M, Aghayan SA, Javidnia J, Mizani A, Moosazadeh M, Shams N, Hosseini SA, Hosseininejad Z, Nayeri Chegeni T, Badali H, Daryani A (2020) A systematic review of *Toxoplasma gondii* genotypes and feline: Geographical distribution trends. Transbound Emerg Dis 67:46–64

Anderson BE, Neuman MA (1997) *Bartonella* spp. as emerging human pathogens. Clin Microbiol Rev 10:203–219

Anderson K (1955) Nine Man-eaters and One Rogue. Allen & Unwin, Sydney

Anderson RM, Vegvari C, Hollingsworth TD, Pi L, Maddren R, Ng CW, Baggaley RF (2021) The SARS-CoV-2 pandemic: remaining uncertainties in our understanding of the epidemiology and

transmission dynamics of the virus, and challenges to be overcome. Interface Focus 11(6): 20210008
Angelakis E, Munasinghe A, Yaddehige I, Liyanapathirana V, Thevanesam V, Bregliano A, Socolovschi C, Edouard S, Fournier PE, Raoult D, Parola P (2012) Detection of rickettsioses and Q fever in Sri Lanka. Am J Trop Med Hyg 86:711–712
Aramini JJ, Stephen C, Dubey JP (1998) *Toxoplasma gondii* in Vancouver Island cougars (*Felis concolor vancouverensis*): serology and oocyst shedding. J Parasitol 84:438–440
Aramini JJ, Stephen C, Dubey JP, Engelstoft C, Schwantje H, Ribble CS (1999) Potential contamination of drinking water with *Toxoplasma gondii* oocysts. Epidemiol Infect 122:305–315
Araújo AC, Villela MM, Sena-Lopes Â, Farias NADR, Faria LMJ, Avila LFDC, Berne MEA, Borsuk S (2018) Seroprevalence of *Toxoplasma gondii* and *Toxocara canis* in a human rural population of Southern Rio Grande do Sul. Rev Inst Med Trop Sao Paulo 60:e28
Araújo FS, Barcelos RM, Mendes TAO, Mafra C (2021) Molecular evidence of *Rickettsia felis* in *Phereoeca* sp. Rev Bras Parasitol Vet 30(1):e015620
Arvand M, Feil EJ, Giladi M, Boulouis HJ, Viezens J (2007) Multi-locus sequence typing of *Bartonella henselae* isolates from three continents reveals hypervirulent and feline-associated clones. PLoS One 19(2):e1346
Auer H, Walochnik J (2020) Toxocariasis and the clinical spectrum. Adv Parasitol 109:111–130
AVMA (American Veterinary Medical Association) (2012) U.S. Pet Ownership & Demographics Sourcebook.
Aylló T, Paulo P, Diniz VP, Breitschwerdt EB, Villaescusa A, Rodríguez-Franco F et al (2012) Vector-borne diseases in client-owned and stray cats from Madrid, Spain. Vector Borne Zoonotic Dis 12:143–150
Azam D, Ukpai OM, Said A, Abd-Allah GA, Morgan ER (2012) Temperature and the development and survival of infective *Toxocara canis* larvae. Parasitol Res 110:649–656
Azzag N, Haddad N, Durand B, Petit E, Ammouche A, Chomel B, Boulouis HJ (2012) Population structure of *Bartonella henselae* in Algerian urban stray cats. PLoS One 7:e43621
Baer JG (1951) Ecology of animal parasites. The University of Illinois Press, Ubana
Bakhshani A, Maleki M, Haghparast A, Parande Shirvan S, Borji H (2019) A survey on *Toxocara cati* eggs on the hair of stray cats: A potential risk factor for human toxocariasis in Northeastern Iran. Comp Immunol Microbiol Infect Dis 64:10–13
Balasundaram MB, Andavar R, Palaniswamy M, Venkatapathy N (2010) Outbreak of acquired ocular toxoplasmosis involving 248 patients. Arch Ophthalmol 128:28–32
Ballweber LR, Panuska C, Huston CL, Vasilopulos R, Pharr GT, Mackin A (2009) Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. Vet Parasitol 160:306–310
Ballweber LR, Xiao L, Bowman DD, Kahn G, Cama VA (2010) Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol 26:180–189
Baneth G, Thamsborg SM, Otranto D, Guillot J, Blaga R, Deplazes P, Solano-Gallego L (2016) Major Parasitic Zoonoses Associated with Dogs and Cats in Europe. J Comp Pathol 155(1 Suppl 1):S54–S74
Bao L, Song Z, Xue J, Gao H, Liu J, Wang J, Guo Q, Zhao B, Qu Y, Qi F, Gong S, Liu M, Lv Q, Li D, Han Y, Zhao W, Deng S, Liu Y, Xiang Z, Yang B, Deng W, Yu H, Cong Z, Wei Q, Xu J, Gao GF, Qin C (2021) Susceptibility and attenuated transmissibility of SARS-CoV-2 in domestic cats. J Infect Dis 223(8):1313–1321
Baril L, Ancelle T, Goulet V, Thulliez P, Tirard-Fleury V, Carme B (1999) Risk factors for *Toxoplasma infection* in pregnancy: a case-control study in France. Scand J Infect Dis 31: 305–309
Barimani M, Mosallanejad B, Ghorbanpoor M, Esmaeilzadeh S (2019) Molecular detection of *Chlamydia felis* in cats in Ahvaz, Iran. Arch Razi Inst 74(2):119–126
Barnes A, Bell SC, Isherwood DR, Bennett M, Carter SD (2000) Evidence of *Bartonella henselae* infection in cats and dogs in the United Kingdom. Vet Rec 147(24):673–7. PMID: 11132671
Barroso-Arévalo S, Barneto A, Ramos ÁM, Rivera B, Sánchez R, Sánchez-Morales L, Pérez-Sancho M, Buendía A, Ferreras E, Ortiz-Menéndez JC, Moreno I, Serres C, Vela C, Risalde MÁ, Domínguez L, Sánchez-Vizcaíno JM (2021) Large-scale study on virological and

serological prevalence of SARS-CoV-2 in cats and dogs in Spain. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14366. Epub ahead of print

Barrs VR, Peiris M, Tam KWS, Law PYT, Brackman CJ, To EMW, Yu VYT, Chu DKW, Perera RAPM, Sit THC (2020) SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. Emerg Infect Dis 26(12):3071–3074

Barua S, Hoque M, Adekanmbi F, Kelly P, Jenkins-Moore M, Torchetti MK, Chenoweth K, Wood T, Wang C (2021) Antibodies to SARS-CoV-2 in dogs and cats, USA. Emerg Microbes Infect 10(1):1669–1674

Barutzki D, Schaper R (2003) Endoparasites in dogs and cats in Germany 1999 – 2002. Parasitol Res 90:148–150

Barutzki D, Schaper R (2011) Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. Parasitol Res 109:S45–S60

Bashor L, Gagne RB, Bosco-Lauth AM, Bowen RA, Stenglein M, VandeWoude S (2021) SARS-CoV-2 evolution in animals suggests mechanisms for rapid variant selection. Proc Natl Acad Sci U S A 118(44):e2105253118

Batz MB, Hoffmann S, Morris JG. Ranking the risks: The 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida. https://www.epi.ufl.edu/media/epiufledu/Ranking-the-Risks-REPORT.pdf. Accessed 10 Jan 2021

Bayliss DB, Morris AK, Horta MC, Labruna MB, Radecki SV, Hawley JR, Brewer MM, Lappin MR (2009) Prevalence of *Rickettsia* species antibodies and *Rickettsia* species DNA in the blood of cats with and without fever. J Feline Med Surg 11:266–270

Beaver PC (1969) The nature of visceral larva migrans. J Parasitol 55:3–12

Behar A, McCormick LJ, Perlman SJ (2010) *Rickettsia felis* infection in a common household insect pest, *Liposcelis bostrychophila* (Psocoptera: Liposcelidae). Appl Environ Microbiol 76(7): 2280–2285

Benavides JA, Megid J, Campos A, Hampson K (2020) Using Surveillance of Animal Bite Patients to Decipher Potential Risks of Rabies Exposure From Domestic Animals and Wildlife in Brazil. Front Public Health 8:318

Benenson MW, Takafuji ET, Lemon SM, Greenup RL, Sulzer AJ (1982) Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. N Engl J Med 307:666–669

Berger A, Huber I, Merbecks SS, Ehrhard I, Konrad R, Hörmansdorfer S, Hogardt M, Sing A (2011a) Toxigenic *Corynebacterium ulcerans* in woman and cat. Emerg Infect Dis 17:1767–1769

Berger A, Schmidt-Wieland T, Huber I, Sing A (2011b) Human diphtheria infection from a cat bite. Ann Intern Med 155:646–648

Berger A, Hogardt M, Konrad R, Sing A (2014) Detection methods for laboratory diagnosis of diphtheria. In: Burkovski A (ed) *Corynebacterium diphtheriae* and related toxigenic species. Genomics, Pathogenicity and Applications. Springer, Berlin/Heidelberg, pp 171–205

Berger A, Dangel A, Peters M et al (2019) *Tox*-positive *Corynebacterium ulcerans* in hedgehogs, Germany. Emerg Microbes Infect 8(1):211–217

Bergh K, Bevanger L, Hanssen I, Løseth K (2002) Low prevalence of *Bartonella henselae* infections in Norwegian domestic and feral cats. APMIS 110:309–314

Bergmans AM, Schellekens JF, van Embden JD, Schouls LM (1996) Predominance of two *Bartonella henselae* variants among cat-scratch disease patients in the Netherlands. J Clin Microbiol 34:254–260

Bergmans AM, de Jong CM, van Amerongen G, Schot CS, Schouls LM (1997) Prevalence of *Bartonella* species in domestic cats in The Netherlands. J Clin Microbiol 35:2256–2261

Bernabeu-Wittel M, del Toro MD, Nogueras MM, Muniain MA, Cardeñosa N, Márquez FJ, Segura F, Pachón J (2006) Seroepidemiological study of *Rickettsia felis, Rickettsia typhi*, and *Rickettsia conorii* infection among the population of southern Spain. Eur J Clin Microbiol Infect Dis 25:375–381

Bertranpetit E, Jombart T, Paradis E, Pena H, Dubey J, Su C, Mercier A, Devillard S, Ajzenberg D (2017) Phylogeography of *Toxoplasma gondii* points to a South American origin. Infect Genet Evol 48:150–155

Beser J, Toresson L, Eitrem R, Troell K, Winiecka-Krusnell J, Lebbad M (2015) Possible zoonotic transmission of *Cryptosporidium felis* in a household. Infect Ecol Epidemiol 5:28463

Beugnet F, Bourdeau P, Chalvet-Monfray K, Cozma V, Farkas R, Guillot J, Halos L, Joachim A, Losson B, Miró G, Otranto D, Renaud M, Rinaldi L (2014) Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit Vectors 7:291

Bigna JJ, Tochie JN, Tounouga DN, Bekolo AO, Ymele NS, Youda EL, Sime PS, Nansseu JR (2020) Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. Sci Rep 10:12102

Bisseru B, Woodruff AW, Hutchinson RI (1966) Infection with adult *Toxocara canis*. Br Med J 1 (5503):1583–1584

Blanco Ramos JR, Oteo Revuelta JA, Martinez de Artola V, Ramalle Gomara E, Garcia Pineda A, Ibarra Cucalon V et al (1998) Seroepidemiology of *Bartonella henselae* infection in a risk group. Rev Clin Esp 198:805–809

Blanton LS, Walker DH (2017) Flea-Borne Rickettsioses and Rickettsiae. Am J Trop Med Hyg 96 (1):53–56

Boillat M, Hammoudi PM, Dogga SK, Pagès S, Goubran M, Rodriguez I, Soldati-Favre D (2020) Neuroinflammation-associated aspecific manipulation of mouse predator fear by *Toxoplasma gondii*. Cell Rep 30:320–334

Boklund A, Hammer AS, Quaade ML, Rasmussen TB, Lohse L, Strandbygaard B, Jørgensen CS, Olesen AS, Hjerpe FB, Petersen HH, Jensen TK, Mortensen S, Calvo-Artavia FF, Lefèvre SK, Nielsen SS, Halasa T, Belsham GJ, Bøtner A (2021) SARS-CoV-2 in Danish mink farms: Course of the epidemic and a descriptive analysis of the outbreaks in 2020. Animals (Basel) 11(1):164

Bonmarin I, Guiso N, Le Flèche-Matéos A, Patey O, Patrick AD, Levy-Bruhl D (2009) Diphtheria: a zoonotic disease in France? Vaccine 27:4196–4200

Bonnet JM, Begg NT (1999) Control of diphtheria: guidance for consultants in communicable disease control. Commun Dis Public Health 2:242–249

Bosco-Lauth AM, Hartwig AE, Porter SM, Gordy PW, Nehring M, Byas AD, VandeWoude S, Ragan IK, Maison RM, Bowen RA (2020) Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. Proc Natl Acad Sci U S A 117(42):26382–26388

Both L, Collins S, de Zoysa A, White J, Mandal S, Efstratiou A (2015) Molecular and epidemiological review of toxigenic diphtheria infections in England between 2007 and 2013. J Clin Microbiol 53(2):567–572

Boulouis HJ, Chomel BB, Guillaume G, Benoît D, Chang CC, Monteil M, Kasten RW, Jack A, Nadia H (2020 Aug) Multiple locus variable number tandem repeat analysis for the characterization of wild feline *Bartonella* species and subspecies. Vet Microbiol 247:108788

Bowman DD (2020) History of *Toxocara* and the associated larva migrans. Adv Parasitol 109:17–38

Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, Marion SA (1997) Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma Investigation Team. Lancet 350:173–177

Boyer K, Hill D, Mui E, Wroblewski K, Karrison T, Dubey JP, Sautter M, Noble AG, Withers S, Swisher C, Heydemann P, Hosten T, Babiarz J, Lee D, Meier P, McLeod R (2011) Toxoplasmosis Study Group. Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. Clin Infect Dis 53:1081–1089

Bouchouicha R, Durand B, Monteil M, Chomel BB, Berrich M, Arvand M, Birtles RJ, Breitschwerdt EB, Koehler JE, Maggi R, Maruyama S, Kasten R, Petit E, Boulouis HJ, Haddad N (2009) Molecular epidemiology of feline and human *Bartonella henselae* isolates. Emerg Infect Dis 15:813–816

Bradbury RS, Hobbs CV (2020) *Toxocara* seroprevalence in the USA and its impact for individuals and society. Adv Parasitol 109:317–339

Braga Mdo S, Diniz PP, André MR, Bortoli CP, Machado RZ (2012) Molecular characterisation of *Bartonella* species in cats from São Luís, state of Maranhão, north-eastern Brazil. Mem Inst Oswaldo Cruz 107:772–777

Branley J, Wolfson C, Waters P, Gottlieb T, Bradbury R (1996) Prevalence of *Bartonella henselae* bacteremia, the causative agent of cat scratch disease, in an Australian cat population. Pathology 28:262–265

Braun KM, Moreno GK, Halfmann PJ, Hodcroft EB, Baker DA, Boehm EC, Weiler AM, Haj AK, Hatta M, Chiba S, Maemura T, Kawaoka Y, Koelle K, O'Connor DH, Friedrich TC (2021) Transmission of SARS-CoV-2 in domestic cats imposes a narrow bottleneck. PLoS Pathog 17(2):e1009373

Breitschwerdt EB (2008) Feline bartonellosis and cat scratch disease. Vet Immunol Immunopathol 123:167–171

Breitschwerdt EB, Kordick DL (2000) *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev 13: 428–438

Bressan M, Rampazzo A, Kuratli J, Marti H, Pesch T, Borel N (2021) Occurrence of *Chlamydiaceae* and *Chlamydia felis pmp9* typing in conjunctival and rectal samples of Swiss stray and pet cats. Pathogens 10(8):951

Brown LD, Christofferson RC, Banajee KH, Del Piero F, Foil LD, Macaluso KR (2015) Cofeeding intra- and interspecific transmission of an emerging insect-borne rickettsial pathogen. Mol Ecol 24(21):5475–5489

Brown LD, Macaluso KR (2016) *Rickettsia felis*, an emerging flea-borne rickettsiosis. Curr Trop Med Rep 3:27–39

Browning GF (2004) Is *Chlamydophila felis* a significant zoonotic pathogen? Aust Vet J 82:695–696

Burnett AJ, Shortt SG, Isaac-Renton J, King A, Werker D, Bowie WR (1998) Multiple cases of acquired toxoplasmosis retinitis presenting in an outbreak. Ophthalmology 105:1032–1037

Busch U, Hörmansdorfer S, Schranner S, Huber I, Bogner KH, Sing A (2007) Enterohemorrhagic *Escherichia coli* excretion by child and her cat. Emerg Infect Dis 13:348–349

Cacciò S, Pinter E, Fantini R, Mezzaroma I, Pozio E (2002) Human infection with *Cryptosporidium felis*: case report and literature review. Emerg Infect Dis 8:85–86

Calvet GA, Pereira SA, Ogrzewalska M, Pauvolid-Corrêa A, Resende PC, Tassinari WS, Costa AP, Keidel LO, da Rocha ASB, da Silva MFB, Dos Santos SA, Lima ABM, de Moraes ICV, Mendes Junior AAV, Souza TDC, Martins EB, Ornellas RO, Corrêa ML, Antonio IMDS, Guaraldo L, Motta FDC, Brasil P, Siqueira MM, Gremião IDF, Menezes RC (2021) Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de Janeiro, Brazil. PLoS One 16(4):e0250853

Cama VA, Bern C, Roberts J, Cabrera L, Sterling CR, Ortega Y, Gilman RH, Xiao L (2008) *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. Emerg Infect Dis 14:1567–1574

Caravedo Martinez MA, Ramírez-Hernández A, Blanton LS (2021) Manifestations and Management of Flea-Borne Rickettsioses. Res Rep Trop Med 12:1–14

Carithers HA (1985) Cat-scratch disease: an overview based on a study of 1200 patients. Am J Dis Child 139:1124–1133

Carlin EP, Tyungu DL (2020) *Toxocara*: Protecting pets and improving the lives of people. Adv Parasitol 109:3–16

Carme B, Demar M, Ajzenberg D, Dardé ML (2009) Severe acquired toxoplasmosis caused by wild cycle of Toxoplasma gondii, French Guiana. Emerg Infect Dis 15:656–658

Carvelli A, Iacoponi F, Scaramozzino P (2006) A Cross-Sectional Survey to Estimate the Cat Population and Ownership Profiles in a Semirural Area of Central Italy. Biomed Res Int. 2016; 2016:3796872Case JB, Chomel B, Nicholson W, Foley JE. Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. J Feline Med Surg 8:111–117

Case SM, Son MB (2021) COVID-19 in pediatrics. Rheum Dis Clin North Am 47(4):797–811

Case JB, Chomel B, Nicholson W, Foley JE (2006) Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. J Feline Med Surg 8 (2):111–7. https://doi.org/10.1016/j.jfms.2005.10.004. Epub 2006 Jan 23. PMID: 16434226.

CDC (Centers for Disease Control and Prevention). Healthy Pets, Healthy People. September 2021. http://www.cdc.gov/healthypets/pets/cats.html. Accessed 11 Oct 2021

Chacin-Bonilla L, Sanchez-Chavez Y, Monsalve F, Estevez J (2001) Seroepidemiology of toxoplasmosis in amerindians from western Venezuela. Am J Trop Med Hyg 65:131–135
Chaloner GL, Harrison TG, Coyne KP, Aanensen DM, Birtles RJ (2011) Multilocus sequence typing of *Bartonella henselae* in the United Kingdom indicates that only a few, uncommon sequence types are associated with zoonotic disease. J Clin Microbiol 49:2132–2137
Chan T, Klaus J, Meli ML, Hofmann-Lehmann R (2021) SARS-CoV-2 Infektionen bei Katzen, Hunden und anderen Tieren: Erkenntnisse zur Infektion und Daten aus der Schweiz [SARS-CoV-2 infections in cats, dogs, and other animal species: Findings on infection and data from Switzerland]. Schweiz Arch Tierheilkd 163(12):821–835
Chang CC, Lee CC, Maruyama S, Lin JW, Pan MJ (2006) Cat-scratch disease in veterinary-associated populations and in its cat reservoir in Taiwan. Vet Res 37:565–577
Chaudhuri RN, Saha TK (1959) Tropical eosinophilia. Experiments with *Toxocara canis*. Lancet 2 (7101):493–494
Chen J, Liu Q, Liu GH, Zheng WB, Hong SJ, Sugiyama H, Zhu XQ, Elsheikha HM (2018) Toxocariasis: a silent threat with a progressive public health impact. Infect Dis Poverty 7:59
Cheslock MA, Embers ME (2019) Human Bartonellosis: An Underappreciated Public Health Problem? Trop Med Infect Dis 4(2):69
Chetboul V, Foulex P, Kartout K, Klein AM, Sailleau C, Dumarest M, Delaplace M, Gouilh MA, Mortier J, Le Poder S (2021) Myocarditis and subclinical-like infection associated with SARS-CoV-2 in two cats living in the same household in France: a case report with literature review. Front Vet Sci 8:748869
Chiang TY, Kuo MC, Chen CH, Yang JY, Kao CF, Ji DD, Fang CT (2014) Risk factors for acute *Toxoplasma gondii* diseases in Taiwan: a population-based case-control study. PLoS One 9: e90880
Chidumayo NN (2020) Prevalence of Toxocara in dogs and cats in Africa. Adv Parasitol 109:861–871. https://doi.org/10.1016/bs.apar.2020.01.032. Epub 2020 Feb 10. PMID: 32381230.
Chmielewski T, Podsiadły E, Tylewska-Wierzbanowska S (2007) Presence of *Bartonella* spp. in various human populations. Pol J Microbiol 56:33–38
Chomel BB, Kasten R, Adams C, Lambillotte D, Theis J, Goldsmith R, Koss J, Chioino C, Widjana DP, Sutisna P (1993) Serosurvey of some major zoonotic infections in children and teenagers in Bali, Indonesia. Southeast Asian J Trop Med Public Health 24:321–326
Chomel BB, Abbott RC, Kasten RW, Floyd-Hawkins KA, Kass PH, Glaser CA, Pedersen NC, Koehler JE (1995) *Bartonella henselae* prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. J Clin Microbiol 33:2445–2550
Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB (2006) *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis 12:389–394
Chomel BB, McMillan-Cole AC, Kasten RW, Stuckey MJ, Sato S, Maruyama S, Diniz PP, Breitschwerdt EB (2012) Candidatus *Bartonella merieuxii*, a potential new zoonotic *Bartonella* species in canids from Iraq. PLoS Negl Trop Dis 6:e1843
Chou CM, Fan CK (2020) Seroprevalence of *Toxocara* spp. infection in Southeast Asia and Taiwan. Adv Parasitol 109:449–463
Cicuttin GL, Brambati DF, De Gennaro MF, Carmona F, Isturiz ML, Pujol LE, Belerenian GC, Gil H (2014) *Bartonella* spp. in cats from Buenos Aires, Argentina. Vet Microbiol 168:225–228
Cieloszyk J, Goñi P, García A, Remacha MA, Sánchez E, Clavel A (2012) Two cases of zoonotic cryptosporidiosis in Spain by the unusual species *Cryptosporidium ubiquitum* and Cryptosporidium felis. Enferm Infecc Microbiol Clin 30:549–551
Coati N, Schnieder T, Epe C (2004) Vertical transmission of *Toxocara cati* Schrank 1788 (Anisakidae) in the cat. Parasitol Res 92:142–146
Coelho WM, do Amarante AF, de Soutello RV, Meireles MV, Bresciani KD (2009) Occurrence of gastrointestinal parasites in fecal samples of cats in Andradina City, São Paulo. Rev Bras Parasitol Vet 18:46–49. [Article in Portuguese]
Cojocariu IE, Bahnea R, Luca C, Leca D, Luca M (2012) Toxocaroza la adult: studiu epidemiologic privind incidenţa în zona moldovei [Adult toxocariasis]. Rev Med Chir Soc Med Nat Iasi 116: 432–435

Colitti B, Bertolotti L, Mannelli A, Ferrara G, Vercelli A, Grassi A, Trentin C, Paltrinieri S, Nogarol C, Decaro N, Brocchi E, Rosati S (2021) Cross-sectional serosurvey of companion animals housed with SARS-CoV-2-infected owners, Italy. Emerg Infect Dis 27(7):1919–1922

Congdon P, Lloyd P (2011) *Toxocara* infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. Int J Public Health 56:15–24

Cook AJC, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT (2000) Sources of *Toxoplamsa* infection in pregnant women: European multicenter case-control study. European Network on Toxoplasmosis. Br Med J 321:142–147

Corbett EJ (1954) The Temple Tiger and More Man-Eaters of Kumaon. Oxford University Press, London, pp 64–86

Corti MA, Bloemberg GV, Borelli S, Kutzner H, Eich G, Hoelzle L, Lautenschlager S (2012) Rare human skin infection with *Corynebacterium ulcerans*: transmission by a domestic cat. Infection 40:575–578

Crowley SL, Cecchetti M, McDonald RA (2020) Our Wild Companions: Domestic cats in the Anthropocene. Trends Ecol Evol 35:477–483

Dabritz HA, Conrad PA (2010) Cats and *Toxoplasma:* implications for public health. Zoonoses Public Health 57:34–52

Dangel A, Berger A, Rau J, Eisenberg T, Kämpfer P, Margos G, Contzen M, Busse HJ, Konrad R, Peters M, Sting R, Sing A (2020) *Corynebacterium silvaticum* sp. nov., a unique group of NTTB corynebacteria in wild boar and roe deer. Int J Syst Evol Microbiol 70(6):3614–3624

Dantas-Torres F (2020) Toxocara prevalence in dogs and cats in Brazil. Adv Parasitol 109:715–741. https://doi.org/10.1016/bs.apar.2020.01.028. Epub 2020 Feb 20. PMID: 32381224.

Dantas-Torres F, Otranto D (2014) Dogs, cats, parasites, and humans in Brazil: opening the black box. Parasites Vectors 7:22

Das P, Roy SS, MitraDhar K, Dutta P, Bhattacharya MK, Sen A, Ganguly S, Bhattacharya SK, Lal AA, Xiao L (2006) Molecular characterization of *Cryptosporidium* spp. from children in Kolkata, India. J Clin Microbiol 44:4246–4249

Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, Pearson CAB, Russell TW, Tully DC, Washburne AD, Wenseleers T, Gimma A, Waites W, Wong KLM, van Zandvoort K, Silverman JD, CMMID COVID-19 Working Group; COVID-19 Genomics UK (COG-UK) Consortium, Diaz-Ordaz K, Keogh R, Eggo RM, Funk S, Jit M, Atkins KE, Edmunds WJ (2021) Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 372(6538):eabg3055

Davis MF, Innes GK (2021) The Cat's in the Bag: Despite limited cat-to-cat Severe Acute Respiratory Syndrome Coronavirus 2 transmission, One Health surveillance efforts are needed. J Infect Dis 223(8):1309–1312

Day MJ, Breitschwerdt E, Cleaveland S, Karkare U, Khanna C, Kirpensteijn J, Kuiken T, Lappin MR, McQuiston J, Mumford E, Myers T, Palatnik-de-Sousa CB, Rubin C, Takashima G, Thiermann A (2012) Surveillance of zoonotic infectious diseases transmitted by small companion animals. Emerg Infect Dis [Internet] Dec [date cited]

De Craeye S, Francart A, Chabauty J, De Vriendt V, Van Gucht S, Leroux I, Jongert E (2008) Prevalence of Toxoplasma gondii infection in Belgian house cats. Vet. Parasitol 157:128–132

de Galvan-Ramirez ML, Troyo R, Roman S, Calvillo-Sanchez C, Bernal-Redondo R (2012) A systematic review and meta-analysis of *Toxoplasma gondii* infection among the Mexican population. Parasit Vectors 5(271)

de Lucio A, Bailo B, Aguilera M, Cardona GA, Fernández-Crespo JC, Carmena D (2017) No molecular epidemiological evidence supporting household transmission of zoonotic *Giardia duodenalis* and C*ryptosporidium* spp. from pet dogs and cats in the province of Álava, Northern Spain. Acta Trop 170:48–56

de Morais HA, Dos Santos AP, do Nascimento NC, Kmetiuk LB, Barbosa DS, Brandão PE, Guimarães AMS, Pettan-Brewer C, Biondo AW (2020) Natural infection by SARS-CoV-2 in companion animals: A review of case reports and current evidence of their role in the epidemiology of COVID-19. Front Vet Sci 7:591216

de Oliveira AGL, Sudré AP, Bergamo do Bomfim TC, Santos HLC (2021) Molecular characterization of *Cryptosporidium* spp. in dogs and cats in the city of Rio de Janeiro, Brazil, reveals potentially zoonotic species and genotype. PLoS One 16(8):e0255087

de Santis AC, Raghavan M, Caldanaro RJ, Glickman NW, Moore GE, Lewis HB, Schantz PM, Glickman LT (2006) Estimated prevalence of nematode parasitism among pet cats in the United States. J Am Vet Med Assoc 228:885–892

de Zoysa A, Hawkey PM, Engler K, George R, Mann G, Reilly W, Taylor D, Efstratiou A (2005) Characterization of toxigenic *Corynebacterium ulcerans* strains isolated from humans and domestic cats in the United Kingdom. J Clin Microbiol 43:4377–4381

Deng J, Jin Y, Liu Y, Sun J, Hao L, Bai J, Huang T, Lin D, Jin Y, Tian K (2020a) Serological survey of SARS-CoV-2 for experimental, domestic, companion and wild animals excludes intermediate hosts of 35 different species of animals. Transbound Emerg Dis 67(4):1745–1749

Deng J, Liu Y, Sun C, Bai J, Sun J, Hao L, Li X, Tian K (2020b) SARS-CoV-2 Serological Survey of Cats in China before and after the Pandemic. Virol Sin 35(6):846–848

Deshayes S, Bonhomme J, de La Blanchardière A (2016) Neurotoxocariasis: a systematic literature review. Infection 44:565–574

Desmonts G, Couvreur J, Alison F, Baudelot J, Gerbeaux J, Lelong M (1965) Etude épidémiologique sur la toxoplasmose: de l'influence de la cuisson des viandes de boucherie sur la fréquence de l'infection humaine [Epidemiological study on toxoplasmosis: the influence of cooking slaughter-animal meat on the incidence of human infection]. Rev Fr Etud Clin Biol 10:952–958

Despommier D (2003) Toxocariasis: Clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev 16:265–272

Detemmerman L, Rousseaux D, Efstratiou A, Schirvel C, Emmerechts K, Wybo I, Soetens O, Piérard D (2013) Toxigenic *Corynebacterium ulcerans* in human and non-toxigenic *Corynebacterium diphtheriae* in cat. New Microbe New Infect 1:18–19

Di Francesco A, Donati M, Battelli G, Cevenini R, Baldelli R (2004) Seroepidemiological survey for *Chlamydophila felis* among household and feral cats in northern Italy. Vet Rec 155:399–400

Dileepan M, Di D, Huang Q, Ahmed S, Heinrich D, Ly H, Liang Y (2021) Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. Virulence 12(1): 1597–1609

Doorduyn Y, Van Den Brandhof WE, Van Duynhoven YT, Breukink BJ, Wagenaar JA, Van Pelt W (2010) Risk factors for indigenous *Campylobacter jejuni* and *Campylobacter coli* infections in The Netherlands: a case-control study. Epidemiol Infect 138:1391–1404

Dovč A, Vlahović K, Suhadolc-Scholten S, TozonN. (2008) Presence of IgG antibodies against *Chlamydophila felis* in cats positive to FIV and/or FELV. Acta Vet 58:17–23

Downes M, Canty MJ, More SJ (2009) Demography of the pet dog and cat population on the island of Ireland and human factors influencing pet ownership. Prev Vet Med 92:140–149

Driscoll CA, Menotti-Raymond M, Roca AL, Hupe K, Johnson WE, Geffen E, Harley EH, Delibes M, Pontier D, Kitchener AC, Yamaguchi N, O'brien SJ, Macdonald DW (2007) The Near Eastern origin of cat domestication. Science 317(5837):519–523

Dróżdż M, Krzyżek P, Dudek B, Makuch S, Janczura A, Paluch E (2021) Current state of knowledge about role of pets in zoonotic transmission of SARS-CoV-2. Viruses 13(6):1149

Dubey JP (2008) The history of *Toxoplasma gondii*--the first 100 years. J Eukaryot Microbiol 55: 467–475

Dubey JP (2020) The History and Life Cycle of *Toxoplasma gondii*. In: Weiss LM, Kim K (eds) *Toxoplasma gondii.* The model apicomplexan – perspectives and methods, 3rd edn. Academic Press, pp 1–19

Dubey JP (2021) Outbreaks of clinical toxoplasmosis in humans: five decades of personal experience, perspectives and lessons learned. Parasit Vectors 14:263

Dubey JP (2010) *Toxoplasma gondii* infection in chickens (*Gallus domesticus*): Prevalence, clinical disease, diagnosis and public health significance. Zoonoses Public Health 57:60–73

Dubey JP, Lunney JK, Shen SK, Kwok OC, Ashford DA, Thulliez P (1996) Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. J Parasitol 82:438–443

Dubey JP, Jones JL (2008) *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol 38:1257–1278

Dubey JP, Rollor EA, Smith K, Kwok OC, Thulliez P (1997) Low seroprevalence of *Toxoplasma gondii* in feral pigs from a remote island lacking cats. J Parasitol 83:839–841

Dubey JP, Cerqueira-Cézar CK, Murata FHA, Kwok OCH, Yang YR, Su C (2020) All about toxoplasmosis in cats: the last decade. Vet Parasitol 283:109145

Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Su C (2021a) Epidemiologic significance of *Toxoplasma gondii* infections in turkeys, ducks, ratites and other wild birds: 2009-2020. Parasitology 148(1):1–30

Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Su C (2021b) Epidemiological significance of *Toxoplasma gondii* infections in wild rodents: 2009-2020. J Parasitol 107:182–204

Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Villena I (2021c) Congenital toxoplasmosis in humans: an update of worldwide rate of congenital infections. Parasitology 18:1–11

Durant JF, Irenge LM, Fogt-Wyrwas R, Dumont C, Doucet JP, Mignon B et al (2012) Duplex quantitative real-time PCR assay for the detection and discrimination of the eggs of *Toxocara canis* and *Toxocara cati* (Nematoda, Ascaridoidea) in soil and fecal samples. Parasit Vectors 5: 288

Ebani VV, Nardoni S, Maestrini M, Perrucci S, Mancianti F (2021) Serological Survey on the Occurrence of *Rickettsia* spp., *Neospora caninum*, *Bartonella henselae* and *Toxoplasma gondii* in cats from Tuscany (Central Italy). Animals (Basel) 11(6):1842

Ekman CC, Chiossi MF, Meireles LR, Andrade Júnior HF, Figueiredo WM, Marciano MA, Luna EJ (2012) Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of São Paulo, Brazil. Rev Inst Med Trop Sao Paulo 54:239–244

Ekstrand K, Flanagan AJ, Lin IE, Vejseli B, Cole A, Lally AP, Morris RL, Morgan KN (2021) Animal transmission of SARS-CoV-2 and the welfare of animals during the COVID-19 pandemic. Animals (Basel) 11(7):2044

Elwin K, Hadfield SJ, Robinson G, Chalmers RM (2012) The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. Epidemiol Infect 140:673–683

Enemark HL, Starostka TP, Larsen B, Takeuchi-Storm N, Thamsborg SM (2020) Giardia and *Cryptosporidium* infections in Danish cats: risk factors and zoonotic potential. Parasitol Res 119 (7):2275–2286

Engels DW (1999) Classical cats. In: The Rise and Fall of the Sacred Cat. Routledge Chapman & Hall, London

English ED, Striepen B (2019) The cat is out of the bag: How parasites know their hosts. PLoS Biol 17(9):e3000446

Epe C, Coati N, Schnieder T (2004) Results of parasitological examinations of faecal samples from horses, ruminants, pigs, dogs, cats, hedgehogs and rabbits between 1998 and 2002. Dtsch Tierarztl Wochenschr 111:243–247

Esch KJ, Petersen CA (2013) Transmission and epidemiology of zoonotic protozoal diseases of companion animals. Clin Microbiol Rev 26:58–85

Eslahi AV, Badri M, Khorshidi A, Majidiani H, Hooshmand E, Hosseini H, Taghipour A, Foroutan M, Pestehchian N, Firoozeh F, Riahi SM, Zibaei M (2020) Prevalence of *Toxocara* and *Toxascaris* infection among human and animals in Iran with meta-analysis approach. BMC Infect Dis 20(1):20

Fahrion AS, Schnyder M, Wichert B, Deplazes P (2011) *Toxocara* eggs shed by dogs and cats and their molecular and morphometric species-specific identification: is the finding of *T. cati* eggs shed by dogs of epidemiological relevance? Vet Parasitol 177:186–189

Fakhri Y, Gasser RB, Rostami A, Fan CK, Ghasemi SM, Javanian M, Bayani M, Armoon B, Moradi B (2018) *Toxocara* eggs in public places worldwide - A systematic review and meta-analysis. Environ Pollut 242(Pt B):1467–1475

Fan CK, Hung CC, Du WY, Liao CW, Su KE (2004) Seroepidemiology of *Toxocara canis* infection among mountain aboriginal schoolchildren living in contaminated districts in eastern Taiwan. Trop Med Int Health 9:1312–1318

Farmer A, Beltran T, Choi YS (2017) Prevalence of *Toxocara* species infection in the U.S.: Results from the National Health and Nutrition Examination Survey, 2011-2014. PLoS Negl Trop Dis 11(7):e0005818

Fava NMN, Cury MC, Santos HA, Takeuchi-Storm N, Strube C, Zhu XQ, Taira K, Odoevskaya I, Panovag O, Mateus TL, Nejsum P (2020) Phylogenetic relationships among *Toxocara* spp. and *Toxascaris* sp. from different regions of the world. Vet Parasitol 282:109133

FEDIAF (The European Pet Food Industry). Facts and figures 2012. http://www.fediaf.org/facts-figures/. Accessed 23 Jan 2014

FEDIAF (The European Pet Food Industry). Facts and figures 2020. https://www.fediaf.org/images/FEDIAF_Facts_and_Figures_2020.pdf. Accessed 22 Sept 2021

Fedorova OS, Fedotova MM, Zvonareva OI, Mazeina SV, Kovshirina YV, Sokolova TS, Golovach EA, Kovshirina AE, Konovalova UV, Kolomeets IL, Gutor SS, Petrov VA, Hattendorf J, Ogorodova LM, Odermatt P (2020) *Opisthorchis felineus* infection, risks, and morbidity in rural Western Siberia, Russian Federation. PLoS Negl Trop Dis 14(6):e0008421

Ferasin L, Fritz M, Ferasin H, Becquart P, Corbet S, Ar Gouilh M, Legros V, Leroy EM (2021) Infection with SARS-CoV-2 variant B.1.1.7 detected in a group of dogs and cats with suspected myocarditis. Vet Rec 189(9):e944

Fernández-Bellon H, Rodon J, Fernández-Bastit L, Almagro V, Padilla-Solé P, Lorca-Oró C, Valle R, Roca N, Grazioli S, Trogu T, Bensaid A, Carrillo J, Izquierdo-Useros N, Blanco J, Parera M, Noguera-Julián M, Clotet B, Moreno A, Segalés J, Vergara-Alert J (2021) Monitoring natural SARS-CoV-2 infection in lions (*Panthera leo*) at the Barcelona Zoo: Viral dynamics and host responses. Viruses 13(9):1683

Fillaux J, Magnaval JP (2013) Laboratory diagnosis of human toxocariasis. Vet Parasitol 193:327–336

Fischhoff IR, Castellanos AA, Rodrigues JPGLM, Varsani A, Han BA (2021) Predicting the zoonotic capacity of mammals to transmit SARS-CoV-2. Proc Biol Sci 288(1963):20211651

Flatt A, Shetty N (2013) Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population. Eur J Public Health 23: 648–652

Flegr J, Havlicek J, Kodym P, Maly M, Smahel Z (2002) Increased risk of traffic accidetns in subjects with latent toxoplasmosis: a retrospective case-control study. BMC Infect Dis 2:11

Flegr J (2013) Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*-human model in studying the manipulation hypothesis. J Exp Biol 216:127–133

Flegr J, Prandota J, Sovičková M, Israili ZH (2014) Toxoplasmosis--a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PLoS One 9(3): e90203

Flegr J, Horáček J (2020) Negative Effects of latent toxoplasmosis on mental health. Front Psychiatry 10:1012

Foroutan-Rad M, Khademvatan S, Majidiani H, Aryamand S, Rahim F, Malehi AS (2016) Seroprevalence of *Toxoplasma gondii* in the Iranian pregnant women: A systematic review and meta-analysis. Acta Trop 158:160–169

Frean J, Arndt S, Spencer D (2002) High rate of *Bartonella henselae* infection in HIV-positive outpatients in Johannesburg, South Africa. Trans R Soc Trop Med Hyg 96:549–550

Frenkel JK, Parker BB (1996) An Apparent Role of Dogs in the Transmission of *Toxoplasma gondii*: The Probable Importance of Xenosmophilia. Ann NY Acad Sci 791:402–407

Fritz M, Rosolen B, Krafft E, Becquart P, Elguero E, Vratskikh O, Denolly S, Boson B, Vanhomwegen J, Gouilh MA, Kodjo A, Chirouze C, Rosolen SG, Legros V, Leroy EM (2021) High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health 11:100192. https://doi.org/10.1016/j.onehlt.2020.100192. Epub ahead of print

Fu CJ, Chuang TW, Lin HS, Wu CH, Liu YC, Langinlur MK, Lu MY, Hsiao WW, Fan CK (2014) Seroepidemiology of Toxocara canis infection among primary schoolchildren in the capital area of the Republic of the Marshall Islands. BMC Infect Dis 14:261

Galal L, Hamidović A, Dardé ML, Mercier M (2019) Diversity of *Toxoplasma gondii* strains at the global level and its determinants. Food Waterborne Parasitol 15:e00052

Gamboa MI (2005) Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. J Helminthol 79:327–331

Garigliany M, Van Laere AS, Clercx C, Giet D, Escriou N, Huon C, van der Werf S, Eloit M, Desmecht D (2020) SARS-CoV-2 Natural Transmission from human to cat, Belgium, March 2020. Emerg Infect Dis 26(12):3069–3071

Gasser RB (2013) A perfect time to harness advanced molecular technologies to explore the fundamental biology of *Toxocara* species. Vet Parasitol 193:353–364

Gatei W, Wamae CN, Mbae C, Waruru A, Mulinge E, Waithera T, Gatika SM, Kamwati SK, Revathi G, Hart CA (2006) Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. Am J Trop Med Hyg 75:78–82

Gaudreault NN, Trujillo JD, Carossino M, Meekins DA, Morozov I, Madden DW, Indran SV, Bold D, Balaraman V, Kwon T, Artiaga BL, Cool K, García-Sastre A, Ma W, Wilson WC, Henningson J, Balasuriya UBR, Richt JA (2020) SARS-CoV-2 infection, disease and transmission in domestic cats. Emerg Microbes Infect 9(1):2322–2332

Gaudreault NN, Carossino M, Morozov I, Trujillo JD, Meekins DA, Madden DW, Cool K, Artiaga BL, McDowell C, Bold D, Balaraman V, Kwon T, Ma W, Henningson J, Wilson DW, Wilson WC, Balasuriya UBR, García-Sastre A, Richt JA (2021) Experimental re-infected cats do not transmit SARS-CoV-2. Emerg Microbes Infect 10(1):638–650

Germonpré M, Sablin MV, Stevens RE, Hedges REM, Hofreiter M, Stiller M, Després VR (2009) Fossil dogs and wolves from Palaeolithic sites in Belgium, the Ukraine and Russia: osteometry, ancient DNA and stable isotopes. J Archaeol Sci 36:473–490

Ghai RR, Carpenter A, Liew AY, Martin KB, Herring MK, Gerber SI, Hall AJ, Sleeman JM, VonDobschuetz S, Behravesh CB (2021) Animal reservoirs and hosts for emerging alphacoronaviruses and betacoronaviruses. Emerg Infect Dis 27(4):1015–1022

Gibbons LM, Jacobs DE, Sani RA (2001) *Toxocara malaysiensis* n. sp. (Nematoda: Ascaridoidea) from the domestic cat (*Felis catus* Linnaeus, 1758). J Parasitol 87:660–665

Gil H, Escudero R, Pons I, Rodríguez-Vargas M, García-Esteban C, Rodríguez-Moreno I, García-Amil C, Lobo B, Valcárcel F, Pérez A, Jiménez S, Jado I, Juste R, Segura F, Anda P (2013) Distribution of *Bartonella henselae* variants in patients, reservoir hosts and vectors in Spain. PLoS One 8:e68248

Gil H, Cano L, de Lucio A, Bailo B, de Mingo MH, Cardona GA, Fernández-Basterra JA, Aramburu-Aguirre J, López-Molina N, Carmena D (2017) Detection and molecular diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in sheltered dogs and cats in Northern Spain. Infect Genet Evol 50:62–69

Giraldo-Ramirez S, Rendon-Marin S, Jaimes JA, Martinez-Gutierrez M, Ruiz-Saenz J (2021) SARS-CoV-2 clinical outcome in domestic and wild cats: A systematic review. Animals (Basel) 11(7):2056

Girard YA, Swift P, Chomel BB, Kasten RW, Fleer K, Foley JE, Torres SG, Johnson CK (2012) Zoonotic vector-borne bacterial pathogens in California mountain lions (*Puma concolor*), 1987-2010. Vector Borne Zoonotic Dis 12:913–921

Gonzales JL, de Jong MCM, Gerhards NM, Van der Poel WHM (2021) The SARS-CoV-2 Reproduction Number R0 in Cats. Viruses 13(12):2480. https://doi.org/10.3390/v13122480. PMID: 34960749; PMCID: PMC8704225

Goodrich JM, Quigley KS, Lewis JC, Astafiev AA, Slabi EV, Miquelle DG, Smirnov EN, Kerley LL, Armstrong DL, Quigley HB, Hornocker MG (2012) Serosurvey of free-ranging Amur tigers in the Russian Far East. J Wildl Dis 48:186–189

Gorog DA, Storey RF, Gurbel PA, Tantry US, Berger JS, Chan MY, Duerschmied D, Smyth SS, Parker WAE, Ajjan RA, Vilahur G, Badimon L, Berg JMT, Cate HT, Peyvandi F, Wang TT, Becker RC (2022) Current and novel biomarkers of thrombotic risk in COVID-19: a consensus statement from the International COVID-19 Thrombosis Biomarkers Colloquium. Nat Rev Cardiol 1–21

Goryoka GW, Cossaboom CM, Gharpure R, Dawson P, Tansey C, Rossow J, Mrotz V, Rooney J, Torchetti M, Loiacono CM, Killian ML, Jenkins-Moore M, Lim A, Poulsen K, Christensen D,

Sweet E, Peterson D, Sangster AL, Young EL, Oakeson KF, Taylor D, Price A, Kiphibane T, Klos R, Konkle D, Bhattacharyya S, Dasu T, Chu VT, Lewis NM, Queen K, Zhang J, Uehara A, Dietrich EA, Tong S, Kirking HL, Doty JB, Murrell LS, Spengler JR, Straily A, Wallace R, Barton Behravesh C (2021) One Health Investigation of SARS-CoV-2 Infection and Seropositivity among Pets in Households with Confirmed Human COVID-19 Cases-Utah and Wisconsin, 2020. Viruses 13(9):1813. https://doi.org/10.3390/v13091813. PMID: 34578394; PMCID: PMC8472995.

Gow AG, Gow DJ, Hall EJ, Langton D, Clarke C, Papasouliotis K (2009) Prevalence of potentially pathogenic enteric organisms in clinically healthy kittens in the UK. J Feline Med Surg 11:655–662

Gower CM, Scobie A, Fry NK, Litt DJ, Cameron JC, Chand MA, Brown CS, Collins S, White JM, Ramsay ME, Amirthalingam G (2020) The changing epidemiology of diphtheria in the United Kingdom, 2009 to 2017. Euro Surveill 25(11):1900462

Gracenea M, Gomes MS, Torres J (2009) Prevalence of intestinal parasites in shelter dogs and cats in the metropolitan area of Barcelona (Spain). Acta Parasitol 54:73–77

Groër MW, Yolken RH, Xiao JC, Beckstead JW, Fuchs D, Mohapatra SS, Seyfang A, Postolache TT (2011) Prenatal depression and anxiety in *Toxoplasma gondii*-positive women. Am J Obstet Gynecol 204(433):e1–e7

Guimaraes AM, Brandão PE, Moraes W, Kiihl S, Santos LC, Filoni C, Cubas ZS, Robes RR, Marques LM, Neto RL, Yamaguti M, Oliveira RC, Catão-Dias JL, Richtzenhain LJ, Messick JB, Biondo AW, Timenetsky J (2010) Detection of *Bartonella* spp. in neotropical felids and evaluation of risk factors and hematological abnormalities associated with infection. Vet Microbiol 142:346–351

Gurfield AN, Boulouis HJ, Chomel BB, Kasten RW, Heller R, Bouillin C, Gandoin C, Thibault D, Chang CC, Barrat F, Piemont Y (2001) Epidemiology of *Bartonella* infection in domestic cats in France. Vet Microbiol 80:185–198

Guzel M, Celebi B, Yalcin E, Koenhemsi L, Mamak N, Pasa S, Aslan O (2011) A serological investigation of *Bartonella henselae* infection in cats in Turkey. J Vet Med Sci 73:1513–1516

Gyang PV, Akinwale OP, Lee YL, Chuang TW, Orok AB, Ajibaye O, Liao CW, Chen PC, Chou CM, Huang YC, Barghouth U, Fan CK (2015) Seroprevalence, disease awareness, and risk factors for *Toxocara canis* infection among primary schoolchildren in Makoko, an urban slum community in Nigeria. Acta Trop 146:135–140

Hacker E, Antunes CA, Mattos-Guaraldi AL, Burkovsky A, Tauch A (2016) *Corynebacterium ulcerans*, an emerging human pathogen. Future Microbiol 11:1191–1208

Halánová M, Sulinová Z, Cisláková L, Trbolová A, Páleník L, Weissová T, Halán M, Kalinová Z, Holičková M. *Chlamydophila felis* in cats--are the stray cats dangerous source of infection? Zoonoses Public Health 2011; 58: 519-522

Halánová M, Petrová L, Halán M, Trbolová A, Babinská I, Weissová T (2019) Impact of way of life and environment on the prevalence of *Chlamydia felis* in cats as potentional sources of infection for humans. Ann Agric Environ Med 26(2):222–226

Halfmann PJ, Hatta M, Chiba S, Maemura T, Fan S, Takeda M, Kinoshita N, Hattori SI, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Imai M, Kawaoka Y (2020) Transmission of SARS-CoV-2 in domestic cats. N Engl J Med 383(6):592–594

Hall AJ, Cassiday PK, Bernard KA, Bolt F, Steigerwalt AG, Bixler D, Pawloski LC, Whitney AM, Iwaki M, Baldwin A, Dowson CG, Komiya T, Takahashi M, Hinrikson HP, Tondella ML (2010) Novel *Corynebacterium diphtheriae* in domestic cats. Emerg Infect Dis 16:688–691

Hamer SA, Ghai RR, Zecca IB, Auckland LD, Roundy CM, Davila E, Busselman RE, Tang W, Pauvolid-Corrêa A, Killian ML, Jenkins-Moore M, Torchetti MK, Robbe Austerman S, Lim A, Akpalu Y, Fischer RSB, Barton Behravesh C, Hamer GL (2021a) SARS-CoV-2 B.1.1.7 variant of concern detected in a pet dog and cat after exposure to a person with COVID-19, USA. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14122. Epub ahead of print

Hamer SA, Pauvolid-Corrêa A, Zecca IB, Davila E, Auckland LD, Roundy CM, Tang W, Torchetti MK, Killian ML, Jenkins-Moore M, Mozingo K, Akpalu Y, Ghai RR, Spengler JR, Barton

Behravesh C, Fischer RSB, Hamer GL (2021b) SARS-CoV-2 Infections and viral isolations among serially tested cats and dogs in households with infected owners in Texas, USA. Viruses 13(5):938

Han T, Ryu B, Lee S, Song Y, Jeong Y, Kim I, Kim J, Kim E, Lee W, Lee H, Hwang H (2021) Management following the first confirmed case of SARS-CoV-2 in a domestic cat associated with a massive outbreak in South Korea. One Health 13:100328

Harms A, Dehio C (2012) Intruders below the radar: molecular pathogenesis of *Bartonella* spp. Clin Microbiol Rev 25:42–78

Hartley JC, Stevenson S, Robinson AJ, Littlewood JD, Carder C, Cartledge J, Clark C, Ridgway GL (2001) Conjunctivitis due to *Chlamydophila felis* (*Chlamydia psittaci* feline pneumonitis agent) acquired from a cat: case report with molecular characterization of isolates from the patient and cat. J Infect 43:7–11

Hatanaka A, Tsunoda A, Okamoto M, Ooe K, Nakamura A, Miyakoshi M, Komiya T, Takahashi M (2003) *Corynebacterium ulcerans* diphtheria in Japan. Emerg Infect Dis 9:752–753

Hayashi E, Tuda J, Imada M, Akao N, Fujita K (2005) The high prevalence of asymptomatic *Toxocara* infection among schoolchildren in Manado, Indonesia. Southeast Asian J Trop Med Public Health 36:1399–1406

Haywood MJ, Vijendren A, Acharya V, Mulla R, Panesar MJ (2017) Multidisciplinary approach to the management of a case of classical respiratory diphtheria requiring percutaneous endoscopic gastrostomy feeding. BMJ Case Report

Heller R, Artois M, Xemar V, De Briel D, Gehin H, Jaulhac B, Monteil H, Piemont Y (1997) Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in stray cats. J Clin Microbiol 35:1327–1331

Herrmann DC, Pantchev N, Vrhovec MG, Barutzki D, Wilking H, Fröhlich A, Lüder CG, Conraths FJ, Schares G (2010) Atypical *Toxoplasma gondii* genotypes identified in oocysts shed by cats in Germany. Int J Parasitol 40:285–292

Hiby E, Eckman H, MacFarlaine I (2013) Cat population management. In: Turner D, Bateson P (eds) The Domestic Cat: The Biology of its Behaviour. Cambridge University Press, Cambridge, pp 215–230

Hidalgo M, Montoya V, Martínez A, Mercado M, De la Ossa A, Vélez C, Estrada G, Pérez JE, Faccini-Martínez AA, Labruna MB, Valbuena G (2013) Flea -borne rickettsioses in the north of Caldas province, Colombia. Vector Borne Zoonotic Dis 13:289–294

Hill D, Dubey JP (2002) *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin Microbiol Infect 8:634–640

Hill D, Coss C, Dubey JP, Wroblewski K, Sautter M, Hosten T, Muñoz-Zanzi C, Mui E, Withers S, Boyer K, Hermes G, Coyne J, Jagdis F, Burnett A, McLeod P, Morton H, Robinson D, McLeod R (2011) Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. J Parasitol 97: 328–337

Hill DE, Chirukandoth S, Dubey JP (2005) Biology and epidemiology of *Toxoplasma gondii* in man and animals. Anim Health Res Rev 6:41–61

Hill SL, Cheney JM, Taton-Allen GF, Reif JS, Bruns C, Lappin MR (2000) Prevalence of enteric zoonotic organisms in cats. J Am Vet Med Assoc 216:687–692

Hirai-Yuki A, Komiya T, Suzaki Y, Ami Y, Katsukawa C, Takahashi M, Yamamoto A, Yamada YK (2013) Isolation and characterization of toxigenic *Corynebacterium ulcerans* from 2 closed colonies of cynomolgus macaques (Macaca fascicularis) in Japan. Comp Med 63:272–278

Hoelzer K, Moreno Switt AI, Wiedmann M (2011) Animal contact as a source of human non-typhoidal salmonellosis. Vet Res 42:34

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181(2):271–280.e8

Hoffmeister B, Glaeser S, Flick H, Pornschlegel S, Suttorp N, Bergmann F (2007) Cerebral toxocariasis after consumption of raw duck liver. Am J Trop Med Hyg 76:600–602

Hogg RA, Wessels J, Hart J, Efstratiou A, De Zoysa A, Mann G, Allen T, Pritchard GC (2009) Possible zoonotic transmission of toxigenic *Corynebacterium ulcerans* from companion animals in a human case of fatal diphtheria. Vet Rec 165:691–692

Holland CV (2017) Knowledge gaps in the epidemiology of *Toxocara*: the enigma remains. Parasitology 144:81–94

Holst BS, Englund L, Palacios S, Renström L, Berndtsson LT (2006) Prevalence of antibodies against coronavirus and *Chlamydophila felis* in Swedish cats. J Feline Med Surg 8:207–211

Homayouni MM, Razavi SM, Shaddel M, Asadpour M (2019) Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia intestinalis* in household dogs and cats from Shiraz, Southwestern Iran. Vet Ital 55(4):311–318

Hoque MM, Barua S, Kelly PJ, Chenoweth K, Kaltenboeck B, Wang C (2020) Identification of *Rickettsia felis* DNA in the blood of domestic cats and dogs in the USA. Parasit Vectors 13(1): 581

Hosie MJ, Epifano I, Herder V, Orton RJ, Stevenson A, Johnson N, MacDonald E, Dunbar D, McDonald M, Howie F, Tennant B, Herrity D, Da Silva FA, Streicker DG (2021a) COVID-19 Genomics UK (COG-UK) consortium, Willett BJ, Murcia PR, Jarrett RF, Robertson DL, Weir W. Detection of SARS-CoV-2 in respiratory samples from cats in the UK associated with human-to-cat transmission. Vet Rec 188(8):e247

Hosie MJ, Hofmann-Lehmann R, Hartmann K, Egberink H, Truyen U, Addie DD, Belák S, Boucraut-Baralon C, Frymus T, Lloret A, Lutz H, Marsilio F, Pennisi MG, Tasker S, Thiry E, Möstl K (2021b) Anthropogenic infection of cats during the 2020 COVID-19 pandemic. Viruses 13(2):185

Hoste L, Van Paemel R, Haerynck F (2021) Multisystem inflammatory syndrome in children related to COVID-19: a systematic review. Eur J Pediatr 180(7):2019–2034

Huang C, Jiang Y, Yan J (2021) Comparative analyses of ACE2 and TMPRSS2 gene: Implications for the risk to which vertebrate animals are susceptible to SARS-CoV-2. J Med Virol 93(9): 5487–5504

Huang Q, Zhan X, Zeng X-T (2020) COVID-19 Pandemic: stop panic abandonment of household pets. J. Travel Med 27. https://doi.org/10.1093/jtm/taaa046

Hwang J, Gottdenker NL (2013) *Bartonella* species in raccoons and feral cats, Georgia, USA. Emerg Infect Dis 19:1167–1168

Ikotun K, Sowemimo O, Chou CM, Ajenifuja K, Chuang TW, Asaolu S, Akinwale O, Gyang V, Nwafor T, Henry E, Fan CK (2020) High seroprevalence of *Toxocara* antibodies in pregnant women attending an antenatal clinic at a university hospital in Ile-Ife, Nigeria. *Trans R Soc Trop Med Hyg* 114:301–307

International Companion Animal Management Coalition. Humane Cat Population Management Guidance. Stray and Feral Animal Populations Collection. 1. 2011. https://www.wellbeingintlstudiesrepository.org/strfapop/1. Accessed 10 Jan 2022

Iredell J, Blanckenberg D, Arvand M, Grauling S, Feil EJ, Birtles RJ (2003) Characterization of the natural population of *Bartonella henselae* by multilocus sequence typing. J Clin Microbiol 41: 5071–5079

Islam A, Ferdous J, Sayeed MA, Islam S, Kaisar Rahman M, Abedin J, Saha O, Hassan MM, Shirin T (2021) Spatial epidemiology and genetic diversity of SARS-CoV-2 and related coronaviruses in domestic and wild animals. PLoS One 16(12):e0260635

Ito Y, Itoh N, Kimura Y, Kanai K (2016) Molecular detection and characterization of *Cryptosporidium* spp. among breeding cattery cats in Japan. Parasitol Res 115(5):2121–2123

Ito Y, Itoh N, Iijima Y, Kimura Y (2017) Molecular prevalence of *Cryptosporidium* species among household cats and pet shop kittens in Japan. JFMS Open Rep 3(2):2055116917730719

Jackson LA, Perkins BA, Wenger JD (1993) Cat scratch disease in the United States: an analysis of three national databases. Am J Public Health 83:1707–1711

Jairak W, Charoenkul K, Chamsai E, Udom K, Chaiyawong S, Bunpapong N, Boonyapisitsopa S, Tantilertcharoen R, Techakriengkrai N, Surachetpong S, Tangwangvivat R, Suwannakarn K, Amonsin A (2021) First cases of SARS-CoV-2 infection in dogs and cats in Thailand. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14383. Epub ahead of print

Jairak W, Charoenkul K, Chamsai E, Udom K, Chaiyawong S, Hangsawek A, Waenkaew S, Mungaomklang A, Tangwangvivat R, Amonsin A (2022) Survey of SARS-CoV-2 in dogs and cats in high-risk areas during the second wave of COVID-19 outbreak, Thailand. Zoonoses Public Health. https://doi.org/10.1111/zph.12907. Epub ahead of print

Jameson P, Greene C, Regnery R, Dryden M, Marks A, Brown J, Cooper J, Glaus B, Greene R (1995) Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. J Infect Dis 172:1145–1149

Jara LM, Ferradas C, Schiaffino F, Sánchez-Carrión C, Martínez-Vela A, Ulloa A, Isasi-Rivas G, Montalván A, Sarmiento LG, Fernández M, Zimic M (2021) Evidence of neutralizing antibodies against SARS-CoV-2 in domestic cats living with owners with a history of COVID-19 in Lima - Peru. One Health 13:100318

Jarosz W, Mizgajska-Wiktor H, Kirwan P, Konarski J, Rychlicki W, Wawrzyniak G (2010) Developmental age, physical fitness and *Toxocara* seroprevalence amongst lower-secondary students living in rural areas contaminated with *Toxocara* eggs. Parasitology 137:53–63

Jarosz W, Durant JF, Irenge LMWB, Fogt-Wyrwas R, Mizgajska-Wiktor H, Gala JL (2021) Optimized DNA-based identification of *Toxocara* spp. eggs in soil and sand samples. Parasit Vectors 14:426

Jenkins EJ (2020) *Toxocara s*pp. in dogs and cats in Canada. Adv Parasitol 109:641–653

Jennings G, Monaghan A, Xue F, Mockler D, Romero-Ortuño R (2021) A systematic review of persistent symptoms and residual abnormal functioning following acute COVID-19: ongoing symptomatic phase vs. post-COVID-19 syndrome. J Clin Med 10(24):5913

Jiang W, Roellig DM, Lebbad M, Beser J, Troell K, Guo Y, Li N, Xiao L, Feng Y (2020) Subtype distribution of zoonotic pathogen *Cryptosporidium felis* in humans and animals in several countries. Emerg Microbes Infect 9(1):2446–2454

Johnson WE, O'Brien SJ (1997) Phylogenetic Reconstruction of the Felidae Using 16S rRNA and NADH-5 Mitochondrial Genes. J Mol Evol 44(Suppl. 1):S98–S116

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O'Brien SJ (2006) The Late Miocene Radiation of Modern *Felidae*: A Genetic Assessment. Science 311:73–77

Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG (2009) Risk factors for *Toxoplasma gondii* infection in the United States. Clin Infect Dis 49:878–884

Jores NL (2004) Zur Kulturgeschichte der Hauskatze unter besonderer Berücksichtigung ihrer Erkankungen. Mensch & Buch, Berlin

Joseph AK, Wood CW, Robson JM, Paul SL, Morris AJ (1997) *Bartonella henselae* bacteraemia in domestic cats from Auckland. N Z Vet J 45:185–187

Jung BK, Song H, Lee SE, Kim MJ, Cho J, Shin EH, Chai JY (2017) Seroprevalence and risk factors of *Toxoplasma gondii* infection among cat sitters in Korea. Korean J Parasitol 55:203–206

Kaabia N, Letaief A (2009) Characterization of rickettsial diseases in a hospital-based population in central Tunisia. Ann N Y Acad Sci 1166:167–171

Kamada T, Hatanaka A, Tasaki A, Honda K, Tsunoda A, Kitamura K (2012) Case of acute pharyngitis caused by *Corynebacterium ulcerans* in Ibaraki Prefecture. Nihon Jibiinkoka Gakkai Kaiho 115:682–686. [Article in Japanese]

Kamrani A, Parreira VR, Greenwood J, Prescott JF (2008) The prevalence of *Bartonella, Hemoplasma*, and *Rickettsia felis* infections in domestic cats and in cat fleas in Ontario. Can J Vet Res 72(5):411–419

Kang YH, Cong W, Qin SY, Shan XF, Gao YH, Wang CF, Qian AD (2016) First Report of *Toxoplasma gondii*, *Dirofilaria immitis*, and *Chlamydia felis* infection in stray and companion cats in Northeastern and Eastern China. Vector Borne Zoonotic Dis 16(10):654–658

Kang K, Chen Q, Gao Y, Yu KJ (2021) Detection of SARS-CoV-2 B.1.617.2 (Delta) variant in three cats owned by a confirmed COVID-19 patient in Harbin, China. Vet Med Sci. https://doi.org/10.1002/vms3.715. Epub ahead of print. PMID: 34958181.

Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J (1996) Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. Am J Epidemiol 144:405–412

Karamali K, Elliott M, Hopkins C (2022) COVID-19 related olfactory dysfunction. Curr Opin Otolaryngol Head Neck Surg 30(1):19–25

Karikalan M, Chander V, Mahajan S, Deol P, Agrawal RK, Nandi S, Rai SK, Mathur A, Pawde A, Singh KP, Sharma GK (2021) Natural infection of Delta mutant of SARS-CoV-2 in Asiatic lions of India. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14290. Epub ahead of print

Kataoka Y, Irie Y, Sawada T, Nakazawa M (2010) A 3-year epidemiological surveillance of *Escherichia coli* O157:H7 in dogs and cats in Japan. J Vet Med Sci 72:791–794

Keller J, Wieland B, Wittwer M, Stephan R, Perreten V (2007) Distribution and genetic variability among *Campylobacter* spp. isolates from different animal species and humans in Switzerland. Zoonoses Public Health 54:2–7

Keller M, Hagag IT, Balzer J, Beyer K, Kersebohm JC, Sadeghi B, Wernike K, Höper D, Wylezich C, Beer M, Groschup MH (2021) Detection of SARS-CoV-2 variant B.1.1.7 in a cat in Germany. Res Vet Sci 140:229–232

Kelly PJ, Lucas H, Eremeeva ME, Dirks KG, Rolain JM, Yowell C, Thomas R, Douglas T, Dasch GA, Raoult D (2010) *Rickettsia felis*, West Indies. Emerg Infect Dis 16:570–571

Kelly PJ, Matthewman LA, Hayter D, Downey S, Wray K, Bryson NR, Raoult D (1996) *Bartonella (Rochalimaea) henselae* in southern Africa--evidence for infections in domestic cats and implications for veterinarians. J S Afr Vet Assoc 67:182–187

Ketzis JK, Lucio-Forster A (2020) Toxocara canis and Toxocara cati in domestic dogs and cats in the United States, Mexico, Central America and the Caribbean: A review. Adv Parasitol 109:655–714. https://doi.org/10.1016/bs.apar.2020.01.027. Epub 2020 Feb 10. PMID: 32381223.

Khan A, Fux B, Su C, Dubey JP, Darde ML, Ajioka JW, Rosenthal BM, Sibley LD (2007) Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. Proc Natl Acad Sci U S A 104:14872–14877

Kho KL, Koh FX, Hasan LI, Wong LP, Kisomi MG, Bulgiba A, Nizam QN, Tay ST (2017) Rickettsial seropositivity in the indigenous community and animal farm workers, and vector surveillance in Peninsular Malaysia. Emerg Microbes Infect 6(4):e18

Kitchener AC, Breitenmoser-Würsten C, Eizirik E, Gentry A, Werdelin L, Wilting A, Yamaguchi N, Abramov AV, Christiansen P, Driscoll C, Duckworth JW, Johnson W, Luo SJ, Meijaard E, O'Donoghue P. Sanderson J, Seymour K, Bruford M, Groves C, Hoffmann M, Nowell K, Timmons Z, Tobe S. A revised taxonomy of the *Felidae*. The final report of the Cat Classification Task Force of the IUCN/ SSC Cat Specialist Group. Cat News Special Issue 11/2017. https://repository.si.edu/bitstream/handle/10088/32616/A_revised_Felidae_Taxonomy_CatNews.pdf

Klaus J, Zini E, Hartmann K, Egberink H, Kipar A, Bergmann M, Palizzotto C, Zhao S, Rossi F, Franco V, Porporato F, Hofmann-Lehmann R, Meli ML (2021a) SARS-CoV-2 infection in dogs and cats from Southern Germany and Northern Italy during the first wave of the COVID-19 pandemic. Viruses 13(8):1453

Klaus J, Meli ML, Willi B, Nadeau S, Beisel C, Stadler T, Eth Sars-CoV-Sequencing Team, Egberink H, Zhao S, Lutz H, Riond B, Rösinger N, Stalder H, Renzullo S, Hofmann-Lehmann R (2021b) Detection and genome sequencing of SARS-CoV-2 in a domestic Cat with respiratory signs in Switzerland. Viruses 13(3):496

König C, Meinel DM, Margos G, Konrad R, Sing A (2014) Multilocus sequence typing of *Corynebacterium ulcerans* provides evidence for zoonotic transmission and for increased prevalence of certain sequence types among toxigenic strains. J Clin Microbiol 52(12):4318–4324

Kolbekova P, Kourbatova E, Novotna M, Kodym P, Flegr J (2007) New and old risk-factors for *Toxoplasma gondii* infection: prospective cross-sectional study among military personnel in the Czech Republic. Clin Microbiol Infect 13:1012–1017

Kong L, Peng HJ (2020) Current epidemic situation of human toxocariasis in China. Adv Parasitol 109:433–448

Konrad R, Hörmansdorfer S, Sing A (2015) Possible human-to-human transmission of toxigenic *Corynebacterium ulcerans*. Clin Microbiol Infect 21(8):768–771

Kostopoulou D, Claerebout E, Arvanitis D, Ligda P, Voutzourakis N, Casaert S, Sotiraki S (2017) Abundance, zoonotic potential and risk factors of intestinal parasitism amongst dog and cat populations: The scenario of Crete, Greece. Parasit Vectors 10(1):43

Krafft E, Denolly S, Boson B, Angelloz-Pessey S, Levaltier S, Nesi N, Corbet S, Leterrier B, Fritz M, Leroy EM, Gouilh MA, Cosset FL, Kodjo A, Legros V (2021) Report of one-year prospective surveillance of SARS-CoV-2 in dogs and cats in France with various exposure risks: Confirmation of a low prevalence of shedding, detection and complete sequencing of an Alpha variant in a cat. Viruses 13(9):1759

Kumasaka K, Arashima Y, Yanai M, Hosokawa N, Kawano K (2001) Survey of veterinary professionals for antibodies to *Bartonella henselae* in Japan. Rinsho Byori 49:906–910

Kyei G, Ayi I, Boampong JN, Turkson PK (2015) Sero-Epidemiology of *Toxocara canis* Infection in Children Attending Four Selected Health Facilities in the Central Region of Ghana. Ghana Med J 49:77–83

Labruna MB, Ogrzewalska M, Moraes-Filho J, Lepe P, Gallegos JL, López J (2007) *Rickettsia felis* in Chile. Emerg Infect Dis 13:1794–1795

Lappin MR, Hawley J (2009) Presence of *Bartonella* species and *Rickettsia* species DNA in the blood, oral cavity, skin and claw beds of cats in the United States. Vet Dermatol 20:509–514

Lappin MR, Elston T, Evans L, Glaser C, Jarboe L, Karczmar P, Lund C, Ray M (2019) 2019 AAFP Feline Zoonoses Guidelines. J Feline Med Surg 21:1008–1021

Lartigue MF, Monnet X, Le Flèche A, Grimont PA, Benet JJ, Durrbach A, Fabre M, Nordmann P (2005) *Corynebacterium ulcerans* in an immunocompromised patient with diphtheria and her dog. J Clin Microbiol 43:999–1001

La Scola B, Meconi S, Fenollar F, Rolain JM, Roux V, Raoult D (2002) Emended description of *Rickettsia felis* (Bouyer et al. 2001), a temperature-dependent cultured bacterium. Int J Syst Evol Microbiol 52:2035–2041

Le TH, Anh NT, Nguyen KT, Nguyen NT, Thuy do TT, Gasser RB (2016) *Toxocara malaysiensis* infection in domestic cats in Vietnam--An emerging zoonotic issue? Infect Genet Evol 37: 94–98

Lecová L, Hammerbauerová I, Tůmová P, Nohýnková E (2020) Companion animals as a potential source of *Giardia intestinalis* infection in humans in the Czech Republic - A pilot study. Vet Parasitol Reg Stud Reports 21:100431

Legendre KP, Macaluso KR (2017) *Rickettsia felis*: A Review of transmission mechanisms of an emerging pathogen. Trop Med Infect Dis 2(4):64

Lehmann T, Marcet PL, Graham DH, Dahl ER, Dubey JP (2006) Globalization and the population structure of Toxoplasma gondii. Proc Natl Acad Sci U S A 103:11423–11428

Levin AT, Hanage WP, Owusu-Boaitey N, Cochran KB, Walsh SP, Meyerowitz-Katz G (2020) Assessing the age specificity of infection fatality rates for COVID-19: systematic review, meta-analysis, and public policy implications. Eur J Epidemiol 35(12):1123–1138

Levy JK, Crawford PC, Lappin MR, Dubovi EJ, Levy MG, Alleman R et al (2008) Infectious diseases of dogs and cats on Isabela Island, Galapagos. J Vet Intern Med 22:60–65

Li J, Dan X, Zhu K, Li N, Guo Y, Zheng Z, Feng Y, Xiao L (2019a) Genetic characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dogs and cats in Guangdong, China. Parasit Vectors 12(1):571

Li J, Ryan U, Guo Y, Feng Y, Xiao L (2021) Advances in molecular epidemiology of cryptosporidiosis in dogs and cats. Int J Parasitol 51(10):787–795

Li W, Liu X, Gu Y, Liu J, Luo J (2019b) Prevalence of *Cryptosporidium, Giardia, Blastocystis*, and trichomonads in domestic cats in East China. J Vet Med Sci 81(6):890–896

Li W, Li Y, Song M, Lu Y, Yang J, Tao W, Jiang Y, Wan Q, Zhang S, Xiao L (2015) Prevalence and genetic characteristics of *Cryptosporidium, Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. Vet Parasitol 208(3-4):125–134

Li W, Chomel BB, Maruyama S, Guptil L, Sander A, Raoult D, Fournier PE (2006) Multispacer typing to study the genotypic distribution of *Bartonella henselae* populations. J Clin Microbiol 44:2499–2506

Li W, Raoult D, Fournier PE (2007) Genetic diversity of *Bartonella henselae* in human infection detected with multispacer typing. Emerg Infect Dis 13:1178–1183

Lim MY, Brady H, Hambling T, Sexton K, Tompkins D, Slaney D (2012) *Rickettsia felis* infections, New Zealand. Emerg Infect Dis 18:167–169

Liu A, Gong B, Liu X, Shen Y, Wu Y, Zhang W, Cao J (2020) A retrospective epidemiological analysis of human *Cryptosporidium* infection in China during the past three decades (1987-2018). PLoS Negl Trop Dis 14(3):e0008146

Liu EW, Chastain HM, Shin SH, Wiegand RE, Kruszon-Moran D, Handali S, Jones JL (2018) Seroprevalence of Antibodies to *Toxocara* Species in the United States and Associated Risk Factors, 2011-2014. Clin Infect Dis 66(2):206–212

Liu Y, Rocklöv J (2021) The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. J Travel Med 28(7):taab124

Lindblom A, Severinson K, Nilsson K (2010) *Rickettsia felis* infection in Sweden: report of two cases with subacute meningitis and review of the literature. Scand J Infect Dis 42:906–909

Llorente MT, Clavel A, Goñi MP, Varea M, Seral C, Becerril R, Suarez L, Gómez-Lus R (2007) Genetic characterization of *Cryptosporidium* species from humans in Spain. Parasitol Int 56: 201–205

Llorente MT, Clavel A, Varea M, Pilar Goñi M, Sahagún J, Olivera S (2006) *Cryptosporidium felis* infection, Spain. Emerg Infect Dis 12:1471–1472

Löe J, Röskaft E (2004) Large carnivores and human safety: A review. Ambio 33:283–288

López-Osorio S, Penagos-Tabares F, Chaparro-Gutiérrez JJ (2020) Prevalence of *Toxocara* spp. in dogs and cats in South America (excluding Brazil). Adv Parasitol 109:743–778

Lötsch F, Obermüller M, Mischlinger J, Mombo-Ngoma G, Groger M, Adegnika AA, Agnandji ST, Schneider R, Auer H, Ramharter M (2016) Seroprevalence of *Toxocara* spp. in a rural population in Central African Gabon. Parasitol Int 65(6 Pt A):632–634

Lötsch F, Grobusch MP (2020) Seroprevalence of *Toxocara* spp. antibodies in humans in Africa: A review. Adv Parasitol 109:483–499

Low JC, Tennant B, Munro D (1996) Multiple-resistant *Salmonella typhimurium* DT104 in cats. Lancet 348:1391

Lucio-Forster A, Griffiths JK, Cama VA, Xiao L, Bowman DD (2010) Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. Trends Parasitol 26:174–179

Lucio-Forster A, Mizhquiri Barbecho JS, Mohammed HO, Kornreich BG, Bowman DD (2016) Comparison of the prevalence of *Toxocara* egg shedding by pet cats and dogs in the U.S.A., 2011-2014. Vet Parasitol Reg Stud Reports 5:1–13

Lukashev AN, Ruzina MN, Akhmadishina LV (2020) *Toxocara* prevalence in dogs, cats and the environment in Russia. Adv Parasitol 109:801–817

Ma G, Holland CV, Wang T, Hofmann A, Fan CK, Maizels RM, Hotez PJ, Gasser RB (2018) Human toxocariasis. Lancet Infect Dis 18:e14–e24

Macpherson CN (2013) The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. Int J Parasitol 43:999–1008

Maggi RG, Ericson M, Mascarelli PE, Bradley JM, Breitschwerdt EB (2013) *Bartonella henselae* bacteremia in a mother and son potentially associated with tick exposure. Parasit Vectors 6:101

Magnaval JF, Michault A, Calon N, Charlet JP (1994) Epidemiology of human toxocariasis in La Réunion. Trans R Soc Trop Med Hyg 88:531–533

Mahajan S, Karikalan M, Chander V, Pawde AM, Saikumar G, Semmaran M, Lakshmi PS, Sharma M, Nandi S, Singh KP, Gupta VK, Singh RK, Sharma GK (2022) Systemic infection of SARS-CoV-2 in free ranging Leopard (*Panthera pardus fusca*) in India. bioRxiv.01.11.475327. https://doi.org/10.1101/2022.01.11.475327

Maina AN, Knobel DL, Jiang J, Halliday J, Feikin DR, Cleaveland S, Ng'ang'a Z, Junghae M, Breiman RF, Richards AL, Njenga MK (2012) *Rickettsia felis* infection in febrile patients, western Kenya, 2007-2010. Emerg Infect Dis 18:328–331

Mansueto P, Pepe I, Cillari E, Arcoleo F, Micalizzi A, Bonura F, Seidita A, Palillo L, Di Gregorio MF, Affronti M, Di Rosa S, Rini G, Vitale G (2012) Prevalence of antibodies anti-*Bartonella henselae* in western Sicily: children, blood donors, and cats. J Immunoassay Immunochem 33: 18–25

Marini RP, Cassiday PK, Venezia J, Shen Z, Buckley EM, Peters Y, Taylor N, Dewhirst FE, Tondella ML, Fox JG (2014) *Corynebacterium ulcerans* in ferrets. Emerg Infect Dis 20: 159–161

Marosevic DV, Berger A, Kahlmeter G, Payer SK, Hörmansdorfer S, Sing A (2020) Antimicrobial susceptibility of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* in Germany 2011-17. J Antimicrob Chemother 75(10):2885–2893

Marrie TJ, Peeling RW, Reid T, De Carolis E (2003) Canadian Community-Acquired Pneumonia Investigators. Chlamydia species as a cause of community-acquired pneumonia in Canada. Eur Respir J 21(5):779–84. https://doi.org/10.1183/09031936.03.00095403. PMID: 12765420.

Maurin M, Fenollar F, Mediannikov O, Davoust B, Devaux C, Raoult D (2021) Current status of putative animal sources of SARS-CoV-2 infection in humans: wildlife, domestic animals and pets. Microorganisms 9(4):868

Martorelli Di Genova B, Wilson SK, Dubey JP, Knoll LJ (2019) Intestinal delta-6-desaturase activity determines host range for *Toxoplasma* sexual reproduction. PLoS Biol 17(8):e3000364

Mathavarajah S, Dellaire G (2020) Lions, tigers and kittens too: ACE2 and susceptibility to COVID-19. Evol Med Public Health 2020(1):109–113

Matos O, Alves M, Xiao L, Cama V, Antunes F (2004) *Cryptosporidium felis* and *C. meleagridis* in persons with HIV, Portugal. Emerg Infect Dis 10:2256–2257

Mattos-Guaraldi AL, Sampaio JL, Santos CS, Pimenta FP, Pereira GA, Pacheco LG, Miyoshi A, Azevedo V, Moreira LO, Gutierrez FL, Costa JL, Costa-Filho R, Damasco PV, Camello TC, Hirata R Jr (2008) First detection of *Corynebacterium ulcerans* producing a diphtheria-like toxin in a case of human with pulmonary infection in the Rio de Janeiro metropolitan area, Brazil. Mem Inst Oswaldo Cruz 103:396–400

Mawuntu AHP, Johar E, Anggraeni R, Feliana F, Bernadus JBB, Safari D, Yudhaputri FA, Dhenni R, Dewi YP, Kato C, Powers AM, Rosenberg R, Soebandrio A, Myint KSA (2020) *Rickettsia felis* identified in two fatal cases of acute meningoencephalitis. PLoS Negl Trop Dis 14(2):e0007893

McAloose D, Laverack M, Wang L, Killian ML, Caserta LC, Yuan F, Mitchell PK, Queen K, Mauldin MR, Cronk BD, Bartlett SL, Sykes JM, Zec S, Stokol T, Ingerman K, Delaney MA, Fredrickson R, Ivančić M, Jenkins-Moore M, Mozingo K, Franzen K, Bergeson NH, Goodman L, Wang H, Fang Y, Olmstead C, McCann C, Thomas P, Goodrich E, Elvinger F, Smith DC, Tong S, Slavinski S, Calle PP, Terio K, Torchetti MK, Diel DG (2020) From people to *Panthera*: Natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. mBio 11(5): e02220–e02220

McElroy KM, Blagburn BL, Breitschwerdt EB, Mead PS, McQuiston JH (2010) Flea-associated zoonotic diseases of cats in the USA: bartonellosis, flea-borne rickettsioses, and plague. Trends Parasitol 26:197–204

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) Food-related illness and death in the United States. Emerg Infect Dis 5:607–625

Mediannikov O, Fenollar F, Bassene H, Tall A, Sokhna C, Trape JF, Raoult D (2013a) Description of "yaaf", the vesicular fever caused by acute *Rickettsia felis* infection in Senegal. J Infect 66: 536–540

Mediannikov O, Socolovschi C, Edouard S, Fenollar F, Mouffok N, Bassene H, Diatta G, Tall A, Niangaly H, Doumbo O, Lekana-Douki JB, Znazen A, Sarih M, Ratmanov P, Richet H, Ndiath MO, Sokhna C, Parola P, Raoult D (2013b) Common epidemiology of *Rickettsia felis* infection and malaria, Africa. Emerg Infect Dis 19:1775–1783

Meekins DA, Gaudreault NN, Richt JA (2021) Natural and experimental SARS-CoV-2 infection in domestic and wild animals. Viruses 13(10):1993

Meinel DM, Margos G, Konrad R, Krebs S, Blum H, Sing A (2014) Next generation sequencing analysis of nine *Corynebacterium ulcerans* isolates reveals zoonotic transmission and a novel putative diphtheria toxin-encoding pathogenicity island. Genome Med 6(11):113

Meira AR (2010) Alfonso Splendore: facetas da vida do descobridor do *Toxoplasma*. (Alfonso Splendore: facets of life of the *Toxoplasma* discoverer). Sci Med (Porto Alegre) 20(1):9–12. (in Portuguese)

Mekaru SR, Marks SL, Felley AJ, Chouicha N, Kass PH (2007) Comparison of direct immunofluorescence, immunoassays, and fecal flotation for detection of *Cryptosporidium* spp. and *Giardia*

spp. in naturally exposed cats in 4 Northern California animal shelters. J Vet Intern Med 21:959–965

Meng XZ, Li MY, Lyu C, Qin YF, Zhao ZY, Yang XB, Ma N, Zhang Y, Zhao Q (2021) The global prevalence and risk factors of *Cryptosporidium* infection among cats during 1988-2021: A systematic review and meta-analysis. Microb Pathog 158:105096

Mehta OP, Bhandari P, Raut A, Kacimi SEO, Huy NT (2021) Coronavirus Disease (COVID-19): comprehensive review of clinical presentation. Front Public Health 8:582932

Meyerowitz-Katz G, Merone L (2020) A systematic review and meta-analysis of published research data on COVID-19 infection fatality rates. Int J Infect Dis 101:138–148

Michelitsch A, Hoffmann D, Wernike K, Beer M (2020) Occurrence of antibodies against SARS-CoV-2 in the domestic cat population of Germany. Vaccines (Basel) 8(4):772

Michelitsch A, Schön J, Hoffmann D, Beer M, Wernike K (2021) The second wave of SARS-CoV-2 circulation-antibody detection in the domestic cat population in Germany. Viruses 13(6):1009

Mietze A, Morick D, Köhler H, Harrus S, Dehio C, Nolte I, Goethe R (2011) Combined MLST and AFLP typing of *Bartonella henselae* isolated from cats reveals new sequence types and suggests clonal evolution. Vet Microbiol 148:238–245

Millán J, Rodríguez A (2009) A serological survey of common feline pathogens in free-living European wildcats (*Felis silvestris*) in central Spain. Eur J Wildl Res 55:285–291

Milne G, Webster JP, Walker M (2020) Toward improving interventions against toxoplasmosis by identifying routes of transmission using sporozoite-specific serological tools. Clin Infect Dis 71: e686–e693

Mishra A, Kumar N, Bhatia S, Aasdev A, Kanniappan S, Sekhar AT, Gopinadhan A, Silambarasan R, Sreekumar C, Dubey CK, Tripathi M, Raut AA, Singh VP (2021) SARS-CoV-2 Delta variant among Asiatic Lions, India. Emerg Infect Dis 27(10):2723–2725

Miyashita N, Fukano H, Mouri K, Fukuda M, Yoshida K, Kobashi Y, Niki Y, Oka M (2005) Community-acquired pneumonia in Japan: a prospective ambulatory and hospitalized patient study. J Med Microbiol 54:395–400

Mizgajska-Wiktor H, Jarosz W, Fogt-Wyrwas R, Drzewiecka A (2017) Distribution and dynamics of soil contamination with *Toxocara canis* and *Toxocara cati* eggs in Poland and prevention measures proposed after 20 years of study. Vet Parasitol 234:1–9

Möbius N (2013) Identifikation von Zoonoseerregern bei Haustieren und Bewertung des humanen Infektionsrisikos. http://elib.tiho-hannover.de/dissertations/moebiusn_ss13.pdf. Accessed 29 Jan 2014

Möller J, Musella L, Melnikov V, Geißdörfer W, Burkovski A, Sangal V (2020) Phylogenomic characterisation of a novel corynebacterial species pathogenic to animals. Antonie Van Leeuwenhoek. 113(8):1225–1239

Mohebali M, Hassanpour G, Zainali M, Gouya MM, Khayatzadeh S, Parsaei M, Sarafraz N, Hassanzadeh M, Azarm A, Salehi-Vaziri M, Sasani F, Heidari Z, Jalali T, Pouriayevali MH, Shoja Z, Ahmadi Z, Sadjadi M, Tavakoli M, Azad-Manjiri S, Karami C, Zarei Z (2022) SARS-CoV-2 in domestic cats (Felis catus) in the northwest of Iran: Evidence for SARS-CoV-2 circulating between human and cats. Virus Res 310:198673. https://doi.org/10.1016/j.virusres.2022.198673. Epub ahead of print

Mokrani K, Tebbal S, Raoult D, Fournier PE (2012) Human rickettsioses in the Batna area, eastern Algeria. Ticks Tick Borne Dis 3:364–366

Molia S, Chomel BB, Kasten RW, Leutenegger CM, Steele BR, Marker L, Martenson JS, Keet DF, Bengis RG, Peterson RP, Munson L, O'Brien SJ (2004) Prevalence of *Bartonella* infection in wild African lions (*Panthera leo*) and cheetahs (*Acinonyx jubatus*). Vet Microbiol 100:31–41

Montazeri M, Mikaeili Galeh T, Moosazadeh M, Sarvi S, Dodangeh S, Javidnia J, Sharif M, Daryani A (2020) The global serological prevalence of *Toxoplasma gondii* in felids during the last five decades (1967-2017): a systematic review and meta-analysis. Parasit Vectors 13:82

Morato EP, Leomil L, Beutin L, Krause G, Moura RA, Pestana de Castro AF (2009) Domestic cats constitute a natural reservoir of human enteropathogenic *Escherichia coli* types. Zoonoses Public Health 56:229–237

Morelli S, Crisi PE, Di Cesare A, De Santis F, Barlaam A, Santoprete G, Parrinello C, Palermo S, Mancini P, Traversa D (2019) Exposure of client-owned cats to zoonotic vector-borne pathogens: Clinic-pathological alterations and infection risk analysis. Comp Immunol Microbiol Infect Dis 66:101344

Morelli S, Diakou A, Di Cesare A, Colombo M, Traversa D (2021) Canine and Feline Parasitology: Analogies, Differences, and Relevance for Human Health. Clin Microbiol Rev 34(4):e0026620

Morgan ER, Azam D, Pegler K (2013) Quantifying sources of environmental contamination with Toxocara spp. eggs. Vet Parasitol 193(4):390–7. https://doi.org/10.1016/j.vetpar.2012.12.034. Epub 2012 Dec 22. PMID: 23333071.

Morgan U, Weber R, Xiao L, Sulaiman I, Thompson RC, Ndiritu W, Lal A, Moore A, Deplazes P (2000) Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya, and the United States. J Clin Microbiol 38:1180–1183

Morganti G, Veronesi F, Stefanetti V, Di Muccio T, Fiorentino E, Diaferia M, Santoro A, Passamonti F, Gramiccia M (2019) Emerging feline vector-borne pathogens in Italy. Parasit Vectors 12(1):193

Morgenthal D, Hamel D, Arndt G, Silaghi C, Pfister K, Kempf VAJ et al (2012) Prevalence of haemotropic *Mycoplasma* spp., *Bartonella* spp. and *Anaplasma phagocytophilum* in cats in Berlin/Brandenburg (Northeast Germany). Berl Munch Tierarztl Wochenschr 125:418–427

Mosbacher ME, Klotz S, Klotz J, Pinnas JL (2011) *Bartonella henselae* and the potential for arthropod vector-borne transmission. Vector Borne Zoonotic Dis 11:471–477

Musso N, Costantino A, La Spina S, Finocchiaro A, Andronico F, Stracquadanio S, Liotta L, Visalli R, Emmanuele G (2020) New SARS-CoV-2 infection detected in an Italian pet cat by RT-qPCR from deep pharyngeal swab. Pathogens 9(9):746

Müller A, Walker R, Bittencourt P, Machado RZ, Benevenute JL, Do Amaral RB et al (2017) Prevalence, hematological findings and genetic diversity of *Bartonella* spp. in domestic cats from Valdivia, southern Chile. Parasitology 144:773–782

Müller A, Reiter M, Schötta AM, Stockinger H, Stanek G (2016) Detection of *Bartonella* spp. in Ixodes ricinus ticks and *Bartonella* seroprevalence in human populations. Ticks Tick Borne Dis 7:763–767

Mughini Gras L, Smid JH, Wagenaar JA, Koene MG, Havelaar AH, Friesema IH, French NP, Flemming C, Galson JD, Graziani C, Busani L, Pelt VAN, W. (2013) Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. Epidemiol Infect 41: 2526–2535

Munday BL (1972) Serological evidence of *Toxoplasma* infection in isolated groups of sheep. Res Vet Sci 13:100–102

Murray JK, Browne WJ, Roberts MA, Whitmarsh A, Gruffydd-Jones TJ (2010) Number and ownership profiles of cats and dogs in the UK. Vet Rec 166:163–168

Muz MN, Erat S, Mumcuoglu KY (2021) Protozoan and microbial pathogens of house cats in the province of Tekirdag in Western Turkey. Pathogens 10(9):1114

Nasisse MP, Guy JS, Stevens JB, English RV, Davidson MG (1993) Clinical and laboratory findings in chronic conjunctivitis in cats: 91 cases (1983-1991). J Am Vet Med Assoc 203:834–837

Negri EC, Santarém VA, Rubinsky-Elefant G, Giuffrida R (2013) Anti-*Toxocara* spp. antibodies in an adult healthy population: serosurvey and risk factors in Southeast Brazil. *Asian Pac*. J Trop Biomed 3(3):211–216

Neira V, Brito B, Agüero B, Berrios F, Valdés V, Gutierrez A, Ariyama N, Espinoza P, Retamal P, Holmes EC, Gonzalez-Reiche AS, Khan Z, van de Guchte A, Dutta J, Miorin L, Kehrer T, Galarce N, Almonacid LI, Levican J, van Bakel H, García-Sastre A, Medina RA (2021) A household case evidences shorter shedding of SARS-CoV-2 in naturally infected cats compared to their human owners. Emerg Microbes Infect 10(1):376–383

Nelson CA, Saha S, Mead PS (2016) Cat-scratch disease in the United States, 2005–2013. Emerg Infect Dis 22:1741–1746

Newman A, Smith D, Ghai RR, Wallace RM, Torchetti MK, Loiacono C, Murrell LS, Carpenter A, Moroff S, Rooney JA, Barton BC (2020) First reported cases of SARS-CoV-2 infection in

companion animals - New York, March-April 2020. MMWR Morb Mortal Wkly Rep 69(23): 710–713
Ng-Nguyen D, Hii SF, Hoang MT, Nguyen VT, Rees R, Stenos J, Traub RJ (2020) Domestic dogs are mammalian reservoirs for the emerging zoonosis flea-borne spotted fever, caused by *Rickettsia felis*. Sci Rep 10(1):4151
Nicholas N (1999) A Conundrum of Cats: Pards and their Relatives in Byzantium. Greek, Roman, and Byzantine Studies 40:253–298
Nicoletti A, Bartoloni A, Sofia V, Mantella A, Nsengiyumva G, Frescaline G, Preux PM (2007) Epilepsy and toxocariasis: a case-control study in Burundi. Epilepsia 48:894–899
Nijsse R, Mughini-Gras L, Wagenaar JA, Franssen F, Ploeger HW (2015) Environmental contamination with *Toxocara* eggs: a quantitative approach to estimate the relative contributions of dogs, cats and foxes, and to assess the efficacy of advised interventions in dogs. Parasit Vectors 8:397
Nishiura H, Ito K, Anzai A, Kobayashi T, Piantham C, Rodríguez-Morales AJ (2021) Relative reproduction number of SARS-CoV-2 Omicron (B.1.1.529) compared with Delta variant in South Africa. J Clin Med 11(1):30. https://doi.org/10.3390/jcm11010030
Noah DL, Kramer CM, Verbsky MP, Rooney JA, Smith KA, Childs JE (1997) Survey of veterinary professionals and other veterinary conference attendees for antibodies to *Bartonella henselae* and *B. quintana*. J Am Vet Med Assoc 210:342–344
Noden BH, Davidson S, Smith JL, Williams F (2017) First Detection of *Rickettsia typhi* and *Rickettsia felis* in fleas collected from client-owned companion animals in the Southern Great Plains. J Med Entomol 54(4):1093–1097
Nogareda F, Le Strat Y, Villena I, De Valk H, Goulet V (2014) Incidence and prevalence of *Toxoplasma gondii* infection in women in France, 1980-2020: model-based estimation. Epidemiol Infect 142(8):1661–1670
Nogueras MM, Cardeñosa N, Sanfeliu I, Muñoz T, Font B, Segura F (2006) Serological evidence of infection with *Rickettsia typhi* and *Rickettsia felis* among the human population of Catalonia, in the northeast of Spain. Am J Trop Med Hyg 74:123–126
Nogueras MM, Pons I, Sanfeliu I, Sala M, Segura F (2014) Serosurvey of *Rickettsia typhi* and *Rickettsia felis* in HIV-infected patients. Microbiol Immunol 58(4):257–259
Nowotny N, Deutz A, Fuchs K, Schuller W, Hinterdorfer F, Auer H, Aspöck H (1997) Prevalence of swine influenza and other viral, bacterial, and parasitic zoonoses in veterinarians. J Infect Dis 176:1414–1415
Nutter FB, Dubey JP, Levine JF, Breitschwerdt EB, Ford RB, Stoskopf MK (2004) Seroprevalences of antibodies against *Bartonella henselae* and *Toxoplasma gondii* and fecal shedding of *Cryptosporidium* spp, *Giardia* spp, and *Toxocara cati* in feral and pet domestic cats. J Am Vet Med Assoc 225:1394–1398
Öge H, Öge S, Özbakış G, Gürcan S (2014) Comparison of *Toxocara* eggs in hair and faecal samples from owned dogs and cats collected in Ankara, Turkey. Vet Parasitol 206:227–231
Oh YI, Seo KW, Kim DH, Cheon DS (2021) Prevalence, co-infection and seasonality of fecal enteropathogens from diarrheic cats in the Republic of Korea (2016-2019): a retrospective study. BMC Vet Res 17:367
OIE (World Organisation for Animal Health) (2021) SARS-CoV-2 in animals. Situation reports. https://www.oie.int/en/what-we-offer/emergency-and-resilience/covid-19/#ui-id-3. Accessed 11 Jan 2022
Okaro U, Addisu A, Casanas B, Andreson B (2017 Jul) *Bartonella* Species, an Emerging Cause of Blood-Culture-Negative Endocarditis. Clin Microbiol Rev 30(3):709–746
Omonijo AO, Kalinda C, Mukaratirwa S (2019) A systematic review and meta-analysis of canine, feline and human *Toxocara* infections in sub-Saharan Africa. J Helminthol 94:e96
Oreshkova N, Molenaar RJ, Vreman S, Harders F, Oude Munnink BB, Hakze-van der Honing RW, Gerhards N, Tolsma P, Bouwstra R, Sikkema RS, Tacken MG, de Rooij MM, Weesendorp E, Engelsma MY, Bruschke CJ, Smit LA, Koopmans M, van der Poel WH, Stegeman A (2020) SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill. 25(23):2001005

Ostanello F, Gherardi A, Caprioli A, La Placa L, Passini A, Prosperi S (2005) Incidence of injuries caused by dogs and cats treated in emergency departments in a major Italian city. Emerg Med J 22:260–262

Otero D, Alho AM, Nijsse R, Roelfsema J, Overgaauw P, Madeira de Carvalho L (2018) Environmental contamination with *Toxocara* spp. eggs in public parks and playground sandpits of Greater Lisbon, Portugal. J Infect Public Health 11(1):94–98

Overgaauw PA (1997) Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. Crit Rev Microbiol 23:233–251

Overgaauw PA, van Zutphen L, Hoek D, Yaya FO, Roelfsema J, Pinelli E, van Knapen F, Kortbeek LM (2009) Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. Vet Parasitol 163:115–122

Overgaauw PA, van Knapen F (2013) Veterinary and public health aspects of *Toxocara* spp. Vet Parasitol 193:398–403

Overgaauw P, Nijsse R (2020) Prevalence of patent *Toxocara* spp. infections in dogs and cats in Europe from 1994 to 2019. Adv Parasitol 109:779–800

Paller VG, de Chavez ER (2014) *Toxocara* (Nematoda: Ascaridida) and other soil-transmitted helminth eggs contaminating soils in selected urban and rural areas in the Philippines. ScientificWorldJournal 2014:386232

Paltrinieri S, Giordano A, Stranieri A, Lauzi S (2021) Feline infectious peritonitis (FIP) and coronavirus disease 19 (COVID-19): Are they similar? Transbound Emerg Dis 68(4):1786–1799

Paludo ML, Falavigna DL, Elefant GR, Gomes ML, Baggio ML, Amadei LB, Falavigna-Guilherme AL (2007) Frequency of *Toxocara* infection in children attended by the health public service of Maringá, south Brazil. Rev Inst Med Trop Sao Paulo 49:343–348

Palmer CS, Thompson RC, Traub RJ, Rees R, Robertson ID (2008) National study of the gastrointestinal parasites of dogs and cats in Australia. Vet Parasitol 151:181–190

Pandat S, Zhu Z, Fuentes-Rojas S, Schurmann P (2021) Arrhythmias in COVID-19. Methodist Debakey Cardiovasc J 17(5):73–82

Paris JK, Wills S, Balzer HJ, Shaw DJ, Gunn-Moore DA (2014) Enteropathogen co-infection in UK cats with diarrhoea. BMC Vet Res 10:13

Parkhe P, Verma S (2021) Evolution, interspecies transmission, and zoonotic significance of animal coronaviruses. Front Vet Sci 8:719834

Parliament of the Commonwealth of Australia (2020) Tackling the feral cat pandemic: a plan to save Australian wildlife. Report of the inquiry into the problem of feral and domestic cats in Australia. www.aph.gov.au/Parliamentary_Business/Committees/House/Environment_and_Energy/Feralanddomesticcats/Report. Accessed 8 Oct 2021

Parola P (2011) *Rickettsia felis*: from a rare disease in the USA to a common cause of fever in sub-Saharan Africa. Clin Microbiol Infect 17:996–1000

Patania OM, Chiba S, Halfmann PJ, Hatta M, Maemura T, Bernard KA, Kawaoka Y, Crawford LK (2021) Pulmonary lesions induced by SARS-CoV-2 infection in domestic cats. Vet Pathol. https://doi.org/10.1177/03009858211066840. Epub ahead of print

Patterson EI, Elia G, Grassi A, Giordano A, Desario C, Medardo M, Smith SL, Anderson ER, Prince T, Patterson GT, Lorusso E, Lucente MS, Lanave G, Lauzi S, Bonfanti U, Stranieri A, Martella V, Solari Basano F, Barrs VR, Radford AD, Agrimi U, Hughes GL, Paltrinieri S, Decaro N (2020) Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat Commun 11(1):6231

Pawełczyk A, Bednarska M, Kowalska JD, Uszyńska-Kałuża B, Radkowski M, Welc-Falęciak R (2019) Seroprevalence of six pathogens transmitted by the *Ixodes ricinus* ticks in asymptomatic individuals with HIV infection and in blood donors. Sci Rep 9(1):2117

Pedersen MG, Mortensen PB, Norgaard-Pedersen B, Postolache TT (2012) *Toxoplasma gondii* Infection and Self-directed Violence in Mothers. Arch Gen Psychiatry 69:1123–1130

Pedraza-Díaz S, Amar C, Iversen AM, Stanley PJ, McLauchlin J (2001) Unusual cryptosporidium species recovered from human faeces: first description of *Cryptosporidium felis* and *Cryptosporidium* 'dog type' from patients in England. J Med Microbiol 50(3):293–296

Pérez JE, Estrada GI, Zapata Y, Hidalgo M, Serna CC, Castro DC, González C (2021) Frequency of antibodies and seroconversion against *Rickettsia* spp in patients consulting health institutions in the department of Caldas, Colombia, 2016-2019. Biomedica 41(Sp. 2):103–117

Persad AK, LeJeune JT (2014) Animal reservoirs of Shiga Toxin-producing *Escherichia coli*. Microbiol Spectr 2:EHEC-0027-2014

Peterhans K, Gnoske LC, Gnoske TP (2001) The science of "man-eating" among lions (*Panthera leo*) with a reconstruction of the natural history of the "man-eaters" of Tsavo. J East African Nat Hist 90:1–40

Petersen E, Vesco G, Villari S, Buffolano W (2010) What do we know about risk factors for infection in humans with *Toxoplasma gondii* and how can we prevent infections? Zoonoses Public Health 57:8–17

Pérez-Osorio CE, Zavala-Velázquez JE, Arias León JJ, Zavala-Castro JE (2008) *Rickettsia felis* as emergent global threat for humans. Emerg Infect Dis 14:1019–1023

Petney TN, Andrews RH, Saijuntha W, Wenz-Mücke A, Sithithaworn P (2013) The zoonotic, fish-borne liver flukes *Clonorchis sinensis, Opisthorchis felineus* and *Opisthorchis viverrini*. Int J Parasitol 43:1031–1046

Phasuk N, Punsawad C (2020) Seroprevalence of *Toxocara canis* infection and associated risk factors among primary schoolchildren in rural Southern Thailand. Trop Med Health 48:23

Philbey AW, Brown FM, Mather HA, Coia JE, Taylor DJ (2009) Salmonellosis in cats in the United Kingdom: 1955 to 2007. Vet Rec 164:120–122

PHLS. Toxigenic Corynebacterium ulcerans in cats. Comm Dis Rep CDR Wkly [serial online] 2002

Pinto-Ferreira F, Caldart ET, Pasquali AKS, Mitsuka-Breganó R, Freire RL, Navarro IT (2019) Patterns of transmission and sources of infection in outbreaks of human toxoplasmosis. Emerg Infect Dis 25:2177–2182

Piplani S, Singh PK, Winkler DA, Petrovsky N (2021) In silico comparison of SARS-CoV-2 spike protein-ACE2 binding affinities across species and implications for virus origin. Sci Rep 11(1): 13063

Pomorska-Mól M, Turlewicz-Podbielska H, Gogulski M, Ruszkowski JJ, Kubiak M, Kuriga A, Barket P, Postrzech M (2021) A cross-sectional retrospective study of SARS-CoV-2 seroprevalence in domestic cats, dogs and rabbits in Poland. BMC Vet Res 17(1):322

Ponce-Macotela M, Martínez-Gordillo MN (2020) *Toxocara*: Seroprevalence in Mexico. Adv Parasitol 109:341–355

Pons I, Sanfeliu I, Nogueras MM, Sala M, Cervantes M, Amengual MJ, Segura F (2008) Seroprevalence of *Bartonella* spp. infection in HIV patients in Catalonia, Spain. BMC Infect Dis 8(58)

Pons I, Sanfeliu I, Quesada M, Anton E, Sampere M, Font B, Pla J, Segura F (2005) Prevalence of *Bartonella henselae* in cats in Catalonia, Spain. Am J Trop Med Hyg 72:453–457

Postolache TT, Wadhawan A, Rujescu D, Hoisington AJ, Dagdag A, Baca-Garcia E, Lowry CA, Okusaga OO, Brenner LA (2021) *Toxoplasma gondii*, suicidal behavior, and intermediate phenotypes for suicidal behavior. Front Psychiatry 12:665682

Poulsen CS, Skov S, Yoshida A, Skallerup P, Maruyama H, Thamsborg SM, Nejsum P (2015) Differential serodiagnostics of *Toxocara canis* and *Toxocara cati*--is it possible? Parasite Immunol 37:204–207

Pozio E, Armignacco O, Ferri F, Gomez Morales MA (2013) *Opisthorchis felineus*, an emerging infection in Italy and its implication for the European Union. Acta Trop 126:54–62

Prestrud KW, Asbakk K, Fuglei E, Mørk T, Stien A, Ropstad E, Tryland M, Gabrielsen GW, Lydersen C, Kovacs KM, Loonen MJ, Sagerup K, Oksanen A (2007) Serosurvey for *Toxoplasma gondii* in arctic foxes and possible sources of infection in the high Arctic of Svalbard. Vet Parasitol 150(1-2):6–12

Pretorius AM, Kuyl JM, Isherwood DR, Birtles RJ (2004) *Bartonella henselae* in African lion, South Africa. Emerg Infect Dis 10:2257–2258
Prutsky G, Domecq JP, Mori L, Bebko S, Matzumura M, Sabouni A, Shahrour A, Erwin PJ, Boyce TG, Montori VM, Malaga G, Murad MH (2013) Treatment outcomes of human bartonellosis: a systematic review and meta-analysis. Int J Infect Dis 17(10):e811–e819
Public Health England (PHE) – Diphtheria Guidelines Working Group: Public health control and management of diphtheria (in England and Wales). 2015
Raccurt CP (2007) La cryptosporidiose zoonosique humaine due à *Cryptosporidium felis* dans le monde. Parasite 14:15–20
Rai SK, Uga S, Ono K, Nakanishi M, Shrestha HG, Matsumura T (1996) Seroepidemiological study of *Toxocara* infection in Nepal. Southeast Asian J Trop Med Public Health 27:286–290
Ramakrishnan C, Maier S, Walker RA, Rehrauer H, Joekel DE, Winiger RR, Basso WU, Grigg ME, Hehl AB, Deplazes P, Smith NC (2019) An experimental genetically attenuated live vaccine to prevent transmission of *Toxoplasma gondii* by cats. Sci Rep 9(1):1474
Rambozzi L, Menzano A, Mannelli A, Romano S, Isaia MC (2007) Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors. J Feline Med Surg 9:392–396
Ramírez-Ocampo S, Cotte-Alzate JD, Escobedo ÁA, Rodríguez-Morales AJ (2017) Prevalence of zoonotic and non-zoonotic genotypes of *Giardia intestinalis* in cats: a systematic review and meta-analysis. Infez Med 25(4):326–338
Rath PM, von Recklinghausen G, Ansorg R (1997) Seroprevalence of immunoglobulin G antibodies to *Bartonella henselae* in cat owners. Eur J Clin Microbiol Infect Dis 16:326–327
Rau J, Eisenberg T, Peters M, Berger A, Kutzer P, Lassnig H, Hotzel H, Sing A, Sting R, Contzen M (2019) Reliable differentiation of a non-toxigenic tox gene-bearing *Corynebacterium ulcerans* variant frequently isolated from game animals using MALDI-TOF MS. Vet Microbiol 237:108399
Ravicini S, Pastor J, Hawley J, Brewer M, Castro-López J, Beall M, Lappin MR (2016) Prevalence of selected infectious disease agents in stray cats in Catalonia, Spain. JFMS Open Rep 2(1): 2055116916634109
Rawal BD (1959) Toxoplasmosis: a dye-test survey on sera from vegetarians and meat eaters in Bombay. Trans R Soc Trop Med Hyg 53:61–63
Regier Y, Rourke OF, Kempf VA (2016) *Bartonella* spp. - a chance to establish One Health concepts in veterinary and human medicine. Parasit Vectors 9(1):261
Regier Y, Komma K, Weigel M, Kraiczy P, Laisi A, Pulliainen AT, Hain T, Kempf VAJ (2019) Combination of microbiome analysis and serodiagnostics to assess the risk of pathogen transmission by ticks to humans and animals in central Germany. Parasit Vectors 12(1):11
Reif KE, Macaluso KR (2009) Ecology of *Rickettsia felis*: a review. J Med Entomol 46:723–736
Reis C, Cote M, Le Rhun D, Lecuelle B, Levin ML, Vayssier-Taussat M, Bonnet SI (2011) Vector competence of the tick *Ixodes ricinus* for transmission of *Bartonella birtlesii*. PLoS Negl Trop Dis 5:e1186
Renvoisé A, Mediannikov O, Raoult D (2009) Old and new tick-borne rickettsioses. Int Health 1 (1):17–25. https://doi.org/10.1016/j.inhe.2009.03.003. PMID: 24036291.
Reynolds MG, Holman RC, Curns AT, O'Reilly M, McQuiston JH, Steiner CA (2005) Epidemiology of cat-scratch disease hospitalizations among children in the United States. Pediatr Infect Dis J 24:700–704
Richards AL, Jiang J, Omulo S, Dare R, Abdirahman K, Ali A, Sharif SK, Feikin DR, Breiman RF, Njenga MK (2010) Human Infection with *Rickettsia felis,* Kenya. Emerg Infect Dis 16:1081–1086
Richter SH (1981) Ormar I hundum (Worms in dogs). Dýraverndarinn 67:15–16. (in Icelandic)
Riegel P, Ruimy R, de Briel D, Prévost G, Jehl F, Christen R, Monteil H (1995) Taxonomy of *Corynebacterium diphtheriae* and related taxa, with recognition of *Corynebacterium ulcerans* sp. nov. nom. rev. FEMS Microbiol Lett 126:271–276
Rivolta S (1884) Sopra una specie di *Distoma* nel gatto e nel cane. Giorn Anat Fisiol Patol Anim 16: 20–28
Rodan KS, Buckley JJ (1969) Infection with adult *Toxocara cati*. Br Med J 2(5650):188

Roghmann MC, Faulkner CT, Lefkowitz A, Patton S, Zimmerman J, Morris JG Jr (1999) Decreased seroprevalence for *Toxoplasma gondii* in Seventh Day Adventists in Maryland. Am J Trop Med Hyg 60:790–792

Rojas-Lopez L, Elwin K, Chalmers RM, Enemark HL, Beser J, Troell K (2020) Development of a *gp60*-subtyping method for *Cryptosporidium felis*. Parasit Vectors 13(1):39

Rolain JM, Brouqui P, Koehler JE, Maguina C, Dolan MJ, Raoult D (2004) Recommendations for treatment of human infections caused by *Bartonella* species. Antimicrob Agents Chemother 48: 1921–1933

Rostami A, Ma G, Wang T, Koehler AV, Hofmann A, Chang BCH, Macpherson CN, Gasser RB (2019) Human toxocariasis - A look at a neglected disease through an epidemiological 'prism'. Infect Genet Evol 74:104002

Rostami A, Sepidarkish M, Ma G, Wang T, Ebrahimi M, Fakhri Y, Mirjalali H, Hofmann A, Macpherson CNL, Hotez PJ, Gasser RB (2020) Global prevalence of *Toxocara* infection in cats. Adv Parasitol 109:615–639

Rotstein DS, Taylor SK, Bradley J, Breitschwerdt EB (2000) Prevalence of *Bartonella henselae* antibody in Florida panthers. J Wildl Dis 36:157–160

Rowan AN (2018) Companion Animal Statistics in the USA. www.wellbeingintlstudiesrepository.org/cgi/viewcontent.cgi?article=1002&context=demscapop. Accessed 8 Oct 2021

Rozental T, Ferreira MS, Guterres A, Mares-Guia MA, Teixeira BR, Gonçalves J, Bonvicino CR, D'Andrea PS, de Lemos ER (2017) Zoonotic pathogens in Atlantic Forest wild rodents in Brazil: *Bartonella* and *Coxiella* infections. Acta Trop 168:64–73

Rubinsky-Elefant G, da Silva-Nunes M, Malafronte RS, Muniz PT, Ferreira MU (2008) Human toxocariasis in rural Brazilian Amazonia: seroprevalence, risk factors, and spatial distribution. Am J Trop Med Hyg 79:93–98

Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU (2010) Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann Trop Med Parasitol 104:3–23

Ruiz-Arrondo I, Portillo A, Palomar AM, Santibáñez S, Santibáñez P, Cervera C, Oteo JA (2021) Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: A case of an asymptomatic cat with SARS-CoV-2 in Europe. Transbound Emerg Dis 68(2):973–976

Rumi MV, Irino K, Deza N, Huguet MJ, Bentancor AB (2012) First isolation in Argentina of a highly virulent Shiga toxin-producing *Escherichia coli* O145:NM from a domestic cat. J Infect Dev Ctries 6:358–363

Ryan U, Fayer R, Xiao L (2014) *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141(13):1667–1685

Ryan UM, Feng Y, Fayer R, Xiao L (2021) Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia* - a 50 year perspective (1971-2021). Int J Parasitol S0020-7519(21):00296–00294

Sachse K, Bavoil PM, Kaltenboeck B, Stephens RS, Kuo CC, Rosselló-Móra R, Horn M (2015) Emendation of the family *Chlamydiaceae*: proposal of a single genus, *Chlamydia*, to include all currently recognized species. Syst Appl Microbiol 38(2):99–103

Sailleau C, Dumarest M, Vanhomwegen J, Delaplace M, Caro V, Kwasiborski A, Hourdel V, Chevaillier P, Barbarino A, Comtet L, Pourquier P, Klonjkowski B, Manuguerra JC, Zientara S, Le Poder S (2020) First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis 67(6):2324–2328

Sandholt B (ed) (2004) Dýralæknatal Búfjársjúkdómar og saga. Dýralæknafélag Íslands, (in Icelandic)

Santín M, Trout JM, Vecino JA, Dubey JP, Fayer R (2006) *Cryptosporidium, Giardia* and *Enterocytozoon bieneusi* in cats from Bogota (Colombia) and genotyping of isolates. Vet Parasitol 141:334–339

Saunders J, Parast L, Babey SH, Miles JV (2017) Exploring the differences between pet and non-pet owners: Implications for human-animal interaction research and policy. PLoS One 12(6): e0179494

Scaramozzino P, Condoleo R, Martini E, Bossu T, Aquilani S, Spallucci V, Aquilini E, Marozzi S (2018) Behaviour and eating habits as determinants for human opisthorchiasis in the Bolsena Lake area, Italy. Folia Parasitol (Praha) 2018(65):013

Schrank F (1788) Verzeichniß der bisher hinlänglich bekannten Eingeweidewürmer, nebst einer Abhandlung über ihre Anverwandtschaften. Johann Baptist Strobl, München

Schuhegger R, Kugler R, Sing A (2008) Pitfalls with diphtheria-like illness due to toxigenic *Corynebacterium ulcerans*. Clin Infect Dis 47:288

Schuhegger R, Schoerner C, Dlugaiczyk J, Lichtenfeld I, Trouillier A, Zeller-Peronnet V, Busch U, Berger A, Kugler R, Hormansdorfer S, Sing A (2009) Pigs as source for toxigenic *Corynebacterium ulcerans*. Emerg Infect Dis 15:1314–1315

Schulz C, Wylezich C, Wernike K, Gründl M, Dangel A, Baechlein C, Hoffmann D, Röhrs S, Hepner S, Ackermann N, Sing A, Pink I, Länger B, Volk HA, Becher P, Sutter G, Neubauer-Juric A, von Köckritz-Blickwede M, Beer M, Volz A (2021a) Prolonged SARS-CoV-2 RNA shedding from therapy cat after cluster outbreak in retirement home. Emerg Infect Dis 27(7):1974–1976

Schulz C, Martina B, Mirolo M, Müller E, Klein R, Volk H, Egberink H, Gonzalez-Hernandez M, Kaiser F, von Köckritz-Blickwede M, Osterhaus A (2021b) SARS-CoV-2-specific antibodies in domestic cats during first COVID-19 wave, Europe. Emerg Infect Dis 27(12):3115–3118. https://doi.org/10.3201/eid2712.211252. Epub ahead of print

Segalés J, Puig M, Rodon J, Avila-Nieto C, Carrillo J, Cantero G, Terrón MT, Cruz S, Parera M, Noguera-Julián M, Izquierdo-Useros N, Guallar V, Vidal E, Valencia A, Blanco I, Blanco J, Clotet B, Vergara-Alert J (2020) Detection of SARS-CoV-2 in a cat owned by a COVID-19-affected patient in Spain. Proc Natl Acad Sci U S A 117(40):24790–24793

Serpell JA (2000) Domestication and history of the cat. In: Turner DC, Bateson P (eds) The Domestic Cat: The Biology of its Behaviour, 2nd edn. Cambridge University Press, Cambridge, pp 179–192

Shafiei R, Rahimi MT, Emameh RZ, Mirzaei M, Perez-Cordon G, Ahmadpour E (2020) Status of human toxocariasis, a neglected parasitic zoonosis in Iran: a systematic review from past to current. Trop Doct 50:285–291

Shepherd SM, Mills A, Shoff WH (2014) Human attacks by large felid carnivores in captivity and in the wild. Wilderness Environ Med 25:220–230

Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y, Tan W, Wu G, Chen H, Bu Z (2020) Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science 368(6494): 1016–1020

Shpynov SN, Fournier PE, Pozdnichenko NN, Gumenuk AS, Skiba AA (2018) New approaches in the systematics of rickettsiae. New Microbes New Infect 23:93–102

Shukla R, Giraldo P, Kraliz A, Finnigan M, Sanchez AL (2006) *Cryptosporidium* spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario. Can Vet J 47:1179–1184

Sianto L, Chaves SAM, Antunes-Ferreira N, Silva ARM (2017) *Toxocara* eggs in an 18th century Franciscan from Portugal. The challenge of differentiating between parasitism and chance in Paleoparasitology. Int J Paleopathol 18:47–51

Sila T, Sunghan J, Laochareonsuk W, Surasombatpattana S, Kongkamol C, Ingviya T, Siripaitoon P, Kositpantawong N, Kanchanasuwan S, Hortiwakul T, Charernmak B, Nwabor OF, Silpapojakul K, Chusri S (2022) Suspected Cat-to-Human Transmission of SARS-CoV-2, Thailand, July-September 2021. Emerg Infect Dis 28(7):1485–1488. https://doi.org/10.3201/eid2807.212605. Epub 2022 Jun 6. PMID: 35666777.

Sing A, Heesemann J (2008) 10 Jahre Konsiliarlaboratorium für Diphtherie: Zur Charakterisierung von *C.-diphtheriae*-verdächtigen Isolaten. Epid. Bull: 23–25 [in German]

Skerget M, Wenisch C, Daxboeck F, Krause R, Haberl R, Stuenzner D (2003) Cat or dog ownership and seroprevalence of ehrlichiosis, Q fever, and cat-scratch disease. Emerg Infect Dis 9:1337–1340

Smith MH, Beaver PC (1953) Persistence and distribution of *Toxocara* larvae in the tissues of children and mice. Pediatrics 12:491–497

Socolovschi C, Mediannikov O, Sokhna C, Tall A, Diatta G, Bassene H, Trape JF, Raoult D (2010) *Rickettsia felis*-associated uneruptive fever, Senegal. Emerg Infect Dis 16:1140–1142

Söderlund R, Jernberg C, Trönnberg L, Pääjärvi A, Ågren E, Lahti E (2019) Linked seasonal outbreaks of *Salmonella* Typhimurium among passerine birds, domestic cats and humans, Sweden, 2009 to 2016. Euro Surveill 24:1900074

Solano-Gallego L, Hegarty B, Espada Y, Llull J, Breitschwerdt E (2006) Serological and molecular evidence of exposure to arthropod-borne organisms in cats from northeastern Spain. Vet Microbiol 118:274–277

Soltani S, Kahvaz MS, Soltani S, Maghsoudi F, Foroutan M (2020) Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in patients undergoing hemodialysis and healthy group. BMC Res Notes 13(1):551

Spada E, Vitale F, Bruno F, Castelli G, Reale S, Perego R, Baggiani L, Proverbio D (2021) A pre- and during pandemic survey of Sars-Cov-2 infection in stray colony and shelter cats from a high endemic area of Northern Italy. Viruses 13(4):618

Spain CV, Scarlett JM, Wade SE, McDonough P (2001) Prevalence of enteric zoonotic agents in cats less than 1 year old in central New York State. J Vet Intern Med 15:33–38

Stevanovic V, Vilibic-Cavlek T, Tabain I, Benvin I, Kovac S, Hruskar Z, Mauric M, Milasincic L, Antolasic L, Skrinjaric A, Staresina V, Barbic L (2021) Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. Transbound Emerg Dis 68(4):1767–1773

Sting R, Ketterer-Pintur S, Contzen M, Mauder N, Süss-Dombrowski C (2015) Toxigenic *Corynebacterium ulcerans* isolated from a free-roaming red fox (*Vulpes vulpes*). Berl Munch Tierarztl Wochenschr 128(5-6):204–208

Stranieri A, Lauzi S, Giordano A, Galimberti L, Ratti G, Decaro N, Brioschi F, Lelli D, Gabba S, Amarachi NL, Lorusso E, Moreno A, Trogu T, Paltrinieri S (2021) Absence of SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies in stray cats. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14200. Epub ahead of print

Strube C, Heuer L, Janecek E (2013) *Toxocara* spp. infections in paratenic hosts. Vet Parasitol 193:375–389

Strube C, Raulf MK, Springer A, Waindok P, Auer H (2020) Seroprevalence of human toxocarosis in Europe: A review and meta-analysis. Adv Parasitol 109:375–418

Stuckey MJ, Boulouis HJ, Cliquet F, Picard-Meyer E, Servat A, Aréchiga-Ceballos N, Echevarría JE, Chomel BB (2017) Potentially Zoonotic *Bartonella* in Bats from France and Spain. Emerg Infect Dis 23(3):539–541

Stützer B, Hartmann K (2012) Chronic Bartonellosis in cats: what are the potential implications? J Feline Med Surg 14:612–621

Su C, Khan A, Zhou P, Majumdar D, Ajzenberg D, Dardé ML, Zhu XQ, Ajioka JW, Rosenthal BM, Dubey JP, Sibley LD (2012) Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proc Natl Acad Sci U S A 109:5844–5849

Swerczek TW, Nielsen SW, Helmboldt CF (1971) Transmammary passage of *Toxocara cati* in the cat. Am J Vet Res 32:89–92

Sykes JE (2005) Feline chlamydiosis. Clin Tech Small Anim Pract 20:129–134

Synowiec A, Szczepański A, Barreto-Duran E, Lie LK, Pyrc K (2021) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): a systemic infection. Clin Microbiol Rev 34(2):e00133–e00120

Taghipour A, Khazaei S, Ghodsian S, Shajarizadeh M, Olfatifar M, Foroutan M, Eslahi AV, Tsiami A, Badri M, Karanis P (2021) Global prevalence of *Cryptosporidium* spp. in cats: A systematic review and meta-analysis. Res Vet Sci 137:77–85

Talan DA, Citron DM, Abrahamian FM, Moran GJ, Goldstein EJ (1999) Bacteriologic analysis of infected dog and cat bites. N Engl J Med 340:85–92

Taleb M, Oumessoum A, Tail G (2013) Diagnosis of prevalent rickettsioses in Algeria using indirect micro-immunofluorescence. Online J BioSci Inf 5:1

Tangtrongsup S, Scorza AV, Reif JS, Ballweber LR, Lappin MR, Salman MD (2020) Seasonal distributions and other risk factors for *Giardia duodenalis* and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand. Prev Vet Med 174:104820

Tateno M, Nishio T, Sakuma M, Nakanishi N, Izawa M, Asari Y, Okamura M, Maruyama S, Miyama TS, Setoguchi A, Endo Y (2013) Molecular epidemiologic survey of *Bartonella, Ehrlichia*, and *Anaplasma* infections in Japanese Iriomote and Tsushima leopard cats. J Wildl Dis 49:646–652

Tauni MA, Osterlund A (2000) Outbreak of *Salmonella* Typhimurium in cats and humans associated with infection in wild birds. J Small Anim Pract 41:339–341

Taylan-Ozkan A (2020) Sources and seroprevalence of toxocariasis in Turkey. Adv Parasitol 109: 465–482

Taylor DJ, Efstratiou A, Reilly WJ (2002) Diphtheria toxin production by *Corynebacterium ulcerans* from cats. Vet Rec 150:355

Temmam S, Barbarino A, Maso D, Behillil S, Enouf V, Huon C, Jaraud A, Chevallier L, Backovic M, Pérot P, Verwaerde P, Tiret L, van der Werf S, Eloit M (2020) Absence of SARS-CoV-2 infection in cats and dogs in close contact with a cluster of COVID-19 patients in a veterinary campus. One Health 10:100164

TerWee J, Sabara M, Kokjohn K, Sandbulte J, Frenchick P, Dreier KJ (1998) Characterization of the systemic disease and ocular signs induced by experimental infection with *Chlamydia psittaci* in cats. Vet. Microbiol 59:259–281

Teutsch SM, Juranek DD, Sulzer A, Dubey JP, Sikes RK (1979) Epidemic toxoplasmosis associated with infected cats. N Engl J Med 300:695–699

Thépault A, Rose V, Queguiner M, Chemaly M, Rivoal K (2020) Dogs and cats: reservoirs for highly diverse *Campylobacter jejuni* and a potential source of human exposure. Animals (Basel) 10(5):838

The Humane Society of the United States. Pets by the numbers. https://humanepro.org/page/pets-by-the-numbers. Accessed 10 Jan 2022

Thompson DE, Bundy DA, Cooper ES, Schantz PM (1986) Epidemiological characteristics of *Toxocara canis* zoonotic infection of children in a Caribbean community. Bull World Health Organ 64:283–290

Tiwari TS, Golaz A, Yu DT, Ehresmann KR, Jones TF, Hill HE, Cassiday PK, Pawloski LC, Moran JS, Popovic T, Wharton M (2008) Investigations of 2 cases of diphtheria-like illness due to toxigenic *Corynebacterium ulcerans*. Clin Infect Dis 46:395–401

Tong WH, Pavey C, O'Handley R, Vyas A (2021) Behavioral biology of *Toxoplasma gondii* infection. Parasit Vectors 14:77

Torgerson PR, Mastroiacovo P (2013) The global burden of congenital toxoplasmosis. A systematic review. Bull World Health Organ 91:501–508

Torres Lde F, Ribeiro D, Hirata R Jr, Pacheco LG, Souza MC, dos Santos LS, dos Santos CS, Salah M, da Costa MM, Ribeiro MG, Selim SA, Azevedo VA, Mattos-Guaraldi AL (2013) Multiplex polymerase chain reaction to identify and determine the toxigenicity of *Corynebacterium* spp with zoonotic potential and an overview of human and animal infections. Mem Inst Oswaldo Cruz 108:272–279

Torrey EF, Yolken RH (2013) *Toxoplasma* oocysts as a public health problem. Trends Parasitol 29: 380–384

Trataris AN, Rossouw J, Arntzen L, Karstaedt A, Frean J (2012) *Bartonella* spp. in human and animal populations in Gauteng, South Africa, from 2007 to 2009. Onderstepoort J Vet Res 79: 452

Traversa D (2012) Pet roundworms and hookworms: a continuing need for global worming. Parasit Vectors 5:91

Tsukahara M, Tsuneoka H, Goto M, Iwamoto A (1999) Seroprevalence of *Bartonella henselae* among HIV-1 infected patients in Japan. Kansenshogaku Zasshi 73:1241–1242

Tyungu DL, McCormick D, Lau CL, Chang M, Murphy JR, Hotez PJ, Mejia R, Pollack H (2020) *Toxocara* species environmental contamination of public spaces in New York City. PLoS Negl Trop Dis 14(5):e0008249

Tzannes S, Batchelor DJ, Graham PA, Pinchbeck GL, Wastling J, German AJ (2008) Prevalence of *Cryptosporidium, Giardia* and *Isospora* species infections in pet cats with clinical signs of gastrointestinal disease. J Feline Med Surg 10:1–8

Uga S, Matsuo J, Kimura D, Rai SK, Koshino Y, Igarashi K (2000) Differentiation of *Toxocara canis* and *T. cati* eggs by light and scanning electron microscopy. Vet Parasitol 92(4):287–294

Ukwah BN, Ezeonu IM, Ezeonu CT, Roellig D, Xiao L (2017) *Cryptosporidium* species and subtypes in diarrheal children and HIV-infected persons in Ebonyi and Nsukka, Nigeria. J Infect Dev Ctries 11(2):173–179

Urakawa T, Seto J, Yamamoto A, Nakajima T, Goto S (2013) Subcutaneous abscess formation in the upper extremity caused by toxigenic *Corynebacterium ulcerans*. J Med Microbiol 62:489–493

USDA (US Animal and Plant Health Inspection Service). 2020 Cases of SARS-CoV-2 in animals in the United States. See https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/sa_one_health/sars-cov-2-animals-us. Accessed 12 Jan 2022

van Aart AE, Velkers FC, Fischer EAJ, Broens EM, Egberink H, Zhao S, Engelsma M, Hakze-van der Honing RW, Harders F, de Rooij MMT, Radstake C, Meijer PA, Oude Munnink BB, de Rond J, Sikkema RS, van der Spek AN, Spierenburg M, Wolters WJ, Molenaar RJ, Koopmans MPG, van der Poel WHM, Stegeman A, Smit LAM (2021) SARS-CoV-2 infection in cats and dogs in infected mink farms. Transbound Emerg. https://doi.org/10.1111/tbed.14173. Epub ahead of print

van der Leij WJR, Broens EM, Hesselink JW, Schuurman N, Vernooij JCM, Egberink HF (2021) Serological screening for antibodies against SARS-CoV-2 in Dutch shelter cats. Viruses 13(8): 1634

van Immerseel F, Pasmans F, De Buck J, Rychlik I, Hradecka H, Collard JM, Wildemauwe C, Heyndrickx M, Ducatelle R, Haesebrouck F (2004) Cats as a risk for transmission of antimicrobial drug-resistant *Salmonella*. Emerg Infect Dis 10:2169–2174

VanWormer E, Fritz H, Shapiro K, Mazet JA, Conrad PA (2013) Molecules to modeling: *Toxoplasma gondii* oocysts at the human-animal-environment interface. Comp Immunol Microbiol Infect Dis 36:217–231

Villanueva-Saz S, Giner J, Tobajas AP, Pérez MD, González-Ramírez AM, Macías-León J, González A, Verde M, Yzuel A, Hurtado-Guerrero R, Pardo J, Santiago L, Paño-Pardo JR, Ruíz H, Lacasta DM, Sánchez L, Marteles D, Gracia AP, Fernández A (2021) Serological evidence of SARS-CoV-2 and co-infections in stray cats in Spain. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14062. Epub ahead of print

Vocelle LA (2017) Revered and reviled: a complete history of the domestic cat. Revised edition. Great Cat Publications

Vollaire MR, Radecki SV, Lappin MR (2005) Seroprevalence of *Toxplasma gondii* antibodies in clinically ill cats in the United States. Am J Vet Res 66:874–877

Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM (2007) Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. Proc Natl Acad Sci U S A 104:6442–6447

Wagner KS, White JM, Crowcroft NS, De Martin S, Mann G, Efstratiou A (2010) Diphtheria in the United Kingdom, 1986-2008: the increasing role of *Corynebacterium ulcerans*. Epidemiol Infection 138:1519–1530

Wagner KS, White JM, Lucenko I, Mercer D, Crowcroft NS, Neal S, Efstratiou A (2012) Diphtheria Surveillance Network. Diphtheria in the postepidemic period, Europe, 2000-2009. Emerg Infect Dis 18:217–225

Waindok P, Kann S, Aristizabal A, Dib JC, Strube C (2021) *Toxocara* Seroprevalence and Risk Factor Analysis in Four Communities of the Wiwa, an Indigenous Tribe in Colombia. Microorganisms 9:1768

Wake K, Kikuchi J, Uchida M, Nemoto M, Kaji Y, Yokoyama T, Suzuki H, Fukushima A, Yamamoto A, Iwaki M, Ono K (2021) Transmission of toxigenic *Corynebacterium ulcerans* infection with airway obstruction from cats to a human. Acute Med Surg 8(1):e705

Wallace GD (1969) Serologic and epidemiologic observations on toxoplasmosis on three Pacific atolls. Am J Epidemiol 90:103–111

Weber A, Schwarzkopf A (2003) Heimtierhaltung – Chancen und Risiken für die Gesundheit. Robert Koch-Institut Berlin. http://edoc.rki.de/documents/rki_fv/reUzuR53Jx9JI/PDF/25uDLpnVUj7Y_53.pdf. Accessed 29 Jan 2014

Waap H, Gomes J, Nunes T (2014) Parasite communities in stray cat populations from Lisbon, Portugal. J Helminthol 88(4):389–395

Wang S, Yao Z, Li H, Li P, Wang D, Zhang H, Xie Q, Zhang Z, Li X (2020) Seroprevalence and risk factors of *Toxoplasma gondii* infection in primary school children in Henan province, central China. Parasite 27:23

Weber A, Wachowitz R, Weigl U, Schäfer-Schmidt R (1995) Nachweishäufigkeit von Salmonellen in Kotproben von Hunden und Katzen in Nordbayern von 1975 bis 1994. [Occurrence of Salmonella in fecal samples of dogs and cats in northern Bavaria from 1975 to 1994]. Berl Munch Tierarztl Wochenschr 108: 401–404. [Article in German]

Weese JS (2011) Bacterial enteritis in dogs and cats: diagnosis, therapy, and zoonotic potential. Vet Clin North Am Small Anim Pract 41:287–309

Wei Y, Aris P, Farookhi H, Xia X (2021) Predicting mammalian species at risk of being infected by SARS-CoV-2 from an ACE2 perspective. Sci Rep 11(1):1702

Wellinghausen N, Sing A, Kern WV, Perner S, Marre R, Rentschler J (2002) A fatal case of necrotizing sinusitis due to toxigenic *Corynebacterium ulcerans*. Int J Med Microbiol 292:59–63

Westgarth C, Pinchbeck GL, Bradshaw JWS, Dawson S, Gaskell RM, Christley RM (2010) Factors associated with cat ownership in a community in the UK. Vet Rec 166:354–357

WHOa. Animal bites. February 2018. https://www.who.int/en/news-room/fact-sheets/detail/animal-bites. Accessed 12 Oct 2021

WHO World Health Organization: Immunization, Vaccines and Biologicals. Vaccine preventable diseases Vaccines monitoring system 2020 Global Summary Reference Time Series: DIPHTHERIA. Accessed 15 Oct 2020

WHO. Foodborne Disease Burden Epidemiology Reference Group (FERG) 2007–2015. WHO estimates of the global burden of foodborne diseases [Internet]. World Health Organization; 2015. http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/. Accessed 14 Dec 2021

WHO-China Joint Mission. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf. Accessed 18 Jan 2022

Wielinga PR, de Vries A, van der Goot TH, Mank T, Mars MH, Kortbeek LM, van der Giessen JW (2008) Molecular epidemiology of *Cryptosporidium* in humans and cattle in The Netherlands. Int J Parasitol 38:809–817

Williams M, Izzard L, Graves SR, Stenos J, Kelly JJ (2011) First probable Australian cases of human infection with *Rickettsia felis* (cat-flea typhus). Med J Aust 194:41–43

Wölfel S, Speck S, Essbauer S, Thoma BR, Mertens M, Werdermann S, Niederstrasser O, Petri E, Ulrich RG, Wölfel R, Dobler G (2017) High seroprevalence for indigenous spotted fever group rickettsiae in forestry workers from the federal state of Brandenburg, Eastern Germany. Ticks Tick Borne Dis 8(1):132–138

Won KY, Kruszon-Moran D, Schantz PM, Jones JL (2008) National seroprevalence and risk factors for zoonotic *Toxocara* spp. infection. Am J Trop Med Hyg 79:552–557

Wons J, Meiller R, Bergua A, Bogdan C, Geißdörfer W (2017) Follicular Conjunctivitis due to *Chlamydia felis*-case report, review of the literature and improved molecular diagnostics. Front Med (Lausanne) 17(4):105

Woodruff AW, Savigny DH, Hendy-Ibbs PM (1982) Toxocaral and toxoplasmal antibodies in cat breeders and in Icelanders exposed to cats, but not to dogs. Br Med J 284:309–310

Wright JG, Tengelsen LA, Smith KE, Bender JB, Frank RK, Grendon JH, Rice DH, Thiessen AM, Gilbertson CJ, Sivapalasingam S, Barrett TJ, Besser TE, Hancock DD, Angulo FJ (2005) Multidrug-resistant *Salmonella* Typhimurium in four animal facilities. Emerg Infect Dis 11: 1235–1241

Wu SM, Huang SY, Xu MJ, Zhou DH, Song HQ, Zhu XQ (2013) *Chlamydia felis* exposure in companion dogs and cats in Lanzhou, China: a public health concern. BMC Vet Res 9:104
Xiao L, Feng Y (2008) Zoonotic cryptosporidiosis. FEMS Immunol Med Microbiol 52:309–323
Xu H, Jin Y, Wu W, Li P, Wang L, Li N, Feng Y, Xiao L (2016) Genotypes of *Cryptosporidium* spp., *Enterocytozoon bieneusi* and *Giardia duodenalis* in dogs and cats in Shanghai, China. Parasit Vectors 9:121
Yamamoto K, Chomel BB, Lowenstine LJ, Kikuchi Y, Phillips LG, Barr BC, Swift PK, Jones KR, Riley SP, Kasten RW, Foley JE, Pedersen NC (1998) *Bartonella henselae* antibody prevalence in free-ranging and captive wild felids from California. J Wildl Dis 34:56–63
Yan C, Fukushi H, Matsudate H, Ishihara K, Yasuda K, Kitagawa H, Yamaguchi T, Hirai K (2000) Seroepidemiological investigation of feline chlamydiosis in cats and humans in Japan. Microbiol Immunol 44:155–160
Yang R, Ying JL, Monis P, Ryan U (2015) Molecular characterisation of *Cryptosporidium* and *Giardia* in cats (*Felis catus*) in Western Australia. Exp Parasitol 155:13–18
Yang WH, Hsu MS, Shu PY, Tsai KH, Fang CT (2021) Neglected human *Rickettsia felis* infection in Taiwan: A retrospective seroepidemiological survey of patients with suspected rickettsioses. PLoS Negl Trop Dis 15(4):e0009355
Yanagihara M, Tsuneoka H, Sugasaki M, Nojima J, Ichihara K (2010) Multispacer typing of *Bartonella henselae* isolates from humans and cats, Japan. Emerg Infect Dis 16:1983–1985
Yazdanpanah F, Garg A, Shadman S, Asmarz HY (2021) Literature Review of COVID-19, pulmonary and extrapulmonary Disease. Am J Med Sci 361(5):567–574
Ye G, Yang L, Xu L, Pan Z, Dong Z (2021) Neurological presentations caused by *Rickettsia felis* infection. Br J Hosp Med (Lond) 82(6):1–2
Yoshimura Y, Tachikawa N, Komiya T, Yamamoto A (2014) A case report and epidemiological investigation of axillary lymph node abscess caused by *Corynebacterium ulcerans* in an HIV-1-positive patient. Epidemiol Infect 142(7):1541–1544
Yoshiuchi R, Matsubayashi M, Kimata I, Furuya M, Tani H, Sasai K (2010) Survey and molecular characterization of *Cryptosporidium* and *Giardia* spp. in owned companion animal, dogs and cats, in Japan. Vet Parasitol 174(3-4):313–316
Younus M, Wilkins MJ, Davies HD, Rahbar MH, Funk J, Nguyen C, Siddiqi AE, Cho S, Saeed AM (2010) The role of exposures to animals and other risk factors in sporadic, non-typhoidal *Salmonella* infections in Michigan children. Zoonoses Public Health 57:e170–e176
Yuan C, Zhu C, Wu Y, Pan X, Hua X (2011) Bacteriological and molecular identification of *Bartonella* species in cats from different regions of China. PLoS Negl Trop Dis 5:e1301
Zakikhany K, Efstratiou A (2012) Diphtheria in Europe: current problems and new challenges. Future Microbiol 7:595–607
Zangwill KM (2013) Cat scratch disease and other *Bartonella* infections. Adv Exp Med Biol 764: 159–166
Zavala-Castro J, Zavala-Velázquez J, Walker D, Pérez-Osorio J, Peniche-Lara G (2009) Severe human infection with *Rickettsia felis* associated with hepatitis in Yucatan, Mexico. Int J Med Microbiol 299:529–533
Zavala-Velázquez JE, Ruiz-Sosa JA, Sánchez-Elias RA, Becerra-Carmona G, Walker DH (2000) *Rickettsia felis* rickettsiosis in Yucatán. Lancet 356(9235):1079–1080
Zendri F, Isgren CM, Sinovich M, Richards-Rios P, Hopkins KL, Russell K, Groves N, Litt D, Fry NK, Timofte D (2021) Case Report: Toxigenic *Corynebacterium ulcerans* Diphtheria-Like Infection in a Horse in the United Kingdom. Front Vet Sci 8:650238
Zeng Z, Wang C, Liu C, Wang B, Meng X, Chen Y, Guo S (2021) Follow-up of a *Rickettsia felis* encephalitis: Some new insights into clinical and imaging features. Int J Infect Dis 104: 300–302
Zhang L, Shan A, Mathew B, Yin J, Fu X, Zhang J, Lu J, Xu J, Dumler JS (2008) Rickettsial seroepidemiology among farm workers, Tianjin, People's Republic of China. Emerg Infect Dis 14:938–940

Zhang Q, Zhang H, Gao J, Huang K, Yang Y, Hui X, He X, Li C, Gong W, Zhang Y, Zhao Y, Peng C, Gao X, Chen H, Zou Z, Shi ZL, Jin M (2020) A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg Microbes Infect 9(1):2013–2019

Zhao S, Schuurman N, Li W, Wang C, Smit LAM, Broens EM, Wagenaar JA, van Kuppeveld FJM, Bosch BJ, Egberink H (2021) Serologic screening of Severe Acute Respiratory Syndrome Coronavirus 2 infection in cats and dogs during first coronavirus disease wave, the Netherlands. Emerg Infect Dis 27(5):1362–1370

Zhu S, Shapiro K, VanWormer E (2021) Dynamics and epidemiology of *Toxoplasma gondii* oocyst shedding in domestic and wild felids. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14197. Epub ahead of print

Zobba R, Chessa G, Mastrandrea S, Parpaglia MLP, Patta C, Masala G (2009) Serological and molecular detection of *Bartonella* spp. in humans, cats and dogs from northern Sardinia, Italy. Clin Microbiol Infect 15:134–135

Zoccola R, Beltramo C, Magris G, Peletto S, Acutis P, Bozzetta E, Radovic S, Zappulla F, Porzio AM, Gennero MS, Dondo A, Pasqualini C, Griglio B, Ferrari A, Ru G, Goria M (2021) First detection of an Italian human-to-cat outbreak of SARS-CoV-2 Alpha variant - lineage B.1.1.7. One Health 13:100295

Public Health and Rodents: A Game of Cat and Mouse

29

Bastiaan G. Meerburg

Contents

Abstract

Rodents are the most abundant order of living mammals, distributed on every continent except Antarctic and represent 43% of all mammalian species. Beside causing food losses and infrastructural damage, rodents can harbor pathogens that may cause serious problems to human and animal health. Unfortunately, rodent-associated problems are not an issue of the past as some may have thought, even not in the developed world. This chapter describes four factors that determine the risk and severity of human infection by zoonotic pathogens of rodents: human behavior, human health condition, rodent ecology & behavior, and pathogen ecology & persistence. It provides an overview of these factors, their interrelation and also some directions for further research. Main conclusion of this chapter is that although science has come a long way already and we have won some small victories over the rodents, the game of cat (i.e., humans) and mouse is far from being settled.

Keywords

Bank vole · Rodent species · *Yersinia pestis* · Rodent population · Zoonotic pathogen

B. G. Meerburg (✉)
Livestock Research, Wageningen University & Research Centre, Wageningen, The Netherlands

Dutch Pest & Wildlife Expertise Centre (KAD), Wageningen, The Netherlands
e-mail: Bastiaan.Meerburg@wur.nl

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_24

29.1 Introduction

The order of *Rodentia* is the most abundant and diversified order of living mammals and represents in total about 43% of all mammalian species (Wilson and Reeder 1993; Huchon et al. 2002). Rodents are distributed on every continent except Antarctica and include many of the most abundant mammals. For many centuries, opportunistic rodent species have been considered as serious pests because of the damage they cause to crops, stored produce or infrastructure and the role they play in the transmission of pathogens to humans and livestock. Improved public sanitation conditions like safe drinking water, the introduction of sewers and the development of efficient anticoagulant rodenticides in the 1950s resulted in an improved public health situation and created the illusion that rodent-associated problems in the developed world had become an issue of the past.

More recently, however, the concern about rodents in both the developing and developed world has grown again because of various reasons. These reasons are the following:

- The distribution and abundance of various rodent species may be significantly affected by changes in land use (e.g., reforestation projects, urbanization).
- Climatic change may improve living conditions for certain rodent populations.
- Growing outdoor leisure activities increase the exposure of humans to rodents and their excrements and hence the transmission risk of rodent-borne pathogens.
- In some countries the government has receded from rodent control and put it out to contract to private companies. This has led to a serious lack of insight in the spreading and abundance of rodent populations, which is important to monitor the potential introduction and spread of rodent-borne pathogens.
- The human world population is growing rapidly and thus more food is needed. Rodents are responsible for huge pre- and postharvest losses (Meerburg et al. 2009b; Htwe et al. 2012).
- Environmental concerns, toxicological safety regulations, and budget reductions have diminished rodent surveillance and rodenticide-based control in many countries.
- The increasing extent of resistance of rodents against second-generation rodenticides has reduced the efficacy and flexibility of rodent control (Pelz 2007; Buckle et al. 2013; Endepols et al. 2012; Meerburg et al. 2014).
- Rodents still play an important role in spreading (re-)emerging zoonotic diseases (Meerburg et al. 2009a).

29.2 Rodents: Both Reservoirs and Carriers

Rodent presence can have serious implications for public health and be potentially hazardous as they amplify pathogens from their environment by forming reservoirs of zoonotic disease (Webster and Macdonald 1995; Gratz 1994). With reservoirs it is meant that rodents can harbor disease-causing organisms and thus serve as potential

sources of disease outbreaks, but always via a vector (tick, sand-fly etc.). Besides as reservoirs, rodents can also act as carriers, which means that rodents that show no or limited disease symptoms but harbor the disease-causing agent are capable of passing it directly onto humans (Meerburg et al. 2009a).

Two main transmission routes of pathogens can be distinguished (Meerburg et al. 2009a): the direct route (when rodents are carriers) or the indirect route (when rodents function as reservoir and transmit a pathogen through means of a vector), see Fig. 1. In the latter, this vector is often an arthropod, but can also occasionally be other animals, such as livestock. Rodents that are (either by accident or on purpose) ingested by livestock can transfer pathogens. When food originating from this livestock is not thoroughly cooked, this may lead to human morbidity (Meerburg et al. 2004).

If we now look at the risks and severity that are imposed by rodents to human health, there are several factors that are of importance (Fig. 2).

The first one is human behavior. People with frequent outdoor leisure activities or which fulfil specific occupations (e.g., in the military, animal trapping, or forestry) or those that live in degraded environments will be more exposed to rodent-borne zoonoses than others (Clement et al. 1997; Muliæ and Ropac 2002; Hukic et al. 2010; Sauvage et al. 2007; Bonnefoy et al. 2008). Exposure is the key word here, thus, for example, also people that keep rodents as pets may experience higher risks of zoonotic infection. The risk of keeping pet rodents will be discussed more in detail later in this chapter.

It is good to understand that especially for commensal rodents human behavior and rodent ecology and behavior are often strongly linked. If humans dispose their garbage in a wrong way, this will provide opportunities for growth of commensal rodent populations. Moreover, following the widespread closures of food-related businesses due to efforts to curtail the spread of the SARS-CoV2 pandemic, public health authorities reported increased sightings of rats in close vicinity of people (Parsons et al. 2020), although the signals about an increasing or decreasing number of rat reports differs between countries.

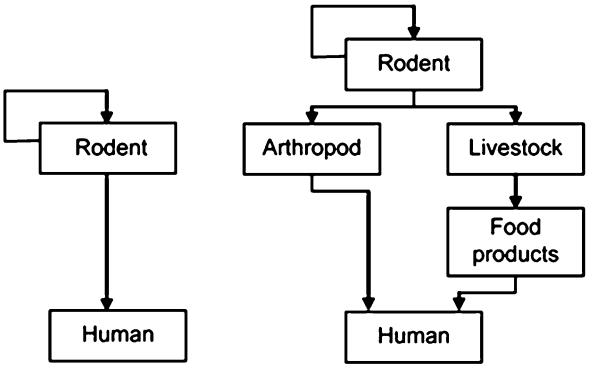

Fig. 1 The two main transmission routes, the direct route (left) and the indirect route (right). (Reproduced from Meerburg et al. 2009a)

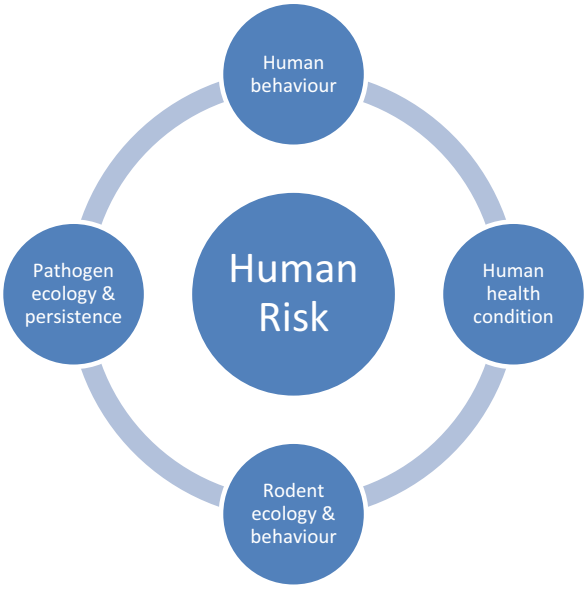

Fig. 2 Four factors determine the risk and severity of human infections by zoonotic pathogens of rodents: human behavior, human health condition, rodent ecology & behavior, and pathogen ecology & persistence

The second factor is the human health condition. Generally, zoonoses pose unique transmission and disease risks if people are not in good health, such as immunocompromised persons, neonates, the elderly, or pregnant women (Mani and Maguire 2009; Hemsworth and Pizer 2006) or may effect persons in specific age groups. As an example Streptobacillus moniliformis may be mentioned, the primary cause of rat bite fever in North America. Children under 12 years of age are mainly infected, and demonstrate an acute syndrome of fever, rash, and polyarthritis. Some years ago, a fatal case-report was reported, a 14-month-old-boy, who was exposed to filthy living conditions and whose family had pet ferrets. Presumably, the boy was bitten by rodents as autopsy revealed a possible bite mark (Banerjee et al. 2011).

Also aspects such as vaccination coverage may influence the factor human health condition. From a number of studies, it is known that wild rodents can be reservoirs for orthopoxviruses (Tryland et al. 1998; Kinnunen et al. 2011). More recently, pet rats were discovered as a new potential source of local outbreaks with cowpox. However, smallpox-vaccinated patients tend to develop less severe reactions and heal more quickly (Vogel et al. 2012). Thus, there is a direct link between actions of public health services (vaccination) and the recovery rate (severity of infection) of infected persons.

A third factor of importance is rodent ecology and behavior. As mentioned in the introduction, rodent ecology may differ over the years, depending on climatic factors, feed abundance, and predation (Witmer and Proulx 2010). Moreover, differences in the ecology and behavior of rodent species may emerge depending whether they are in a rural or urban environment (Feng and Himshworth 2014).

Unfortunately, the knowledge about behavior of rodent pest species is still quite limited. Rodent management could become more efficient and effective when concentrating efforts on areas where rodents perceive the least levels of predation risk (Krijger et al. 2017).

During a study in Namibia, mice entered buildings during the post-harvest stage, which may represent a period of food shortage for these mice in the field (Monadjem et al. 2011). If these species are coincidently contracted with zoonotic pathogens, this may increase the risk of human infection. In a study from Cambodia, it was demonstrated that the rainy season is favorable for transmission of leptospires in rodents, particularly in rain-fed fields (Ivanova et al. 2012). Here, the human risk of contracting *Leptospira* spp. is determined strongly by the ecology of the rodents: in rice-fields, forest, secondary forests, and their interface with agricultural fields the potential of humans for contracting leptospirosis infection is the highest (Ivanova et al. 2012).

But the link between rodent ecology and human health risks is not depending solely on one rodent species. Also the presence of other non-reservoir rodent species is important. In a recent study from Panama it was demonstrated that hantavirus prevalence in wild reservoir (rodent) populations and reservoir population density increased when small-mammal species diversity was reduced (Suzán et al. 2009). These authors thus claim that high biodiversity is important to reduce transmission of zoonotic pathogens among wildlife hosts (Suzán et al. 2009). Also host relationships form part of the rodent ecology and there can be significant differences in such relations within the same rodent species. In a study where host-tick relationships of the yellow-necked mouse (*Apodemus flavicollis*), a critical host in the maintenance of the zoonotic disease tick-borne encephalitis, were investigated it was demonstrated that the transmission potential was not evenly distributed among the yellow-necked mice population. The authors found that 20% of hosts most infested with ticks were accountable for 80% of the transmission potential, and that these hosts were identified as the sexually mature males of high body mass (Perkins et al. 2003). This leads to the impression that control efforts targeted at this host group would reduce the transmission potential significantly.

In the past, seasons of exceptionally high rainfall were thought to increase rodent populations (because plant growth would lead to abundant seeds and insects) and thus outbreaks of some rodent-borne diseases (Engelthaler et al. 1997; Brown and Ernest 2002). However, we now start to discover that such relationships between rodent population dynamics and precipitation are complex and non-linear. This was also the main conclusion after some scientists studied the El Niño phenomenon in deserts of southwestern North-America (Brown and Ernest 2002). In agricultural contexts, it is also difficult to predict exactly the breeding ecology of species. A recent study from the Philippines compared two rodent species *R. argentiventer* and *R. tanezumi* during four cropping seasons (two dry and two wet). The expectation was that *R. tanezumi* breeding would occur throughout the season, whereas the breeding of *R. argentiventer* would be strongly cued to the generative stage of rice crops (Htwe et al. 2012). However, it was found

that their breeding ecology was exactly similar, with the onset of the breeding season at the tillering stage of the rice crops. The conception of adult females was highest during booting and ripening of the rice and the highest litter size occurred at booting and ripening of the rice (Htwe et al. 2012). Such information is essential in order to optimize the management of rodents in order to reduce harvest losses and pathogen transmission risks.

The fourth factor is pathogen ecology and persistence. Many of the mechanisms that mediate pathogen ecology and persistence only start being uncovered. Concerning hantaviruses in rodents, several host factors, including sex steroids, glucocorticoids, and genetic factors, are reported to alter host susceptibility and may contribute to the persistence of hantaviruses in rodents (Easterbrook and Klein 2008). Moreover, because of the recent discovery of structural and non-structural proteins in humans that suppress type I interferon responses, it is now thought that immune responses of rodent hosts could be mediated directly by this virus (Easterbrook and Klein 2008). In laboratory rats it was observed that *Leptospira interrogans* serovar Copenhageni initially disseminates extensively throughout the host, prior to clearance from all tissues except the kidneys, suggesting that the kidneys are immune privileged sites and that this is not caused by tissue tropism (Athanazio et al. 2008). In a study in black rats (*Rattus rattus*) in Madagascar, an important difference in plague resi *s*tance between rat populations from the plague focus (central highlands) and those from the plague-free zone (low altitude area) was confirmed to be widespread (Tollenaere et al. 2010). Moreover, these authors discovered that sex-influenced plague susceptibility, with males slightly more resistant than females (Tollenaere et al. 2010). It is difficult whether this phenomenon is caused by rodent ecology, pathogen ecology, or a combination of both. This is also the case with other findings. In Belgium, a close association between the distribution of hantavirus-infected bank voles and wet habitat types was found (Verhagen et al. 1986). In another, more recent, study from this country, a direct relation between climate and the incidence of human cases of nephropathia epidemica (NE) due to Puumala virus (PUUV) infection was found. High summer and autumn temperatures, 2 years and 1 year respectively before NE occurrence, relate to high NE incidence (Tersago et al. 2009). In the United States, human cases of Hantavirus Pulmonary Synodrome (HPS) were clustered seasonally and temporally by biome type and geographic location. In this study, exposure sites were most frequently found in pinyon-juniper woodlands, grasslands, and Great Basin desert scrub lands, at elevations of 1800 m to 2500 m (Engelthaler et al. 1997). This might be caused by presence of rodent reservoir hosts in these areas, but perhaps also because of favorable environmental conditions for pathogen survival. Pathogens do not only persist in the host itself, but may also survive for longer periods of time throughout the environment. For example, *Yersinia pestis* biotype Orientalis remains viable and fully virulent after 40 weeks in the soil and is then able to continue its role in plague epidemiology (Ayyadurai et al. 2008). Moreover, if factors such as pH, viscosity, and salt concentration are optimal, *Leptospira* spp. are thought to be able to survive in fresh water under low-nutrient conditions for over 100 days (Trueba et al. 2004).

29.3 Conclusion

It is clear that for eradication strategies, more work has to be done on the pathogenesis of the various zoonotic pathogens which can be transferred by rodents. Especially the further development of genetic tools could lead to a better understanding of the virulence and survival mechanisms that are used by pathogens to ensure their persistence in different ecological niches and host reservoirs.

Often, there is a relation between the different factors and complex relationships between pathogen prevalence and rodent density appear likely. In North-Western Europe, the main disease-causing hantavirus species is the Puumala virus (PUUV). The reservoir species for PUUV is the bank vole (*Myodes glareolus*), which exists in specific habitats. The risk for PUUV transfer from the bank vole to the human population via excretion of the virus in the environment is dependent on a myriad of biotic and abiotic risk factors, either rodent-, virus-, or human-related, that vary in time and space. In a study from Finland, the effect of PUUV infection on the winter survival of bank voles was investigated (Kallio et al. 2007). These authors demonstrate that PUUV infected bank voles had a significantly lower overwinter survival probability than antibody negative bank voles. Thus, the pathogen is able to influence the host population dynamics. During a study on the ecology and demographics of hantavirus infections in rodent populations in the Walker River Basin of Nevada and California, it was found that antibody prevalence could vary within repeatedly sampled sites from 0 to 50% over the course of several months (Boone et al. 1998). In Tanzania, an African rodent (*Mastomys natalensis*) is thought to be the principal source of human infections with *Leptospira* spp. In a study where the dynamics of infection were modelled and in which the climatic conditions in central Tanzania were included, a strong seasonality was visualized in the force of infection on humans with a peak in the abundance of infectious mice between January and April in agricultural environments (Holt et al. 2006). In urban environments, however, dynamics were predicted to be more stable and the period of high numbers of infectious animals runs from February to July (Holt et al. 2006). In countries in Northern-Europe (Germany, Denmark), there are also regional differences visible in the level of encountered *Leptospira* spp. infected-rats (Runge et al. 2013; Krøjgaard et al. 2009). Why these differences occur, is not yet fully understood.

As mentioned before, the risk of transmitting zoonotic pathogens to humans is largest if the exposure risk is maximal. Handlers and owners of pet rodents are often in direct contact with them and may experience significant risks. Some years ago, there was an outbreak of 28 cases of multidrug-resistant *S. enterica* Serotype Typhimurium in the United States. After the outbreak, 22 patients were interviewed. Of them, 13 (59%) had had contact with rodents purchased from retail pet stores (Swanson et al. 2007), while 2 patients (9%) acquired salmonellosis through secondary transmission from a primary patient who had been exposed to rodents. Moreover, 7 patients (32%) had no identified rodent exposure. Matching isolates were obtained from one submitted urine specimen and 27 stool specimens from patients (Swanson et al. 2007). These authors warn that consumers and animal workers should be aware that rodents can shed salmonellae and should expect rodent

excrements to be potentially infectious. Thus, handling of pet rodents may result in health risks, especially for children. When handling pet rodents, their cages, or bedding, the hands should be thoroughly rinsed with water and soap. Animal vendors should be aware if substantial diarrhea-associated complications or death occurs among rodents intended for sale (Swanson et al. 2007).

Some years earlier, an human infection with Lymphocytic Choriomeningitis Virus (LCMV) in the United States was found by the CDC to be associated with pet rodents (hamsters and guinea pigs). Here, the risks extended also beyond the owners of these pets. In this particular case, LCMV was responsible for the death of three immunocompromised persons (organ transplant recipients) who received these organs from pet rodent owners (Anonymous 2005). More recently, workers at a rodent breeding facility in the United States were confronted with a LCMV infection. In total, 52 current and former employees of the facility were tested, and 13 of them (25%) demonstrated a recent LCMV infection (Anonymous 2012).

Exotic rodents may introduce pathogens that were previously unknown to continents. For example, in 2003 a monkeypox outbreak in pet distribution facilities in the USA occurred after import of infected African prairie dogs (Anonymous 2003). In total, monkeypox was confirmed in 35 persons, of which none died, but the outbreak required vaccination of 30 persons with smallpox vaccine.

Commercially-traded wild prairie dogs were also responsible for an outbreak caused by *Francisella tularensis* type B in Texas. Antibodies to this pathogen were found in one person that was exposed, thus leading to the first evidence of tularemia transmission from prairie dog to human (Avashia et al. 2002). Problematic was that in the period June–July 2002, more than 1000 prairie dogs were distributed from the facility where the pathogen emerged, to locations in 10 other US states and 7 other countries (Avashia et al. 2002). These had to be traced back and were all euthanized. However, this case underlines the health risks to humans who handle wild-caught animals and underscores the speed of transportation of exotic species and their pathogens over the globe (Avashia et al. 2002).

A human cowpox virus infection is an uncommon and potentially fatal skin disease, which is confined to major parts of Europe. Patients may sporadically contract the pathogen by contact with infected cows, cats, or small rodents. However, recently there is also a report from Germany (Munich), where 8 patients were infected by pet rats they had purchased at a local supplier (Vogel et al. 2012). Thus, pet rats can be considered as a novel potential source of local outbreaks of human cowpox virus infections.

Also, dermatophytes can be transferred to humans by rodents. In Switzerland, for example, 9 isolates of the fast-growing dermatophyte species *Arthroderma benhamiae* were isolated from 8 children and 1 adult. Eight of the 9 patients had had previous contact with rodents, mostly with guinea pigs (Fumeaux et al. 2004). In another study, where the frequency and types of dermatophytes among both Guinea pigs and rabbits were determined (Kraemer et al. 2012), *Trichophyton mentagrophytes* was determined to be the most common dermatophyte in pet Guinea pigs and rabbits, but asymptomatic carriers were regularly observed only in Guinea pigs. Consequently, pet guinea pigs carrying dermatophytes can be considered as a serious zoonotic risk for their owners, especially children (Kraemer et al. 2013).

An Australian patient who experienced an infection with *Streptobacillus moniliformis*, the causative agent of rat-bite fever, obtained this pathogen not because she was bitten by rats, but because she had had contact with her pets, including cuddling and kissing them (Papanicolas et al. 2012). This is a risk as *S.* moniliformis forms part of the commensal flora of the rat's oropharynx (Elliott 2007).

But not only handling or keeping pet rodents can impose a risk. Also commensal rodent species (species that live in or around a house or a farm) may lead to health risks. The risk of bites by rats inflicted in urban environments (often in substandard dwellings) and the spread of infection to humans is substantial. In the United States, there a hundreds of rat bite reports each year, while the number may even be underreported by a factor of at least ten (Bonnefoy et al. 2008; Hirschhorn and Hodge 1999). Next to rat bites, ectoparasites that are associated with these rodents can spread additional infectious organisms. The rodents are sometimes also carrying endoparasites or other pathogens which may contaminate the local environment. A literature review on helminths in rodents in South East Asia showed that the highest helminth species richness was found in *Rattus tanezumi*, *Rattus norvegicus*, and *Rattus argentiventer*, which are found in more human-dominated habitats such as agricultural areas or human settlements (Chaisiri et al. 2010). In a study in Tokyo, Japan, 17% of the brown rats (*Rattus norvegicus*) from urban areas carried leptospires in their kidneys and cases in human patients could directly be linked to these rats via DNA-analysis (Koizumi et al. 2009). Moreover, rodents in agro-ecological surroundings can be infected with *Salmonella* spp. and *Campylobacter* spp. and transfer these bacteria to livestock or amplify their number in the farm environment (Meerburg and Kijlstra 2007). In this way, a resident infected rodent population could lead to continuously returning infections in the farm environment, with all the negative consequences for both livestock and farmers. The exact risk dimension of livestock-pathogen-human-wildlife interactions is not yet known for many pathogens. Two pathogens may serve as an example here: *Coxiella burnetii*, the causative agent of Q-fever, and Hepatitis E virus (HEV). Concerning *Coxiella burnetii,* it has been implicated in many studies that rodents function as reservoirs for Q-fever, but their exact role in pathogen maintenance, geographic spread, and transmission still remains to be clarified (Meerburg and Reusken 2011; Webster et al. 1995). Problematic in determining the exact contribution of rodents is that basic wildlife and domestic cycles of *C. burnetii* infection can operate independently, but will overlap in many instances as many areas in the world are occupied by both domestic and wild animals (Meerburg and Reusken 2011), which makes it hard to unravel their exact contribution. In a recent study from Japan (Kanai et al. 2012) in which wild *Rattus norvegicus* were caught near a pig farm where HEV was present, it was demonstrated that in these rodents there was a relatively high prevalence (17.9%). Consequently, these authors conclude that *R. norvegicus* may be a carrier of swine HEV in endemic regions, but that the HEV contamination risk due to rats in human habitats remains unclear (Kanai et al. 2012).

Consequently, there remains much work for scientists to be done. Concerning the factor human behavior, the use of Geographical Information System (GIS) technology could prove to be a useful tool for the identification of endemic foci and high-risk areas

for numerous pathogens that are transmitted by rodents. Such technology was recently tested in a study in Cyprus (Psaroulaki et al. 2010), where rats were used as disease sentinels and tested for seropositivity on six microbial agents. In the Philippines, dogs were responsible for human schistosomiasis infection, but the authors claim that rats could be useful as schistosomiasis sentinels to monitor infection levels in the environment (Carabin et al. 2014). By optimizing this technology, more information could be acquired about possible outbreak areas, which facilitates informing the general public by public health officials.

When considering the factor human health condition, one should remember that the world population will increase the coming decades and also that the average age of the world population will increase. Thus, the number of people that may experience significant health effects when infected by zoonoses is growing. We do not yet know the exact dimension of the problem, but it is something to keep in mind.

High resolution remote-sensing could also prove useful to monitor the factor rodent ecology and behavior. This was recently done in Kazakhstan, where great gerbil burrow systems were observed by means of satellite images (Addink et al. 2010). The occupancy rate of these burrows is a strong indicator for the probability of a plague outbreak. By monitoring the density of great gerbil burrow systems, or locating new or expanding foci, a direct contribution could be made to surveillance and control efforts (Addink et al. 2010). Of course, with such techniques it is not possible to monitor the ecology and behavior of all rodent species. To gain more insight into the population dynamics and habitat preferences of rodents, field studies will remain necessary. By collection of small mammals in several habitat types, an action which was recently undertaken in Albania (Rogozi et al. 2012), one can gain more knowledge of the reservoir ecology in a country, and thus acquire more possibilities for reliable risk assessments for rodent-borne diseases. Moreover, also rodent identification via molecular methods, e.g., molecular barcoding using short genetic markers (Galan et al. 2012) may be useful as this will lead to a quicker and more accurate species identification. The previous will also prove its worth, if rodent dynamics and ecology will change in the future because of climatic change. The use of digital monitoring techniques, e.g., traps that are directly linked to GSM, Wifi, or other types of networks, will provide data that is very relevant for control of commensal rodents.

Concerning the factors pathogen ecology and persistence, there are also new opportunities. Fecal samples of wild rodents that were collected in California and Virginia were surveyed in order to obtain an initial unbiased measure of the viral diversity in the enteric tract (Phan et al. 2011). Viral RNA and DNA were randomly amplified. Phylogenetic analyses of full and partial viral genomes revealed many previously uncharacterized viral species, genera, and families, and close genetic similarities between some rodent and human viruses even reflected past zoonoses (Phan et al. 2011). In another recent study, a comparative approach was used to study microparasite species richness across rodent species according to the latitude where they occur (Bordes et al. 2011). The results demonstrated that virus species richness increased toward tropical latitudes, and that rodent litter size seemed to decrease when microparasite species richness increased independently from the latitude. The

authors thus claim that rodent species in the tropics harbor higher parasite species loads effectively, at least in terms of species richness for viruses, and that parasite species richness influences rodent life-history traits (Bordes et al. 2011). This information is also important for reliable risk assessments.

Finally, we may conclude that humankind has come a long way. We increased our knowledge and understanding and have gained some small victories over the rodents. However, there is no definitive victory over them yet, and although they are not able to defeat the cats (in this case the humans), they do still pretty well in avoiding capture. Let's hope that further scientific progress will lead to a better understanding about rodents and their risk for public health and that the contest between cat and mouse may end in a favorable way for humankind.

References

Addink EA, De Jong SM, Davis SA, Dubyanskiy V, Burdelov LA, Leirs H (2010) The use of high-resolution remote sensing for plague surveillance in Kazakhstan. Remote Sens Environ 114(3): 674–681

Anonymous (2003) Update: multistate outbreak of monkeypox-Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. Morb Mortal Wkly Rep (MMWR) (Centers for Disease Control and Prevention) 52(27):642–646

Anonymous (2005) Update: interim guidance for minimizing risk for human lymphocytic choriomeningitis virus infection associated with pet rodents. Morb Mortal Wkly Rep (MMWR) (Centers for Disease Control and Prevention) 54(32):799–801

Anonymous (2012) Notes from the field: Lymphocytic Choriomeningitis virus infections in employees of a Rodent breeding facility—Indiana, May–June 2012. Morb Mortal Wkly Rep (MMWR) 61(32):622–623

Athanazio DA, Silva EF, Santos CS, Rocha GM, Vannier-Santos MA, McBride AJA et al (2008) *Rattus norvegicus* as a model for persistent renal colonization by pathogenic Leptospira interrogans. Acta Trop 105(2):176–180

Avashia SBPJ, Lindley CM, Schriefer ME, Gage KL, Cetron M et al (2004) First reported prairie dog-to-human tularemia transmission, Texas, 2002. Emerg Infect Dis 10(3):483–486

Ayyadurai S, Houhamdi L, Lepidi H, Nappez C, Raoult D, Drancourt M (2008) Long-term persistence of virulent *Yersinia pestis* in soil. Microbiology 154(9):2865–2871

Banerjee P, Ali Z, Fowler DR (2011) Rat bite fever, a fatal case of *Streptobacillus moniliformis* infection in a 14-month-old boy. J Forensic Sci 56(2):531–533

Bonnefoy X, Kampen H, Sweeney K (2008) Public health significance of urban pests: World Health Organization – Regional Office for Europe

Boone JD, Otteson EW, McGwire KC, Villard P, Rowe JE, St Jeor SC (1998) Ecology and demographics of hantavirus infections in rodent populations in the Walker River Basin of Nevada and California. Am J Trop Med Hyg 59(3):445–451

Bordes F, Guégan JF, Morand S (2011) Microparasite species richness in rodents is higher at lower latitudes and is associated with reduced litter size. Oikos 120(12):1889–1896

Brown JH, Ernest SKM (2002) Rain and Rodents: complex dynamics of desert consumers. BioScience 52(11):979–987. (2013/08/07)

Buckle A, Endepols S, Klemann N, Jacob J (2013) Resistance testing and the effectiveness of difenacoum against Norway rats (*Rattus norvegicus*) in a tyrosine139cysteine focus of anticoagulant resistance, Westphalia, Germany. Pest Manag Sci 69(2):233–239

Carabin H, McGarvey ST, Sahlu I, Tarafder MR, Joseph L et al (2014) *Schistosoma japonicum* in Samar, the Philippines: infection in dogs and rats as a possible risk factor for human infection. Epidemiol Infect 143(8):1767–1776

Chaisiri K, Chaeychomsri W, Siruntawineti J, Bordes F, Herbreteau V, Morand S (2010) Human-dominated habitats and helminth parasitism in Southeast Asian murids. 107(4):931–937
Clement J, Heyman P, McKenna P, Colson P, Avsic-Zupanc T (1997) The hantaviruses of Europe: from the bedside to the bench. Emerg Infect Dis 3(2):205–211
Easterbrook JD, Klein SL (2008) Immunological mechanisms mediating Hantavirus persistence in rodent reservoirs. PLoS Pathog 4(11):e1000172
Elliott SP (2007) Rat bite fever and Steptobacillus moniliformis. Clin Microbiol Rev 20(1):13–22
Endepols S, Klemann N, Jacob J, Buckle AP (2012) Resistance tests and field trials with bromadiolone for the control of Norway rats (*Rattus norvegicus*) on farms in Westphalia, Germany. Pest Manag Sci 68(3):348–354
Engelthaler DM, Mosley DG, Cheek JE, Levy CE, Komatsu KK, Ettestad P et al (1997) Climatic and environmental patterns associated with hantavirus pulmonary syndrome, Four Corners region, United States. Emerg Infect Dis 3(2):205–211. 1999;5(1):87–94
Feng AYT, Himshworth CG (2014) The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). Urban Ecosyst 17:149–162
Fumeaux J, Mock M, Ninet B, Jan I, Bontems O, Léchenne B et al (2004) First report of *Arthroderma benhamiae* in Switzerland. Dermatology 208(3):244–250
Galan M, Pagès M, Cosson J-F (2012) Next-generation sequencing for Rodent barcoding: species identification from fresh, degraded and environmental samples. PLoS One 7(11):e48374
Gratz NG (1994) Rodents as carriers of disease. In: Buckle AP, Smith RH (eds) Rodent pests and their control. CAB International, Oxford, pp 85–108
Hemsworth S, Pizer B (2006) Pet ownership in immunocompromised children – a review of the literature and survey of existing guidelines. Eur J Oncol Nurs 10(2):117–127
Hirschhorn R, Hodge R (1999) Identification of risk factors in rat-bite incidents involving humans. Pediatrics 104:e35
Holt J, Davis S, Leirs H (2006) A model of Leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. Acta Trop 99(2–3): 218–225
Htwe NM, Singleton GR, Hinds LA, Propper CR, Sluydts V (2012) Breeding ecology of rice field rats, *Rattus argentiventer* and *R. tanezumi* in lowland irrigated rice systems in the Philippines. Agric Ecosyst Environ 161:39–45
Huchon D, Madsen O, Sibbald MJJB, Ament K, Stanhope MJ, Catzeflis F et al (2002) Rodent phylogeny and a timescale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. Mol Biol Evol 19:1053–1065
Hukic M, Nikolic J, Valjevac A, Seremet M, Tesic G, Markotic A (2010) A serosurvey reveals Bosnia and Herzegovina as a Europe's hotspot in hantavirus seroprevalence. Epidemiol Infect 138(08):1185–1193
Ivanova S, Herbreteau V, Blasdell K, Chaval Y, Buchy P, Guillard B et al (2012) *Leptospira* and Rodents in Cambodia: environmental determinants of infection. Am J Trop Med Hyg 86(6): 1032–1038
Kallio ER, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Koskela E et al (2007) Endemic hantavirus infection impairs the winter survival of its rodent host. Ecology 88(8):1911–1916. (2013/08/07)
Kanai Y, Miyasaka S, Uyama S, Kawami S, Kato-Mori Y, Tsujikawa M et al (2012) Hepatitis E virus in Norway rats (*Rattus norvegicus*) captured around a pig farm. BMC Res Notes 5(1):4
Kinnunen PM, Henttonen H, Hoffmann B, Kallio ER, Korthase C, Laakkonen J et al (2011) Orthopox virus infections in Eurasian wild rodents. Vector-Borne Zoonotic Dis 11(8):1133–1140
Koizumi N, Muto M, Tanikawa T, Mizutani H, Sohmura Y, Hayashi E et al (2009) Human leptospirosis cases and the prevalence of rats harbouring *Leptospira interrogans* in urban areas of Tokyo, Japan. J Med Microbiol 58(9):1227–1230
Kraemer A, Mueller RS, Werckenthin C, Straubinger RK, Hein J (2012) Dermatophytes in pet Guinea pigs and rabbits. Vet Microbiol 157(1–2):208–213
Kraemer A, Hein J, Heusinger A, Mueller RS (2013) Clinical signs, therapy and zoonotic risk of pet guinea pigs with dermatophytosis. Mycoses 56(2):168–172

Krijger IM, Belmain SR, Singleton GR, Groot Koerkamp PW, Meerburg BG (2017) The need to implement the landscape of fear within rodent pest management strategies. Pest Manag Sci 73 (12):2397–2402. https://doi.org/10.1002/ps.4626

Krøjgaard LH, Villumsen S, Markussen MDK, Jensen JS, Leirs H, Heiberg A-C (2009) High prevalence of Leptospira spp. in sewer rats (*Rattus norvegicus*). Epidemiol Infect 137(11):1586–1592

Mani I, Maguire JH (2009) Small animal zoonoses and immuncompromised pet owners. Top Companion Anim Med 24(4):164–174

Meerburg BG, Kijlstra A (2007) Role of rodents in transmission of *Salmonella* and *Campylobacter*. J Sci Food Agric 87(15):2774–2781

Meerburg BG, Reusken CBEM (2011) The role of wild rodents in spread and transmission of *Coxiella burnetii* needs further elucidation. Wildl Res 38(7):617–625

Meerburg BG, Bonde M, Brom FWA, Endepols S, Jensen AN, Leirs H et al (2004) Towards sustainable management of rodents in organic animal husbandry. NJAS-Wag J Life Sci 52(2): 195–205

Meerburg B, Singleton G, Kijlstra A (2009a) Rodent-borne diseases and their risks for public health. Crit Rev Microbiol 35(3):221–270

Meerburg BG, Singleton GR, Leirs H (2009b) The year of the Rat ends—time to fight hunger! Pest Manag Sci 65(4):351–352

Meerburg BG, van Gent-Pelzer MP, Schoelitsz B, Van der Lee TA (2014) Distribution of anticoagulant rodenticide resistance in *Rattus norvegicus* in the Netherlands according to Vkorc1 mutations. Pest Manag Sci 70(11):1761–1766

Monadjem A, Mahlaba TA, Dlamini N, Eiseb SJ, Belmain SR, Mulungu LS et al (2011) Impact of crop cycle on movement patterns of pest rodent species between fields and houses in Africa. Wildl Res 38(7):603–609

Muliæ R, Ropac D (2002) Epidemiologic characteristics and military implications of hemorrhagic fever with renal syndrome in Croatia. Croatian Med J 43(5):581–586

Papanicolas LE, Holds JM, Bak N (2012) Lessons from practice: meningitis and pneumonitis caused by pet rodents. Med J Aust 196(3):202–203

Parsons MH, Kiyokawa Y, Richardson JL, Stryjek R, Byers KE et al (2020) Rats and the COVID-19 pandemic: early data on the global emergence of rats in response to social distancing. MedRxiv 2020.07.05.20146779

Pelz H-J (2007) Spread of resistance to anticoagulant rodenticides in Germany. Int J Pest Manage 53 (4):299–302

Perkins SE, Cattadori IM, Tagliapietra V, Rizzoli AP, Hudson PJ (2003) Empirical evidence for key hosts in persistence of a tick-borne disease. Int J Parasitol 33(9):909–917

Phan TG, Kapusinszky B, Wang C, Rose RK, Lipton HL, Delwart EL (2011) The fecal viral flora of wild rodents. PLoS Pathog 7(9):e1002218

Psaroulaki A, Antoniou M, Toumazos P, Mazeris A, Ioannou I, Chochlakis D et al (2010) Rats as indicators of the presence and dispersal of six zoonotic microbial agents in Cyprus, an island ecosystem: a seroepidemiological study. Trans R Soc Trop Med Hyg 104(11):733–739

Rogozi E, Bego F, Papa A, Mersini K, Bino S (2012) Distribution and ecology of small mammals in Albania. Int J Environ Health Res 23(3):258–268. (2013/08/07)

Runge M, von Keyserlingk M, Braune S, Becker D, Plenge-Bönig A, Freise JF et al (2013) Distribution of rodenticide resistance and zoonotic pathogens in Norway rats in Lower Saxony and Hamburg, Germany. Pest Manag Sci 69(3):403–408

Sauvage F, Langlais M, Pontier D (2007) Predicting the emergence of human hantavirus disease using a combination of viral dynamics and rodent demographic patterns. Epidemiol Infect 135 (01):46–56

Suzán G, Marcé E, Tomasz Giermakowski J, Mills JN, Ceballos G, Ostfeld RS et al (2009) Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. PLoS One 4(5):e5461

Swanson SJ, Snider C, Braden CR, Boxrud D, Wünschmann A, Rudroff JA et al (2007) Multidrug-resistant *Salmonella enterica* serotype *Typhimurium* associated with pet rodents. N Engl J Med 356(1):21–28

Tersago K, Verhagen R, Servais A, Heyman P, Ducoffre G, Leirs H (2009) Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. Epidemiol Infect 137(02):250–256

Tollenaere C, Rahalison L, Ranjalahy M, Duplantier JM, Rahelinirina S, Telfer S et al (2010) Susceptibility to *Yersinia pestis* experimental infection in Wild Rattus rattus, reservoir of plague in Madagascar. EcoHealth 7(2):242–247

Trueba G, Zapata S, Madrid K, Cullen P, Haake D (2004) Cell aggregation: a mechanism of pathogenic Leptospira to survive in fresh water. Int Microbiol 7:35–40

Tryland M, Sandvik T, Mehl R, Bennett M, Traavik T, Olsvik O (1998) Serosurvey for orthopoxviruses in rodents and shrews from Norway. J Wildl Dis 34(2):240–250

Verhagen R, Leirs H, Tkachenko E, Groen G (1986) Ecological and epidemiological data on Hantavirus in bank vole populations in Belgium. Arch Virol 91(3–4):193–205

Vogel S, Sárdy M, Glos K, Korting HC, Ruzicka T, Wollenberg A (2012) The Munich outbreak of cutaneous cowpox infection: transmission by infected pet rats. Acta Derm Venereol 92(2): 126–131

Webster JP, Macdonald DW (1995) Parasites of wild brown rats (*Rattus norvegicus*) on UK farms. Parasitology 111:247–255

Webster JP, Lloyd G, Macdonald DW (1995) Q fever (*Coxiella burnetii*) reservoir in wild brown rat (*Rattus norvegicus*) populations in the UK. Parasitology 110(Pt 1):31–35

Wilson DE, Reeder DM (1993) Mammal species of the world. Smithsonian Institution Press, Washington, DC

Witmer G, Proulx G (2010) Rodent outbreaks in North America. In: Singleton GR, Belmain SR, Brown PR, Hardy B (eds) Rodent outbreaks: ecology and impacts. International Rice Research Institute, Los Banos, pp 253–267

Camel-Related Zoonoses: A Light on "Ship of the Desert"

30

Camels and Public Health

Alireza Sazmand and Alireza Nourian

Contents

Abstract

With a worldwide population of over 37 million, camels are an important source of meat, milk, and labor in many regions of the world, mainly in Africa and Asia. The one-humped camel, also known as dromedary (*Camelus dromedarius*), accounts for approximately 95% of the whole population of camelids and is distributed in at least 47 countries of the world. Despite being extremely resistant to harsh environmental conditions, camels can get infected with several zoonotic pathogens, thus posing a public health's risk. In this chapter most important parasitic, bacterial, viral, and fungal zoonoses related to camels are discussed.

Keywords

Bacteria · Bacterial · Camel · Camelids · *Camelus dromedarius* · Disease · Dromedary · Fungal · Fungi · Infection · Neglected · One-Health · One-Humped Camel · Parasite · Parasitic · Public Health · Viral · Virus · Zoonoses · Zoonotic

A. Sazmand (✉) · A. Nourian
Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
e-mail: alireza.sazmand@basu.ac.ir; nourian@basu.ac.ir

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_48

30.1 Introduction

One-humped camels, also known as dromedary (*Camelus dromedarius*) are specifically adapted to live in hot, arid areas of the world, notably the Middle East, Africa, and India. With a worldwide population of over 37 million, camels are an important source of meat and milk in many marginal, desert areas of the world where they survive under harsh conditions, with a considerable feral population in Australia (Camel-Scan 2019; Diall et al. 2022; FAOSTAT 2019) (Fig. 1).

Although compared with other animal species (*e.g.*, dogs), the domestication of the dromedary camel took place rather late in human history, approximately 3000 years ago (MacHugh et al. 2017), nowadays this livestock species is distributed in at least 47 countries, playing a crucial role in their economy; for example, in the year 2019, camels produced about 3,111,462 tons of milk and 653,135 tons of meat (FAOSTAT 2019). Unique physiological peculiarities of dromedaries in their circulatory system, respiratory system, water economy mechanism, heat tolerance, etc. enable them to survive almost 1 week with little or no food and water (Ouajd and Kamel 2009), making them suitable also for trade and trafficking over longer distances in arid areas. Indeed, they are utilized since ancient times for transportation of people, goods, warfare, and as draft animals including in agriculture and in local industry. Furthermore, they provide food (meat and dairy products) with great nutritional value, wool and leather in regions of the globe where the common

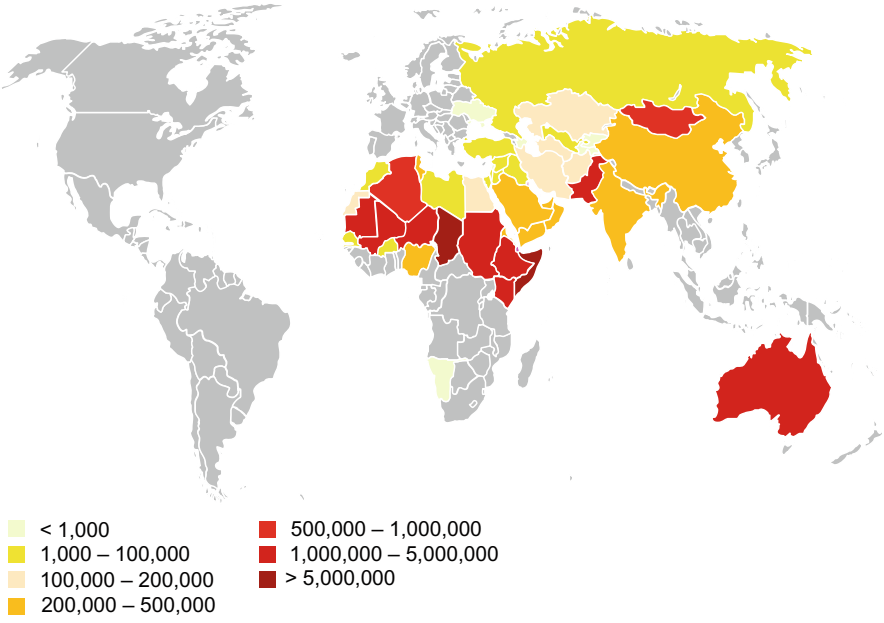

Fig. 1 Map of dromedary camel population by 47 countries in 2019. (Camel-Scan 2019; FAOSTAT 2019)

ruminant livestock species (cattle, sheep, and goats) cannot be used for these purposes. Therefore, as they are important food sources in semi-arid and arid zones, the picture of camelids transformed from "ship of the desert" to a "food security livestock" species. One evidence is that between the years 2009 and 2019, the world's camel population increased by 29% compared to only 7%, 12%, 19%, and -12% for cattle, sheep, goats, and pigs, respectively (FAOSTAT 2019). This trend recognizes the economic value of this livestock species as a food source, consequently implying the need for research on its pathogens and their zoonotic implications. This is also emphasized by the expansion of the human population in some developing countries by at least double their current size. It has been estimated that the world human population will reach 9.7 billion in 2050, mainly in developing countries of the southern hemisphere (United Nations 2019), which will parallel an increased need for food resources in these areas, including camel meat and milk. Overall, this could make camels an increasingly important source for zoonotic disease transmission to humans, especially in resource-poor communities with improper sanitation and medical access.

30.2 Public Health Impact of Camels

Adult camels are large animals standing about 2 m at the shoulders and weighing up to 600 kg. They may kill or injure humans by bites, falls from their backs, kicks, or collisions with motor vehicles; however, the relative magnitude of each mechanism has never been extensively studied. Furthermore, the incidence of camel-related injuries is not known worldwide but scant information is available, such as the study of Abu-Zidan et al. in Al-Ain city, United Arab Emirates, where the incidence of hospitalized camel-related injured patients in the city was estimated as 6.88 per 100,000 population per year (Abu-Zidan et al. 2012b). In Saudi Arabia more than 600 camel–vehicle collisions occur each year, out of which the fatality rate is 0.25; about six times that of all types of traffic accidents in the country (Al-Ghamdi and AlGadhi 2004).

Camel bite injuries occur more often during the rutting season (November–March) when they behave unpredictably. Injuries are usually severe and are including penetrating and crushing injuries by the camel jaw and blunt injuries when patients are picked up and thrown away (Abu-Zidan et al. 2012a). The head and neck sustain frequent and severe injuries which may involve facial wounds, skull fractures, intracranial bleeding, and cervical neurovascular injuries (Balac et al. 2019). Patients who sustain camel bites are prone to infection with *Staphylococcus*, *Aeromonas*, *Pasteurella*, and *Actinobacillus* species. Infection rates from camel bites have been reported to be as high as 86%. Furthermore, patients are at risk for tetanus and rabies (Calleo et al. 2018). Other causes of deaths associated with camels involve kicking, stomping, kneeling or sitting on a victim, or biting and shaking and throwing. Lethal mechanisms include hemorrhage from vascular injuries and internal organ disruption, crush asphyxia, and blunt craniospinal injuries (Gilbert and Byard 2021).

While direct impact of camels on human health is obvious, the zoonotic health risks of camels to humans are indirect and much less visible. As stated before, camels are extremely resistant to harsh environmental conditions; however, dromedaries can get infected with several zoonotic pathogens, thus posing a public health's risk. Literature review on camel-borne zoonotic diseases revealed that the majority of publications within 1970 and 2018 focused on four pathogens, i.e., Middle East respiratory syndrome (MERS), hydatidosis, brucellosis, and Rift Valley fever (Zhu et al. 2019). In this chapter 14 most discussed parasites (*Echinococcus granulosus* sensu lato, *Toxoplasma gondii*, *Cryptosporidium* spp., *Trypanosoma* spp., *Balantidium coli*, *Sarcoptes scabei*), bacteria (*Brucella* spp., *Mycobacterium tuberculosis* complex, *Yersinia pestis*), viruses (Middle East respiratory syndrome, Crimean–Congo hemorrhagic fever, Rift Valley fever, West Nile virus), and ringworm causing fungi related to zoonoses will be addressed.

30.3 Major Zoonotic Pathogens of Camels

30.3.1 Parasites

Relatively few parasites of camels are specific for this host species (Schuster 2018), whereas many others that infect camels are (i) non–zoonotic but with a large host range, or, (ii) of zoonotic concern. Camel echinococcosis is the most studied zoonotic parasitic infection affecting humans but *Toxoplasma gondii*, *Cryptosporidium* spp., *Fasciola* spp., *Trichinella* spp., and *Linguatula serrata* originating from camels are also considered as major public health risks (Zhu et al. 2019).

Transmission of zoonotic parasites includes different routes of infection such as fecal contamination (*e.g.*, *Cryptosporidium* spp., *Giardia duodenalis*, *Balantidium coli*, *Blastocystis* spp., *Enterocytozoon* spp., or consumption of raw or undercooked infected tissues and milk (*e.g.*, *Toxoplasma gondii*, *Trichinella* spp., *Linguatula serrata*). In addition, camels serve as reservoir hosts for *Trypanosoma* spp. or may be infected by gastropod–borne trematodes (*e.g.*, *Fasciola* spp., *Dicrocoelium dendriticum* and *Schistosoma* spp.) or metacestode larvae of zoonotic tapeworms, such as *Echinococcus granulosus* sensu lato (Sazmand and Joachim 2017; Sazmand et al. 2019b). Moreover, camels are blood source for several hematophagous ectoparasites, such as ticks and fleas, which ultimately transmit zoonotic viral and bacterial pathogens (*e.g.*, Crimean–Congo hemorrhagic fever virus, *Coxiella burnetii*, *Rickettsia* spp., *Bartonella* spp., and *Yersinia pestis*) (Bahari et al. 2021; Sazmand et al. 2019a; Wernery et al. 2014).

30.3.1.1 *Echinococcus granulosus* sensu lato

Cystic echinococcosis (CE) is a major zoonotic infection worldwide caused by larval stage of the tapeworm *Echinococcus granulosus* sensu lato. It causes considerable medical costs and economic losses in endemic areas (Deplazes et al. 2017). More than one million people are affected with echinococcosis at any one time, and it is estimated that echinococcosis to be the cause of 19,300 deaths and around 871,000

disability-adjusted life-years (DALYs) globally each year. Annual global costs associated with CE including treating cases and losses to the livestock industry are estimated to be 3 billion USD (WHO 2015). These costs might comprise 0.01% to 0.04% of nation's gross domestic product (GDP) in low- and middle-income countries (Harandi et al. 2012). Transmission typically occurs between canid definitive hosts and intermediate hosts such as human through the ingestion of parasite eggs shed in the feces of infected definitive hosts in the environment or dog's coat (Thompson 2017).

Among species and genotypes of *Echinococcus* (Vuitton et al. 2020), G6 genotype of *E. canadensis* that was formerly known as "camel strain" is particularly well adapted to camels but dromedaries are also important in the epidemiology of *E. granulosus* sensu stricto (G1–G3) and *E. ortleppi* (G5) (Sazmand et al. 2019b). Interestingly, it has been suggested that *E. canadensis* may have an affinity for the brain in humans (Sadjjadi et al. 2013; Shirmen et al. 2018). A recent article estimated that 23.75% of camels across the world harbor hydatid cysts (Anvari et al. 2021). In a study from Africa where pooled CE prevalence in different intermediate host species was compared, camels had the highest prevalence (Ohiolei et al. 2020). Cysts are commonly found in the lungs and, to a lesser extent, the liver of camels resulting in carcass condemnation and, subsequently, great economic losses. In Iran where CE is endemic, the annual monetary burden of CE has been estimated at 232.3 million USD, out of which the economic loss due to condemnation of infected camel livers amounts to approximately 600,000 USD (Harandi et al. 2012).

Major obstacles in controlling CE include the inability of local authorities and owners in poor environments to handle the costs of deworming treatment of dogs, to maintain proper abattoirs, to control stray dog populations, to vaccinate lambs, and the lack of public education (Otranto et al. 2017).

30.3.1.2 *Cryptosporidium* spp.

A wide range of gastrointestinal protozoan parasites such as *Eimeria* spp., *Cystoisoispora orlovi* and camel-specific *Sarcosystis* species develop exclusively in camels (Sazmand et al. 2012; Hamidinejat et al. 2013; Dubey and Schuster 2018); however, there are scientific data about infections of camels with several species of protozoan parasites of zoonotic importance including *Cryptosporidium* spp. (Phylum: Protozoa; Subphylum: Sporozoa; Class: Gregarinomorphea; Subclass: Cryptogregaria; Order Cryptogregarida; Family: Cryptosporidiidae).

Cryptosporidium spp. are highly successful parasites causing diarrhoeal disease in both people and animals worldwide. Due to their large host range, high oocyst output from infected individuals, water- and food-borne transmission routes, and low infectious dose from as low as one oocyst these protozoan parasites are responsible for >eight million cases of food-borne illness annually (Innes et al. 2020). Considering the fact that one infected neonatal calf sheds around 40 billion infectious oocysts in feces during the acute infection, we can understand the massive environmental contamination and a huge risk for other vulnerable hosts such as humans (Nydam et al. 2001). In children younger than 5 years, *Cryptosporidium* is responsible for 12,868,500 DALYs considering both acute effects of diarrhea and

associated growth faltering (Khalil et al. 2018). Outbreaks of cryptosporidiosis associated with contaminated water supplies can result in significant economic and health impacts. Costs associated with two outbreaks in Wisconsin and Ireland were estimated as high as 96.2 million and 22.44 million USD, respectively (Chyzheuskaya et al. 2017).

At least 48 *Cryptosporidium* species are considered valid and > 100 genotypes yet to be formally described, due to lack of biological and/or genetic data (Ježková et al. 2021). Over 20 species and genotypes have been reported in human patients (Feng et al. 2018). In dromedary camels so far, infection with *C. parvum* subtype IIaA17G2R1, a common zoonotic subtype reported in humans and animals worldwide, which is genetically related to the *C. hominis* of the If subtype family, *C. andersoni*, *Cryptosporidium* rat genotype IV and *Cryptosporidium* camel genotype have been confirmed (Baroudi et al. 2018; El-Alfy et al. 2019; Gu et al. 2016; Zahedi et al. 2018). Furthermore, natural infection of closely related species *Camelus bactrianus* (two-humped camel) with *C. occultus*, *C. ubiquitum*, *C. bovis*, and *C. muris* (Cao et al. 2020; Wang et al. 2021) suggests that dromedaries might also get infected with a wider range of species and genotypes than we know today. There is only one documentation on zoonosis linked with camels from Iran where 24 of 100 people in long-term contact with camels were found infected with *Cryptosporidium* spp. (Sazmand et al. 2012). Although *C. parvum* and *C. andersoni* identified in camels are potentially infectious for humans, no confirmed direct association between camels and human infections have been reported, in contrast to other livestock such as cattle (Lal et al. 2016) warranting further investigations.

30.3.1.3 *Toxoplasma gondii*

Due to its exceptionally wide range of warm- and cold-blooded hosts, *T. gondii* is one of the most successful zoonotic parasites on earth. One third of the world's human population are infected with this cosmopolitan food– and waterborne parasite, albeit with high heterogeneity between countries and regions (Djurković-Djaković et al. 2019). In the USA alone, toxoplasmosis accounts for 32,700 DALYs annually, being also responsible for 8% of foodborne–illnesses hospitalizations with 86,700 confirmed patients and 330 deaths (Scallan et al. 2015; Scallan et al. 2011). Like other livestock, camels acquire *T. gondii* infections through ingestion of sporulated oocysts shed by cats or wild felids in the environment (Hamidinejat et al. 2013). Antibodies against *T. gondii* in sera of dromedaries from different countries have been determined using various techniques, reporting seroprevalences as high as 67% (Dubey 2021; Gebremedhin et al. 2014; Nourian 1992). It has been estimated that 36% of camels in Africa have anti-*T. gondii* antibodies (Tonouhewa et al. 2017). Our knowledge about clinical and congenital toxoplasmosis in camels, however, is limited to few reports and the significance of infection is probably underestimated (Hagemoser et al. 1990; Ishag 2003; Ishag and Majid 2008; Riley et al. 2017). *Toxoplasma gondii* cysts have been isolated from camel meat (Gebremedhin et al. 2014) but predilection sites of *Toxoplasma* cysts have not been comprehensively investigated in this host species. The rooted habits of nomadic populations of some African and Asian communities of raw camel liver

consumption (Bin Saeed et al. 2005; Gebremedhin et al. 2014) could represent a risk factor for infection of humans, as *T. gondii* is frequently isolated from the livers of domestic ruminants and horses (Belluco et al. 2016). In addition, consumption of camel milk is becoming increasingly popular in recent years, as it is richer in vitamin C and iron than cow's milk, with suggested therapeutic effects on type 1 diabetes and reduction of allergies in children (Boughattas 2017). The implication of unpasteurized camel milk as a source of human toxoplasmosis (Medani and Mohamed 2016) suggests that consuming raw milk or dairy products derived from it (*e.g.*, Shubat, a beverage of fermented camel milk, sparkling white with a sour flavor, popular in Central Asia) could be a risk for human health. Little is known about the genetic characteristics of *T. gondii* genotypes infecting camels. Some surveys showed the occurrence of all three conventionally defined clonal lineages (Types I, II and III) in camel meat and milk (El-Alfy et al. 2019; Elfadaly et al. 2017; Tavakoli Kareshk et al. 2018). All of these types have also been isolated from human patients (Ajzenberg et al. 2009). Since the conventional nomenclature of *Toxoplasma* isolates does not sufficiently delineate the plethora of existing genotypes (Shwab et al. 2014), multilocus PCR-RFLP genotyping should be applied to elucidate potential links with disease manifestations in people consuming meat and dairy products of camels.

30.3.1.4 *Trypanosoma* spp.

Camels are affected by several *Trypanosoma* species (Roettcher et al. 1987). *Trypanosoma evansi*, the etiologic agent of "Surra" is the more prevalent trypanosome species of camels (Desquesnes et al. 2013). It was the first trypanosome to be described and identified as the causative agent of mammalian trypanosomosis. The earliest report on *T. evansi* was published by Griffith Evans who associated it with an endemic disease in equids in the Dera Ismail Khan district of Punjab in Pakistan (Evans 1880). While *T. evansi* is the more prevalent trypanosome species of camels, *T. brucei*, *T. congolense*, and *T. vivax* are found at lower infection rates (Al Malki and Hussien 2022; Birhanu et al. 2015; Dirie et al. 1989; Mossaad et al. 2017). However, in a recent report that described *T. vivax* for the first time in dromedaries of central desert of Iran this infection was more prevalent than *T. evansi* (Asghari and Rassouli 2021).

Due to a partial loss of *T. evansi* mitochondrial DNA, which occurred during its segregation from *T. brucei* (Lai et al. 2008) and acquiring the capacity for mechanical transmission by virtually all biting flies its geographical distribution is potentially unlimited. *Trypanosoma evansi* affects a wide range of domestic and wild mammals in Africa, Asia, and South America (Desquesnes et al. 2022; Sazmand et al. 2022), and recent outbreaks of infection among camelid populations on the Canary Islands, in mainland Spain, and France demonstrated the potential of the parasite to spread rapidly even in non–endemic areas (Gutierrez et al. 2010). In dromedaries, the infection may cause significant morbidity and great impairment of productivity and mortality (Sazmand et al. 2016; Sazmand et al. 2011). It is assumed that the spread of *T. evansi* among camels with the consequence of fatal anemia weakened the Arab–African Muslim forces in their prolonged battle against Christendom, as they relied heavily on camels and equids for transport and economy

(Clarence-Smith 2013). *Trypanosoma evansi* has its highest prevalence in camels compared to other animal hosts such as buffaloes, cattle, dogs, equids, and small ruminants (Aregawi et al. 2019), but in contrast to other livestock species, the economic burden of this infection has not been evaluated in camels (Reid 2002). Human cases of *T. evansi* infection have been reported from India, Sri Lanka, Egypt, Thailand, and Indonesia (Joshi et al. 2005; Sawitri et al. 2019; Sengupta et al. 2022; Truc et al. 2013; Van Vinh Chau et al. 2016). For a decade it was hypothesized that human susceptibility to *T. evansi* could be linked to insufficient or missing levels of human trypanocide apolipoprotein L1 (APOL1), a trypanocidal component of normal human serum (Vanhollebeke et al. 2006). However, report of infection in a patient with no previous immunological risk, 2 wild–type APOL1 alleles and a normal serum APOL1 concentration confirmed that *T. evansi* is a true zoonosis with a risk of infection for the general population (Van Vinh Chau et al. 2016).

30.3.1.5 *Balantidium coli*

Balantidiasis caused by *B. coli* is a zoonotic disease with domestic and wild pigs, non-human primates and humans as reservoirs. Cysts of this large ciliated protozoan live in cecum and colon of the hosts, are shed in feces, and transmitted to susceptible hosts via fecal-oral route. *Balantidium coli* is the only ciliated protozoan that is pathogenic for humans, being most common in the Philippines, but is also reported in Central and South America, Papua New Guinea, and parts of Western Asia (Chalmers 2014). Although the worldwide prevalence is estimated at 0.02 to 1%, it varies widely by geographic location as, for instance, in New Guinea and Altiplano area of Bolivia, infection rates of as high as 28% and 29% have been reported (Schuster and Ramirez-Avila 2008). Indeed, human populations living in close proximity to domestic pigs are naturally resistant and mostly without any clinical manifestation, though a case fatality rate of 30% has been reported in acute balantidiasis with intestinal perforation or fulminating hemorrhagic dysentery and shock (Schuster and Ramirez-Avila 2008). Rare cases of balantidial appendicitis, and extension to extraintestinal sites causing, for example, urinary tract infection and vaginitis, and lung infections, have been reported (Chalmers 2014). Because of the pleomorphism of balantidial trophozoites and the host range, taxonomy of this genus is controversial.

In absence of pig raising, such as in Middle-Eastern countries, camels play a major epidemiological role in the transmission of *B. coli* (Cox 2005). As for other mammalian hosts (Nakauchi 1999), balantidia from camels, provisionally named *Balantidium cameli* (Hegner 1934), are now referred to as *B. coli*. The infection is widespread in camel populations and infection rates up to 53% have been reported from Algeria, Bahrain, Egypt, India, Iran, Iraq, Nigeria, and Saudi Arabia (Ahmed et al. 2020a; El-Khabaz et al. 2019). Infected camels can shed very large number of cysts and trophozoites *e.g.,* 15,000 per gram (Tajik et al. 2013), therefore, may have a significant role in transmission of the infection to people in contact with them. There is only one documentation on zoonosis linked with camels from Iraq where 10 of 25 camel breeders and 50% of their camels were found infected with *B. coli* (Hussein et al. 2016). Recent studies on the genetic diversity of *Balantidium* spp. and *Balantidium* – like cyst – forming ciliates, such as *Buxtonella*, suggest that genetic

analyses are needed to explain the real spectrum of intestinal ciliates as the cysts are morphologically indistinguishable. *Buxtonella sulcata,* another ciliate with world-wide distribution, is mainly found in the cecum of cattle but also of camels (Taylor et al. 2016). Finding of *Buxtonella*-like ciliates in primates opened the hypothesis that *Buxtonella* may also be a pathogen in humans (Pomajbíková et al. 2013), and the possible transmission from camels to humans should be further investigated.

30.3.1.6 *Sarcoptes scabei*

Camels can be infested by a wide range of external parasites such as mites and tick that irritate, injure, or debilitate them. Camel mange caused by *Sarcoptes scabiei* var. *cameli* is a worldwide major threat to camel health and production. It is extremely contagious and the infestation rate can reach up to 83% in camels, causing loss of condition, decreased work tolerance, and, in extreme cases, death (Al-Rawashdeh et al. 2000; Wernery et al. 2014). Initially camels rub the affected areas against inanimate objects or against one another causing erythema with numerous papules and nodules. With advancement of the infection alopecia to complete baldness patches are observed, and in severe cases the skin is thickened, wrinkled, and thrown into folds, is covered with thick crusts, and shows extensive fissuring and cracking. In terminal stages of the disease, camels decrease their food and water intake and become emaciated. There is marked edema of the legs, especially on the footpads, and the skin becomes soft, and pits on pressure. Urination and defecation are not affected, but the animals are unable to work or to walk for long distances and eventually die (Nayel and Abu-Samra 1986).

Sarcoptic mange is considered second only to Surra trypanosomosis in terms of losses in camels (Pegram and Higgins 1992). Overcrowding, temperature, and the skin microclimate have been suggested as important factors in the epizootiology of the disease in Sudan (Nayel and Abu-Samra 1986). Furthermore, camels older than 2 years, female camels, and winter season were found to be the higher risk of exposure in Egypt (Ahmed et al. 2020b). Transmission of *S. scabiei* var. *cameli* to humans, particularly camel attendants and riders, is well–known since ancient times (Tadjbakhsh 1994). Although *S. scabiei* of camels' origin cannot multiply in the human host, it causes pseudoscabies that usually takes place during milking, handling, or riding. In herdsmen, the lesions are observed mainly in the interdigital spaces of the hands, the flexor surface of the wrists, the forearms, the elbows, and axillary folds. In the case of camel riders, the lesions occur between the thighs. Treatment of both animals and the camel handlers can help in controlling this zoonotic problem (Schillinger 1987).

30.3.2 Viruses

30.3.2.1 Middle East Respiratory Syndrome (MERS)

The highly lethal Middle East respiratory syndrome (MERS) was initially reported in April, 2012 (WHO 2021a). The causative coronavirus, MERS-CoV was first isolated from a man with severe pneumonia in Saudi Arabia, which was died of

multiple organ dysfunctions (Zaki et al. 2012). The virus which is enlisted in the WHO blueprint list for highly concerned pathogens (WHO 2020b), is circulating among societies, and several outbreaks of the disease have been occurred in the Middle East (WHO 2019) and South Korea (Kim et al. 2017). By the end of July 2021, a total of 2578 laboratory-confirmed human cases have been reported globally including 888 deaths, majority of which have been occurred in Arabian Peninsula (WHO 2021b). The clinical manifestation of the disease may range from mild to severe, which the latter usually occurs in individuals older than 65 years. The infection is mainly limited to the respiratory tract; however, also, the viral particles have been detected in the kidney of one autopsied human body (Memish et al. 2020). MERS-CoV is a zoonotic virus with dromedary camels being considered as the host reservoir for the pathogen (Drosten et al. 2014). Experimentally infected camels could shed the virus through nasal secretions with rhinorrhea as the only clinical sign of the disease (Adney et al. 2014). Although nosocomial transmission of MERS-CoV in health-care facilities has been proposed to be responsible for almost half of the cases reported to WHO (Hui et al. 2018), humans may acquire the infection through direct or indirect contact with infected patients or camels (Azhar et al. 2014; Conzade et al. 2018). Infected camels may shed the pathogen via nasal and eye discharge and feces. People may also get the infection through consumption of various raw or undercooked camel products such as milk, meat, urine, and blood. Individuals with underlying medical conditions are at high risk of severe illness (WHO 2018b). To prevent further outbreaks of the disease, it is crucial to maintain good hygiene, especially among individuals working in health care and people in contact with camels. The genome of MERS or MERS-like coronaviruses have been detected in viscera or droppings of bats in Switzerland, Italy, and South Korea (Hardmeier et al. 2021; Kim et al. 2016; Moreno et al. 2017), implicating the potential role of bats in the spread of the pathogen to other parts of the planet.

30.3.2.2 Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever (CCHF), an important tick-borne zoonotic disease is caused by a virus from genus *Nairovirus* of the family *Bunyaviridae* (OIE 2021), which has been detected in arid regions of Eastern Europe, throughout the Mediterranean, northwestern China, central Asia, Africa, the Middle East, and the Indian subcontinent (Hoogstraal 1979; Wernery et al. 2014). The virus is chiefly spread by ticks of genus *Hyalomma*, and circulates in a tick-vertebrate-tick cycle, however also, vertically and horizontally within the arthropod vector (OIE 2021). The presence of pathogen has been confirmed in a wide range of wild and domestic animals such as sheep, goats, cattle, camels, horses, pigs, dogs, hedgehogs, chicken, ostriches, *and* vectors including ticks and biting midges of the genus *Culicoides* (Causey et al. 1970; Hassanein et al. 1997; Khamassi Khbou et al. 2021; Mostafavi et al. 2013; Schwarz et al. 1996). Animals usually act as reservoirs with no obvious clinical signs of the disease, hence, the infection has little if any considerable effect on the animal health and animal production industry; however, the virus can cause a lethal disease in humans (OIE 2021), with the case-fatality ratio of as high as 73% (Schwarz et al. 1997). Despite many

investigations, the pathogenesis of CCHF is not fully understood. The main cells affected are endothelial and immune cells, and the main organs with histopathological changes are liver and spleen (Akıncı et al. 2013). Human can be infected by tick bite or through contact with tissues, blood, and body fluids of infected animals or other individuals (Bente et al. 2013). The sexual route of viral transmission among people has also been suggested (Ergonul and Battal 2014). The presence of the CCHFV has been confirmed in the camels of different countries. While the importance of CCHFV for camels is not elucidated yet, it has been shown that CCHFV strains in some regions are specifically associated with camels and camel ticks (Camp et al. 2021; Khalafalla et al. 2021). In one study on the UAE camels, CCHFV antibodies were detected in the serum of 67% of animals, and the majority of infesting ticks were *Hyalomma dromedarii* (Camp et al. 2020). In a study from Iran, antibodies against CCHFV were found in 5.3% of camels and genome of the virus was detected in 10.2% of ticks collected from camels (Champour et al. 2016). As the CCHFV is widespread in many regions of the world (Msimang et al. 2021), it is suggested that exposure to the virus in people who work in camel-related settings is more common than generally assumed, therefore, public education on the risk factors associated with the infection are needed to control the disease. Besides, the detection of CCHFV RNA in ticks of migratory birds from different countries of Asia, Africa, and Europe (Leblebicioglu et al. 2014; Mancuso et al. 2019; Palomar et al. 2013), as well as finding the CCHFV reactive antibodies in migratory bats of Africa (Müller et al. 2016), indicate the very possibility of CCHFV transportation to other countries and continents by these flying animals.

30.3.2.3 Rift Valley Fever

Rift Valley fever (RVF) is an arthropod-borne viral zoonosis which is endemic in sub-Saharan African countries and the Arabian Peninsula (Khalafalla 2016). The causative virus belongs to the genus *Phlebovirus* from the family *Bunyaviridae* (Flick and Bouloy 2005). RVFV affects ruminants (*e.g.,* cattle, sheep, and goats), camels, and humans. The virus can produce an acute to peracute disease in animals; however, the infection in camels is mostly manifested by abortion. Both New World and Old World camelids can become infected. The presence of serum RVFV-specific antibodies in blood serum of up to 57% of camels have been documented (Britch et al. 2013), and the virus has been isolated from camels (Faye et al. 2014). In at least one outbreak in Mauritania, in which 13 people died, dromedary camels played a major role in the epidemiology and transmission of RVF to humans (El Mamy et al. 2011). The disease has also been detected in young llamas and alpacas of South Africa (Wernery et al. 2014). RVF epidemics have been occurring for more than 70 years in southern and eastern Africa before 2000 in which for the first time, the virus infected humans and animals out of Africa in Saudi Arabia and Yemen (Shoemaker et al. 2002). In one study in the UAE, out of 1119 dairy dromedary camels, four adults (0.35%) were seropositive with ELISA (Wernery et al. 2008). The disease causes serious economic costs to animal owners through loss of production and death. The infection may even lead to 100% abortion in animals. It is believed that RVF outbreaks usually occur following heavy raining, which indicate

the appearance of a very high population of vectors, mainly the mosquitoes of *Anopheles*, *Culex*, *Aedes,* and *Mansonia* genera (Wernery et al. 2014). Humans can get the infection mainly through contact with infected animal carcasses (Hoogstraal et al. 1979) with a fatality rate of approximately 1% (WHO 2018a). In most cases, the clinical disease in humans is similar to influenza, and may easily be confused with malaria. The severe disease may be manifested in the forms of ocular, meningo-encephalitic, and hemorrhagic, the latter being fatal (CDC 2020). No licensed vaccine is available for human use. However, several vaccines have been developed for livestock, albeit not for use outside the endemic areas (Faburay et al. 2017). Camels imported from countries with confirmed RVF outbreaks should be tested for the infection. People should avoid contact with blood, body fluids, and tissues of infected animals, and all animal products should be cooked before consuming. Protection against blood-sucking insects especially mosquitoes is of importance (CDC 2020).

30.3.2.4 West Nile Virus

The zoonotic West Nile Virus (WNV) is a member of the Japanese Encephalitis virus (JEV) serocomplex, and belongs to the *Flavivirus* genus of the family *Flaviviridae* (Fall et al. 2017). The WNV normally exists in nature circulating between birds and mosquitos, with *Culex* spp. as the principal vectors for the pathogen. Human and other mammals are accidental hosts. The virus was first isolated in 1937 from a woman in Uganda (Smithburn et al. 1940), after which, it has been detected in all continents. In infected human, the clinical manifestation ranges from asymptomatic to encephalitis (and resulted paralysis) and death. The nervous symptoms of WNV may easily be confused with similar infections such as viral encephalitis and bacterial meningitis (Rossi et al. 2010). Less than 1% of infected individuals develop a serious illness (CDC 2021), and the infection is more life-threatening in immuno-compromised and old people. Among animals, birds are the most susceptible to the infection; however, serum antibodies against WNV has been frequently detected in many other animals. For instance, using ELISA method, some personnel and different birds, mammals, and reptiles from the Yum-Ká zoo in Mexico were found seropositive for the WNV (Hidalgo-Martinez et al. 2008). Although there have been several serologically confirmed WNV infection in camels from the Middle East, North Africa, and Europe (Mentaberre et al. 2013; Touil et al. 2012; Wernery and Wernery 1990), the virus was isolated for the first time in 2016 from a dromedary camel calf in the UAE. Pallor of skeletal and myocardial muscles, massive lung congestion, and colitis were seen at necropsy (Joseph et al. 2016). The presence of virus in camels may lead to spread of the infection to the neighboring human and animal populations. It has been proposed that geographic correlation of seropositive animals with positive veterinarians suggests predictability of the risk in humans (Venter et al. 2017). While there are several vaccines available for immunizing horses against WNV, no licensed vaccine exists for use in humans and camels (Saiz 2020). It is essential to detect new cases in birds and other animals including horses and camels to provide early warning for veterinary and public health authorities (WHO 2017).

30.3.3 Bacteria

30.3.3.1 *Brucella* spp.

Brucellosis, a contagious disease caused by a zoonotic bacterium of the genus *Brucella*, is of greatest concern, not only because it is zoonotic but also due to its severe economic losses for farmers and ranchers across the world in terms of lost milk, reduced fertility, stillbirths, and abortions (Tibary et al. 2006; Wernery 2014). Humans generally acquire the disease through direct contact with infected animals, by eating or drinking contaminated animal products, or by inhaling airborne agents (Hekmatimoghaddam et al. 2013). Camels can be infected with different biovars of either *B. abortus* and *B. melitensis*, both of which being the main causative agents of human brucellosis (Abbas and Agab 2002).

Camel brucellosis is endemic in all camel-rearing countries with exception of Australia (Gwida et al. 2012). In East Africa, the seroprevalence of brucellosis can reach to 40% at the herd level; however, in a recent study, *B. melitensis* was isolated from lymph nodes of two seronegative camels highlighting the challenges in the diagnosis and control of camel brucellosis (Dadar and Alamian 2020). Human infection occurs via the mucous membranes, mostly through consumption of raw milk but also through cutaneous abrasions (Wernery 2014). Several outbreaks of human infection linked to consumption of traditionally (Garcell et al. 2016) and even commercially sold camel milk (Bardenstein et al. 2021) have been reported. Furthermore, *B. abortus* was detected in the camel liver (Bahari et al. 2021) suggesting that eating raw or undercooked liver – which is popular in some African and Asian regions – can be a health hazard. As clinical signs of brucellosis are mild and the antibody concentrations are low (Gwida et al. 2012), dromedaries might act as silent carriers of the pathogen. In the absence of standardized serological camel-specific tests for active and passive case findings, education of the society such as heat treatment of the milk and avoiding mixed farming of camels with ruminants are advocated.

30.3.3.2 Tuberculosis

The zoonotic disease, tuberculosis (TB), remains among the most serious important public health problems of the twenty-first century (Furin et al. 2019). TB is a chronic granulomatous disease, which is one of the top 10 causes of death worldwide, and the deadliest infectious disease of humankind. It is estimated that about 25% of the world's population is infected with the causative *Mycobacterium* bacilli (WHO 2020a), belonging to the *Mycobacterium tuberculosis* complex (MTC). TB has infected ten million persons worldwide in 2019, and about 1.4 million individuals were died of the disease in the same year (Fukunaga et al. 2021). The same year, approximately 140,000 new human cases of zoonotic TB occurred globally. According to the most recent comprehensive report of the WHO, most people who become infected with TB are living in South-East Asia, Africa, and the Western Pacific (WHO 2020a). While *M. tuberculosis* is responsible for the infection in most human cases, *M. bovis*, the causative agent of bovine TB also can affect people through drinking raw milk and inhalation of infectious droplets (Thoen et al. 2006).

TB infects many vertebrates including camels. Camelids are being considered as a source of TB, as the infection is detected in many countries around the world, *e.g.*, Ethiopia (Gumi et al. 2012), Kenya (Lamuka et al. 2018), India (Ranjan et al. 2018), UAE (Kinne et al. 2006), Spain (Infantes-Lorenzo et al. 2020), and America (Bush et al. 1990). Since 1997, Australia is officially free of *M. bovis* as the most common cause of TB in camels (Brown 2004).

Although is not clear yet, the principal mode of TB transmission between camelids is suggested to be via infected aerosols (Twomey et al. 2009). Disseminated infection of cow and camel with *T. bovis* highlights the probability of TB transmission between livestock species (Ahmad et al. 2019). Despite the very low occurrence of natural cases of TB in the South American camelids (Fowler and Bravo 2010), llamas and alpacas have shown to be very susceptible to *M. bovis* in experimental infections (Stevens et al. 1998), implicating the lack of exposure to the pathogen in their normal habitats (Wernery et al. 2014). In camelids, the lungs and associated thoracic lymph nodes are the most frequently affected organs, where extensive caseonecrotic lesions occur (Wernery and Kinne 2012). Camel farming in many parts of the world is no longer limited to nomadic conditions; therefore keeping camels in close proximity to other animals and humans may increase the risk of zoonotic transmission of the pathogen (Mustafa 1987). To control the disease, all infected animals should be removed from the herd, and further introduction of infection to other animals has to be prevented; however, before the infection is controlled in reservoir hosts, TB will not be eradicated in camels (Thoen et al. 2006).

30.3.3.3 Plague

The deadly zoonotic disease, plague, is an important infectious illness affected many countries around the world. The disease is caused by bacterium *Yersinia pestis*, which is responsible for several devastating pandemics in human history. Based on the European sources, at least two pandemics have been occurred in Europe, the second of which was called "Black Death" and killed about one-third of the continent's population (Mordechai et al. 2019; Slack 1989). The third pandemic was occurred in China and spread to other parts of the world (Tan et al. 2002). In recent history, Africa has faced the vast majority of world plague cases (Nyirenda et al. 2016), and the last outbreak occurred in late 2017 in the island of Madagascar with 202 deaths (Mead 2018).

The causative agent of plague is mainly transmitted by rodents' related fleas. Rodents are the main reservoirs for *Y. pestis*, and camels can be infected with the pathogen. The role of camel in the plague epidemiology has been known for centuries (Gatt Rutter and Mack 1963). In recent decades, *Y. pestis* infection in camels has been reported from several countries such as Libya (Christie et al. 1980), Jordan (Arbaji et al. 2005), Saudi Arabia (Bin Saeed et al. 2005), Afghanistan (Leslie et al. 2011), and Kazakhstan (Lowell et al. 2007). The common clinical manifestations of plague in camels are cutaneous, pulmonary, and septicemic (Wernery et al. 2014). Camels can become infected following contact with dead rodents or rodent-contaminated carnivore carcasses or their excrement (Andrey et al. 2004). Since *Y. pestis* has been isolated from *Xenopsylla cheopis* rat fleas captured near camel pens (Bin Saeed et al. 2005), it

is assumed that this arthropod may act as vector for plague in camels, which in turn, can infect humans directly or carry infected fleas close to humans. Infection of camels with plague has been suspected for a long time (Fedorov 1960), and the role of this animal species in outbreaks in different countries have been documented although infection might show no overt symptoms (Stenseth et al. 2008).

Transmission of plague form camels to humans has been reported in in Kazakhstan, where from 1907 to 2001, human plague was acquired from camels in 400 instances (Aikimbayew et al. 2003). Several outbreaks of plague in humans are believed to have been in association with the consumption of raw or undercooked camel meat or liver in Central Asia (Leslie et al. 2011) and the Middle East (Arbaji et al. 2005; Bin Saeed et al. 2005). Plague is a re-emerging threat to the modern world, and prevention is the best defense strategy against the disease. Vaccination is an efficient tool for protection of human and animals live in high-risk areas. Although several vaccine candidates exist, no licensed plague vaccine is available yet (Sun and Singh 2019).

30.3.4 Fungi

30.3.4.1 Ringworm

Superficial fungal infections are zoonotic diseases caused by taxonomically related fungi known as dermatophytes, which involve the skin, hair, and nails. Infection of the skin is called ringworm (tinea, dermatophytosis), a ring-shaped skin patch with worm-like edges. The causative microorganisms are a group of fungi that require keratin for their growth. Around 40 species of dermatophytes have been described and are classified in three genera of *Trichophyton*, *Microsporum*, and *Epidermophyton* (Weitzman and Summerbell 1995). Although zoophilic dermatophytes such as *Microsporum canis* and *Trichophyton mentagrophytes* are primary animal pathogens, they can infect human beings, too (Zachary 2017). Generally, dermatophtes grow in dead and keratinized cells of stratum corneum or growing hair, and the growth stops when reaching the live tissue (Wernery et al. 2014). These pathogens produce a number of diverse lesions with characteristic distributions, including Tinea capitis, Tinea barbae, Tinea corporis, Tinea cruris, Tinea pedis, and Tinea versicolor (Kumar et al. 2021). Infection can be transmitted to others through direct or indirect contact with infected humans or animals including camels, and contaminated fomites (Wernery et al. 2014). In camelids, the infection is more common in Old World camels under 3 years of age, and is rare in New World camels (Fowler and Bravo 2010). In affected camel herds, up to 80% of calves may show clinical signs of the disease. Dermatophytes are more common in warm and humid environments, and young animals are more susceptible than adults (Wilson 1998). Mixed infection of *T. verrucosum* and *Nocardia asteroides* in dromedary camels has been reported from Iran (Khosravi et al. 2007). Clinical appearances of camel dermatophytoses are quite various. The typical clinical features are either small, round grey-white lesions occurring on the legs, head and neck of young animals, or generalized lesions similar to of mange (Manefield and Tinson 1997).

Histopathological manifestations of the lesions are hyperkeratosis, parakeratosis, and acanthosis in the cornified layer of the skin, stratum corneum, and dermal inflammation (Zachary 2017). It has been estimated that chlamydospores of *T. verrucosum* and *T. mentagrophytes* can remain viable for up to 4.5 years in skin debris and fomites (Fowler and Bravo 2010), therefore public knowledge on the different aspects of the disease and particularly the zoonotic nature of the infection is important.

30.4 Conclusions

This chapter introduced the main parasitic, bacterial, viral, and fungal zoonotic diseases of camels as important livestock animals especially in developing countries. Infective zoonotic agents may be transmitted to humans directly via close contact to the infected camels, consumption of their products, or indirectly through invertebrate vectors. The role of camels in the epidemiology of zoonotic diseases is not fully understood, thus further investigation through transmissible infections between these animals and humans is warranted. In addition, the knowledge and awareness of the communities/individuals in close contact with these animals should be raised through education, in order to lessen the risk of zoonotic infections from animals to humans and vice versa.

30.5 Cross-References

- ▶ Brucellosis
- ▶ Crimean-Congo Hemorrhagic Fever Virus: An Emerging and Re-emerging Pathogen of Public Health Concern
- ▶ Cryptosporidium and Cryptosporidiosis: Trickle or Treat?
- ▶ Cystic and Alveolar Echinococcosis: Fraternal Twins Both in Search of Optimal Treatment
- ▶ Toxoplasmosis: A Widespread Zoonosis Diversely Affecting Humans and Animals
- ▶ Vector-Borne Zoonoses
- ▶ West Nile Virus: From Africa to Europe, America, and Beyond

References

Abbas B, Agab H (2002) A review of camel brucellosis. Prev Vet Med 55:47–56. https://doi.org/10.1016/s0167-5877(02)00055-7

Abu-Zidan FM, Eid HO, Hefny AF, Bashir MO, Branicki F (2012a) Camel bite injuries in United Arab Emirates: a 6 year prospective study. Injury 43:1617–1620. https://doi.org/10.1016/j.injury.2011.10.039

Abu-Zidan FM, Hefny AF, Eid HO, Bashir MO, Branicki FJ (2012b) Camel-related injuries: prospective study of 212 patients. World J Surg 36:2384–2389. https://doi.org/10.1007/s00268-012-1673-2

Adney DR et al (2014) Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerg Infect Dis 20:1999–2005. https://doi.org/10.3201/eid2012.141280

Ahmad I, Kudi CA, Magaji AA, Yakubu Y, Salisu MD, Shuaibu S, Daninna ZM (2019) Disseminated tuberculosis in a cow and a dromedary bull-camel in Zamfara state in Nigeria. Vet Med Sci 5:93–98. https://doi.org/10.1002/vms3.132

Ahmed A et al (2020a) *Balantidium coli* in domestic animals: an emerging protozoan pathogen of zoonotic significance. Acta Trop 203:105298. https://doi.org/10.1016/j.actatropica.2019.105298

Ahmed MA et al (2020b) Epidemiological and histopathological investigation of sarcoptic mange in camels in Egypt. Animals 10:1485. https://doi.org/10.3390/ani10091485

Aikimbayew A, Meka-Mechenko T, Temiralieva G, Bekenov J, Sagiyev Z, Kaljan K, Mukhambetova A (2003) Plague peculiarities in Kazakhstan at the present time. Przeglad Epidemiol 57:593–598

Ajzenberg D et al (2009) Genotype of 88 *toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. J Infect Dis 199:1155–1167. https://doi.org/10.1086/597477

Akıncı E, Bodur H, Leblebicioglu H (2013) Pathogenesis of Crimean-Congo hemorrhagic fever. Vector Borne Zoonotic Dis 13:429–437. https://doi.org/10.1089/vbz.2012.1061

Al-Ghamdi AS, AlGadhi SA (2004) Warning signs as countermeasures to camel–vehicle collisions in Saudi Arabia. Accid Anal Prev 36:749–760. https://doi.org/10.1016/j.aap.2003.05.006

Al Malki JS, Hussien NA (2022) Molecular characterization of *Trypanosoma evansi*, *T. vivax* and *T. congolense* in camels (*Camelus dromedarius*) of KSA. BMC Veterinary Research 18(1):45. https://doi.org/10.1186/s12917-022-03148-0

Al-Rawashdeh OF, Al-Ani FK, Sharrif LA, Al-Qudah KM, Al-Hami Y, Frank N (2000) A survey of camel (*Camelus dromedarius*) diseases in Jordan. J Zoo Wildl Med 31:335–338. https://doi.org/10.1638/1042-7260(2000)031[0335:ASOCCD]2.0.CO;2

Andrey PA, Luther EL, Gerald BP (2004) Intraspecific diversity of *Yersinia pestis*. Clin Microbiol Rev 17:434–464. https://doi.org/10.1128/CMR.17.2.434-464.2004

Anvari D et al (2021) The global status and genetic characterization of hydatidosis in camels (*Camelus dromedarius*): a systematic literature review with meta-analysis based on published papers. Parasitology 148:259–273. https://doi.org/10.1017/S0031182020001705

Arbaji A, Kharabsheh S, Al-Azab S, Al-Kayed M, Amr ZS, Abu Baker M, Chu MC (2005) A 12-case outbreak of pharyngeal plague following the consumption of camel meat, in North–Eastern Jordan. Ann Trop Med Parasitol 99:789–793. https://doi.org/10.1179/136485905X65161

Aregawi WG, Agga GE, Abdi RD, Büscher P (2019) Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasit Vectors 12:67. https://doi.org/10.1186/s13071-019-3311-4

Asghari MM, Rassouli M (2021) First identification of *Trypanosoma vivax* among camels (*Camelus dromedarius*) in Yazd, Central Iran, jointly with *Trypanosoma evansi*. Parasitol Int 86:102450. https://doi.org/10.1016/j.parint.2021.102450

Azhar EI, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, Madani TA (2014) Evidence for camel-to-human transmission of MERS coronavirus. N Engl J Med 370:2499–2505. https://doi.org/10.1056/NEJMoa1401505

Bahari A, Azami S, Goudarztalejerdi A, Karimi S, Esmaeili S, Chomel BB, Sazmand A (2021) Molecular detection of zoonotic pathogens in the blood and tissues of camels (*Camelus dromedarius*) in central desert of Iran. Yale J Biol Med 94:249–258

Balac K, Al-Ali MA, AlMahmoud T, Abu-Zidan FM (2019) Globe rupture caused by a camel bite. Trauma Case Rep 21:100202. https://doi.org/10.1016/j.tcr.2019.100202

Bardenstein S, Gibbs RE, Yagel Y, Motro Y, Moran-Gilad J (2021) Brucellosis outbreak traced to commercially sold camel milk through whole-genome sequencing, Israel. Emerg Infect Dis 27: 1728–1731. https://doi.org/10.3201/eid2706.204902

Baroudi D et al (2018) Divergent *Cryptosporidium parvum* subtype and *Enterocytozoon bieneusi* genotypes in dromedary camels in Algeria. Parasitol Res 117:905–910. https://doi.org/10.1007/s00436-017-5734-1

Belluco S, Mancin M, Conficoni D, Simonato G, Pietrobelli M, Ricci A (2016) Investigating the determinants of *Toxoplasma gondii* prevalence in meat: a systematic review and meta-regression. PLoS One 11:e0153856. https://doi.org/10.1371/journal.pone.0153856

Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M (2013) Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antivir Res 100:159–189. https://doi.org/10.1016/j.antiviral.2013.07.006

Bin Saeed AA, Al-Hamdan NA, Fontaine RE (2005) Plague from eating raw camel liver. Emerg Infect Dis 11:1456–1457. https://doi.org/10.3201/eid1109.050081

Birhanu H et al (2015) Epidemiology of *Trypanosoma evansi* and *Trypanosoma vivax* in domestic animals from selected districts of Tigray and Afar regions, Northern Ethiopia. Parasit Vectors 8: 212–212. https://doi.org/10.1186/s13071-015-0818-1

Boughattas S (2017) *Toxoplasma* infection and milk consumption: meta-analysis of assumptions and evidences. Crit Rev Food Sci Nutr 57:2924–2933. https://doi.org/10.1080/10408398.2015.1084993

Britch SC et al (2013) Rift valley fever risk map model and seroprevalence in selected wild ungulates and camels from Kenya. PLoS One 8:e66626. https://doi.org/10.1371/journal.pone.0066626

Brown A (2004) A review of camel diseases in Central Australia. Department of Business, Industry and Resource Development. https://dpir.nt.gov.au/__data/assets/pdf_file/0014/233222/tb314.pdf. Accessed 29 Sept 2021

Bush M, Montali R, Phillips L, Holobaugh PA (1990) Bovine tuberculosis in a Bactrian camel herd: clinical, therapeutic, and pathologic finding. J Zoo Wildl Med 2:171–179

Calleo VJ, Curtin PO, Biondich AS (2018) Camel bites: a case study with a summary of current epidemiology, injury patterns, and treatment modalities. Wilderness Environ Med 29:380–382. https://doi.org/10.1016/j.wem.2018.02.009

Camel-Scan (2019) Large populations of feral camels in Australia. Feral Scan, Australia. https://www.feralscan.org.au/camelscan/default.aspx. Accessed 29 Sept 2021

Camp JV et al (2020) Crimean-Congo hemorrhagic fever virus endemicity in United Arab Emirates, 2019. Emerg Infect Dis 26:1019–1021. https://doi.org/10.3201/eid2605.191414

Camp JV et al (2021) Association of dromedary camels and camel ticks with reassortant Crimean-Congo hemorrhagic fever virus, United Arab Emirates. Emerg Infect Dis 27:2471–2474. https://doi.org/10.3201/eid2709.210299

Cao Y et al (2020) Genetic diversity of *Cryptosporidium* in bactrian camels (*Camelus bactrianus*) in Xinjiang, Northwestern China. Pathogens 9:946. https://doi.org/10.3390/pathogens9110946

Causey OR, Kemp GE, Madbouly MH, David-West TS (1970) Congo virus from domestic livestock, African hedgehog, and arthropods in Nigeria. Am J Trop Med Hyg 19:846–850. https://doi.org/10.4269/ajtmh.1970.19.846

CDC Centers for Disease Control and Prevention (2020) Rift Valley Fever (RVF). https://www.cdc.gov/vhf/rvf/symptoms/index.html. Accessed 29 Sept 2021

CDC Centers for Disease Control and Prevention (2021) West Nile virus. https://www.cdc.gov/westnile/index.html. Accessed 29 Sept 2021

Chalmers RM (2014) Balantidium coli. In: Percival SL, Yates MV, Williams DW, Chalmers RM, Gray NF (eds) Microbiology of waterborne diseases, 2nd edn. Elsevier, London, pp 277–286

Champour M et al (2016) Crimean-Congo hemorrhagic fever in the one-humped camel (*Camelus dromedarius*) in East and Northeast of Iran. J Arthropod-Borne Dis 10:168–177

Christie AB, Chen TH, Elberg SS (1980) Plague in camels and goats: their role in human epidemics. J Infect Dis 141:724–726. https://doi.org/10.1093/infdis/141.6.724

Chyzheuskaya A, Cormican M, Srivinas R, O'Donovan D, Prendergast M, O'Donoghue C, Morris D (2017) Economic assessment of waterborne outbreak of cryptosporidiosis. Emerg Infect Dis 23:1650. https://doi.org/10.3201/eid2310.152037

Clarence-Smith WG (2013) The historical spread of *Trypanosoma evansi* (Surra) in camels: a factor in the weakening of Islam? In: Emery E (ed) Selected papers from the first international conference of camel cultures: historical traditions, present threats and future prospects. RN Books, London, pp 87–94

Conzade R et al (2018) Reported direct and indirect contact with dromedary camels among laboratory-confirmed MERS-CoV cases. Viruses 10. https://doi.org/10.3390/v10080425

Cox FEG (2005) Human balantidiasis in Iran: are camels reservoir hosts? Trends Parasitol 21:553. https://doi.org/10.1016/j.pt.2005.09.002, author reply 554–555

Dadar M, Alamian S (2020) Isolation of *Brucella melitensis* from seronegative camel: potential implications in brucellosis control. Prev Vet Med 185:105194. https://doi.org/10.1016/j.prevetmed.2020.105194

Deplazes P et al (2017) Global distribution of alveolar and cystic echinococcosis. Adv Parasitol 95: 315–493. https://doi.org/10.1016/bs.apar.2016.11.001

Desquesnes M, Holzmuller P, Lai D-H, Dargantes A, Lun Z-R, Jittaplapong S (2013) *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. Biomed Res Int 2013:194176. https://doi.org/10.1155/2013/194176

Desquesnes M, Gonzatti M, Sazmand A, Thévenon S, Bossard G, Boulangé A, Gimonneau G, Truc P, Herder S, Ravel S, Sereno D, Jamonneau V, Jittapalapong S, Jacquiet P, Solano P, Berthier D (2022) A review on the diagnosis of animal trypanosomoses. Parasites & Vectors 15(1):64. https://doi.org/10.1186/s13071-022-05190-1

Diall O, Desquesnes M, Faye B, Dia ML, Jacquiet P, Sazmand A, Otranto D, Touratier L (2022) Development of a control strategy towards elimination of *Trypanosoma evansi* infection (surra) in camels in Africa. Acta Tropica 234106583-S0001706X22002753 106583. https://doi.org/10.1016/j.actatropica.2022.106583

Dirie MF, Wallbanks K, Aden AA, Bornstein S, Ibrahim M (1989) Camel trypanosomiasis and its vectors in Somalia. Vet Parasitol 32:285–291. https://doi.org/10.1016/0304-4017(89)90039-3

Djurković-Djaković O, Dupouy-Camet J, Van der Giessen J, Dubey JP (2019) Toxoplasmosis: overview from a one health perspective. Food Waterborne Parasitol 12:e00054. https://doi.org/10.1016/j.fawpar.2019.e00054

Drosten C, Kellam P, Memish ZA (2014) Evidence for camel-to-human transmission of MERS coronavirus. N Engl J Med 371:1359–1360. https://doi.org/10.1056/NEJMc1409847

Dubey JP (2021) Toxoplasmosis of animals and humans, 3rd edn. CRC Press, Boca Raton

Dubey J, Schuster R (2018) A review of coccidiosis in old world camels. Vet Parasitol 262:75–83. https://doi.org/10.1016/j.vetpar.2018.08.008

El Mamy ABO et al (2011) Unexpected rift valley fever outbreak, northern Mauritania. Emerg Infect Dis 17:1894–1896. https://doi.org/10.3201/eid1710.110397

El-Alfy E-S, Abu-Elwafa S, Abbas I, Al-Araby M, Al-Kappany Y, Umeda K, Nishikawa Y (2019) Molecular screening approach to identify protozoan and trichostrongylid parasites infecting one-humped camels (*Camelus dromedarius*). Acta Trop 197:105060. https://doi.org/10.1016/j.actatropica.2019.105060

Elfadaly HA, Hassanan N, Shaapan RM, Hassanain MA, Barakat AM, Abdelrahman KA (2017) Molecular detection and genotyping of *Toxoplasma gondii* from Egyptian isolates. Asian J Epidemiol 10:37–44. https://doi.org/10.3923/aje.2017.37.44

El-Khabaz KA, Abdel-Hakeem SS, Arfa MI (2019) Protozoan and helminthes parasites endorsed by imported camels (*Camel dromedaries*) to Egypt. J Parasit Dis 43:607–615. https://doi.org/10.1007/s12639-019-01138-y

Ergonul O, Battal I (2014) Potential sexual transmission of Crimean-Congo hemorrhagic fever infection. Japan J Infect Dis 67:137–138. https://doi.org/10.7883/yoken.67.137

Evans G (1880) Report on 'Surra' disease in the Dera Ismail Khan District, November 13th, 1880, Military Department, No. 493, p 4467

Faburay B, LaBeaud AD, McVey DS, Wilson WC, Richt JA (2017) Current status of rift valley fever vaccine development. Vaccine 5:29. https://doi.org/10.3390/vaccines5030029
Fall G et al (2017) Biological and phylogenetic characteristics of West African lineages of West Nile virus. PLoS Negl Trop Dis 11:e0006078. https://doi.org/10.1371/journal.pntd.0006078
FAOSTAT (2019) Food and Agriculture Organization of the United Nations statistics division. FAO, Rome
Faye O et al (2014) Reemergence of Rift Valley fever, Mauritania, 2010. Emerg Infect Dis 20:300–303. https://doi.org/10.3201/eid2002.130996
Fedorov VN (1960) Plague in camels and its prevention in the USSR. Bull World Health Organ 23: 275–281
Feng Y, Ryan UM, Xiao L (2018) Genetic diversity and population structure of *Cryptosporidium*. Trends Parasitol 34:997–1011. https://doi.org/10.1016/j.pt.2018.07.009
Flick R, Bouloy M (2005) Rift valley fever virus. Curr Mol Med 5:827–834. https://doi.org/10.2174/156652405774962263
Fowler ME, Bravo PW (2010) Infectious diseases. In: Medicine and surgery of camelids, 3rd edn. Blackwell Publishing, Ames, pp 173–230. https://doi.org/10.1002/9781118785706.ch7
Fukunaga R, Glaziou P, Harris JB, Date A, Floyd K, Kasaeva T (2021) Epidemiology of tuberculosis and progress toward meeting global targets – worldwide, 2019. MMWR Morb Mortal Wkly Rep 70:427–430. https://doi.org/10.15585/mmwr.mm7012a4
Furin J, Cox H, Pai M (2019) Tuberculosis. Lancet 393:1642–1656. https://doi.org/10.1016/S0140-6736(19)30308-3
Garcell HG, Garcia EG, Pueyo PV, Martín IR, Arias AV, Serrano RNA (2016) Outbreaks of brucellosis related to the consumption of unpasteurized camel milk. J Infect Public Health 9: 523–527. https://doi.org/10.1016/j.jiph.2015.12.006
Gatt Rutter TE, Mack R (1963) Diseases of camels. Part 1: bacterial and fungal diseases. Vet Bull Weybridge 33:119–124
Gebremedhin EZ et al (2014) First report of *Toxoplasma gondii* in camels (*Camelus dromedarius*) in Ethiopia: bioassay and seroepidemiological investigation. BMC Vet Res 10:222–222. https://doi.org/10.1186/s12917-014-0222-7
Gilbert JD, Byard RW (2021) Camel-related deaths – a forensic overview. Am J Forensic Med Pathol 42:46–50. https://doi.org/10.1097/PAF.0000000000000606
Gu Y et al (2016) Investigation on *Cryptosporidium* infections in wild animals in a zoo in Anhui Province. J Zoo Wildl Med 47:846–854. https://doi.org/10.1638/2015-0301.1
Gumi B et al (2012) Zoonotic transmission of tuberculosis between pastoralists and their livestock in South-East Ethiopia. EcoHealth 9:139–149. https://doi.org/10.1007/s10393-012-0754-x
Gutierrez C, Desquesnes M, Touratier L, Büscher P (2010) *Trypanosoma evansi*: recent outbreaks in Europe. Vet Parasitol 174:26–29. https://doi.org/10.1016/j.vetpar.2010.08.012
Gwida M, El-Gohary A, Melzer F, Khan I, Rösler U, Neubauer H (2012) Brucellosis in camels. Res Vet Sci 92:351–355. https://doi.org/10.1016/j.rvsc.2011.05.002
Hagemoser W, Dubey J, Thompson J (1990) Acute toxoplasmosis in a camel. J Am Vet Med Assoc 196:347
Hamidinejat H et al (2013) Occurrence of anti- *Toxoplasma gondii* and *Neospora caninum* antibodies in camels (*Camelus dromedarius*) in the center of Iran. Turk J Vet Anim Sci 37: 277–281. https://doi.org/10.3906/vet-1110-21
Hamidinejat H, Hekmatimoghaddam S, Jafari H, Sazmand A, Haddad P, Derakhshan ML, Mirabdollahi S (2013) Prevalence and distribution patterns of *Sarcocystis* in camels (*Camelus dromedarius*) in Yazd province Iran. Journal of Parasitic Diseases 37(2):163–165. https://doi.org/10.1007/s12639-012-0150-z
Harandi MF, Budke CM, Rostami S (2012) The monetary burden of cystic echinococcosis in Iran. PLoS Negl Trop Dis 6(e1915). https://doi.org/10.1371/journal.pntd.0001915
Hardmeier I et al (2021) Metagenomic analysis of fecal and tissue samples from 18 endemic bat species in Switzerland revealed a diverse virus composition including potentially zoonotic viruses. PLoS One 16:e0252534. https://doi.org/10.1371/journal.pone.0252534

Hassanein KM, el-Azazy OM, Yousef HM (1997) Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia. Trans R Soc Trop Med Hyg 91:536–537. https://doi.org/10.1016/s0035-9203(97)90014-6

Hegner R (1934) Specificity in the genus *Balantidium* based on size and shape of body and macronucleus, with descriptions of six new species. Am J Epidemiol 19:38–67. https://doi.org/10.1093/oxfordjournals.aje.a117992

Hekmatimoghaddam S, Sadeh M, Khalili MB, Mollaabedin M, Sazmand A (2013) Comparison of PCR, Wright agglutination test and blood culture for diagnosis of brucellosis in suspected patients. Pak J Biol Sci 16:1589–1592. https://doi.org/10.3923/pjbs.2013.1589.1592

Hidalgo-Martinez M et al (2008) Prevalence of West Nile virus infection in animals from two state zoos Tabasco. Salud Publica Mex 50:76–85. https://doi.org/10.1590/s0036-36342008000100014

Hoogstraal H (1979) The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol 15:307–417. https://doi.org/10.1093/jmedent/15.4.307

Hoogstraal H, Meegan JM, Khalil GM, Adham FK (1979) The Rift Valley fever epizootic in Egypt 1977–78. 2. Ecological and entomological studies. Trans R Soc Trop Med Hyg 73:624–629. https://doi.org/10.1016/0035-9203(79)90005-1

Hui DS, Azhar EI, Kim YJ, Memish ZA, Oh MD, Zumla A (2018) Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis 18:e217–e227. https://doi.org/10.1016/s1473-3099(18)30127-0

Hussein AG, Al-Samarai FR, Asal SN (2016) Impact of age and gender of camels (*Camelus dromedarius*) and their breeders on the prevalence of *Balantidium coli*. J Biosci Agric Res 8:734–738. https://doi.org/10.18801/jbar.080216.87

Infantes-Lorenzo JA et al (2020) Tuberculosis caused by *Mycobacterium caprae* in a camel (*Camelus dromedarius*). BMC Vet Res 16:435. https://doi.org/10.1186/s12917-020-02665-0

Innes EA, Chalmers RM, Wells B, Pawlowic MC (2020) A one health approach to tackle cryptosporidiosis. Trends Parasitol 36:290–303. https://doi.org/10.1016/j.pt.2019.12.016

Ishag MY (2003) Studies on *Toxoplasma* and *Sarcocystis* from camels (*Camelus dromedaries*) in the Sudan. PhD thesis, University of Khartoum, Sudan

Ishag MY, Majid A (2008) Association of diarrhea with congenital toxoplasmosis in calf-camel (*Camelus dromedarius*). Int J Trop Med 3:10–11

Ježková J et al (2021) *Cryptosporidium myocastoris* n. sp. (Apicomplexa: Cryptosporidiidae), the species adapted to the nutria (*Myocastor coypus*). Microorganisms 9(813). https://doi.org/10.3390/microorganisms9040813

Joseph S et al (2016) First isolation of West Nile virus from a dromedary camel. Emerg Microb Infect 5:e53. https://doi.org/10.1038/emi.2016.53

Joshi PP et al (2005) Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. Am J Trop Med Hyg 73:491–495. https://doi.org/10.4269/ajtmh.2005.73.491

Khalafalla AI (2016) Emerging infectious diseases in camelids. In: Bayry J (ed) Emerging and re-emerging infectious diseases of livestock. Springer, Cham, pp 425–441. https://doi.org/10.1007/978-3-319-47426-7_20

Khalafalla AI et al (2021) Identification of a novel lineage of Crimean-Congo haemorrhagic fever virus in dromedary camels, United Arab Emirates. J Gen Virol 102:001473. https://doi.org/10.1099/jgv.0.001473

Khalil IA et al (2018) Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analyses study. Lancet Glob Health 6:e758. https://doi.org/10.1016/S2214-109X(18)30283-3

Khamassi Khbou M et al (2021) Presence of antibodies to Crimean Congo haemorrhagic fever virus in sheep in Tunisia, North Africa. Vet Med Sci. https://doi.org/10.1002/vms3.597

Khosravi AR, Shokri H, Niasari-Naslaji A (2007) An outbreak of a mixed infection of *Trichophyton verrucosum* and Nocardia asteroides in dromedary camels in Iran. J Camel Pract Res 14:109–112

Kim HK et al (2016) Detection of severe acute respiratory syndrome-like, middle east respiratory syndrome-like bat coronaviruses and group H rotavirus in faeces of Korean bats. Transbound Emerg Dis 63:365–372. https://doi.org/10.1111/tbed.12515

Kim KH, Tandi TE, Choi JW, Moon JM, Kim MS (2017) Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak in South Korea, 2015: epidemiology, characteristics and public health implications. J Hosp Infect 95:207–213. https://doi.org/10.1016/j.jhin.2016.10.008

Kinne J, Johnson B, Jahans KL, Smith NH, Ul-Haq A, Wernery U (2006) Camel tuberculosis-a case report. Trop Anim Health Prod 38:207–213. https://doi.org/10.1007/s11250-006-4366-8

Kumar V, Abbas AK, Aster JC (2021) Robbins & Cotran pathologic basis of disease, 10th edn. Elsevier, Philadelphia

Lai D-H, Hashimi H, Lun Z-R, Ayala FJ, Lukeš J (2008) Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. Proc Natl Acad Sci U S A 105:1999–2004

Lal A, Dobbins T, Bagheri N, Baker MG, French NP, Hales S (2016) Cryptosporidiosis risk in New Zealand children under 5 years old is greatest in areas with high dairy cattle densities. EcoHealth 13:652–660. https://doi.org/10.1007/s10393-016-1187-8

Lamuka PO, Njeruh FM, Gitao GC, Matofari J, Bowen R, Abey KA (2018) Prevalence of bovine and avian tuberculosis in camel herds and associated public health risk factors in Isiolo County, Kenya. Trop Anim Health Prod 50:937–945. https://doi.org/10.1007/s11250-017-1486-2

Leblebicioglu H, Eroglu C, Erciyas-Yavuz K, Hokelek M, Acici M, Yilmaz H (2014) Role of migratory birds in spreading Crimean-Congo hemorrhagic fever, Turkey. Emerg Infect Dis 20:1331–1334. https://doi.org/10.3201/eid2008.131547

Leslie T et al (2011) Outbreak of gastroenteritis caused by *Yersinia pestis* in Afghanistan. Epidemiol Infect 139:728–735. https://doi.org/10.1017/s0950268810001792

Lowell JL et al (2007) Phenotypic and molecular characterizations of *Yersinia pestis* isolates from Kazakhstan and adjacent regions. Microbiology 153:169–177. https://doi.org/10.1099/mic.0.29059-0

MacHugh DE, Larson G, Orlando L (2017) Taming the past: ancient DNA and the study of animal domestication. Ann Rev Anim Biosci 5:329–351. https://doi.org/10.1146/annurev-animal-022516-022747

Mancuso E et al (2019) Crimean-Congo hemorrhagic fever virus genome in tick from migratory bird, Italy. Emerg Infect Dis 25:1418–1420. https://doi.org/10.3201/eid2507.181345

Manefield GW, Tinson AH (1997) Camels: a compendium. University of Sydney Post Graduate Foundation in Veterinary Science, Sydney

Mead PS (2018) Plague in Madagascar – a tragic opportunity for improving public health. N Engl J Med 378:106–108. https://doi.org/10.1056/NEJMp1713881

Medani M, Mohamed H (2016) Camel's milk as a source of human toxoplasmosis in Butana area-Sudan. Int J Infect Dis 45:471–472. https://doi.org/10.1016/j.ijid.2016.02.996

Memish ZA, Perlman S, Van Kerkhove MD, Zumla A (2020) Middle East respiratory syndrome. Lancet 395:1063–1077. https://doi.org/10.1016/s0140-6736(19)33221-0

Mentaberre G et al (2013) A transversal study on antibodies against selected pathogens in dromedary camels in the Canary Islands, Spain. Vet Microbiol 167:468–473. https://doi.org/10.1016/j.vetmic.2013.07.029

Mordechai L, Eisenberg M, Newfield TP, Izdebski A, Kay JE, Poinar H (2019) The Justinianic plague: an inconsequential pandemic? Proc Natl Acad Sci U S A 116:25546–25554. https://doi.org/10.1073/pnas.1903797116

Moreno A et al (2017) Detection and full genome characterization of two beta CoV viruses related to Middle East respiratory syndrome from bats in Italy. Virol J 14:239. https://doi.org/10.1186/s12985-017-0907-1

Mossaad E et al (2017) *Trypanosoma vivax* is the second leading cause of camel trypanosomosis in Sudan after *Trypanosoma evansi*. Parasit Vectors 10:176. https://doi.org/10.1186/s13071-017-2117-5

Mostafavi E, Chinikar S, Moradi M, Bayat N, Meshkat M, Khalili Fard M, Ghiasi SM (2013) A case report of Crimean Congo hemorrhagic fever in ostriches in Iran. Open Virol J 7:81–83. https://doi.org/10.2174/1874357901307010081

Msimang V et al (2021) Risk factors associated with exposure to Crimean-Congo haemorrhagic fever virus in animal workers and cattle, and molecular detection in ticks, South Africa. PLoS Negl Trop Dis 15:e0009384. https://doi.org/10.1371/journal.pntd.0009384

Müller MA et al (2016) Evidence for widespread infection of African bats with Crimean-Congo hemorrhagic fever-like viruses. Sci Rep 6:26637. https://doi.org/10.1038/srep26637

Mustafa IE (1987) Bacterial disease of dromedaries and bacterian camels. Rev Sci Tech 6:391–405. https://doi.org/10.20506/rst.6.2.294

Nakauchi K (1999) The prevalence of *Balantidium coli* infection in fifty-six mammalian species. J Vet Med Sci 61:63–65. https://doi.org/10.1292/jvms.61.63

Nayel NM, Abu-Samra MT (1986) Sarcoptic mange in the one-humped camel (*Camelus dromedarius*). A clinico-pathological and epizootiological study of the disease and its treatment. J Arid Environ 10:199–211. https://doi.org/10.1016/S0140-1963(18)31240-0

Nourian A (1992) Serological study of toxoplasmosis in camels of Fars Province. DVM dissertation. Shiraz University, Iran

Nydam DV, Wade SE, Schaaf SL, Mohammed HO (2001) Number of *Cryptosporidium parvum* oocysts or *Giardia* spp cysts shed by dairy calves after natural infection. Am J Vet Res 62:1612–1615. https://doi.org/10.2460/ajvr.2001.62.1612

Nyirenda SS, Hang'ombe BM, Kilonzo BS (2016) Factors that precipitated human plague in Zambia from 1914 to 2014 – an overview for a century (100 years). J Zoonot Dis 1:1–14

Ohiolei JA et al (2020) Prevalence and distribution of *Echinococcus* spp. in wild and domestic animals across Africa: a systematic review and meta-analysis. Transbound Emerg Dis 67:2345–2364. https://doi.org/10.1111/tbed.13571

OIE (2021) Manual of diagnostic tests and vaccines for terrestrial animals 2021. In: Manual of diagnostic tests and vaccines for terrestrial animals 2021. World Organisation for Animal Health, Paris, pp 399–406

Otranto D, Dantas-Torres F, Mihalca AD, Traub RJ, Lappin M, Baneth G (2017) Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis. Trends Parasitol 33:813–825. https://doi.org/10.1016/j.pt.2017.05.013

Ouajd S, Kamel B (2009) Physiological particularities of dromedary (*Camelus dromedarius*) and experimental implications. Scand J Lab Anim Sci 36:19–29. https://doi.org/10.23675/sjlas.v36i1.165

Palomar AM et al (2013) Crimean-Congo hemorrhagic fever virus in ticks from migratory birds, Morocco. Emerg Infect Dis 19:260–263. https://doi.org/10.3201/eid1902.121193

Pegram R, Higgins AJ (1992) Camel ectoparasites: a review. Paper presented at the Proceeding of the First International Camel Conference, Dubai

Pomajbíková K et al (2013) Novel insights into the genetic diversity of *Balantidium* and *Balantidium*-like cyst-forming ciliates. PLoS Negl Trop Dis 7:e2140. https://doi.org/10.1371/journal.pntd.0002140

Ranjan R, Narnaware SD, Nath K, Sawal RK, Patil NV (2018) Rapid diagnosis of tuberculosis in dromedary camel (*Camelus dromedarius*) using lateral flow assay-based kit. Trop Anim Health Prod 50:907–910. https://doi.org/10.1007/s11250-017-1502-6

Reid SA (2002) *Trypanosoma evansi* control and containment in Australasia. Trends Parasitol 18:219–224. https://doi.org/10.1016/S1471-4922(02)02250-X

Riley J, Garner MM, Kiupel M, Hammond EE (2017) Disseminated toxoplasmosis in a captive adult dromedary camel (*Camelus dromedarius*). J Zoo Wildl Med 48:937–940. https://doi.org/10.1638/2016-0057.1

Roettcher D, Schillinger D, Zweygarth E (1987) Trypanosomiasis in the camel (*Camelus dromedarius*). Rev Sci Tech 6:463–470

Rossi SL, Ross TM, Evans JD (2010) West Nile virus. Clin Lab Med 30:47–65. https://doi.org/10.1016/j.cll.2009.10.006

Sadjjadi S, Mikaeili F, Karamian M, Maraghi S, Sadjjadi F, Shariat-Torbaghan S, Kia E (2013) Evidence that the *Echinococcus granulosus* G6 genotype has an affinity for the brain in humans. Int J Parasitol 43:875–877. https://doi.org/10.1016/j.ijpara.2013.06.008

Saiz JC (2020) Animal and human vaccines against West Nile virus. Pathogens 9:1073. https://doi.org/10.3390/pathogens9121073

Sawitri DH, Wardhana AH, Sadikin M, Wibowo H (2019) Detection of Surra (trypanosomiasis) positivity in humans in an outbreak area of Indonesia. Med J Indonesia 28:196–202. https://doi.org/10.13181/mji.v28i2.1767

Sazmand A, Joachim A (2017) Parasitic diseases of camels in Iran (1931–2017) – a literature review. Parasite 24:21. https://doi.org/10.1051/parasite/2017024

Sazmand A, Rasooli A, Nouri M, Hamidinejat H, Hekmatimoghaddam S (2011) Serobiochemical alternations in subclinically affected dromedary camels with *Trypanosoma evansi* in Iran. Pak Vet J 31:223–226

Sazmand A, Rasooli A, Nouri M, Hamidinejat H, Hekmatimoghaddam S (2012) Prevalence of *Cryptosporidium* spp. in camels and involved people in Yazd Province, Iran. Iran J Parasitol 7: 80–84

Sazmand A et al (2016) Molecular identification of hemoprotozoan parasites in camels (*Camelus dromedarius*) of Iran. Iran J Parasitol 11:568–573

Sazmand A et al (2019a) Vector-borne bacteria in blood of camels in Iran: new data and literature review. Comp Immunol Microbiol Infect Dis 65:48–53. https://doi.org/10.1016/j.cimid.2019.04.004

Sazmand A, Joachim A, Otranto D (2019b) Zoonotic parasites of dromedary camels: so important, so ignored. Parasit Vectors 12:610. https://doi.org/10.1186/s13071-019-3863-3

Sazmand A, Desquesnes M, Otranto D (2022) *Trypanosoma evansi*. Trends in Parasitology 38 (6):489–490. S1471492222000010. https://doi.org/10.1016/j.pt.2021.12.010

Scallan E et al (2011) Foodborne illness acquired in the United States – major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.P11101

Scallan E, Hoekstra R, Mahon B, Jones T, Griffin P (2015) An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. Epidemiol Infect 143:2795–2804. https://doi.org/10.1017/S0950268814003185

Schillinger D (1987) Mange in camels – an important zoonosis. Rev Sci Tech 6:479–480

Schuster RK (2018) Parasites of dromedaries and bactrian camels – a review part 1: stenoxenous parasites. J Camel Pract Res 25:1–8. https://doi.org/10.5958/2277-8934.2018.00001.2

Schuster FL, Ramirez-Avila L (2008) Current world status of *Balantidium coli*. Clin Microbiol Rev 21:626–638. https://doi.org/10.1128/CMR.00021-08

Schwarz TF et al (1996) Polymerase chain reaction for diagnosis and identification of distinct variants of Crimean-Congo hemorrhagic fever virus in the United Arab Emirates. Am J Trop Med Hyg 55:190–196. https://doi.org/10.4269/ajtmh.1996.55.190

Schwarz TF, Nsanze H, Ameen AM (1997) Clinical features of Crimean-Congo haemorrhagic fever in the United Arab Emirates. Infection 25:364–367. https://doi.org/10.1007/BF01740819

Sengupta PP, Jacob Atru SS, Chandu GS, Das S (2022) Silent *Trypanosoma evansi* infection in humans from India revealed by serological and molecular surveys and characterized by variable surface glycoprotein gene sequences. Acta Tropica 229106369–S0001706X22000687 106369 https://doi.org/10.1016/j.actatropica.2022.106369

Shirmen O et al (2018) Cerebral cystic echinococcosis in Mongolian children caused by *Echinococcus canadensis*. Parasitol Int 67:584–586. https://doi.org/10.1016/j.parint.2018.05.006

Shoemaker T et al (2002) Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000–01. Emerg Infect Dis 8:1415–1420. https://doi.org/10.3201/eid0812.020195

Shwab EK, Zhu X-Q, Majumdar D, Pena HF, Gennari SM, Dubey JP, Su C (2014) Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitology 141:453–461. https://doi.org/10.1017/S0031182013001844

Slack P (1989) The black death past and present. 2. Some historical problems. Trans R Soc Trop Med Hyg 83:461–463. https://doi.org/10.1016/0035-9203(89)90247-2

Smithburn KC, Hughes TP, Burke AW, Paul JH (1940) A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 1:471–492. https://doi.org/10.4269/ajtmh.1940.s1-20.471

Stenseth NC et al (2008) Plague: past, present, and future. PLoS Med 5:e3. https://doi.org/10.1371/journal.pmed.0050003

Stevens JB, Thoen CO, Rohonczy EB, Tessaro S, Kelly HA, Duncan JR (1998) The immunological response of llamas (*Lama glama*) following experimental infection with *Mycobacterium bovis*. Canad J Vet Res 62:102–109

Sun W, Singh AK (2019) Plague vaccine: recent progress and prospects. npj Vaccines 4:11. https://doi.org/10.1038/s41541-019-0105-9

Tadjbakhsh H (1994) Traditional methods used for controlling animal diseases in Iran. Rev Sci Tech 13:599–614

Tajik J, Nourollahi Fard SR, Paidar A, Anousheh S, Dehghani E (2013) Balantidiasis in a dromedarian camel. Asian Pac J Trop Dis 3:409–412. https://doi.org/10.1016/S2222-1808(13)60093-6

Tan J, Liu Y, Shen E, Zhu W, Wang W, Li R, Yang L (2002) Towards "the atlas of plague and its environment in the People's republic of China": idea, principle and methodology of design and research results. Huan Jing Ke Xue 23:1–8

Tavakoli Kareshk A et al (2018) The first survey of isolation and molecular typing of *Toxoplasma gondii* by bioassay and PCR method in BALB/C mice in camels from eastern Iran. Iran J Parasitol 13:382–391

Taylor MA, Coop RL, Wall RL (2016) Veterinary parasitology, 4th edn. Wiley Blackwell, Oxford/Ames

Thoen C, Lobue P, de Kantor I (2006) The importance of *Mycobacterium bovis* as a zoonosis. Vet Microbiol 112:339–345. https://doi.org/10.1016/j.vetmic.2005.11.047

Thompson R (2017) Biology and systematics of *Echinococcus*. Adv Parasitol 95:65–109. https://doi.org/10.1016/bs.apar.2016.07.001

Tibary A, Fite C, Anouassi A, Sghiri A (2006) Infectious causes of reproductive loss in camelids. Theriogenology 66:633–647. https://doi.org/10.1016/j.theriogenology.2006.04.008

Tonouhewa ABN et al (2017) *Toxoplasma gondii* infection in meat animals from Africa: systematic review and meta-analysis of sero-epidemiological studies. Vet World 10:194. https://doi.org/10.14202/vetworld.2017.194-208

Touil N, Cherkaoui Z, Lmrabih Z, Loutfi C, Harif B, El Harrak M (2012) Emerging viral diseases in dromedary camels in the Southern Morocco. Transbound Emerg Dis 59:177–182. https://doi.org/10.1111/j.1865-1682.2011.01282.x

Truc P et al (2013) Atypical human infections by animal trypanosomes. PLoS Negl Trop Dis 7:e2256–e2256. https://doi.org/10.1371/journal.pntd.0002256

Twomey DF et al (2009) Suspected transmission of *Mycobacterium bovis* between alpacas. Vet Rec 165:121–122. https://doi.org/10.1136/vetrec.165.4.121

United Nations (2019) Probabilistic population projections rev. 1 based on the world population prospects 2019 rev. 1. http://population.un.org/wpp/

Van Vinh Chau N et al (2016) A clinical and epidemiological investigation of the first reported human infection with the zoonotic parasite *Trypanosoma evansi* in Southeast Asia. Clin Infect Dis 62:1002–1008. https://doi.org/10.1093/cid/ciw052

Vanhollebeke B et al (2006) Human *Trypanosoma evansi* infection linked to a lack of apolipoprotein L-I. N Engl J Med 355:2752–2756. https://doi.org/10.1056/NEJMoa063265

Venter M et al (2017) West Nile virus lineage 2 in horses and other animals with neurologic disease, South Africa, 2008–2015. Emerg Infect Dis 23:2060–2064. https://doi.org/10.3201/eid2312.162078

Vuitton DA et al (2020) International consensus on terminology to be used in the field of echinococcoses. Parasite 27:41. https://doi.org/10.1051/parasite/2020024

Wang L, Cao L, Zheng S, Chang Y, Zhang K, Zhang S, Zhang L (2021) Molecular identification and biological characterization of *Cryptosporidium muris* from camels (*Camelus bactrianus*) in China. Parasit Vectors 14:365. https://doi.org/10.1186/s13071-021-04862-8

Weitzman I, Summerbell RC (1995) The dermatophytes. Clin Microbiol Rev 8:240–259. https://doi.org/10.1128/cmr.8.2.240

Wernery U (2014) Camelid brucellosis: a review. Rev Sci Tech 33:839–857. https://doi.org/10.20506/rst.33.3.2322

Wernery U, Kinne J (2012) Tuberculosis in camelids: a review. Rev Sci Tech 31:899–906. https://doi.org/10.20506/rst.31.3.2161

Wernery U, Wernery R (1990) Seroepidemiologic studies of the detection of antibodies to *Brucella*, *Chlamydia*, *Leptospira*, BVD/MD virus, IBR/IPV virus and enzootic bovine leukosis virus (EBL) in dromedary mares (*Camelus dromedarius*). Dtsch Tierarztl Wochenschr 97:134–135

Wernery U, Thomas R, Raghavan R, Syriac G, Joseph S, Georgy N (2008) Seroepidemiological studies for the detection of antibodies against 8 infectious diseases in dairy dromedaries of the United Arab Emirates using modern laboratory techniques – part II. J Camel Pract Res 15:139–145

Wernery U, Kinne J, Schuster RK (2014) Camelid infectious disorders. OIE (World Organisation for Animal Health), Paris

WHO (2015) WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007–2015. World Health Organization, Geneva

WHO (2017) West Nile virus. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/west-nile-virus. Accessed 29 Sept 2021

WHO (2018a) Rift Valley fever. World Health Organization. https://www.who.int/en/news-room/fact-sheets/detail/rift-valley-fever. Accessed 29 Sept 2021

WHO (2018b) Update on MERS-CoV transmission from animals to humans, and interim recommendations for at-risk groups. World Health Organization. https://www.who.int/news/item/26-01-2018-update-on-mers-cov-transmission-from-animals-to-humans-and-interim-recommendations-for-at-risk-groups. Accessed 29 Sept 2021

WHO (2019) Middle East respiratory syndrome coronavirus (MERS-CoV). World Health Organization. https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov). Accessed 29 Sept 2021

WHO (2020a) Global tuberculosis report 2020. World Health Organization. https://www.who.int/publications/i/item/9789240013131. Accessed 29 Sept 2021

WHO (2020b) A research and development blueprint for action to prevent epidemics. World Health Organization. https://www.who.int/blueprint/en. Accessed 29 Sept 2021

WHO (2021a) Middle East respiratory syndrome coronavirus (MERS-CoV). World Health Organization. https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers. Accessed 29 Sept 2021

WHO (2021b) Middle East respiratory syndrome coronavirus (MERS-CoV) – Saudi Arabia. World Health Organization. https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON333. Accessed 29 Sept 2021

Wilson RT (1998) The tropical agriculturalist: camels. Macmillan Education, London

Zachary JF (2017) Pathologic basis of veterinary disease, 6th edn. Elsevier, St. Louis

Zahedi A, Lee GK, Greay TL, Walsh AL, Blignaut DJ, Ryan UM (2018) First report of *Cryptosporidium parvum* in a dromedary camel calf from Western Australia. Acta Parasitol 63:422–427. https://doi.org/10.1515/ap-2018-0049

Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA (2012) Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 367:1814–1820. https://doi.org/10.1056/NEJMoa1211721

Zhu S, Zimmerman D, Deem SL (2019) A review of zoonotic pathogens of dromedary camels. EcoHealth 16:356–377. https://doi.org/10.1007/s10393-019-01413-7

Animal Bites and Zoonoses: From A to Z – Alligators to Zebras

31

Ellie J. C. Goldstein and Fredrick M. Abrahamian

Contents

Abstract

Worldwide, animal bite-related injuries to humans are a common daily occurrence. Injuries can range from minor puncture wounds to extensive crush injuries and even amputations and death. Increasing population, continued spread of habitation, and the popularity of owning various types of both traditional and nontraditional pets have made it easier for humans to have contact with various

E. J. C. Goldstein (✉)
David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

R. M. Alden Research Laboratory, Santa Monica, CA, USA

F. M. Abrahamian
David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Faculty, Department of Emergency Medicine, Olive View-UCLA Medical Center, Sylmar, CA, USA

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_26

types of animals. In general, the oral flora of the biting animal will be concordant with the bacteria isolated from the wound. These oral flora organisms often originate from the normal oral residents and environmental flora as well as the skin and intestinal bacteria of the animals' food sources and prey.

Keywords

Aerobes · Anaerobes · Animal · Avulsion · *Bacteroides* · Bite · *Capnocytophaga* · Cat · Dog · *Eikenella* · Emergency · *Fusobacterium* · Human · Injury · Laceration · *Pasteurella* · Pet · Puncture · *Staphylococcus* · *Streptococcus*

31.1 Introduction

A wide variety of domestic and wild animals are reported to bite people worldwide each year causing injuries that range from minor infections to debilitating and even lethal injuries. The increasing population, continued spread of habitation, and popularity of owning various types of both traditional and nontraditional pets have made it easier for humans to have contact with various types of animals. It is therefore not surprising that this increased exposure can consequently result in more bite injuries.

Most bite wounds are minor injuries that go unreported. Patients often self-administer first aid and usually do not seek or require medical attention. In rural areas and non-industrialized settings, medical care is often hard to obtain and not readily available. In contrast, residents of industrialized countries, especially those in urban and suburban areas, often seek care when incurring moderate to severe bite injuries in an emergency department or in a physician's office. When these injuries are reported, it is usually from small, limited studies that focus on a specific aspect or complication of injury or concentrate on unusual or resistant organisms. These small often retrospective studies form the only basis for the medical decision-making and treatment of bite wounds that is currently employed worldwide.

Bite wounds can consist of lacerations, evulsions, punctures, scratches, and crush injuries. Although the majority of patients never seek or do not require extensive medical care, awareness of the magnitude of the infectious complications from bites is necessary. The bacteria associated with bite infections may come from the environment, the victim's skin flora, or most frequently, the oral flora of the biter which can also be influenced by the microbiome of their ingested prey and other food.

The public health implications for animal bite wounds include not only the cost of therapy but that of resultant physical disability, both acutely and in the long term, days lost from work or school, costs of hospitalization, and insurance claims. According to an analysis of homeowners' insurance claims data in 2021, the average cost of dog bites per claim was $49,025. This reflects an increase of 39% in the average cost per claim nationally from 2012 to 2021 (https://www.iii.org/article/spotlight-on-dog-bite-liability).

31.2 Animal Ownership/Contact

It is estimated that five million households in the United States own at least 1 pet (approximately 70 million own at least 1 dog and 45 million own at least 1 cat) (https://petkeen.com/pet-ownership-statistics/). In addition, based on the 2021–2022 American Pet Products Association's survey, approximately 12 million households own freshwater fish, ten million own pet birds, and six million own small animal or reptiles (https://www.iii.org/fact-statistic/facts-statistics-pet-ownership-and-insurance).

Based on a survey conducted in 2018, approximately 45% of households in the United Kingdom had pets (26% with dogs; 18% with cats) (https://www.pfma.org.uk/pet-population-2018). In a 2021 report, it was estimated that 90 million European Union households (46%) owned at least 1 pet animal (25% cats or dogs) (https://europeanpetfood.org/about/statistics/). According to a report in 2011, there is an estimated 30 million pet cats and 21 million pet dogs in Russia, ranking second only to the United States on the number of pets per capita (https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Pet%20Food%20Market%20Brief_Moscow_Russian%20Federation_4-21-2011.pdf#:~:text=According%20to%20recent%20research%2C%20there%20are%20about%2030,own%20a%20cat%20and%2035%20percent%20a%20dog). In 2010, the Australian Companion Animal Council reported 3.4 million dogs in 36% of households and 2.4 million cats in 23% of Australian households (https://pijaccanada.com/wp-content/uploads/2017/07/Australian-Companion-Animal-Council-report-2010.pdf).

As more people begin to backpack, enjoy ecotourism, and push habitations into more rural areas, there is a potential for increased contact with wild animals. Although no estimates exist for the numbers of these contacts, this growing trend and exposure could certainly result in a higher incidence of bite injuries.

31.2.1 Dog Bites

The Health Care Utilization Project reported on emergency department visits and hospital stays related to dog bites for 2008 noted 316,200 emergency department visits (103.9 per 100,000 population) and 9500 hospital admissions (2.5% of patients with bite injuries) related to dog bites which had increased to 86.3% since 1993 (Emergency Department Visits and Inpatient Stays Involving Dog Bites 2008). Children less than 10 years old had a higher rate of emergency department visits (199.3/100,000 population) than other age groups. Patients residing in rural areas were more likely to visit emergency departments (119.3/100,000 population) and be hospitalized than those from urban settings (29.4/100,000 population). Emergency department visits for dog bites were more common in the Midwest and Northeast United States and lowest in the Western United States. Skin and soft-tissue infections were the cause of 43% of hospitalizations followed by open wounds to the extremities (22.1%).

The average cost of a dog bite hospitalization was $18,200 which was about 50% more than for other injury-related hospital admissions with an average stay of 3.3 days. Approximately 58% of dog bite admissions required a surgical procedure, most commonly debridement of an infected wound followed by suturing of wounds, muscle- and tendon repair-related procedures and incision, and drainage of abscesses. In the United Kingdom, Hospital Episode Statistics recorded 6450 admissions for dog bites or "strikes" in a period of 12 months in 2012, a 5.2% rise compared to the prior 12-month period (https://www.pfma.org.uk/pet-population-2018; https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Pet%20Food%20Market%20Brief_Moscow_Russian%20Federation_4-21-2011.pdf#:~:text=According%20to%20recent%20research%2C%20there%20are%20about%2030,own%20a%20cat%20and%2035%20percent%20a%20dog). The incidence of dog bites worldwide has been estimated by extrapolating similar above information and using population statistics to estimate their occurrence (https://www.dogsbite.org/dog-bite-statistics-study-emergency-visits-involving-dog-bites-ahrq-2008.php).

It has been estimated that one of every two Americans will be bitten in their lifetime, usually by a dog. Dog bites occur in 4.7 million Americans yearly (Sacks et al. 1996) and account for 800,000 medical visits, including approximately 1% of all emergency department visits (Weiss et al. 1998). Most dog bites (85%) are provoked attacks by either the victim's own pet or a dog known to the victim and occur during the warm weather months (Goldstein et al. 1980). Bite wounds that require attention are often those to the extremities, especially the dominant hand. Facial bites are more frequent in children younger than 10 years and lead to 5–10 deaths per year, often because of exsanguination (Lockwood 1997).

Larger dogs can exert more than 450 lbs./in.^{2} of pressure with their jaws, which can lead to extensive crush injuries. Home insurance companies may decline to insure homeowners with certain breeds of dogs that are considered to be more aggressive than others. Even insured owners may not be able to find insurance again, once a dog has been reported as a biter. These breeds often include chows, Rottweilers, Dobermans, Akitas, wolf hybrids, bull terriers, pit bulls, and shepherds.

Patients who present early after an incident often are concerned about crush injuries, care of disfiguring wounds, or the need for rabies or tetanus immunization (Goldstein et al. 1980). Between 2% and 30% of wounds will become infected and may require hospitalization (Goldstein 1992; Brook 1987; Talan et al. 1999). Patients presenting later than 8 hours after injury usually have established infection. Infections can range from localized cellulitis or abscess to septic arthritis, osteomyelitis, tenosynovitis, and rarely, severe sepsis and septic shock. The distribution of bite wounds is shown in Fig. 1 (Talan et al. 1999). Fatal infections may occur in certain compromised hosts, as in patients with asplenia and cirrhosis or on steroids which may be due to *Capnocytophaga canimorsus* (Brenner et al. 1989). Women who have undergone radical or modified radical mastectomy or patients with pre-existing edema of an extremity due to any cause are at increased risk of infection.

Dog bite wound infections are predominantly related to the microbiology of their oral flora (Goldstein et al. 1980; Brook 1987; Talan et al. 1999). Table 1 lists

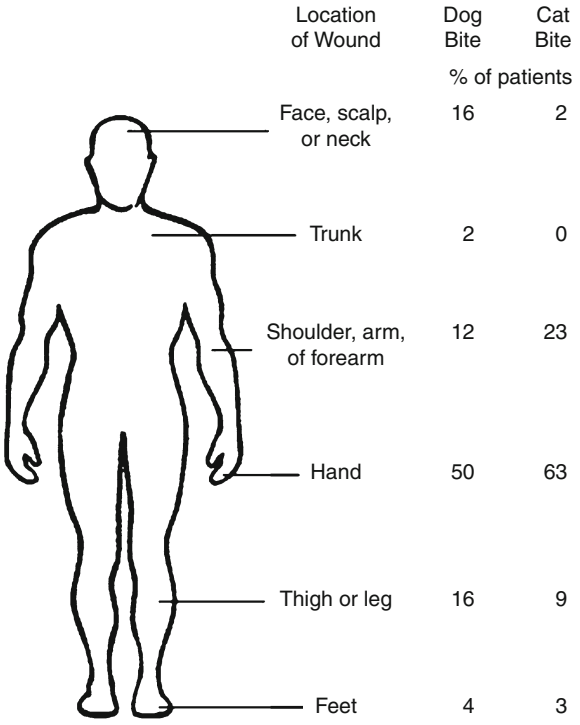

Fig. 1 Anatomic distribution of 50 dog and 57 cat bite wound infections. (Based on reference (Talan et al. 1999) (with permission))

common pathogens found in dog wound infections (Talan et al. 1999). *Pasteurella multocida* is a major and important pathogen but one should note that *Pasteurella canis* is more frequently isolated than *Pasteurella multocida* subspecies *multocida*. Compared to cat bites, this may, in part, account for the greater potential for infection from cat bite wounds. The spectrum of organisms associated with dog bite wound infections is much greater and includes streptococci, staphylococci as *Staphylococcus intermedius* and *Staphylococcus aureus*, and anaerobic organisms (Talan et al. 1999). In one large study, the median number of strains isolated from infected dog bite wounds was 2–7.5. Non-purulent but infected wounds had 2 strains per specimen compared to 7.5 strains for abscesses (Talan et al. 1999). Forty-eight percent of dog bites grew both aerobes and anaerobes, including 67% of abscesses, 62% of purulent wounds, and 13% of non-purulent wounds (Talan et al. 1999). No growth occurred less than 10% of the time which may suggest that other fastidious organisms may be involved in infected bite wounds.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been cultured from a variety of companion animals including dogs, although not yet reported as isolated from infected dog bite wounds in humans. MRSA should be considered as a potential causative secondary invader, especially in patients who are not responding to initially administered antibiotics that often do not exhibit activity against MRSA and those known to be colonized or have had prior infections with MRSA.

Table 1 Common aerobic and anaerobic bacteria isolated from infected dog and cat bite wounds

Organism	Frequency (%)	
	Dog ($n = 50$)	Cat ($n = 57$)
Aerobes		
***Pasteurella* species**	**50**	**75**
P. multocida subspecies *multocida*	12	54
P. multocida subspecies *septica*	10	28
P. canis	26	2
P. dagmatis	4	7
P. stomatis	12	4
P. multocida subspecies *gallicida*	2	0
Pasteurella species, other	2	0
***Staphylococcus* species**	**46**	**35**
S. aureus	20	4
S. epidermidis	18	18
S. warneri	6	11
***Streptococcus* species**	**46**	**46**
S. mitis	22	23
S. mutans	12	11
S. pyogenes	12	0
S. sanguis II	8	12
S. intermedius	6	4
***Neisseria* species**	**32**	**35**
N. weaveri	14	14
***Corynebacterium* species**	**12**	**28**
Group G	6	5
C. minutissimum	4	7
C. aquaticum	2	14
***Moraxella* species**	**10**	**35**
***Enterococcus* species**	**10**	**12**
***Bacillus* species**	**8**	**11**
***Pseudomonas* species**	**6**	**5**
P. aeruginosa	2	0
***Weeksella* species**	**4**	**7**
***Capnocytophaga* species**	**2**	**7**
Anaerobes		
***Fusobacterium* species**	**32**	**33**
F. nucleatum	16	25
F. russii	2	14
***Porphyromonas* species**	**28**	**30**
P. gulae	4	11
P. canoris	4	9
P. macacae	6	7
***Prevotella* species**	**28**	**19**
P. heparinolytica	14	9

(continued)

Table 1 (continued)

Organism	Frequency (%)	
	Dog ($n = 50$)	Cat ($n = 57$)
P. intermedia	8	0
***Propionibacterium* species**	**20**	**18**
P. acnes	14	16
***Bacteroides* species**	**18**	**28**
B. tectus	14	28
B. fragilis	2	2
B. ovatus	2	0
***Peptostreptococcus* species**	**16**	**5**
P. anaerobius	8	5

Adapted from reference (Talan et al. 1999) (with permission) and updated

The antimicrobial treatment of non-infected dog bite wounds that present less than 24 hours after injury remains a controversial issue. The Cochrane reports do not recommend prophylactic antimicrobials for these wounds but the data is flawed as it is based on only a few biased studies with small numbers of patients and incomplete bacteriology (Medeiros and Saconato 2001). Recently, a report (Quinn et al. 2010) on the outcomes of 94 patients and in a cost model using sensitivity analysis across rates of infections from 0% to 16% determined that "if the risk of wound infection was greater than 5%," then antibiotics could decrease that rate and be "cost effective." It is the authors' opinion that all moderate to severe bite wounds, especially those to the hands, injuries associated with moderate to severe swelling (pre-existing or as a result of trauma), and bites in immunocompromised hosts and in proximity to a bone or joint, except those not clinically infected and more than a few days old, should be considered contaminated with potential pathogens and treated.

31.2.2 Cat Bites

Worldwide, cat bites account for 2–50% of injuries related to animal bites. They are second only to dog bites in terms of incidence. In Italy, the incidence of cat-related injuries is 18 per 100,000 population, while in the United States, there are an estimated 400,000 cat bites and 66,000 related visits to emergency departments every year (http://www.who.int/mediacentre/factsheets/fs373/en/).

Wounds inflicted by cats are frequently scratches or tiny but somewhat deep punctures located on the extremities, which are at higher risk of becoming infected (Lucas and Bartlett 1981). Figure 1 shows the comparative distribution of cat bite wounds compared to dog bite wounds (Talan et al. 1999). Deep puncture wounds over or near a joint, especially on the hands, may result in osteomyelitis and septic arthritis. *Pasteurella multocida* has been isolated from 50% to 70% of healthy cats and is a frequent pathogen in cat-associated wounds (Goldstein 1992; Talan et al. 1999).

Patients who present with an infected cat bite often have more severe infections than those with infected dog bites, which may be attributed to the higher incidence of *Pasteurella multocida* and *Pasteurella septica* in cat bites compared to dog bites (Table 1). Cat scratches are likely to get infected from the cats grooming themselves and inoculating *Pasteurella multocida* onto their claws. *Erysipelothrix rhusiopathiae* has been isolated from 4% of cat bite wounds (Talan et al. 1999).

Most cat bite wounds are mixed aerobic infections (64%) with a median number of isolates per wound of 5–7 per specimen. There are 5 isolates per specimen (range, 0–12) for non-purulent but infected wounds compared to 6.5 per specimen (range, 0–13) for purulent wounds and 7 per wound (range, 3–13) for abscesses (Talan et al. 1999). Five percent of infected wounds do not grow a pathogen, suggesting that there are other fastidious organisms present that require special media for isolation. Cougar, tiger, and other feline bites also yield *Pasteurella multocida* (Burdge et al. 1985; Kizer 1989). Tularemia has likewise been transmitted by cat bites (Capellan and Fong 1993). Cat bites may be treated differently in a court of law as cats are more independent and therefore are not subject to the same scrutiny as dog bites.

31.2.3 Venomous Snake Bites

Approximately 600 species of venomous snakes exist worldwide with an estimated five million people bitten annually. In the United States, only 50–70% of venomous snake bite victims are envenomated as the remainder are "dry" bites. Still, considerable morbidity and mortality occur from these injuries. There are an estimated 2.4 million envenomations and 94,000–125,000 deaths annually, resulting in 400,000 amputations and other complications, such as infection, tetanus, scarring, contractures, and psychological consequences. Access to health care and the availability of antivenom decreases the severity of the injuries and improves patient outcomes (http://www.who.int/news-room/fact-sheets/detail/animal-bites).

The majority of snake bites occur in Africa and Southeast Asia and are most common among people living in rural, resource-poor settings, especially agricultural workers, women, and children. The socioeconomic impact and burden of snake bites on these families and communities is increased in these settings (http://www.who.int/news-room/fact-sheets/detail/animal-bites). While snake bites are uncommon in Europe, a review of the literature from 1970 to 2010 found 7992 snake bite reports with an even distribution between Northern, Southern, and Central Europe, including Russia and Turkey (Chippaix 2012). Most bites occurred between May and September, and 15% were considered severe.

Venomous snakes, usually vipers (rattlesnakes, copperheads, cottonmouths, and water moccasins), bite approximately 8000 people in the United States yearly, of which 5 or 6 result in death, usually in children or the elderly, who receive either no or delayed antivenom therapy (Russell 1969). The majority of bites occur in young men in the Southwestern United States between April and September (Gold et al. 2002).

Envenomation can cause extensive tissue destruction and devitalization that predisposes to infection from the snake's normal oral flora. Sparse data exists on the incidence and bacteriology of snakebite infections. In rattlesnakes, the oral flora

appears to be fecal in nature because the live prey usually defecates in the snake's mouth coincident with ingestion. Common oral isolates include *Pseudomonas aeruginosa,* coagulase-negative staphylococci, *Proteus* and *Clostridium* species, *Bacteroides fragilis*, and *Salmonella arizonae (Salmonella* groups IIIa and IIIb) (Russell 1969; Goldstein et al. 1979).

Several treatment guidelines have been published (Lavonas et al. 2011; Walk 2012). In Nigeria, the Health Ministry has attempted to make antivenom more readily available to rural endemic areas and has created a "hub-and-spoke" strategy as a component of "antivenomics" to improve the selection and purchasing of locally appropriate antivenoms (Habib 2013).

31.2.4 Monkey/Simian

Bites from monkeys typically occur in people who keep them as pets, use them for medical research, and those who travel to cities and countries that have a high prevalence of free-roaming monkeys (e.g., Gibraltar, Bali, certain parts of India). Monkey bites account for 2–21% of animal bite injuries (http://www.who.int/mediacentre/factsheets/fs373/en/). In India, for example, monkeys are second to dogs for animal bite injuries. Approximately, 11% of bite wounds in US military personnel stationed in Afghanistan are due to *Macaca mulatta* monkeys, often kept by locals as pets (Mease and Baker 2012).

An emergency department in Los Angeles (Kizer 1979) reported that 1.7% (5/332) of patients who presented during 1975 for bite wounds had monkey bites. Rates of the percentage of monkey bite wounds have been reported from a number of countries and range from 3.2% in India to 0.7% in Israel (Eslamifar et al. 2008; Gross and Torok 1984; Ichhpujani et al. 2008). In the United Kingdom, an animal facility reported 85 (67 incidents in men and 18 in women) monkey bites in handlers over a 6-year period (Tribe and Noren 1983). In addition, persons who visit or work in wilderness areas and national parks worldwide where monkeys reside are also at higher risk. These animals are often considered "mischievous" and will scour for food if hungry. Simple wound management of monkey bites may not prevent potential infectious complications and a protocol for post-bite exposure treatment has been published (Tregle Jr et al. 2011; Newton F. United States Armed Forces 2010).

The spectrum of isolates from humans bitten by monkeys is similar to those isolated from human bite wounds. There is a predominance of α-hemolytic streptococci, enterococci, *Staphylococcus epidermidis*, *Neisseria* and *Haemophilus* species, *Eikenella corrodens*, and anaerobes including *Bacteroides* and *Fusobacterium* species (Goldstein et al. 1995). Monkeys can naturally acquire *Bartonella quintana* (trench fever) and they may potentially act as vectors (O'Rourke et al. 2005). Transmission of viral diseases is a major health and economic concern with monkey contact and bites (Estrep et al. 2010). Non-human primates are susceptible to a variety of pathogens that bear significant homology to human pathogens. These same viruses pose a potential health issue to humans and can include herpes B virus, simian varicella virus, rhesus cytomegalovirus, gamma-herpesviruses, lymphocryptoviruses, herpes saimiri, rhesus macaque rhadinoviruses, and others (Estrep et al. 2010).

In monkeys from Nepal the prevalence of selected enzootic primate-borne viruses through positive antibody response among 39 rhesus monkeys in Katmandu has been reported (Jones-Engel et al. 2006). The various viruses found included simian foamy virus (97.4%), *Cercopithecine herpesvirus* 8 (94.9%), simian virus 40 (89.7%), and *Cercopithecine herpesvirus* 1 (64.1%; also known as B virus). Documented cases of B virus infection in humans have mostly been attributed to monkey bites. However, other less commonly reported modes of transmission have been due to scratches and percutaneous inoculation with infected materials (Centers for Disease Control and Prevention 1998). A fatal case of B virus infection has been reported following a mucocutaneous exposure (Centers for Disease Control and Prevention 1998).

Other reports of bite-related primate-borne viral infections in humans have included simian foamy virus (especially from ape bites) and monkeypox infections (Calattini et al. 2007; Mutombo et al. 1983; Schweizer et al. 1997) and yellow fever virus with the arbovirus carried from one host to another, primarily between monkeys, from monkeys to humans, and from person to person. Sylvatic (or jungle) yellow fever occurs in tropical rainforests where monkeys are infected by wild mosquitoes and then pass the virus to other mosquitoes that feed on them. The infected mosquitoes bite humans entering the forest, resulting in occasional cases of yellow fever. The majority of infections occur in young men working in the forest (e.g., for logging) (http://www.who.int/mediacentre/factsheets/fs100/en/).

Hepatitis A virus can infect various monkey species such as chimpanzees, owl monkeys, cynomolgus monkeys, rhesus monkeys, stump-tailed monkeys, African green monkeys, tamarins, marmosets, and squirrel monkeys. The transmission of human hepatitis A virus from experimentally infected animals to humans has occurred and been documented (http://www.who.int/mediacentre/factsheets/fs100/en/). Still unknown is the susceptibility of humans to true simian hepatitis A virus strains (http://www.who.int/mediacentre/factsheets/fs100/en/).

While man is the natural host for hepatitis E virus, chimpanzees, cynomolgus monkeys, rhesus monkeys, pigtail monkeys, owl monkeys, tamarins, and African green monkeys are reported to be susceptible to natural infection with human strains of hepatitis E virus making transmission possible.

The monkeypox virus can cause a fatal disease in humans. It is similar to human smallpox, although typically much less serious. It occurs primarily in remote villages in Central and West Africa, near tropical rainforests. The monkeypox virus is transmitted to people from a variety of wild animals and it spreads in the human population through human-to-human transmission (http://www.who.int/mediacentre/factsheets/fs100/en/).

Malaria due to *Plasmodium knowlesi* also known as "monkey malaria" can occur in humans while staying in rainforests or their fringe areas in Southeast Asia, within the range of the natural monkey hosts and mosquito vector of this infection. These areas include parts of Cambodia, China, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam. Travelers to forested areas of Southeast Asia where human *Plasmodium knowlesi* infections have been reported should protect themselves against mosquito bites between dusk and dawn to prevent infection and take the usual chemoprophylaxis where indicated (http://www.who.int/mediacentre/factsheets/fs100/en/).

31.2.5 Bears

Bear attacks occur worldwide and are, in part, an ecological conflict, often where humans visit or decide to work or live in areas where these large carnivores inhabit (Cardall and Rosen 2003; De Giorgio et al. 2007; Frosch et al. 2011; Herrero 1970; Herrero and Fleck 1990; Mihailoviv et al. 2011; Nabi et al. 2009; Risholt et al. 1998; Tough and Butt 1993; Vougiouklakis 2006). In Norway, from 1971 to 1995 there were 80 incidents involving human-bear interactions of which there were 4 fatalities and 6 injuries, many of which were due to bites (Risholt et al. 1998). A review of wild animal bites in Kashmir from 2005 to 2007 noted that 51.2% (104/203) were caused by black bears (Nabi et al. 2009). In North America, human injuries from grizzly bears (*Ursus arctos horribilis*) in the national parks have been reported at a rate of one person per two million visitors (Herrero 1970).

From 1900 to 1985, 162 bear-inflicted injuries (approximately 2 attacks per year) were reported in the United States and Canadian national parks (Herrero and Fleck 1990). Although bear-inflicted human injuries and death are uncommon (Floyd 1999), as the remote bear habitat decreases and humans enter wilderness areas for living and recreation, there are more chances of encounters between bears and humans. These have occurred in all hemispheres where bears are resident and some have even occurred in zoos (Mihailoviv et al. 2011).

The bacteriology of bear oral flora and bear bite wounds to humans is limited to a few studies and case reports (Floyd et al. 1990; Kunimoto et al. 2004; Lehtinen et al. 2005; Parry et al. 1983; Rose 1982). The bacteria isolated from bear bite wounds include *Serratia fonticola*, *Serratia marcescens*, *Aeromonas hydrophila*, *Bacillus cereus*, and *Enterococcus durans*. Lehtinen et al. reported a case of a 56-year-old male who sustained several bite wounds from a brown bear (*Ursus arctos*) that grew *Streptococcus sanguinis*, *Neisseria sicca*, *Bacillus* species, and *Mycobacterium fortuitum* (Lehtinen et al. 2005). Those patients that survived a bear attack required air-lifting to nearby medical facilities.

Rabies virus infection in bears has also been reported (Centers for Disease Control and Prevention 1999). However, to our knowledge there have not been any reports of rabies transmission from bears to humans.

31.2.6 Pigs

Pigs are aggressive animals and their bite injuries are a common occupational hazard and may also occur to those who own pigs as pets. Nogalski et al. reported 5.13% (96/1872) of animal-related injuries seen between 2001 and 2004 were related to pigs, either bites or battering or both (Nogalski et al. 2007). These injuries were more often from rural (88.5%; 85/96) areas than urban (11.5%; 11/96). Injuries, often on extremities, commonly occur during capture, transport, or immobilization of the pig (Barnham 1988; Nishioka et al. 1994; Van Demark Sr and Van Demark Jr 1991). Unusual pig bites have included a de-gloving injury to the penis and the prolapsed rectum of a child (Georgiou et al. 2001; Gangopadhyay et al. 2002). In Southeastern

Brazil, a case series from a teaching hospital reported 23 pig bites from 1987 to 1990 and estimated the annual incidence to be 1.5/100,000 population (Nishioka et al. 1994). They reported a male to female ratio of 6.7:1 and a median age of 36 years for victims.

Only a few human wound infections after a pig bite have been reported (Barnham 1988; Ejlertsen et al. 1996; Escande et al. 1996; Goldstein et al. 1990). Organisms isolated from these patients included *Streptococcus agalactiae, Streptococcus equisimilis, Streptococcus suis, Pasteurella aerogenes, Proteus species, Escherichia coli, Bacteroides* species including *Bacteroides fragilis*, *Pasteurella multocida*, coagulase-negative *Staphylococcus*, *Streptococcus milleri*, and *Myroides odoratimimus* (Ejlertsen et al. 1996; Escande et al. 1996; Goldstein et al. 1990; Maraki et al. 2012). Identification of bacteria isolated from pig bites in humans is problematic because they cannot be identified or are misidentified by commercial kits and conventional methods (Lindberg et al. 1998).

Recent studies have shown a high prevalence of nasal MRSA colonization in people commonly in contact with live pigs (Khanna et al. 2008; Köck et al. 2009; Smith et al. 2009; Van Cleef et al. 2010). Molecular characterization of MRSA found in pigs and humans in contact with pigs has revealed a *Staphylococcus aureus* protein A (spa) type t108 and sequence type (ST) 398 (van Belkum et al. 2008; van Loo et al. 2007; Huijsdens et al. 2006). Clonal spread of MRSA and transmission through family members of a pig farmer, his co-workers, and his pigs have been reported (Huijsdens et al. 2006).

Although rare, rabies infection has been reported worldwide in pigs (DuVernoy et al. 2008; Morehouse et al. 1968; Yates et al. 1983; DuVernoy et al. 2008; Luo et al. 2013). However, to our knowledge there have not been any reports of rabies transmission from pigs to humans, although there is a report of a rabid pig biting humans playing golf in India (https://timesofindia.indiatimes.com/city/chennai/Rabid-pig-bites-two-golfers-washerman-done-to-death/articleshow/12092460.cms). Although there are no reports of bite transmission, hepatitis E virus infections in humans have been associated with pig contact and ingestion of raw pig products (Ruggeri et al. 2013; Dalton et al. 2013).

Pigs may also carry *Clostridium difficile* (Fry et al. 2012), and toxigenic PCR ribotypes found in pigs correspond to PCR ribotypes associated with human disease in hospitalized patients in the Netherlands (Koene et al. 2012). Although not a bite-related infection, *Clostridium difficile* disease may potentially be transmitted to humans.

31.2.7 Horses

Humans and horses have shared a close relationship with one another for over thousands of years. Throughout the world, millions of people have had contact with horses through recreation and sporting or for occupational reasons. It is estimated that there are 893,152 pet horses in the United States (https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-pet-ownership.aspx). In the United Kingdom, it is estimated that there are 374,000 horse-

owning households (https://petkeen.com/horse-statistics-uk/). Horse riding is estimated to be a more popular sport than rugby, cricket, or fishing, with greater than 7% of the population riding at least one time annually.

It is estimated that between 3% and 4.5% of all animal bites are due to horses (Langley and Morris 2009). Carithers reported that 5 out of 157 (3%) animal bites seen in children in Jacksonville, Florida, over a 20-month period were due to horse bites (Carithers 1958). In England, over a 2-year period, a local hospital reported 622 patients with horse-related injuries, of which 24 (3.8%) were bite wounds (Edixhoven et al. 1981). In that series, few had extensive muscle damage and most injuries healed uneventfully. In Lublin, Poland (Nogalski et al. 2007), it was noted that 2.4% of animal-related injuries were due to horses and that they occurred in equal frequency in urban and rural areas. In contrast, horse bites accounted for 17% of animal bite injuries and were second only to dog bites (69%) in eastern Turkey, during a period of 2 years (Emet et al. 2009). However, in Pune, India, only 0.4% of bite cases were due to horses (Shetty et al. 2005).

Horses and zebras share the same genus, so we suspect that their oral flora will likely be similar. Most reports of the bacteriology of horse bite wounds in humans have revealed infections to be polymicrobial with a mixture of aerobic and anaerobic organisms (Dibb et al. 1981; Peel et al. 1991; Benaoudia et al. 1994). *Actinobacillus lignieresii* has often been reported in infected wounds of humans bitten by horses (Dibb et al. 1981; Peel et al. 1991; Benaoudia et al. 1994). *Actinobacillus* species, specifically *Actinobacillus suis*, has been found to be a part of normal horse oral and upper respiratory tract floras (Bisgaard et al. 1984; Kim et al. 1976) and has been isolated from a horse bite (Peel et al. 1991). Horses are known to carry MRSA (Hartmann et al. 1997; Van den Eede et al. 2013; Schwaber et al. 2013), including the ST398 and ST568 strains (Gómez-Sanz et al. 2013), but no reports of horse bite infection due to MRSA have yet been reported.

31.2.8 Komodo Dragon

Komodo dragons migrated from Australia to the Indonesian Islands of Rinca, Flores, and Gili Motang. These largest living lizards can reach a length of approximately 10 feet and weigh up to 150 pounds. They are held in captivity in many zoos around the world. They are carnivores and eat mostly carrion but will prey on birds and mammals. Komodo dragons can bite people and some attacks may lead to death.

The myth of the Komodo bite's lethal ability related to its oral bacterial flora has recently been disproven by the finding of venom glans in their oral cavity (Fry et al. 2009). It is postulated that the venom is able to kill smaller, more appropriate sized prey but that the larger prey die from delayed wound sepsis due to open tear wounds and secondary infection obtained at watering holes. Goldstein et al. (2013) studied captive Komodo oral flora and found a variety of aerobic gram-negative rods (1–8 per specimen), especially *Enterobacteriaceae*; aerobic gram-positive bacteria (2–9 per specimen), especially *Staphylococcus sciuri* and *Enterococcus faecalis*; and anaerobes (1–6 per specimen), especially *Clostridia*. As with other carnivores,

captive Komodo oral flora is simply reflective of the gut and skin flora of their recent meals and environment and is unlikely to cause rapid fatal infection. To our knowledge, there have not been any reports on the bacteriology of infected human wounds from Komodo bites.

31.2.9 Alligator/Crocodile

Both the alligator population and human encounters with alligators have increased in the United States (Langley 2005). The same situation exists for crocodiles in Australia and Asia (Caldicott et al. 2005; Gruen 2009; https://www.outback-australia-travel-secrets.com/crocodile-attacks.html). From 1948 to 2004, there were 376 injuries and 15 deaths reported from alligators in the United States (Langley 2005). In 2009, 11 provoked and 8 unprovoked alligator attacks were reported in Florida (Florida Fish and Wildlife Conservation Commission Historic Alligator Bites on Humans in Florida) (Langley 2005). Unprovoked bites were defined as bites on human beings by wild alligators, which were not provoked by handling or intentional harassment. A review of crocodile attacks in Asia notes that the Nile and salt water crocodiles are most likely to prey on humans, the former most commonly in sub-Saharan Africa and the latter in New Guinea, Borneo, and the Solomon Islands. They also note 18 of 31 fatal attacks by salt water crocodiles occurred in Australia between 1970 and 1996 (https://www.outback-australia-travel-secrets.com/crocodile-attacks.html).

The microbiology of human wounds inflicted by alligators or crocodiles is limited to a few case reports where wound cultures grew mainly aquatic environmental organisms as well as bacterial flora from the skin and intestines of prey such as *Aeromonas hydrophila*, *Enterobacter agglomerans*, *Citrobacter diversus*, *Enterococcus* species, and *Clostridium* species (Flandry et al. 1989; Wamisho et al. 2009). In Malawi (Wamisho et al. 2009) a review of five patients bitten by crocodiles showed wound cultures grew *Citrobacter* species and other investigators have isolated *Vibrio vulnificus*, *Citrobacter* species, *Burkholderia pseudomallei*, *Pantoea agglomerans*, *Bacteroides melaninogenicus*, *Aeromonas hydrophila*, *Serratia fanticola*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*. Caimans are very aggressive and bites from Brazil have been reported. It was speculated that the oral flora of the caiman might be similar to that of the alligators in the United States (Hertner 2006). The oral and cloacal flora of wild crocodiles in the Mexican Caribbean included *Aeromonas hydrophila* and also *Salmonella arizonae* and *Salmonella typhi* which could pose a threat to humans from both bites and from the aquatic environment (Charruau et al. 2012).

31.2.10 Rodents/Rats

It is estimated that annually there are 20,000 rat bites in the United States with children the most common victims and most wounds to the face or hands (Hirschhorn and Hodge 1999; Ordog et al. 1985; Elliott 2007; Glaser et al. 2000).

Most cities in the United States are populated by the Norway rat, black rat, or the house mouse. Rats and other rodents have also gained popularity as pets. The Centers for Disease Control and Prevention (CDC) in the United States initiated a limited national surveillance program for animal bites in 1971 and reported that 4.3% of the 196,684 cases were due to rat bites (Moore Jr et al. 1977). In Philadelphia, 622 rat bite cases were confirmed from 1974 to 1996 (Hirschhorn and Hodge 1999). It is estimated that approximately 100 persons are bitten by rats in New York City annually and the Department of Health has a proactive, active surveillance "rat-indexing" program that inspects properties and tries to abate substandard living environments to reduce this number (Bragdon et al. 2012; Childs et al. 1998).

Infections from rat bites occur in less than 2% of bitten individuals. Ordog et al. (Ordog et al. 1985) conducted a prospective study of 50 patients with uninfected rat bite wounds and found that only 1 patient developed an infection. The bacterial isolates they cultured from wounds were mostly skin flora organisms such as *Staphylococcus epidermidis, Bacillus subtilis*, alpha-hemolytic *Streptococcus*, and diphtheroids. In Tanzania, 34 Type-II male diabetic patients with peripheral neuropathy were bitten by rats during sleep which resulted in 4 deaths and 17 minor or major amputations (Abbas et al. 2005). *Actinobacillus equuli* has been isolated from the nasopharynx of laboratory mice and rats and *Actinobacillus lignieresii* has been isolated from the nasopharynx of laboratory rats (Lentsch and Wagner 1980). Cases of rat bite-associated infections in humans with *Corynebacterium kutscheri* (Holmes and Korman 2007) and *Leptospira* (Gollop et al. 1993; Luzzi et al. 1987) have also been reported.

Most attention has been focused on rat bite fever, an ancient disease caused by *Streptobacillus moniliformis*, a fastidious highly pleomorphic, filamentous, gram-negative rod, and *Spirillum minus*, a short, tightly coiled gram-negative rod (Elliott 2007). *Streptobacillus moniliformis* infection is more common in North America, while *Spirillum minus* is more common in Asia. Rat bite fever is rare in the United States and its incidence is unknown since it is not a nationally reportable disease.

From 1996 through 1998, Norway rats (*Rattus norvegicus*) from downtown Los Angeles were examined and seroprevalence rates in rats were 25.9% for *Rickettsia typhi*, 6.7% for Seoul virus, and 73.1% for hepatitis E virus (Smith et al. 2002). Fifty-two percent of blood specimens collected from rats grew *Bartonella elizabethae*-like isolates when cultured. However, in local skid row residents, the prevalence of antibodies to hepatitis E virus was 13.6%, *Bartonella elizabethae* 12.5%, *Bartonella quintana* 9.5%, Seoul virus 0.5%, and *Rickettsia typhi* 0%.

31.2.11 Sharks

The International Shark Attack File reported 118 alleged shark attacks in 2012, of which 80 were confirmed as unprovoked and 16 were provoked. The number of unprovoked attacks has steadily increased since the early 1900s and may reflect increased opportunities of interaction due to water sports and time spent in the seas by humans. Conversely, the shark population has declined worldwide. From 1999 to 2009 there were 455 shark attacks in the United States, and the United States (including Florida, Hawaii, and Puerto Rico) is the most common country where

they occur (http://www.flmnh.ufl.edu/fish/sharks/isaf/isaf.htm). The majority of attacks occurred in Florida (294 attacks), Hawaii (42 attacks), South Carolina (32 attacks), and California (30 attacks). Worldwide, during the same period, 700 shark attacks were reported with 51 (7.3%) being fatal attacks.

The highest numbers of attacks worldwide, in order of decreasing frequency, were reported from Florida, Australia, Hawaii, South Africa, and California. There were 7 fatalities due to unprovoked shark attacks reported in 2012 resulting in a fatality rate of 8.8% (http://www.flmnh.ufl.edu/fish/sharks/isaf/isaf.htm). The US fatality rate was 1.9% compared to 22.2% in the rest of the world. Approximately 60% of incidents occur in surfers and boarders compared to 22% in swimmers and 8% in divers. Shark attacks obviously have an economic impact on the specific vacation spots and beaches involved.

The oral aerobic flora of a male great white shark from Connecticut waters was obtained and various isolates of *Vibrio* species such as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio fluvialis* were recovered. Other isolates included *Pseudomonas putrefaciens*, "gold-pigmented" *Staphylococcus* species, *Citrobacter* species, and *Micrococcus* species (Buck et al. 1984).

Vibrio carchariae has been isolated from an infected wound in a shark bite victim swimming off the South Carolina coast (Pavia et al. 1989), and in two cases of infections following shark bites in Australia, the wound cultures grew *Vibrio parahaemolyticus* and *Aeromonas caviae* and the other *Vibrio alginolyticus*, *Aeromonas hydrophila*, *Proteus* species, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Clostridium freundii*, and an *Enterococcus* species (Royle et al. 1997). Most of these isolates are aquatic organisms and should be considered when selecting antimicrobial prophylaxis (Rtshiladze et al. 2011).

Interaminense et al. (2010) cultured the oral flora of sharks involved in human attacks in Recife, Brazil, and found the majority were enterobacteria such as *Enterobacter*, *Citrobacter*, and *Proteus* species, *Providencia alcalifaciens, Escherichia coli, Moellerella wisconsensis,* and *Leclercia adecarboxylata*. Other gram-negative organisms isolated included *Vibrio* species, *Burkholderia cepacia, Acinetobacter* species, and *Pseudomonas* species. Gram-positive strains were also isolated including coagulase-positive and coagulase-negative *Staphylococcus* species, *Enterococcus* species, *Micrococcus* species, and viridans streptococci.

31.3 Conclusion

In conclusion, worldwide, animal bite-related injuries to humans are a common daily occurrence. Injuries can range from minor puncture wounds to extensive crush injuries and even amputations and death. Increasing population, continued spread of habitation, and the popularity of owning various types of both traditional and nontraditional pets has made it easier for humans to have contact with various types of animals. In general, the oral flora of the biting animal will be concordant with the

bacteria isolated from the wound. These oral flora organisms often originate from the normal oral residents and environmental flora as well as the skin and intestinal bacteria of the animals' food sources and prey.

References

Abbas ZG, Lutale J, Archibald LK (2005) Rodent bites on the feet of diabetes patients in Tanzania. Diabet Med 22:631–633

Barnham M (1988) Pig bite injury and infection: report of seven human cases. Epidemiol Infect 101:641–645

Benaoudia F, Escande F, Simonet M (1994) Infection due to *Actinobacillus lignieresii* after a horse bite. Eur J Clin Microbiol Infect Dis 13:439–440

Bisgaard M, Piechulla K, Ying YT et al (1984) Prevalence of organisms described as *Actinobacillus suis* or haemolytic *Actinobacillus equuli* in the oral cavity of horses. Comparative investigations of strains obtained and porcine strains of *A. suis sensu stricto*. Acta Pathol Microbiol Immunol Scand B 92:291–298

Bragdon C, Kass D, Matte R et al (2012) Evaluation of a neighborhood rat-management program-New York City, December 2007-August 2009. MMWR Morb Mortal Wkly Rep 61: 733–736

Brenner DJ, Hollis DG, Fanning GR et al (1989) *Capnocytophaga canimorsus* sp. nov. (formerly CDC group DF2), a cause of septicemia following dog bite, and *C. cynodegmi* sp. nov., a cause of localized wound infection following dog bite. J Clin Microbiol 27:231–235

Brook I (1987) Microbiology of human and animal bite wounds in children. Pediatr Infect Dis J 6: 29–32

Buck JD, Spotte S, Gadbaw JJ Jr (1984) Bacteriology of the teeth from a great white shark: potential medical implications for shark bite victims. J Clin Microbiol 20:849–851

Burdge DR, Scheifele D, Speert DP (1985) Serious *Pasteurella multocida* infections from lion and tiger bites. J Am Med Assoc 253:3296–3297

Calattini S, Betsem EB, Froment A et al (2007) Simian foamy virus transmission from apes to humans, rural Cameroon. Emerg Infect Dis 13:1314–1320

Caldicott DG, Croser D, Manolis C et al (2005) Crocodile attack in Australia: an analysis of its incidence and review of the pathology and management of crocodilian attacks in general. Wilderness Environ Med 16:143–159

Capellan J, Fong IW (1993) Tularemia from a cat bite: case report and review. Clin Infect Dis 16: 472–475

Cardall TY, Rosen P (2003) Grizzly bear attack. J Emerg Med 24:331–333

Carithers HA (1958) Mammalian bites of children; a problem in accident prevention. AMA J Dis Child 95:150–156

Centers for Disease Control and Prevention (1998) Fatal *Cercopithecine herpesvirus* 1 (B virus) infection following a mucocutaneous exposure and interim recommendations for worker protection. MMWR Morb Mortal Wkly Rep 47:1073–1076

Centers for Disease Control and Prevention (1999) Multiple human exposures to a rabid bear cub at a petting zoo and barnwarming- Iowa, August 1999. MMWR Morb Mortal Wkly Rep 48:761

Charruau P, Pérez-Flores J, Pérez-Juárez JG et al (2012) Oral and cloacal microflora of wild crocodiles *Crocodylus acutus* and *C. moreletii* in the Mexican Caribbean. Dis Aquat Org 98: 27–39

Childs JE, McLafferty SL, Sadek R et al (1998) Epidemiology of rodent bites and prediction of rat infestation in New York City. Am J Epidemiol 148:78–87

Chippaix JP (2012) Epidemiology of snakebites in Europe: a systematic review of the literature. Toxicon 59:86–99

Dalton HR, Hunter JG, Bendall RP (2013) Hepatitis E. Curr Opin Infect Dis 26:471–478
De Giorgio F, Rainio J, Pascali V, Lalu K (2007) Bear attack-a unique fatality in Finland. Forensic Sci Int 173:64–67
Dibb WL, Digranes A, Tønjum S (1981) *Actinobacillus lignieresii* infection after a horse bite. Br Med J (Clin Res Ed) 283:583–584
DuVernoy TS, Mitchell KC, Myers RA et al (2008) The first laboratory-conformed rabid pig in Maryland, 2003. Zoonoses Public Health 55:431–435
Edixhoven P, Sinha SC, Dandy DJ (1981) Horse injuries. Injury 12:279–282
Ejlertsen T, Gahm-Hansen B, Søgaard P et al (1996) *Pasteurella aerogenes* isolated from ulcers or wounds in humans with occupational exposure to pigs: a report of 7 Danish cases. Scand J Infect Dis 28:567–570
Elliott SP (2007) Rat bite fever and *Streptobacillus moniliformis*. Clin Microbiol Rev 20:13–22
Emergency Department Visits and Inpatient Stays Involving Dog Bites, 2008 - Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. https://www.ncbi.nlm.nih.gov/books/NBK52650/ . Accessed 07–02-22
Emet M, Beyhun NE, Kosan Z et al (2009) Animal-related injuries: epidemiological and meteorological features. Ann Agric Environ Med 16:87–92
Escande F, Bailly A, Bone S, Lemozy J (1996) *Actinobacillus suis* infection after a pig bite. Lancet 348:888
Eslamifar A, Ramezani A, Razzaghi-Abyaneh M et al (2008) Animal bites in Teheran, Iran. Arch Iran Med 11:200–202
Estrep RD, Messaoudi I, Wong SW (2010) Simian herpesviruses and their risk to humans. Vaccine 26:B78–B84
Flandry F, Lisecki EJ, Domingue GJ et al (1989) Initial antibiotic therapy for alligator bites: characterization of the oral flora of *Alligator mississippiensis*. South Med J 82:262–266
Floyd T (1999) Bear-inflicted human injury and fatality. Wilderness Environ Med 10:75–87
Floyd T, Manville AM, French SP (1990) Normal oral flora in black bears: guidelines for antimicrobial prophylaxis following bear attacks. J Wilderness Med 1:47–49
Frosch C, Dustiov A, Georgiov G, Nowak C (2011) Case report of a fatal bear attack documented by forensic wildlife genetics. Forensic Sci Int Genet 5:342–344
Fry BG, Wroe S, Teeuwisse W et al (2009) A central role for venom in predation by *Varanus komodooensis* (Komodo Dragon) and the extinct giant *Varanus* (*Megalania*) *priscus*. Proc Natl Acad Sci 106:8969–8974
Fry PR, Thakur S, Abley M, Gebreyes WA (2012) Antimicrobial resistance, toxinotype, and genotypic profiling of *Clostridium difficile* isolates of swine origin. J Clin Microbiol 50: 2366–2372
Gangopadhyay AN, Gupta DK, Dhulkotia A et al (2002) Pig bite of prolapsed rectum in a child. J Pediatr Surg 37:657–658
Georgiou P, Liakopoulos P, Gamatsi E, Komninakis E (2001) Degloving injury of the penis from pig bite. Plast Reconstr Surg 108:805–806
Glaser C, Lewis P, Wong S (2000) Pet-, animal- and vector-borne infections. Pediatr Rev 21: 219–232
Gold B, Dart RC, Barish RA (2002) Bites of venomous snakes. N Engl J Med 347:347–356
Goldstein EJC (1992) Bite wounds and infection. Clin Infect Dis 14:633–640
Goldstein EJC, Citron DM, Gonzalez H et al (1979) Bacteriology of rattlesnake venom and implications for therapy. J Infect Dis 140:818–821
Goldstein EJC, Citron DM, Finegold SM (1980) Dog bite wounds and infection: a prospective clinical study. Ann Emerg Med 9:508–512
Goldstein EJC, Citron DM, Merkin TE, Pickett MJ (1990) Recovery of an unusual *Flavobacterium* IIb-like isolate from a hand infection following pig bite. J Clin Microbiol 28:1079–1081
Goldstein EJ, Pryor EP 3rd, Citron DM (1995) Simian bites and bacterial infection. Clin Infect Dis 20:1551–1552
Goldstein EJC, Tyrrell KL, Citron DM et al (2013) The anaerobic and aerobic bacteriology of the saliva and gingiva from 16 captive Komodo dragons (*Varanus komodoensis*): refuting the "bacteria as venom" model. J Zoo Wildl Med 44:262–272

Gollop JH, Katz AR, Rudoy RC, Sasaki DM (1993) Rat-bite leptospirosis. West J Med 159:76–77
Gómez-Sanz E, Simón C, Ortega C et al (2013, 2013) First detection of methicillin-resistant *Staphylococcus aureus* ST398 and *Staphylococcus pseudintermedius* ST68 from hospitalized equines in Spain. Zoonoses Public Health. https://doi.org/10.1111/zph.12059
Gross EM, Torok V (1984) Monkey-caused injuries in the region served by the beer Sheva district health office, 1978-1982. Israel J Med Sci 20:725–726
Gruen RL (2009) Crocodile attacks in Australia: challenges for injury prevention and trauma care. World J Surg 33:1554–1561
Habib AG (2013) Public health aspects of snakebite care in West Africa: perspectives from Nigeria. J Venom Anim Toxins Incl Trop Dis 9:1–27
Hartmann FA, Trostle SS, Klohnen AA (1997) Isolation of methicillin-resistant *Staphylococcus aureus* from a postoperative wound infection in a horse. J Am Vet Med Assoc 211:590–592
Herrero S (1970) Human injury inflicted by grizzly bears. Science 170:593–598
Herrero S, Fleck S (1990) Injury to people inflicted by black, grizzly, and polar bears: recent trends and new insights. Proc Conf Bears Res Manage 8:25
Hertner G (2006) Caiman bite. Wilderness Environ Med 17:267–270
Hirschhorn RB, Hodge RR (1999) Identification of risk factors in rat bite incidents involving humans. Pediatrics 104:e35
Holmes NE, Korman TM (2007) *Corynebacterium kutscheri* infection of the skin and soft tissue following rat bite. J Clin Microbiol 45:3468–3469
http://www.flmnh.ufl.edu/fish/sharks/isaf/isaf.htm. Accessed 07-02-22
http://www.who.int/mediacentre/factsheets/fs100/en/. Accessed 07-02-22
http://www.who.int/mediacentre/factsheets/fs373/en/. Accessed 07-02-22
http://www.who.int/news-room/fact-sheets/detail/animal-bites. Accessed 07-02-22
https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Pet%20Food%20Market%20Brief_Moscow_Russian%20Federation_4-21-2011.pdf#:~:text=According%20to%20recent%20research%2C%20there%20are%20about%2030,own%20a%20cat%20and%2035%20percent%20a%20dog. Accessed 07-02-22
https://europeanpetfood.org/about/statistics/. Accessed 07-02-22
https://petkeen.com/horse-statistics-uk/. Accessed 07-02-22
https://petkeen.com/pet-ownership-statistics/. Accessed 07-02-22
https://pijaccanada.com/wp-content/uploads/2017/07/Australian-Companion-Animal-Council-report-2010.pdf. Accessed 07-02-22
https://timesofindia.indiatimes.com/city/chennai/Rabid-pig-bites-two-golfers-washerman-done-to-death/articleshow/12092460.cms. Accessed 07-02-22
https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-pet-ownership.aspx. Accessed 07-02-22
https://www.dogsbite.org/dog-bite-statistics-study-emergency-visits-involving-dog-bites-ahrq-2008.php. Accessed 07-02-22
https://www.iii.org/article/spotlight-on-dog-bite-liability. Accessed 07-02-22
https://www.iii.org/fact-statistic/facts-statistics-pet-ownership-and-insurance. Accessed 07-02-22
https://www.outback-australia-travel-secrets.com/crocodile-attacks.html. Accessed 07-02-22
https://www.pfma.org.uk/pet-population-2018. Accessed 07-02-22
Huijsdens XW, van Dijke BJ, Spalburg E et al (2006) Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob 5:26
Ichhpujani RL, Mala C, Veena M et al (2008) Epidemiology of animal bites and rabies cases in India. A multicentric study. J Commun Dis 40:27–36
Interaminense JA, Nascimento DC, Ventura RF et al (2010) Recovery and screening for antibiotic susceptibility of potential bacterial pathogens from the oral cavity of shark species involved in attacks on humans in Recife, Brazil. J Med Microbiol 59:941–947
Jones-Engel L, Engel GA, Heidrich J et al (2006) Temple monkeys and health implications of commensalism, Kathmandu, Nepal. Emerg Infect Dis 12:900–906
Khanna T, Friendship R, Dewey C, Weese JS (2008) Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol 128:298–303
Kim BH, Phillips JE, Atherton JG (1976) *Actinobacillus suis* in the horse. Vet Rec 98:239

Kizer KW (1979) Epidemiological and clinical aspects of animal bite injuries. J Am Col Emerg Phys 8:134–141
Kizer KW (1989) *Pasteurella multocida* infection from a cougar bite. A review of cougar attacks. West J Med 150:87–90
Köck R, Harlizius J, Bressan N et al (2009) Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. Eur J Clin Microbiol Infect Dis 28:1375–1382
Koene MG, Mevius D, Wagenaar JA et al (2012) *Clostridium difficile* in Dutch animals: their presence, characteristics and similarities with human isolates. Clin Microbiol Infect 18:778–784
Kunimoto D, Rennie R, Citron DM, Goldstein EJC (2004) Bacteriology of a bear bite wound to a human: case report. J Clin Microbiol 42:3374–3376
Langley RL (2005) Alligator attacks on humans in the United States. Wilderness Environ Med 16: 119–124
Langley R, Morris T (2009) That horse bit me: zoonotic infections of equines to consider after exposure through the bite or the oral/nasal secretions. J Agromedicine 14:370–381
Lavonas EJ, Ruha AM, Banner W, et al. and Rocky Mountain Poison and Drug Center, Denver Health and Hospital Authority (2011) Unified treatment algorithm for the management of crotaline snakebite in the United States: results of an evidence-informed consensus workshop. BMC Emerg Med 11:2
Lehtinen VA, Kaukonen T, Ikäheimo I et al (2005) *Mycobacterium fortuitum* infection after a brown bear bite. J Clin Microbiol 43:1009
Lentsch RH, Wagner JE (1980) Isolation of *Actinobacillus lignieresii* and *Actinobacillus equuli* from laboratory rodents. J Clin Microbiol 12:351–354
Lindberg J, Frederiksen W, Gahrn-Hansen B, Bruun B (1998) Problems of identification in clinical microbiology exemplified by pig bite wound infections. Zentralbl Bakteriol 288:491–499
Lockwood R (1997) Dog-bite-related fatalities-United States, 1995–1996. MMWR Morb Mortal Wkly Rep 46:463–467
Lucas GL, Bartlett DH (1981) *Pasteurella multocida* infection in the hand. Plast Reconstr Surg 67: 49–53
Luo Y, Zhang Y, Liu X et al (2013) Characterization of a wild rabies virus isolate of porcine origin in China. Infect Genet Evol 17:147–152
Luzzi GA, Milne LM, Waitkins SA (1987) Rat-bite acquired leptospirosis. J Infect 15:57–60
Maraki S, Sarchianaki E, Barbagadakis S (2012) *Myroides odoratimimus* soft tissue infection in an immunocompetent child following a pig bite: case report and literature review. Braz J Infect Dis 16:390–392
Mease LE, Baker KA (2012) Monkey bites among US military members in Afghanistan 2011. Emerg Infect Dis 18:1647–1649
Medeiros I, Saconato H (2001) Antibiotic prophylaxis for mammalian bites. Cochrane Database Syst Rev 2:CD001738
Mihailoviv Z, Savic S, Damjanjuk I et al (2011) A case of fatal Himalayan bear attack in the zoo. J Foresenic Sci 56:806–809
Moore RM Jr, Zehmer RB, Moulthrop JI, Parker RL (1977) Surveillance of animal-bite cases in the United States, 1971-1972. Arch Environ Health 32:267–270
Morehouse LG, Kintner LD, Nelson SL (1968) Rabies in swine. J Am Vet Med Assoc 153: 57–64
Mutombo M, Arita I, Jezek Z (1983) Human monkeypox transmitted by a chimpanzee in a tropical rain-forest area of Zaire. Lancet 1:735–737
Nabi DG, Tak SR, Kangoo KA, Halwai MA (2009) Increasing incidence of injuries and fatalities inflicted by wild animals in Kashmir. Injury 40:87–89
Newton F. United States Armed Forces (2010) Monkey bite exposure treatment protocol. J Spec Oper Med 10:48–49
Nishioka SA, Handa ST, Nunes RS (1994) Pig bite in Brazil: a case series from a teaching hospital. Rev Soc Bras Med Trop 27:15–18

Nogalski A, Jankiewicz L, Cwik G et al (2007) Animal related injuries treated at the Department of Trauma and Emergency Medicine, Medical University of Lublin. Ann Agric Environ Med 14: 57–61

O'Rourke LG, Pitulle C, Hegarty BC et al (2005) *Bartonella quintana* in cynomolgus monkey (*Macaca fascicularis*). Emerg Infect Dis 11:1931–1934

Ordog GJ, Balasubramanium S, Wasserberger J (1985) Rat bites: fifty cases. Ann Emerg Med 14: 126–130

Parry RG, Ziemis R, Reynolds H, Miller S (1983) Brown/Grizzly bear mouth cultures in Alaska. Alaska Med 25:1–2

Pavia AT, Bryan JA, Maher KL et al (1989) *Vibrio carchariae* infection after a shark bite. Ann Intern Med 111:85–86

Peel MM, Hornidge KA, Luppino M et al (1991) *Actinobacillus* spp. and related bacteria in infected wounds of humans bitten by horses and sheep. J Clin Microbiol 29:2535–2538

Quinn JV, McDermott D, Rossi J et al (2010) Randomized controlled trial of prophylactic antibiotics in dog bite wounds with refined cost model. West J Emerg Med 11:435–441

Risholt T, Persen E, Solem OI (1998) Man and polar bear in Svalbard: a solvable ecological conflict? Int J Circumpolar Health 57(Suppl 1):532–534

Rose SC (1982) Bear maulings in Alaska. Alaska Med 24:29–32

Royle JA, Isaacs D, Eagles G et al (1997) Infections after shark attacks in Australia. Pediatr Infect Dis J 16:531–532

Rtshiladze MA, Andersen SP, Nguyen DQ et al (2011) The 2009 Sydney shark attacks: case series and literature review. ANZ J Surg 81:345–351

Ruggeri FM, Di Bartolo I, Ponterio E et al (2013) Zoonotic transmission of hepatitis E virus in industrialized countries. New Microbiol 36:331–344

Russell FE (1969) Clinical aspects of snake venom poisoning in North America. Toxicon 7:33–37

Sacks JJ, Kresnow M, Houston B (1996) Dog bites: how big a problem? Inj Prev 2:52–54

Schwaber MJ, Navon-Venezia S, Masarwa S et al (2013) Clonal transmission of a rare methicillin-resistant *Staphylococcus aureus* genotype between horses and staff at a veterinary teaching hospital. Vet Microbiol 162:907–911

Schweizer M, Falcone V, Gänge J et al (1997) Simian foamy virus isolated from an accidentally infected human individual. J Virol 71:4821–4824

Shetty RA, Chaturvedi S, Singh Z (2005) Profile of animal bite cases in Pune. J Commun Dis 37: 66–72

Smith HM, Reporter R, Rood MP et al (2002) Prevalence study for antibody to rat borne pathogens and other agents among patients using a free clinic in downtown Los Angeles. J Infect Dis 186: 1673–1676

Smith TC, Male MJ, Harper AL et al (2009) Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One 4:e4258. https://doi.org/10.1371/journal.pone.0004258

Talan DA, Citron DM, Abrahamian FM et al (1999) Bacteriologic analysis of infected dog and cat bites. N Engl J Med 340:85–92

Tough SC, Butt JC (1993) A review of fatal bear maulings in Alberta, Canada. Am J Forensic Med Pathol 14:22–27

Tregle RW Jr, Loe CL, Earhart RH 3rd, d'Autremont SB (2011) Cercopithecine herpesvirus 1 risk in a child bitten by Bonnet Macaque monkey. J Emerg Med 41:e89–e90

Tribe GW, Noren E (1983) Incidence of bites from cynomolgus monkeys, in attending animal staff, 1975-80. Lab Anim 17:110

van Belkum A, Melles DC, Peeters JK, et al., and Dutch Working Party on Surveillance and Research of MRSA-SOM (2008) Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and humans. Emerg Infect Dis 14:479–483

Van Cleef BA, Broens EM, Voss A et al (2010) High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in the Netherlands. Epidemiol Infect 138: 756–763

Van Demark RE Sr, Van Demark RE Jr (1991) Swine bites of the hand. J Hand Surg Am 16: 136–138

Van den Eede A, Martens A, Floré K et al (2013) MRSA carriage in the equine community: an investigation of horse-caretaker couples. Vet Microbiol 163:313–318

van Loo I, Huijsdens X, Tiemersma E et al (2007) Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis 13:1834–1839

Vougiouklakis T (2006) Fatal brown bear (*Ursus arctos*) attack: case report and literature review. Am J Forensic Med Pathol 27:266–267

Walk C (2012) British military snake-bite guidelines: pressure immobilization. J R Army Med Corps 158:194–198

Wamisho BL, Bates J, Tompkins M et al (2009) Ward round-crocodile bites in Malawi: microbiological and surgical management. Malawi Med J 21:29–31

Weiss HB, Friedman DJ, Cohen JH (1998) Incidence of dog bite injuries treated in emergency departments. J Am Med Assoc 279:51–53

Yates WDG, Rehmtulla AJ, McIntosh DW (1983) Porcine rabies in Western Canada. Can Vet J 24: 162–163

Part VI

Zoonoses of Wildlife Animals

Zoonotic Pathogens of Reptiles: An Unregarded Slithery Matter

32

Jairo Alfonso Mendoza Roldan, Marialaura Corrente, and Domenico Otranto

Contents

Abstract

Increased urbanization and introduction of exotic species of reptiles may act as drivers for the transmission of zoonotic pathogens through the environment. Reptiles are reservoirs of a wide range of pathogens, including viruses, bacteria, protozoa, helminths, pentastomids, and arthropod parasitic species, some of which may represent a public health concern. Reptiles can also be a source of life-threatening parasitoses through uncooked or raw meat ingestion. The main zoonotic disease associated to reptiles is salmonellosis, yet other less regarded diseases are equally devastating, such as spotted fever, sparganosis, or pentastomiasis.

Keywords

Zoonosis · Reptiles · Parasites · Bacteria

J. A. M. Roldan · M. Corrente
Department of Veterinary Medicine, University of Bari "Aldo Moro", Bari, Italy

D. Otranto (✉)
Department of Veterinary Medicine, University of Bari, Valenzano, BA, Italy
e-mail: domenico.otranto@uniba.it

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_56

32.1 Introduction

Reptiles represent a diverse and successful group of vertebrates, including more than 1200 genera and around 11,000 species (Roll et al. 2017). This class, however, is considered artificial, given the paraphyletic origin of the group, where also birds are included (Pincheira-Donoso et al. 2013). Moreover, non-avian reptiles are divided in four main orders: The largest order being Squamata (i.e., 10,417), which is represented by snakes, lizards, and amphisbaenas. Other orders are represented by *Testudines* (i.e., 351), which are turtles and tortoises; *Crocodylia* (i.e., 24), which includes species of crocodiles, alligators, caimans, and gavials; and, finally, *Rhynchocephalia*, represented by a single species of living fossils named tuataras (*Pincheira-Donoso* et al. 2013). Reptiles are considered a successful class due to having endured with scarcely changes in their morphology, biology, and ecology, since their appearance of reptiles, 310–320 million years ago in the late Carboniferous (Lepetz et al. 2009). However, a dwindling number of species survived the mass extinction events during the Cretaceous-Paleogene period (i.e., around 65 million years ago). Yet, those that survived spread through all biomes, diverging into the high species richness recorded in present times (Mohabey and Samant 2019). Through these epochs represented by millions of years, pathogens (e.g., bacteria, parasites, viruses) coevolved with reptiles to the present diseases associated to this cold-blooded class (Mendoza-Roldan et al. 2021a). Indeed, reptiles are hosts of viral, bacterial, and parasitic pathogens, some of which may have a zoonotic importance (Mitchell 2011; Mendoza-Roldan et al. 2020). For example, *Salmonella* species have been historically associated as the most common cause of zoonotic disease, given their natural affinity with reptiles, especially those with aquatic behavior (i.e., freshwater terrapins; Bertrand et al. 2008; Sodagari et al. 2020). Yet, other bacteria, viruses, and parasites associated to reptiles can also represent a risk to public health. Nonetheless, these other pathogenic agents have been less studied, and information on the biology, ecology, and zoonotic potential of most of them is scarce, being historically neglected even in endemic areas. Furthermore, the transmission pathways of reptile-borne zoonoses have been divided in three main routes: (i) through consumption raw or uncooked meat of reptiles, (ii) environmental contamination or direct contact, and (iii) through reptile zoonotic vector-borne diseases (Mendoza-Roldan et al. 2020, 2021a). These routes are also influenced by other factors, such as geographical area and human level of interaction with reptiles (i.e., herpetophagy, reptile kept as pets, synanthropy). In this chapter, the main reptile-associated zoonotic diseases in different contexts from veterinary and medical perspective are discussed.

32.2 Reptiles in Culture and Religion

Like a boa snake constricting its prey, myths and legends have always wrapped reptiles, associating them with positive or negative beliefs in religion and folklore. This can be observed in the symbolic usage in art and literature in many cultures all over the world. The relationship human beings have developed with reptiles through time are studied in the field of ethnoherpetology (Costa et al. 2021). For example,

while in Western cultures, snakes are mostly feared because of their association with evil in Judeo-Christian religions, in Africa and in Asian cultures, they are often seen as good omens and even worshipped. Accordingly, reptiles are represented as real or imaginary in the mythology and in the religions around the word (Crump 2021). One of the most popular legendary creatures with snake-like traits is the dragon embodying both awe and respect in European as well as East Asian (e.g., China, Japan, Korea) cultures. In Greek mythology, snakes are personified as antagonists of many heroes, such as for the nine-headed Lernaean Hydra that was defeated by Hercules and the three Gorgon sisters with snakes instead of hair.

In the opposite part of the world, in Latin America, Aztec and other Nahua people worship Cipactli, the giant earth crocodile, and in Andean and South American, a serpent-like God known as Amaru (Steele and Allen 2004). Conversely, Christianity and Judaism have historically portrayed reptiles as fearful and evil beings fear. As paradigmatic example, from the very beginning of the ancient testament (Genesis 3:1) the evil, under the shape of a slithery serpent, caused Adam and Eve to be expelled from the Eden.

32.3 In Sickness and in Health: Public Health Impact of Reptiles

In general, having a healthy and species-rich population of reptiles is beneficial for humans. Indeed, some reptiles are of great ecological significance, as they represent in nature either the first (e.g., small lizards and snakes) or the higher level (e.g., crocodiles and large snakes) of the food chain (Cortéz-Gómez et al. 2015). In the latter case, given their aquatic habits and longevity (generally >60 years), crocodilians may be useful as potential indicators of environmental pollution, as they are sentinels of environmental stability and health (Briggs-Gonzalez et al. 2021). However, reptiles may represent public health concerns in some regions. For example, snakebite envenomation is still considered an important yet neglected disease in tropical countries in the developing world (Ruiz de Castañeda et al. 2021). Indeed, more than two million people worldwide, per year, suffer from snakebite accidents, with more than 130,000 deaths every year (Gutiérrez et al. 2017). However, given anthropic pressures, such as rising urbanization, habitat fragmentation, and deforestation, reptiles and their zoonotic pathogens have become even more close contact with human populations. These pathogens are represented by bacteria, such as *Salmonella* spp., and to a lesser extent and importance species from the genera *Aeromonas, Pseudomonas, Citrobacter*, *Vibrio*, *Klebsiella*, *Enterobacter*, *Shewanella*, *Acinetobacter*, *Yersinia*, as well as *Campylobacter*, *Arcobacter*, *Chlamydia*, *Leptospira*, and *Mycobacterium* (Table 1). The beforementioned genera of bacteria are mainly transmitted through the direct contact or environmental contamination through reptiles' secretions/excretions. Moreover, zoonotic bacterial RVBDs are represented by microorganisms of the genera *Aeromonas, Anaplasma, Borrelia*, *Coxiella, Ehrlichia,* and *Rickettsia* (Mendoza-Roldan et al. 2021a). On the other hand, parasitic zoonotic diseases associated to reptiles are mainly represented by food-borne pathogens, such as pentastomids and cestodes, while other zoonotic

Table 1 Main zoonotic reptile-associated bacteria

Pathogen[a]	Animal disease	Human disease	Transmission
Enterobacteria			
Salmonella spp.	Reptiles are carriers and rarely show clinical disease Intermittent shedders	Gastroenteritis Bloody mucoid diarrhea, nausea, vomiting, fever Meningitis, osteomyelitis septicemia	Direct contact handling Ingestion Contaminated water Indirect
Klebsiella spp., *E. coli, Proteus* spp., *Citrobacter* spp., *Yersinia* spp.	Mostly asymptomatic ulcerations, stomatitis	Opportunistic infections	Direct contact handling Ingestion
Non-fermentative, Gram-negative organisms			
Vibrio spp., *Aeromonas* spp., *Pseudomonas* spp.	Mostly asymptomatic	Opportunistic infections	Direct contact handling Ingestion
Campylobacter spp.	Mostly asymptomatic	Infections in immunocompromised patients	Direct contact handling Ingestion Contaminated water
Other			
Mycobacterium spp.[b]	Granulomatous disease ulcerative stomatitis	Granulomatous disease at infection site Disseminated granulomatous disease in immunocompromised patients	Handling, puncture wounds, scratches, inhalation

[a]Name of the genus
[b]Nontuberculous mycobacteria

parasitic agents can be transmitted by direct contact (i.e., *Cryptosporidium*) or by vectors (i.e., *Trypanosomatidae*; Mendoza-Roldan et al. 2020). Finally, also associated to vector transmission, arboviruses may be associated to reptilian reservoirs which, given the low host specificity of mosquitoes, may be then transmitted to humans.

32.4 Zoonotic Pathogens Associated to Reptiles

32.4.1 Bacteria

32.4.1.1 Salmonellosis

The Pathogen

Reptiles are natural reservoirs of *Salmonella*, and they may harbor a wide variety of *Salmonella* serotypes in their intestine, even simultaneously (Chiodini and Sundberg 1981; Willis et al. 2002). The so-called "exotic" *Salmonella* serotypes, belonging to

Salmonella bongori and *Salmonella enterica* subspecies II, IIIa, IIIb, IV, and VI, are mainly isolated from reptiles and from the environment where reptiles live (Pignato et al. 1998). However, in free-living and captive reptiles, the isolation of *Salmonella enterica* subspecies I is also a frequent finding (Mitchell and Shane 2000). *Salmonella* strains are well-adapted to reptiles, eventually causing mostly asymptomatic infections and only occasionally disease and death (Le Souëf et al. 2015). Nonetheless, those strains may retain pathogenicity for warm-blooded animals. Reptiles and amphibians are estimated to account for 6% of all *Salmonella* spp. infections in the United States and Europe (Mermin et al. 2004; De Jong et al. 2005; Corrente et al. 2006; Wilkstrom et al. 2014). Most of reptile-associated salmonellosis (RAS) are mainly reported in children younger than 5 years, elderly people, or immunocompromised persons (CDC 1992a, b, 1999; Woodward et al. 1997; Friedman et al. 1998; Meyer Sauteur et al. 2013; Pees et al. 2013; Ricard et al. 2013; Murphy and Oshin 2015). In those population groups, RAS may be fatal. A literature review conducted by Meyer Sauteur et al. (2013) examined published studies from 1965 to 2012, describing RAS in children aged less than 18 years, with a total of 182 cases identified. The primary reptiles associated with gastrointestinal salmonellosis were turtles; however, exposure to iguanas was significantly more prevalent in children with invasive *Salmonella* diseases, causing septicemia or meningitis. In addition, *Salmonella* multidrug-resistant (MDR) strains emerge as a potential concern for public health safety, with implications of increased disease severity, longer hospitalizations, and higher cost rates. It has been shown that also reptiles may hold MDR *Salmonella* (Seepersadsingh and Adesiyun 2003; Wei et al. 2019). The widespread use of antibiotics against *Salmonella* has been described in the international trade of pet reptiles, to prevent economical losses, as well as in animal welfare in crowded farms and long-distance transport (Goławska et al. 2019).

Epidemiology of Salmonellosis

High prevalence of *S. enterica* has been reported for pet reptiles, estimated to be 48–50% in lizards, 7–10% in chelonians, and 51–83% in snakes (Fagre et al. 2020). Since the difference in prevalence detected may be linked to intermittent excretion, every animal could be potentially considered as a potential shedder (Corrente et al. 2004). In addition, vertical transmission of *Salmonella* spp. in reptiles has been demonstrated (Schröter et al. 2006). The infection to other animals, such as pets, and to humans can be transmitted by both direct and indirect contact, as *Salmonella* strains display good resistance in the environment (Winfield and Groisman 2003). For example, turtles are small enough to be kissed and held by children, which increases the likelihood of direct transmission of *Salmonella*. In addition, indirect transmission of this pathogen can occur through cross-contamination by cleaning reptile habitats in a kitchen sink or bathtub (Corrente et al. 2017).

Prophylaxis. As a high prevalence of carriers is generally found in reptiles, antibiotic prophylaxis does not seem useful and appropriate to solve this problem. Nevertheless, it is important to remind that all *Salmonella* spp. strains isolated from reptiles retain pathogenicity for humans and that some health measures are necessary. For RAS surveillance, the identification of *Salmonella* carriers, the control of health practices, and a good knowledge of the zoonotic risks linked to reptile

husbandry are of outmost importance. In a recent survey related to the attitudes of reptile owners, the prevalence of *Salmonella* spp. colonization was correlated to several factors (Corrente et al. 2017). Indeed, when animals shared the terrarium with other reptiles, they were at least two times more exposed to *Salmonella* spp. than the animals living alone. Regarding the practice of handwashing after contact or husbandry of the animals, the prevalence of *Salmonella* spp. was three times higher in animals handled by owners who were not used to washing their hands. In the same survey, as many as 62% of the interviewed admitted ignoring the risk of RAS and other health problems associated with cold-blooded animals. Accordingly, prevention of RAS must rely mainly on information and education, with the veterinarian health bodies primarily involved in this task.

32.4.1.2 Miscellaneous Bacterial Infections: Other Gram-Negative Microorganisms

The microbiota of reptiles is mainly composed by Gram-negative bacteria. In the gut, the microbiota is influenced by the diet and the environment, especially in captivity, when a shift to gut colonization by zoonotic organisms is observed (Kohl et al. 2017). In fact, the captivity significantly alters both bacterial community membership and structure in lizards kept for 8 weeks. Notably, they showed an invasion of the gut community by *Enterobacter* spp. and *Salmonella* spp., potentially pathogenic bacterial genera (Colston 2017).

Reptiles are prone to infection by a variety of predominately Gram-negative commensal bacteria, including those in the genera *Aeromonas, Klebsiella, Proteus, Pseudomonas,* and *Yersinia* (Table 1).

Among Gram-negative organisms, *Aeromonas* spp., *Pseudomonas* spp., *Citrobacter* spp., and *Vibrio* spp. were reported in reptiles, both from clinical cases (bronchopneumonia, ulcerations, stomatitis, septicemia, etc.) and healthy animals as carriers (Goławska et al. 2019). *Klebsiella* spp., *Enterobacter* spp., *Shewanella* spp., *Acinetobacter* spp., and *Yersinia* spp. were reported as opportunistic bacteria found in Testudines. Both pathogenic and opportunistic bacteria might cause infections in other poikilothermic and homoeothermic animals as well as humans, particularly those with immune deficiency (Goławska et al. 2019).

As for *Salmonella* spp., the zoonotic potential of all those bacteria may be exacerbated by the acquisition of AMR genes. Golawska et al. found in tortoises a high prevalence of MDR *E. coli* and the horizontal genetic transfer of AMR genes among Gram-negative organisms is very frequent (Goławska et al. 2019).

32.4.1.3 The Heterogeneous World of Epsilonproteobacteria

Ectothermic reptiles display a distinct and largely unique Epsilonproteobacteria community, including taxa, which can cause disease in humans. Several *Arcobacter* taxa are widespread among reptiles and often show a broad host range. Similarly, reptiles carry a large diversity of unique and novel *Helicobacter* taxa, which apparently evolved in an ectothermic host. Some species, such as *Campylobacter fetus*, display a distinct intraspecies host dichotomy, with genetically divergent lineages (Gilbert et al. 2019).

The presence of Epsilonproteobacteria in many different reptiles without clinical symptoms indicates that most of them are carried without adverse health effects. Most of the *Arcobacter* and *Campylobacter* species found in reptiles are associated with disease in humans, although infections are often sporadic and mainly affecting immunocompromised hosts (Collado and Figueras 2011).

Campylobacteriosis

The genus is composed by Gram-negative, microaerophilic bacteria that inhabit the intestinal tract of various animals, as either commensals or pathogens (Rukambile et al. 2019). Human campylobacteriosis is the most frequently reported foodborne zoonosis in the European Union (EFSA and ECDC 2018). Sources of human infections are consumption of raw or undercooked meat, unpasteurized milk, and untreated water or direct contact with colonized animals that carry *Campylobacter* spp. asymptomatically. In reptiles, especially chelonians, *Campylobacter* spp. occurrence ranged from 9.7% in Taiwan (Wang et al. 2013) and 10.2% in Italy to 25.3% in the Netherlands (Gilbert et al. 2014).

Currently, *C. fetus* is the only species for which an association between reptile contact and human infection has been demonstrated (De Luca et al. 2020). A novel subspecies of *C. fetus*, named *C. fetus* subs. *Testudinum*, has been described in chelonians (Fitzgerald et al. 2014). Following this discovery, *C. fetus* isolated from human infections has been increasingly identified as subsp. *testudinum* (Choi et al. 2016), and two new other *Campylobacter* species were identified in reptiles. *Campylobacter iguaniorum* was isolated from lizards, such as *Iguana iguana* and *Pogona vitticeps* (Gilbert et al. 2014), and tortoises, like *Stigmochelys pardalis* (Benejat et al. 2014) and *C. geochelonis* (Piccirillo et al. 2016).

Besides from humans, *C. fetus* subsp. *testudinum* has only been isolated from reptiles, with a reported culturing-based prevalence of 5.5–6.7% in reptiles and 7.1–9.7% in chelonians (Wang et al. 2013; Gilbert et al. 2014). Reptile-associated *C. fetus subsp. testudinum* showed a remarkable epidemiology, as all human cases were in men, most of whom were of Asian origin (Patrick et al. 2013).

Infections by *C. fetus* subsp. *testudinum* were in humans >60 years of age or immunocompromised. Humans may contract *C. fetus* subsp. *testudinum* through exposure to reptiles, possibly by ingestion or by contact with feces or the environment.

After the finding of *Campylobacter*, also Arcobacter and *Helicobacter* species have been reported to be isolated from reptiles. *Arcobacter butzleri* and *Helicobacter* species have been isolated from tortoises (Stacy and Wellehan Jr. 2010).

Helicobacter taxa have only been isolated from reptiles and have not been associated with infection in humans thus far, except for a case of bacteremia caused by a urease-negative *Helicobacter* strain, isolated from blood cultures of a 28-year-old man with a severe agammaglobulinemia (Schwarze-Zander et al. 2010).

32.4.1.4 Mycobacteriosis

The genus *Mycobacterium* includes over 200 species classified as tuberculous and not tuberculous mycobacteria (NTM), on the basis of biological and genetic

characteristics (Armstrong and Parrish 2021). NTM have a cosmopolitan distribution and have been isolated from all the major classes of animals. Many of the NTM that can infect animals can also infect humans (Ebani et al. 2012). In reptiles, several species, such as *Mycobacterium chelonae, M. fortuitum, M. intracellulare, M. marinum, M. phlei, M. smegmatis, M. ulcerans, M. confluentis, M. haemophilum, M. hiberniae, M. neoaurum,* and *M. nonchromogenicum*, have been isolated, provoking granulomatous disease and ulcerative stomatitis. Systemic infections have been also reported (Soldati et al. 2004). NTM can be transmitted to humans and herpetofauna through aerosolization of contaminated respiratory secretions or direct skin contact, soil, or water. Reptiles and amphibians can harbor *Mycobacterium* species that can also be found as opportunistic pathogens in humans, especially those who are immunocompromised. Infections by *M. marinum*, *M. chelonae*, and *M. abscessus* have been reported (Soldati et al. 2004). Pet owners should always practice strict disinfection protocols when handling their pets and limit contact when they have an open wound on their hands (Ebani 2017).

32.4.1.5 Chlamydiosis

Several *Chlamydia* species have been isolated from reptiles, such as *C. psittaci*, *C. abortus*, and *C. pneumoniae,* causing granulomatous lesions in various organs and pneumonia. Despite their zoonotic potential, no cases of transmission from reptiles to humans are reported (Ebani 2017).

32.4.1.6 Leptospirosis

Leptospira spp. are organisms diffused worldwide and responsible for a reemerging zoonosis. Data about *Leptospira* spp. and the infection in reptiles are very scant. *Leptospira* spp. serovars are traditionally related to aquatic habitat. Specific antibodies in reptiles living in aquatic habitat such as crocodiles have been detected. However, no symptoms related to leptospirosis were reported. A role of those animals in the epidemiology of leptospirosis may be hypothesized, but no reptile-associated cases in humans have been described (Ebani 2017).

32.4.2 Zoonotic Bacterial Reptile Vector-Borne Diseases (ZBRBVDs)

Reptile vector-borne diseases (RVBDs; Table 2) of zoonotic concern are caused by bacteria, protozoa, and viruses transmitted by arthropod vectors, included in the subclass Acarina (i.e., mites and ticks; Fig. 1) or the order Diptera (i.e., mosquitoes, sand flies, and tsetse flies). Zoonotic bacterial RVBDs (ZBRBVDs) belong to the genera *Aeromonas, Anaplasma, Borrelia, Coxiella, Ehrlichia,* and *Rickettsia*. Moreover, *Bartonella henselae* or a species genetically related to *Bartonella vinsonii* subsp. *berkhoffii* was recorded in marine turtles (Valentine et al. 2007). Of these genera of bacteria, reptiles may play an important role as reservoirs of Lyme disease and spotted fever group *Rickettsia*.

Main vectors of granulocytic anaplasmosis (GA) caused by *Anaplasma phagocytophilum* (i.e., *Ixodes pacificus* in North America and *Ixodes ricinus* in

Table 2 Zoonotic reptiles' vector-borne pathogens

Pathogen	Reptile host	Country	Disease in humans	Vectors
Rickettsia spp.	Lizards Snakes Turtles Tortoises	Algeria Australia Brazil Colombia Chile El Salvador Italy Ghana Japan Madagascar Mexico Netherlands Honduras United States Zambia	**Spotted fever:** African fever, Brazilian spotted fever, Flinders Island spotted fever, Mediterranean spotted fever	Mites and ticks
Aeromonas hydrophila	Snakes	Worldwide	Gastroenteritis, in rare cases, necrotizing fasciitis	Mites
Borrelia spp.	Lizards Tortoises	Australia Japan United states North Africa	Lyme disease Relapsing fever Neuroborreliosis	Ticks
Coxiella burnetii	Monitor Lizards Tortoises	Congo Guinea Bissau Ghana Romania	Q fever	Ticks
Ehrlichia sp.	Monitor Lizards Snakes Tortoises	Japan Malaysia Italy Romania United Kingdom Zambia	Unknown	Ticks
Anaplasma phagocytophilum	Box turtles Monitor lizards Snakes Tortoises	Indonesia Malaysia Poland Romania United States	Granulocytic anaplasmosis	Ticks
Crimean-Congo hemorrhagic fever	Tortoises	Turkey	Crimean-Congo hemorrhagic fever	Ticks
Leishmania spp.	Lizards	China Iran Kenya France Italy Pakistan Spain	Visceral and cutaneous leishmaniasis	Phlebotomine sand flies

(continued)

Table 2 (continued)

Pathogen	Reptile host	Country	Disease in humans	Vectors
Zika virus	Lizards Snakes	Cuba	Fever, rash, conjunctivitis, muscle and joint pain, malaise or headache	Mosquitoes
Eastern equine encephalitis virus (EEEV) Western equine encephalitis (WEE)	Lizards Snakes Turtles	United States	Fever, neurologic disease, including meningitis	Mosquitoes
Chikungunya virus	Crocodiles Lizards Snakes Turtles	United States	Fever and joint pain	Mosquitoes
West Nile Virus	Snakes	Israel	Encephalitis, meningitis	Mosquitoes

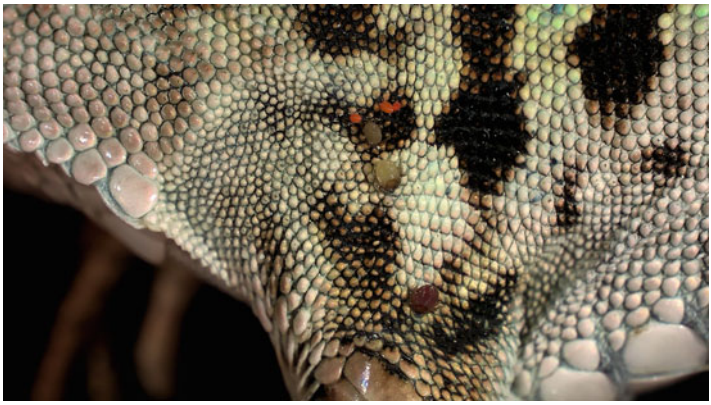

Fig. 1 *Podarcis siculus* lizard parasitized by ticks (*Ixodes ricinus*) and chigger mites (*Neotrombicula autumnalis*)

Europe) occasionally can feed on reptiles, especially in their immature stages (i.e., larvae and nymphs). However, studies have showed that these ectothermic tetrapods play a minor role as reservoirs of GA (Nieto et al. 2009). *Anaplasma* is a genus of Gram-negative bacteria that includes pathogenic species transmitted mainly by ticks. These bacteria can cause severe disease and even death (Rymaszewska and Grenda 2008). Additionally, other species of *Anaplasma* have been molecularly detected in ticks associated to reptiles (e.g., *I. ricinus* in Central and Western Europe; Mendoza-Roldan et al. 2021b). On the other hand, *Ehrlichia* spp. have been detected in different tick of reptiles. For example, *Ehrlichia chaffeensis* and *Candidatus* Neoehrlichia mikurensis were detected in *Amblyomma* ticks from reptiles imported to Japan (Andoh et al. 2015).

32.4.2.1 Lyme Disease and Borrelioses

The *Borrelia* genus comprises spirochete bacteria separated in four main groups (i.e., relapsing fever group, reptilian-*Borrelia* group, monotreme-associated *Borrelia* group, Lyme borreliosis group). Importantly, the Lyme disease group includes more than 20 species that are under the *Borrelia burgdorferi* sensu lato complex. Nine of these species have pathogenic potential (Majláthová et al. 2008; Mendoza-Roldan et al. 2019).

Borrelia lusitaniae is associated with lacertid lizards from Western Europe. Moreover, Lyme disease species are likely associated to these group of lizards, acting as natural reservoirs (Majláthová et al. 2006; Mendoza-Roldan et al. 2019), or being refractory to infection by means of complement-mediated killing effect, which has been described in species of lizards in the United States (Kuo et al. 2000). Indeed, some species of lizards may reduce the presence of bacteria producing a zooprophylactic effect (Tijsse-Klasen et al. 2010).

32.4.2.2 Spotted Fever Group Rickettsioses

Rickettsia bacteria comprise a large group of Gram-negative, intracellular pathogens associated with invertebrate vectors (Abdad et al. 2018). Reptiles have a direct role in the epidemiological cycle of some pathogens within the Rickettsiaceae family (Novakova et al. 2015). Indeed, most of the *Rickettsia* species of zoonotic concern, associated to reptiles' Acarina ectoparasites, are within the spotted fever group (SFG). One of the first solely associated to a reptilian tick *Rickettsia* species was *Rickettsia honei*, the causative agent of Flinders Island spotted fever, in Southern Australia. This species was described infecting *Bothriocroton hydrosauri*, a tick that feeds primarily from lizards and snakes (Stenos et al. 2003; Whiley et al. 2016). Furthermore, eight other species of SFG *Rickettsia* have been detected in ectoparasites and some even in reptiles. In Europe, SFG *Rickettsia* have been identified in reptiles, including species such as *Rickettsia helvetica* and *Rickettsia monacensis* detected in *I. ricinus* ticks, and in blood and tail of lacertid lizards (Mendoza-Roldan et al. 2021b). Other rickettsial agents detected in ticks and mites collected from reptiles are *R. aeschlimannii*, *R. amblyommatis, R. hoogstraalii, R. massiliae*, *R. raoultii*, *R. rhipicephali*, *R. tamurae*, and *R. typhi* (Sánchez-Montes et al. 2019).

On the other hand, *Rickettsia* species have been detected in *Amblyomma*, *Bothriocroton*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Ixodes* ticks and in mites from families *Ixodorhynchidae*, *Macronyssidae*, *Pterygosomatidae*, and *Trombiculidae* (Sánchez-Montes et al. 2019; Mendoza-Roldan et al. 2021a).

32.4.2.3 Other Zoonotic Reptile Vector-Borne Diseases (ZRBVDs)

Protozoa

Vector-borne zoonotic protozoa associated to reptiles are represented by trypanosomatid flagellates, such as *Leishmania* and *Trypanosoma* (Kinetoplastida: *Trypanosomatidae*) (Table 2; Poinar Jr and Poinar 2004). For example, *Trypanosoma brucei*, which causes sleeping sickness in Africa, was detected in monitor lizards from Kenya (Njagu et al. 1999), serving as an important blood

source for the tsetse fly (*Glossina fuscipes fuscipes*) also in Uganda (Waiswa et al. 2006). Indeed, experimental evidence indicates that some species of reptiles could be potential reservoirs of this pathogen (Woo and Soltys 1969).

32.4.2.4 Leishmaniases in Reptiles: Is It a Big Deal?

The species of *Leishmania* that infect reptiles belong to the subclade *Sauroleishmania*, which is a sister clade of the pathogenic species of mammalian-associated *Leishmania*, with around 21 species infecting reptiles, mainly lizards (Ticha et al. 2021). These genetic and phylogenetic similarities suggest that species of *Leishmania* typical of reptiles could transiently infect mammals and vice versa (Klatt et al. 2019). For example, *Leishmania adleri* from lacertid lizards in Kenya may produce cutaneous leishmaniasis in mammals (Coughlan et al. 2017). Also, *Leishmania tarentolae* from geckoes has been molecularly detected in human mummies from Brazil (Novo et al. 2015), human blood (Pombi et al. 2020; Iatta et al. 2021), and shelter dogs in Italy (Mendoza-Roldan et al. 2021c). Additionally, *Sergentomyia minuta* sand fly, the natural vector of *L. tarentolae*, has been detected feeding from humans and other mammals (Abbate et al. 2020). Given the genetic similarity with *Leishmania infantum*, *L. tarentolae* natural infection could, to a certain extent, protect mammals against other pathogenic *Leishmania* spp., yet this issue needs further investigation also considering the promising results of vaccination attempts using this species of *Leishmania* (Klatt et al. 2019). On the other hand, reptiles could potentially have a role as reservoirs of pathogenic *Leishmania* spp. in areas where primary hosts do not occur or where reptiles and natural hosts live in sympatry. For example, *Leishmania tropica*, *Leishmania donovani*, and *Leishmania turanica* were molecularly detected in lizards and snakes from Northwestern China (Chen et al. 2019; Zhang et al. 2019), and *L. infantum* was molecularly detected in lizards collected from dog shelters in Southern Italy (Mendoza-Roldan et al. 2022b), both areas being endemic for leishmaniases.

32.4.3 Viruses

32.4.3.1 COVID-19 and Other Viral Diseases Associated to Reptiles

At the beginning of the COVID-19 pandemic, some studies initially hypothesized that the origin of the SARS-CoV-2 was from snakes, which were kept alive in the Wuhan meat market (Ji et al. 2020). Later, bats and/or pangolins were pointed out as the more probable reservoirs of this viral disease, given that coronavirus species are associated to endothermic tetrapods (i.e., mammals and birds; Gautam et al. 2020). Conversely, snake venom from vipers, such as *Bothrops jararaca*, contains peptides that could be useful for the treatment of COVID-19 (Gouda and Mégarbane 2021) or complications associated to the disease, such as thrombo-cardiovascular disorders (Kalita et al. 2021). Hence, snakes in the COVID-19 pandemic went from villains to potential heroes.

On the other hand, ectothermic tetrapods (i.e., reptiles and amphibians) may play a role as reservoirs for arboviruses (Table 2). Medically important anthropophilic species of mosquitoes, such as *Aedes aegypti* and *Aedes albopictus*, may

opportunistically feed on reptiles, and most of the orders of reptiles have been found by molecular and serological assays infected with arboviruses of zoonotic concern (Marschang 2011). Indeed, reptiles can be reservoirs of important arboviruses, such as Western and Eastern equine encephalitis, Venezuelan equine encephalitis, West Nile virus, and most recently Chikungunya virus (Bingham et al. 2012; Bosco-Lauth et al. 2018). Moreover, reptiles, specifically non-native lizards, can be important blood source of vectors (i.e., *Culex* spp.) of *Flavivirus* (Reeves and Burkett-Cadena 2022). In fact, blood meal identification indicated that arbovirus vectors may predominantly feed on reptiles (Burkett-Cadena et al. 2008). For example, *Culex tarsalis* mosquitoes can feed on garter snakes, which may maintain the virus of the Western equine encephalitis during winter (overwintering). Other reptile-associated viruses are the Japanese encephalitis and Zika viruses (Oya et al. 1983; Bueno et al. 2016).

Conversely, viral zoonotic diseases may be also vectored by ticks, such as *Hyalomma aegyptium*, that may be a putative vectors of Crimean-Congo hemorrhagic fever (CCHF), associated to tortoises. This zoonotic neglected disease is widely distributed through Africa, the Balkans, the Middle East, and Western Asia (Kar et al. 2020), where tortoises and their ticks may play a role in the cryptic transmission cycle (Kar et al. 2020).

32.4.4 Helminths and Other Zoonotic Endoparasites Associated to Reptiles

Reptiles represent essential part of the food webs in many ecosystems (Valencia-Aguilar et al. 2013), both as top predators (i.e., large carnivorous reptile species such as crocodilians, monitor lizards, pythons) and as prey for many carnivores (e.g., smaller Squamata reptile taxa). Therefore, many heteroxenous metazoan parasites cycle through reptiles, using them as intermediate or final hosts (Table 3; Greiner 2003), eventually infesting humans. This may happen when reptiles are used as part of the human diet throughout the world (Klemens and Thorbjarnarson 1995). Thus, reptiles may transmit some zoonotic helminths, mainly through their consumption or usage in medicinal practices (Magnino et al. 2009). For example, nematodes (e.g., *Angiostrongylus cantonensis*, *Anisakis* spp., *Gnathostoma* spp., *Eustrongylides* spp., *Trichinella* spp.), cestodes (e.g., *Spirometra* spp.), trematodes (e.g., *Alaria*), and several pentastomids, including *Armillifer* spp. (*A. armillatus; A. moniliformis; A. grandis, A. agkistrodontis*) and *Raillietiella hemidactyli*, may have a zoonotic potential. Most of the times, parasites do not complete their biological life cycles in humans rather these represent aberrant or dead-end hosts.

32.4.4.1 Nematodes

Along with amphibians, reptiles represent paratenic hosts for *Angiostrongylus cantonensis* (Strongylida, Angiostrongylidae), a metastrongyloid nematode causing eosinophilic meningitis in humans (Barratt et al. 2016). While rats represent the definitive hosts and molluscs the intermediate hosts, reptiles may cumulate infective L3 larvae in the liver and other tissues (Johny et al. 2018), therefore being important

Table 3 Zoonotic helminths and other endoparasites of reptiles

Pathogen	Reptile host	Region	Disease in humans	Transmission
Spirometra spp.	Snakes	Americas, Europe, Asia, Australia	Sparganosis Blindness, paralysis, death	Ingestion of the plerocercoid
Armillifer spp.	Snakes	Asia, Africa	Pentastomiasis Organ damage by larvae	Ingestion of embryonated eggs or larvae
Raillietiella hemidactyli	Lizards	Southeast Asia	Creeping disease Subcutaneous pentastomiasis	Ingestion of embryonated eggs or larvae
Trichinella spp.	Crocodiles Snakes Monitor lizards Turtles	Worldwide	Trichinosis Fever, myalgia, gastrointestinal symptoms	
Anisakis spp.	Worldwide	Crocodiles	Anisakiasis Eosinophilic granulomas	
Gnathostoma spp.	Snakes	Asia Central America	Gnathostomiasis Cutaneous or visceral *larvae migrans* symptoms	
Angiostrongylus cantonensis	Monitor lizards	Worldwide	Neuro-angiostrongyliasis Eosinophilic meningitis	

for the maintenance and amplification of the infection. The consumption of raw meat of varanid lizards was documented as an infection route in Asia (Parameswaran 2006; Johny et al. 2018). As the disease in humans is difficult to identify at a preoperative stage, the anamnesis is pivotal for an expedite diagnosis and treatment.

Within the genus *Trichinella* (Adenophorea, Trichinellidea), two non-encapsulated species – *Trichinella zimbabwensis* and *Trichinella papuae* – have been found in crocodiles and monitor lizards (Pozio et al. 2004). Though this genus includes nematodes with high zoonotic potential and most encapsulated ones are infective only for mammals, the zoonotic role of *Trichinella* spp. of reptiles is not well elucidated. For example, infections in humans due to the consumption of meat of monitor lizards and a turtle have been reported in Thailand, even though the *Trichinella* species involved was not identified (Khamboonruang 1991). Nonetheless, reptiles seem to be the main hosts for *T. zimbabwensis* and *T. papuae* as confirmed also experimentally (Pozio et al. 2004).

32.4.4.2 Sparganosis (Cestoda)

Spirometra (Diphyllobothriidae) are pseudophyllidean tapeworms, which develop as adults in carnivorous mammals (definitive hosts) and as larvae in freshwater crustaceans (i.e., cyclops) and poikilothermic vertebrates, being first and second intermediate hosts, respectively (Denegri 1993). This genus of cestode includes the most reported reptile-borne zoonotic helminths caused mainly by *Spirometra erinaceieuropaei*, *Spirometra*

mansonoides, and *Spirometra proliferum*. Humans may be infected by eating contaminated uncooked meat with infective larval stages (i.e., plerocercoids, also referred to as *sparganum*). In humans, visceral migration of plerocercoids may be disseminated in various organs and tissues (e.g., subcutaneous tissue, muscles, lungs, pleural cavity, urogenital, and abdominal viscera) (Presti et al. 2015). Depending on the invaded organs, sparganosis may be of minor to middle severity, as in the case of subcutaneous migrations, or even life-threatening (e.g., visceral sparganosis) and fatal, when localized in the central nervous system (e.g., ocular and cerebral forms) (Liu et al. 2015). A severe clinical condition in humans is also caused by the proliferative sparganosis, which is caused by the asexual multiplication of plerocercoids in human body (Anantaphruti et al. 2011). Sparganosis is described mainly in Asian countries, associated to the consumption of raw or inadequately cooked meat of snakes, frogs, and tadpoles infected with the plerocercoid forms. In Europe, cases of people infected with sparganosis were reported in foreigners coming from continents where the disease is endemic (Presti et al. 2015). However, humans may get infected even by drinking untreated water with infected copepods (i.e., first intermediate hosts) or transcutaneous, applying the flesh of infected snake or frog as a poultice to a wound as prescribed in Asian traditional medicine (Liu et al. 2015).

32.4.4.3 Pentastomiasis

Pentastomids are a subclass of parasitic arthropods that represent a unique lineage deriving from crustaceans. These exceptional parasites thrive within the respiratory tract of vertebrates, such as Squamata reptiles (snakes and lizards), mammals (canids), birds, and some species that are well-adapted to parasitize fishes (Gomez-Puerta et al. 2020). However, species of pentastomids that represent a zoonotic concern are typically associated with reptiles. Snake-associated pentastomids of the genus *Armillifer* are highly pathogenic to their ophidian host, and several species may cause zoonotic infections in Africa, such as *Armillifer grandis* and *Armillifer armillatus*, and in Asia, particularly *Armillifer moniliformis and Armillifer agkistrodontis* (Chen et al. 2010). Additionally, species of the genus *Porocephalus* (Brookins et al. 2009) have been recorded infecting humans and dogs, whereas *Raillietiella* infecting humans (Tappe et al. 2016). *Armillifer* parasitize snakes in Africa and Asia and *Porocephalus* in America. The life cycles of *Armillifer* and *Porocephalus* are heteroxenous, where adults are large and vermiform and infect the lower respiratory tract of snakes, laying infective eggs with typical larvae, that are excreted in the snakes feces. Intermediate hosts are represented by small mammals (i.e., rodents), in which larvae develop to nymphs in connective tissues and parenchymatous organs (i.e., liver, spleen, lungs) (Tappe et al. 2014). On the other hand, the genus *Raillietiella* (Fig. 2) is cosmopolitan, mainly recorded in in Africa, Asia, Australia, and the Americas (Kelehear et al. 2011). Differently from *Armillifer* and *Porocephalus*, *Raillietiella* spp. develop initially in insects as intermediate hosts (e.g., cockroaches), whereas adults thrive in the lung of lizards or snakes and amphibians (Walden et al. 2020).

Furthermore, species of pentastomids have been recorded in new geographical areas other than those of their original description (Walden et al. 2020), associated to invasive

Fig. 2 *Raillietiella hemidactyli* found in the lung of *Tarentola mauritanica* gecko

reptile species (e.g., *Raillietiella orientalis* in Burmese pythons in United States; Mendoza-Roldan et al. 2020), representing a risk of introduction of new zoonotic species. For example, *Raillietiella hemidactyli* was recorded infecting *Tarentola mauritanica* geckoes in a Southern island of Italy (Mendoza-Roldan et al. 2022).

Disease in Humans

Human pentastomiasis is associated with the consumption of raw reptile flesh, and occasionally infection through contaminated environment by eggs represents a risk in endemic areas (Tappe et al. 2011). Humans act as dead-end hosts where larvae cannot develop, thus they ultimately encyst in connective tissues and parenchymatous organs. Massive infections, which rarely lead to lethal cases, are believed to be associated with ingestion of gravid females of *Armillifer* spp. (Ette et al. 2003). Even though they occur frequently in some areas of West and Central Africa, human infections are usually asymptomatic and are diagnosed incidentally by X-ray, ultrasonography, or autopsy (Tappe et al. 2014). Indeed, postmortem prevalence in humans may be up to 23% in Central Africa and 40% in SA Asia (Burns-Cox et al. 1969). On the other hand, *R. hemidactyli* was reported as a potential causative agent of "creeping disease" in humans from Asia, probably due to *larva migrans* dwelling in the subcutaneous tissue (Dollfus and Canet 1954). This infection was associated to the consumption of raw meat of lizards. However, the ingestion of reptile secretions and feces may represent another transmission route of pentastomids to humans (Mendoza-Roldan et al. 2020).

32.5 Conclusions

Reptile-associated zoonotic diseases have historically received little attention. Although salmonellosis, pentastomiasis, and sparganosis are among the main zoonotic diseases, the role of reptiles as reservoirs and hosts of zoonotic pathogens, mainly for vector-borne diseases (VBDs), has not been fully elucidated.

Furthermore, the recent COVID-19 pandemic has highlighted the risk that anthropic pressures may exert on wild-animal populations, resulting in the emergence of new zoonotic diseases. Certainly, studies focusing on monitoring reptile-borne pathogens are advocated, in order to elucidate the origin of introduced parasites and the role of reptiles as definitive, intermediate, and paratenic hosts in non-endemic areas. This could contribute to reduce the risk of zoonotic transmission and, at the same time, improve welfare and conservation efforts for these cold-blooded animals.

32.6 Cross-References

► Cryptosporidium and Cryptosporidiosis: Trickle or Treat?
► Vector-Borne Zoonoses

References

Abbate JM, Maia C, Pereira A, Arfuso F, Gaglio G, Rizzo M, Caracappa G, Marino G, Pollmeier M, Giannetto S, Brianti E (2020) Identification of trypanosomatids and blood feeding preferences of phlebotomine sand fly species common in Sicily, Southern Italy. PLoS One 15(3):e0229536

Abdad MY, Abou Abdallah R, Fournier PE, Stenos J, Vasoo S (2018) A concise review of the epidemiology and diagnostics of rickettsioses: *Rickettsia* and *Orientia* spp. J Clin Microbiol 56(8):e01728-17

Anantaphruti MT, Nawa Y, Vanvanitchai Y (2011) Human sparganosis in Thailand: an overview. Acta Trop 118(3):171–176

Andoh M, Sakata A, Takano A, Kawabata H, Fujita H, Une Y, Goka K, Kishimoto T, Ando S (2015) Detection of *Rickettsia* and *Ehrlichia* spp. in ticks associated with exotic reptiles and amphibians imported into Japan. PLoS One 10(7):e0133700

Armstrong DT, Parrish N (2021) Current updates on mycobacterial taxonomy, 2018 to 2019. J Clin Microbiol 59(7):e0152820

Barratt J, Chan D, Sandaradura I, Malik R, Spielman D, Lee R, Marriott D, Harkness J, Ellis J, Stark D (2016) *Angiostrongylus cantonensis*: a review of its distribution, molecular biology and clinical significance as a human pathogen. Parasitology 143(9):1087

Benejat L, Gravet A, Sifré E, Ben Amor S, Quintard B, Mégraud F, Lehours P (2014) Characterization of a *Campylobacter fetus*-like strain isolated from the faeces of a sick leopard tortoise (*Stigmochelys pardalis*) using matrix-assisted laser desorption/ionization time of flight as an alternative to bacterial 16S rDNA phylogeny. Lett Appl Microbiol 58(4):338–343

Bertrand S, Rimhanen-Finne R, Weill FX, Rabsch W, Thornton L, Perevoscikovs J, van Pelt W, Heck M (2008) *Salmonella* infections associated with reptiles: the current situation in Europe. Euro Surveill 13(24):18902

Bingham AM, Graham SP, Burkett-Cadena ND, White GS, Hassan HK, Unnasch TR (2012) Detection of eastern equine encephalomyelitis virus RNA in North American snakes. Am J Trop Med Hyg 87(6):1140–1144

Briggs-Gonzalez VS, Basille M, Cherkiss MS, Mazzotti FJ (2021) American crocodiles (*Crocodylus acutus*) as restoration bioindicators in the Florida Everglades. PLoS One 16(5): e0250510

Brookins MD, Wellehan JF, Roberts JF, Allison K, Curran SS, Childress AL, Greiner EC (2009) Massive visceral pentastomiasis caused by *Porocephalus crotali* in a dog. Vet Pathol 46(3):460–463

Bosco-Lauth AM, Hartwig AE, Bowen RA (2018) Reptiles and amphibians as potential reservoir hosts of Chikungunya virus. Am J Trop Med Hyg 98(3):841–844
Bueno MG, Martinez N, Abdalla L, Duarte Dos Santos CN, Chame M (2016) Animals in the Zika virus life cycle: what to expect from Megadiverse Latin American Countries. PLoS Negl Trop Dis 10(12):e0005073
Burkett-Cadena ND, Graham SP, Hassan HK, Guyer C, Eubanks MD, Katholi CR, Unnasch TR (2008) Blood feeding patterns of potential arbovirus vectors of the genus *Culex* targeting ectothermic hosts. Am J Trop Med Hyg 79(5):809–815
Burns-Cox CJ, Prathap K, Clark E, Gillman R (1969) Porocephaliasis in Western Malaysia. Trans R Soc Trop Med Hyg 63(3):409–411
CDC (Centers for Disease Control and Prevention) (1992a) Iguana-associated salmonellosis – Indiana, 1990. Morb Mortal Wkly Rep 4:38–39
CDC (Centers for Disease Control and Prevention) (1992b) Lizard-associated salmonellosis – Utah. Morb Mortal Wkly Rep 41:610–611
CDC (Centers for Disease Control and Prevention) (1999) Reptile-associated salmonellosis – selected states, 1996–1998. Morb Mortal Wkly Rep 48:1009–1013
Chen SH, Liu Q, Zhang YN, Chen JX, Li H, Chen Y, Steinmann P, Zhou XN (2010) Multi-host model-based identification of *Armillifer agkistrodontis* (Pentastomida), a new zoonotic parasite from China. PLoS Negl Trop Dis 4(4):e647
Chen H, Li J, Zhang J, Guo X, Liu J, He J, Song Q, Zhang J, Chen M, Zheng Z, Chen D, Chen J (2019) Multi-locus characterization and phylogenetic inference of *Leishmania* spp. in snakes from Northwest China. PLoS One 14(4):e0210681
Chiodini RJ, Sundberg JP (1981) Salmonellosis in reptiles: a review. Am J Epidemiol 113:494–499
Choi HS, Shin SU, Bae EH, Ma SK, Kim SW (2016) Infectious spondylitis in a patient with chronic kidney disease: identification of *Campylobacter fetus* Subsp. testudinum by 16S ribosomal RNA sequencing. Jpn J Infect Dis 69(6):517–519
Collado L, Figueras MJ (2011) Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. Clin Microbiol Rev 24(1):174–192
Colston TJ (2017) Gut microbiome transmission in lizards. Mol Ecol 26(4):972–974. https://doi.org/10.1111/mec.13987
Cortéz-Gómez AMM, Ruiz-Agudelo CA, Valencia-Aguilar A, Ladle RJ (2015) Ecological functions of neotropical amphibians and reptiles: a review. Univ Sci 20(2):229–245
Costa MKBD, Alves RRDN, Navoni JA, Freire EMX (2021) Ethnozoology of snakebite victims in a risk area in Northeast Brazil. Toxicon 201:155–163
Coughlan S, Mulhair P, Sanders M, Schonian G, Cotton JA, Downing T (2017) The genome of *Leishmania adleri* from a mammalian host highlights chromosome fission in *Sauroleishmania*. Sci Rep 7:43747
Corrente M, Madio A, Friedrich KG, Greco G, Desario C, Tagliabue S, D'Incau M, Campolo M, Buonavoglia C (2004) Isolation of *Salmonella* strains from reptile faeces and comparison of different culture media. J Appl Microbiol 96(4):709–715
Corrente M, Totaro M, Martella V, Campolo M, Lorusso A, Ricci M, Buonavoglia C (2006) Reptile-associated salmonellosis in man, Italy. Emerg Infect Dis 12(2):358–359
Corrente M, Sangiorgio G, Grandolfo E, Bodnar L, Catella C, Trotta A, Martella V, Buonavoglia D (2017) Risk for zoonotic *Salmonella* transmission from pet reptiles: a survey on knowledge, attitudes and practices of reptile-owners related to reptile husbandry. Prev Vet Med 146:73–78
Crump M (2021) Eye of Newt and Toe of Frog, Adder's Fork and Lizard's Leg. University of Chicago Press
De Luca C, Iraola G, Apostolakos I, Boetto E, Piccirillo A (2020) Occurrence and diversity of *Campylobacter* species in captive chelonians. Vet Microbiol 241:108567
De Jong B, Andersson Y, Ekdahl K (2005) Effect of regulation and education on reptile-associated salmonellosis. Emerg Infect Dis 11:398–403
Denegri GM (1993) The complete biological cycle of *Diphyllobothrium erinaceieuropaei* (Cestoidea, Pseudophyllidea) under experimental conditions. Helminthologia 30:177–179

Dollfus RP, Canet J (1954) Sur un pentastomide: *Raillietiella* (*Heymonsia*) *hemidactyli* M. L. Hett 1934; supposé susceptible de parasiter l'homme consécutivement à l'ingestion thérapeutique de lézards vivants. Bulletin de la Société de pathologie exotique et de ses filiales 47(3):401–408

Ebani VV, Fratini F, Bertelloni F, Cerri D, Tortoli E (2012) Isolation and identification of mycobacteria from captive reptiles. Res Vet Sci 93(3):1136–1138

Ebani VV (2017) Domestic reptiles as source of zoonotic bacteria: A mini review. Asian Pac J Trop Med 10(8):723–728

Ette H, Fanton L, Adou Bryn KD, Botti K, Koffi K, Malicier D (2003) Human pentastomiasis discovered postmortem. Forensic Sci Int 137(1):52–54

European Food Safety Authority (EFSA) and European Center for Disease Control (ECDC) (2018) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J 16(12):5500

Fitzgerald C, Tu ZC, Patrick M, Stiles T, Lawson AJ, Santovenia M, Gilbert MJ, van Bergen M, Joyce K, Pruckler J, Stroika S, Duim B, Miller WG, Loparev V, Sinnige JC, Fields PI, Tauxe RV, Blaser MJ, Wagenaar JA (2014) *Campylobacter fetus* subsp. *testudinum* subsp. Nov. Isolated from humans and reptiles. Int J Syst Evol Microbiol 64:2944–2948

Fagre AC, Pabilonia KL, Johnston MS, Morley PS, Burgess BA (2020) Comparison of detection methods for *Salmonella enterica* shedding among reptilian patients at a veterinary teaching hospital. J Vet Diagn Investig 32(1):118–123

Friedman CR, Torigian C, Shillam PJ, Hoffman RE, Heltzel D, Beebe JL, Malcolm G, De Witt WE (1998) An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. J Pediatr 132:802–807

Gautam A, Kaphle K, Shrestha B, Phuyal S (2020) Susceptibility to SARS, MERS, and COVID-19 from animal health perspective. Open Vet J 10(2):164–177

Gilbert MJ, Kik M, Timmerman AJ, Severs TT, Kusters JG, Duim B, Wagenaar JA (2014) Occurrence, diversity, and host association of intestinal *Campylobacter*, *Arcobacter*, and *Helicobacter* in reptiles. PLoS One 9(7):e101599

Gilbert MJ, Duim B, Zomer AL, Wagenaar JA (2019) Living in cold blood: *Arcobacter, Campylobacter*, and *Helicobacter* in reptiles. Front Microbiol 10:1086

Gomez-Puerta LA, Baselly L, Lopez-Urbina MT, Gonzalez AE, Mayor P (2020) Visceral infection by *Porocephalus* spp. (Pentastomida) in Neotropical wild mammals. J Helminthol 94:e141

Goławska O, Zajac M, Maluta A, Pristas P, Hamarová L, Wasyl D (2019) Complex bacterial flora of imported pet tortoises deceased during quarantine: another zoonotic threat? Comp Immunol Microbiol Infect Dis 65:154–159

Gouda AS, Mégarbane B (2021) Snake venom-derived bradykinin-potentiating peptides: a promising therapy for COVID-19? Drug Dev Res 82(1):38–48

Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA (2017) Snakebite envenoming. Nat Rev Dis Primers 3:17063

Greiner EC (2003) Coccidiosis in reptiles. In: Seminars in avian and exotic pet medicine, vol 12, No. 1. WB Saunders, pp 49–56

Iatta R, Mendoza-Roldan JA, Latrofa MS, Cascio A, Brianti E, Pombi M, Gabrielli S, Otranto D (2021) *Leishmania tarentolae* and *Leishmania infantum* in humans, dogs and cats in the Pelagie archipelago, southern Italy. PLoS Negl Trop Dis 15(9):e0009817

Ji W, Wang W, Zhao X, Zai J, Li X (2020) Cross-species transmission of the newly identified coronavirus 2019-nCoV. J Med Virol 92(4):433–440

Johny J, Kumar A, Kolar VV, Hamide A (2018) Eosinophilic meningitis caused by consumption of meat of monitor lizard (*Varanus bengalensis*). Neurol India 66(4):1166

Kalita B, Saviola AJ, Samuel SP, Mukherjee AK (2021) State-of-the-art review – a review on snake venom-derived antithrombotics: potential therapeutics for COVID-19-associated thrombosis? Int J Biol Macromol 192:1040–1057

Kar S, Rodriguez SE, Akyildiz G, Cajimat MNB, Bircan R, Mears MC, Bente DA, Keles AG (2020) Crimean-Congo hemorrhagic fever virus in tortoises and Hyalomma aegyptium ticks in East Thrace Turkey: potential of a cryptic transmission cycle. Parasit Vectors 13(1):201. https://doi.org/10.1186/s13071-020-04074-6

Kelehear C, Spratt DM, Dubey S, Brown GP, Shine R, Salamin N (2011) Using combined morphological allometric and molecular approaches to identify species of the genus *Raillietiella* (Pentastomida). PLoS ONE 6(9):e24936. https://doi.org/10.1371/journal.pone.0024936

Khamboonruang C (1991) The present status of trichinellosis in Thailand, Southeast Asian. J Trop Med Public Health 22 Suppl:312–315

Klatt S, Simpson L, Maslov DA, Konthur Z (2019) *Leishmania tarentolae*: taxonomic classification and its application as a promising biotechnological expression host. PLoS Negl Trop Dis 13(7): e0007424

Klemens MW, Thorbjarnarson JB (1995) Reptiles as a food resource. Biodivers Conserv 4(3):281–298

Kohl KD, Brun A, Magallanes M, Brinkerhoff J, Laspiur A, Acosta JC, Caviedes-Vidal E, Bordenstein SR (2017) Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. Mol Ecol 26(4):1175–1189

Kuo MM, Lane RS, Giclas PC (2000) A comparative study of mammalian and reptilian alternative pathway of complement-mediated killing of the Lyme disease spirochete (*Borrelia burgdorferi*). J Parasitol 86(6):1223–1228

Le Souëf AT, Barry M, Brunton DH, Jakob-Hoff R, Jackson B (2015) Ovariectomy as treatment for ovarian bacterial granulomas in a Duvaucel's gecko (*Hoplodactylus duvaucelii*). N Z Vet J 63 (6):340–344

Lepetz V, Massot M, Chaine AS, Clobert J (2009) Climate warming and the evolution of morphotypes in a reptile. Glob Change Biol 15(2):454–466

Liu LN, Zhang X, Jiang P, Liu RD, Zhou J, He RZ, Cui J, Wang ZQ (2015) Serodiagnosis of sparganosis by ELISA using recombinant cysteine protease *of Spirometra erinaceieuropaei* spargana. Parasitol Res 114(2):753–757

Majláthová V, Majláth I, Derdáková M, Víchová B, Pet'ko B (2006) *Borrelia lusitaniae* and green lizards (*Lacerta viridis*), Karst Region, Slovakia. Emerg Infect Dis 12(12):1895–1901

Majláthová V, Majláth I, Hromada M, Tryjanowski P, Bona M, Antczak M, Peťko B (2008) The role of the sand lizard (*Lacerta agilis*) in the transmission cycle of *Borrelia burgdorferi* sensu lato. Int J Med Microbiol 298:161–167

Magnino S, Colin P, Dei-Cas E, Madsen M, McLauchlin J, Nöckler K, Maradona MP, Tsigarida E, Vanopdenbosch E, Van Peteghem C (2009) Biological risks associated with consumption of reptile products. Int J Food Microbiol 134(3):163–175

Marschang RE (2011) Viruses infecting reptiles. Viruses 3(11):2087–2126

Mendoza-Roldan JA, Colella V, Lia RP, Nguyen VL, Barros-Battesti DM, Iatta R, Dantas-Torres F, Otranto D (2019) *Borrelia burgdorferi* (sensu lato) in ectoparasites and reptiles in southern Italy. Parasit Vectors 12(1):35

Mendoza-Roldan JA, Modry D, Otranto D (2020) Zoonotic parasites of reptiles: a crawling threat. Trends Parasitol 36(8):677–687

Mendoza-Roldan JA, Mendoza-Roldan MA, Otranto D (2021a) Reptile vector-borne diseases of zoonotic concern. Int J Parasitol Parasites Wildl 15:132–142

Mendoza-Roldan JA, Ravindran Santhakumari Manoj R, Latrofa MS, Iatta R, Annoscia G, Lovreglio P, Stufano A, Dantas-Torres F, Davoust B, Laidoudi Y, Mediannikov O, Otranto D (2021b) Role of reptiles and associated arthropods in the epidemiology of rickettsioses: a one health paradigm. PLoS Negl Trop Dis 15(2):e0009090

Mendoza-Roldan JA, Latrofa MS, Iatta R, Manoj RS, Panarese R, Annoscia G, Pombi M, Zatelli A, Beugnet F, Otranto D (2021c) Detection of *Leishmania tarentolae* in lizards, sand flies and dogs in southern Italy, where *Leishmania infantum* is endemic: hindrances and opportunities. Parasit Vectors 14(1):461

Mendoza-Roldan JA, Lia RP, Tarallo VD, Annoscia G, Bezerra-Santos MA, Otranto D (2022a) *Raillietiella hemidactyli* (Pentastomida: Raillietiellidae) in *Tarentola mauritanica* geckoes: a new zoonotic parasite for Europe. Acta Trop 228:106316

Mendoza-Roldan JA, Latrofa MS, Tarallo VD, Manoj RRS, Bezerra-Santos MA, Annoscia G, Iatta R, Otranto D (2022b) Transbound Emerg Dis 69(5):2856–2866. https://doi.org/10.1111/tbed.14438

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, J, Koehler J, Marcus R, and Angulo FJ. (2004) Reptiles, amphibians and human *Salmonella* infection: a population-based, case-control study. Clin Infect Dis 38:S253–S261

Meyer Sauteur PM, Relly C, Hug M, Wittenbrink MM, Berger C (2013) Risk factors for invasive reptile-associated salmonellosis in children. Vector Borne Zoonotic Dis 13(6):419–421

Mitchell MA, Shane SM (2000) Preliminary findings of *Salmonella* spp. in captive green iguanas (*Iguana iguana*) and their environment. Prev Vet Med 45(3–4):297–304

Mitchell MA (2011) Zoonotic diseases associated with reptiles and amphibians: an update. Vet Clin North Am Exot Anim Pract 14(3):439–456, v–vi

Mohabey DM, Samant B (2019) Cretaceous-Paleogene transition of reptilian tetrapods across Deccan volcanism in India. Open J Geol 9(10):–639

Murphy D, Oshin F (2015) Reptile-associated salmonellosis in children aged under 5 years in South West *England*. Arch Dis Child 100(4):364–365

Nieto NC, Foley JE, Bettaso J, Lane RS (2009) Reptile infection with *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. J Parasitol 95(5):1165–1170

Njagu Z, Mihok S, Kokwaro E, Verloo D (1999) Isolation of *Trypanosoma brucei* from the monitor lizard (*Varanus niloticus*) in an endemic focus of Rhodesian sleeping sickness in Kenya. Acta Trop 72(2):137–148

Novakova M, Literak I, Chevez L, Martins TF, Ogrzewalska M, Labruna MB (2015) Rickettsial infections in ticks from reptiles, birds and humans in Honduras. Ticks Tick Borne Dis 6(6):737–742

Novo S, Leles D, Bianucci R, Araujo A (2015) *Leishmania tarentolae* molecular signatures in a 300 hundred-years-old human Brazilian mummy. Parasit Vectors 8(1):72. https://doi.org/10.1186/s13071-015-0666-z

Oya A, Doi R, Shirasaka A, Yabe S, Sasa M (1983) Studies on Japanese encephalitis virus infection of reptiles. I. Experimental infection of snakes and lizards. Jpn J Exp Med 53(2):117–123

Patrick ME, Gilbert MJ, Blaser MJ, Tauxe RV, Wagenaar JA, Fitzgerald C (2013) Human infections with new subspecies of *Campylobacter fetus*. Emerg Infect Dis 19:1678–1680

Parameswaran K (2006) Case series of eosinophilic meningoencephalitis from South India. Ann Indian Acad Neurol 9(4):217

Pees M, Rabsch W, Plenz B, Fruth A, Prager R, Simon S, Schmidt V, Munch S, Braun P (2013) Evidence for the transmission of *Salmonella* from reptiles to children in Germany, July 2010 to October 2011. Euro Surveill 18(46):20634

Piccirillo A, Niero G, Calleros L, Pérez R, Naya H, Iraola G (2016) *Campylobacter geochelonis* sp. nov. isolated from the western Hermann's tortoise (*Testudo hermanni hermanni*). Int J Syst Evol Microbiol 66(9):3468–3476

Pignato S, Giammanco G, Santangelo C, Giammanco GM (1998) Endemic presence of *Salmonella bongori* 48:z35:- causing enteritis in children in Sicily. Res Microbiol 149(6):429–431

Pincheira-Donoso D, Bauer AM, Meiri S, Uetz P (2013) Global taxonomic diversity of living reptiles. PLoS One 8(3):e59741

Poinar G Jr, Poinar R (2004) Evidence of vector-borne disease of Early Cretaceous reptiles. Vector Borne Zoonotic Dis 4(4):281–284

Pombi M, Giacomi A, Barlozzari G, Mendoza-Roldan J, Macrì G, Otranto D, Gabrielli S (2020) Molecular detection of *Leishmania* (*Sauroleishmania*) *tarentolae* in human blood and *Leishmania* (*Leishmania*) *infantum* in *Sergentomyia minuta*: unexpected host-parasite contacts. Med Vet Entomol 34(4):470–475

Pozio E, Owen IL, Marucci G, La Rosa G (2004) *Trichinella papuae* in saltwater crocodiles (*Crocodylus porosus*) of Papua New Guinea. Emerg Infect Dis 10(8):1507–1509

Presti A, Aguirre DT, De Andrés P, Daoud L, Fortes J, Muñiz J (2015) Cerebral sparganosis: case report and review of the European cases. Acta Neurochir 157(8):1339–1343

Reeves LE, Burkett-Cadena ND (2022) Lizards are important hosts for Zoonotic Flavivirus Vectors, Subgenus *Culex*, in the Southern USA. Front Trop Dis:4

Ricard C, Mellentin J, Ben Abdallah Chabchoub R, Kingbede P, Heuclin T, Ramdame A, Bouquet A, Couttenier FS, Hendricx S (2013) Méningite à *Salmonelle* chez un nourrisson due à une tortue domestique. Arch Pediatr 22:605–607

Roll U, Feldman A, Novosolov M, Allison A, Bauer AM, Bernard R, Böhm M, Castro-Herrera F, Chirio L, Collen B, Colli GR, Dabool L, Das I, Doan TM, Grismer LL, Hoogmoed M, Itescu Y, Kraus F, LeBreton M, Lewin A, Martins M, Maza E, Meirte D, Nagy ZT, de C Nogueira C, Pauwels OSG, Pincheira-Donoso D, Powney GD, Sindaco R, Tallowin OJS, Torres-Carvajal O, Trape JF, Vidan E, Uetz P, Wagner P, Wang Y, Orme CDL, Grenyer R, Meiri S (2017) The global distribution of tetrapods reveals a need for targeted reptile conservation. Nat Ecol Evol 1(11): 1677–1682

Ruiz de Castañeda R, Bolon I, Gutiérrez JM (2021) A transdisciplinary approach to snakebite envenoming. Toxicon X 13:100088

Rukambile E, Sintchenko V, Muscatello G, Kock R, Alders R (2019) Infection, colonization and shedding of *Campylobacter* and *Salmonella* in animals and their contribution to human disease: a review. Zoonoses Public Health 66(6):562–578

Rymaszewska A, Grenda S (2008) Bacteria of the genus *Anaplasma*–characteristics of *Anaplasma* and their vectors: a review. Vet Med 53(11):573–584

Sánchez-Montes S, Isaak-Delgado AB, Guzmán-Cornejo C, Rendón-Franco E, Muñoz-García CI, Bermúdez S, Morales-Diaz J, Cruz-Romero A, Romero-Salas D, Dzul-Rosado K, Lugo-Caballero C, Colunga-Salas P, Becker I (2019) *Rickettsia* species in ticks that parasitize amphibians and reptiles: novel report from Mexico and review of the worldwide record. Ticks Tick Borne Dis 10(5):987–994

Schröter M, Speicher A, Hofmann J, Roggentin P (2006) Analysis of the transmission of *Salmonella* spp. through generations of pet snakes. Environ Microbiol 8:556–559

Seepersadsingh N, Adesiyun AA (2003) Prevalence and antimicrobial resistance of *Salmonella* spp. in pet mammals, reptiles, fish aquarium water, and birds in Trinidad. J Vet Med Ser B Infect Dis Vet Public Health 50:488–493

Sodagari HR, Habib I, Shahabi MP, Dybing NA, Wang P, Bruce M (2020) A review of the public health challenges of *Salmonella* and turtles. Vet Sci 7(2):56

Soldati G, Lu ZH, Vaughan L, Polkinghorne A, Zimmermann DR, Huder JB, Pospischil A (2004) Detection of mycobacteria and chlamydiae in granulomatous inflammation of reptiles: a retrospective study. Vet Pathol 41:388–397

Stacy BA, Wellehan JF Jr (2010) Fatal septicemia caused by *Helicobacter* infection in a pancake tortoise (Mala*cochersus tornieri*). J Vet Diagn Investig 22:660–662

Steele P, Allen C (eds) (2004) Handbook of Inca mythology. ABC-CLIO

Stenos J, Graves S, Popov VL, Walker DH (2003) *Aponomma hydrosauri*, the reptile-associated tick reservoir of *Rickettsia honei* on Flinders Island, Australia. Am J Trop Med Hyg 69(3):314–317

Tappe D, Meyer M, Oesterlein A, Jaye A, Frosch M, Schoen C, Pantchev N (2011) Transmission of *Armillifer armillatus* ova at snake farm, The Gambia, West Africa. Emerg Infect Dis 17(2):251–254

Tappe D, Dijkmans AC, Brienen EA, Dijkmans BA, Ruhe IM, Netten MC, van Lieshout L (2014) Imported *Armillifer pentastomiasis*: report of a symptomatic infection in The Netherlands and mini-review. Travel Med Infect Dis 12(2):129–133

Tappe D, Sulyok M, Riu T, Rózsa L, Bodó I, Schoen C, Muntau B, Babocsay G, Hardi R (2016) Co-infections in visceral pentastomiasis, democratic republic of the Congo. Emerg Infect Dis 22 (8):1333–1339

Ticha L, Kykalova B, Sadlova J, Gramiccia M, Gradoni L, Volf P (2021) Development of various *Leishmania* (*Sauroleishmania*) *tarentolae* strains in three *Phlebotomus* species. Microorganisms 9(11):2256

Tijsse-Klasen E, Fonville M, Reimerink JH, Spitzen-van der Sluijs A, Sprong H (2010) Role of sand lizards in the ecology of Lyme and other tick-borne diseases in the Netherlands. Parasit Vectors 3:42

Valencia-Aguilar A, Cortés-Gómez AM, Ruiz-Agudelo CA (2013) Ecosystem services provided by amphibians and reptiles in Neotropical ecosystems. Int J Biodivers Sci Ecosyst Serv Manag 9 (3):257–272

Valentine KH, Harms CA, Cadenas MB, Birkenheuer AJ, Marr HS, Braun-McNeill J, Maggi RG, Breitschwerdt EB (2007) *Bartonella* DNA in loggerhead sea turtles. Emerg Infect Dis 13(6):949–950. https://doi.org/10.3201/eid1306.061551

Waiswa C, Picozzi K, Katunguka-Rwakishaya E, Olaho-Mukani W, Musoke RA, Welburn SC (2006) *Glossina fuscipes fuscipes* in the trypanosomiasis endemic areas of south eastern Uganda: apparent density, trypanosome infection rates and host feeding preferences. Acta Trop 99(1):23–29

Walden HDS, Iredale ME, Childress A, Wellehan JFX Jr, Ossiboff RJ (2020) Case Report: Invasive Pentastomes, *Raillietiella orientalis* (Sambon, 1922), in a Free-Ranging Banded Water Snake (*Nerodia fasciata*) in North Central Florida, USA. Front Vet Sci 7:467

Wang CM, Shia WY, Jhou YJ, Shyu CL (2013) Occurrence and molecular characterization of reptilian *Campylobacter fetus* strains isolated in Taiwan. Vet Microbiol 164:67–76

Whiley H, Custance G, Graves S, Stenos J, Taylor M, Ross K, Gardner M (2016) *Rickettsia* Detected in the Reptile Tick Bothriocroton hydrosauri from the Lizard *Tiliqua rugosa* in South Australia. Pathogens 5(2):41. https://doi.org/10.3390/pathogens5020041

Willis C, Wilson T, Greenwood M, Ward L (2002) Pet reptiles associated with a case of salmonellosis in an infant were carrying multiple strains of *Salmonella*. J Clin Microbiol 40(12):4802–4803

Wilkstrom VO, Fernstrom LL, Melin L, Boqvist S (2014) *Salmonella* isolated from individual reptiles and environmental samples from terraria in private households in Sweden. Acta Vet Scand 56:7

Winfield MD, Groisman EA (2003) Role of Nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl Environ Microbiol 69:3687–3694

Wei Z, Xu X, Yan M, Chang H, Li Y, Kan B, Zeng M (2019) *Salmonella* Typhimurium and *Salmonella enteritidis* infections in sporadic diarrhea in children: source tracing and resistance to third-generation cephalosporins and ciprofloxacin. Foodborne Pathog Dis 16(4):244–255

Woo P, Soltys MA (1969) The experimental infection of reptiles with *Trypanosoma brucei*. Ann Trop Med Parasitol 63(1):35–38

Woodward DL, Khakhria R, Johnson WM (1997) Human salmonellosis associated with exotic pets. J Clin Microbiol 35:2786–2790

Schwarze-Zander C, Becker S, Wenzel J, Rockstroh JK, Spengler U, Yassin AF (2010) Bacteremia caused by a novel *Helicobacter* species in a 28-year-old man with X-linked agammaglobulinemia. J Clin Microbiol 48:4672–4676

Zhang JR, Guo XG, Chen H, Liu JL, Gong X, Chen DL, Chen JP (2019) Pathogenic *Leishmania* spp. detected in lizards from Northwest China using molecular methods. BMC Vet Res 15(1):446

Wild Birds and Zoonotic Pathogens

33

Beware of the Fowl Feces

Nadine A. Vogt

Contents

Abstract

Wild birds are an extraordinarily diverse group of species that occupy a variety of ecological niches. Concerns about the ability of birds to transmit pathogenic organisms have arisen, mainly due to their ability to fly and travel long distances, as well as their tendency to gather and travel in flocks. This chapter will address the most relevant zoonotic agents and related public health risks associated with wild birds, including *Salmonella*, *Campylobacter*, *Chlamydophila psittaci*, and *Escherichia coli*, as well as agents for which wild birds may act as a reservoir species (e.g., West Nile virus), and zoonoses that may be acquired from direct exposure to or handling of wild birds (e.g., mites, ticks). Bacterial, viral, fungal, and parasitic zoonoses with a minor, theoretical, or potential emerging risk of infection will also be briefly discussed (e.g., avian influenza).

N. A. Vogt (✉)
Department of Population Medicine, Ontario Veterinary College, Guelph, ON, Canada

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_47

Keywords

Wild birds · Avifauna · Avian · Wildlife · Zoonoses · Reservoir · Parasites · Foodborne bacteria · Public health · Epidemiology · Whole-genome sequencing · Molecular typing · *Salmonella* · *Campylobacter* · *Escherichia coli* · *Chlamydophila psittaci* · West Nile virus · Avian influenza

33.1 Introduction

An estimated 10,000 different species of birds inhabit the earth (Li and Jiang 2014). With the sheer number and diversity of species that exist, wild birds have adapted to a vast number of ecological niches, and at least one species from the avian kingdom can be found on every continent. The diversity of biological adaptations exhibited by wild birds is impressive, with specializations in vision, smell, speed, and, most importantly, flight (Caspermeyer 2016; Laguë 2017; Ponitz et al. 2014; Potier et al. 2020). Birds (even flightless ones) have hollow bones, feathers, and a unique respiratory system without dead space that allows them to take in oxygen during inspiration as well as expiration. These, along with other physical adaptations, allow birds to migrate exceedingly long distances (Hedenström 2008). In fact, the artic tern (*Sterna paradisaea*) performs a record total migration of approximately 50,000 km every single year, from the Artic to the Antarctic pole (Alerstam et al. 2019). Birds-of-paradise are exotic and colorful birds with incredible displays of courtship that are found in New Guinea and its surrounding islands, and are yet another sight to behold (Irestedt et al. 2009). Members of the Psittacidae family (parrots) also exhibit marked diversity in their coloration and appearance, and certain species have the unique ability to learn and mimic human language, to communicate with us on our own terms (Colbert-White et al. 2011). Birds of prey, or raptors, have particularly specialized adaptations for hunting, with exceptional vision two to five times better than humans (Guzman-Pando and Chacon-Murguia 2021), allowing them to maintain a visual target in a diving stoop at speeds the fastest of any living creature on earth, achieving over 300 km/h for the peregrine falcon (*Falco peregrinus*) (Ponitz et al. 2014; Tucker 1998). Another raptor, the vulture, performs Mother Nature's garbage disposal and is renowned for its ability to consume rotting flesh without any adverse effects and, interestingly, can also safely consume animals that have succumbed to anthrax, botulism, and other infectious diseases (Chung et al. 2015; Zepeda Mendoza et al. 2018).

33.2 Wild Birds in Human History

In addition to their roles in the ecosystem as pollinators, food sources, and agents of seed dispersal and pest control, birds have long been valued in practical, cultural, and religious contexts across the world, throughout human history. Nearly every country has its own recognized national bird, many of these chosen carefully for their

symbolic value. Owls are widely recognized as a symbol of wisdom and knowledge, eagles symbolize royalty and power, and the peacock is used as a symbol of protection against evil spirits in Indigenous cultures. The dove is the ultimate symbol of peace, innocence, and purity. Birds have also appeared in classic folklore and mythology to convey important cultural messages, such as that of renewal and rebirth, as with the immortal phoenix which cyclically dies in a burst of flames and regenerates from its own ashes. Birds have also served practical purposes in human culture, as with pigeon post, the ancient art of falconry, the ancient Japanese tradition of cormorant fishing (i.e., birds trained to retrieve fish) (Kurihara et al. 2020), and the infamous honeyguides, which are a species of African bird that are purportedly self-trained to guide humans to wild beehives (Spottiswoode et al. 2016). Wild birds have been hunted by humans, both for sport and as a source of food, and, in some cases, they have been overhunted and are now gone forever, as with the passenger pigeon (*Ectopistes migratorius*) (Horns and Şekercioğlu 2018). For certain cultures, the use of falconry birds to hunt other animals is a 2000-year-old tradition; the Mongolian rite of passage for young boys involves using golden eagles (*Aquila chrysaetos*) on horseback to take down and capture other apex predators such as foxes and wolves (Bolat 2016). Finally, wild birds are relied upon for more somber purposes in Buddhist sky burial practices in China, Tibet, Mongolia, Bhutan, and surrounding regions, in which the bodies of the deceased are prepared for consumption by vultures; this practice has deep spiritual meaning and significance, but also averts the practical challenges of performing burials or cremations in high-altitude areas which are above the tree line and have permafrost (Lu et al. 2009).

33.3 Public Health Impacts of Wild Birds

Wild birds are widely regarded as sentinels of environmental pollution, since they are often the first class of animal species to show signs of illness from toxins (e.g., DDT or dichloro-diphenyl-trichloroethane, Teflon™), hence the expression, "canary in the coal mine" (Espín and Sánchez-Virosta 2021; Golden and Rattner 2003). Conversely, there is an emerging body of evidence that suggests wild birds may be an important source of pathogenic organisms that cause illness in people (Bengis et al. 2004; Hamer et al. 2012; Sauvala et al. 2021). Over 50 different bacterial pathogens have been isolated from a diversity of wild bird species, including *Salmonella*, *Campylobacter*, *Chlamydophila psittaci*, *Clostridioides difficile* (formerly *Clostridium difficile*), and *Pseudomonas* (Chung et al. 2018; Greig et al. 2015; Rodrigues et al. 2021; Sevilla et al. 2020; Stokes et al. 2021). In some cases, it remains unclear whether wild birds are acting primarily as sentinels of environmental contamination with these pathogenic organisms, or if birds play a more substantial role in the transmission and dissemination of these agents within the environment (Smith et al. 2020). Challenges with sampling of wild birds further complicate the study of potential zoonotic risks posed by birds; most research sampling of wild birds (and indeed, other wildlife as well) is opportunistic, often resulting in limited sample sizes with limited generalizability to the general

population of wild birds being sampled (Vogt et al. 2020; Wobeser 2007). It appears, however, that the prevalence of a given pathogen in a certain bird species can vary greatly depending on the geographic region and season of sampling, as well as access to features of the local environment (e.g., sewage, landfills) (Broman et al. 2002; Chung et al. 2018; Girdwood et al. 1985; Hald et al. 2016; Ito et al. 1988; Kapperud and Rosef 1983; Kirk et al. 2002; Vogt et al. 2019).

In certain cases, identical strains of pathogenic organisms identified in human outbreak cases and in wild birds were also epidemiologically linked (Alley et al. 2002; Collins et al. 2019; Kwan et al. 2014). With the advent of newer, high-resolution typing methods using genomic data, the presence of identical strains in wild birds and humans is consistent with transmission events (Collins et al. 2019; Ford et al. 2019; Söderlund et al. 2019), but, overall, the evidence for a causal relationship for many pathogens remains circumstantial, as the direction of potential transmission between wild birds and humans is difficult to ascertain (Tsiodras et al. 2008). Nonetheless, the increasing burden of human illness due to enteropathogenic organisms of unidentified origin (e.g., *Salmonella*, *Campylobacter*) has prompted researchers to increasingly examine wild birds as a potential source of these particular zoonoses (Fonseca et al. 2020; Lynch et al. 2009). For other zoonoses, such as Lyme disease (caused by the *Borrelia burgdorferi* sensu *lato* species complex) or West Nile virus, wild birds represent a known reservoir species and may thus pose an indirect risk to human health in the presence of competent vector species (Chancey et al. 2015; Newman et al. 2015). Most people rarely come into direct contact with wild birds or wild bird specimens (except for those involved in bird-banding activities or wildlife rehabilitation); thus, it believed that the risk of transmission of most zoonotic diseases from birds to humans is primarily through indirect transmission, via exposure to contaminated, shared environments (Tsiodras et al. 2008). Fungal and bacterial diseases such as histoplasmosis and "parrot fever" (caused by *Chlamydophila psittaci*) may be acquired from wild birds through inhalation of aerosolized particles (Andersen and Vanrompay 2000; Deepe Jr. 2018). Zoonotic diseases acquired through direct contact (i.e., ticks, mites, *Erysipelas*) likely only represent a risk to those who regularly handle wild birds (Tsiodras et al. 2008). The many theoretical environmental pathways of transmission are complex and the major known and suspected routes are illustrated in Fig. 1. The gregarious nature and ability of certain bird species to produce copious amounts of fecal material, as starlings (*Sturnus vulgaris*) do, may contribute to environmental contamination with pathogens harbored by wild birds, particularly in agricultural contexts (Clark 2014). In these types of contexts, wild birds may directly contribute to the burden of infection/colonization of pathogens among livestock species (e.g., cattle, poultry), with potential downstream implications for human health (Cabe 2021; Clark 2014; Hald et al. 2016). In general, the species of wild birds most likely to contribute to environmental contamination are those which are often found in large numbers in urban areas shared by humans and wildlife, especially aquatic environments (Clark 2014; Minette 1986). As such, waterfowl, pigeons, and certain passerine (i.e., songbird) species have been the focus of much research investigating zoonotic disease risks associated with wild birds (Tsiodras et al. 2008).

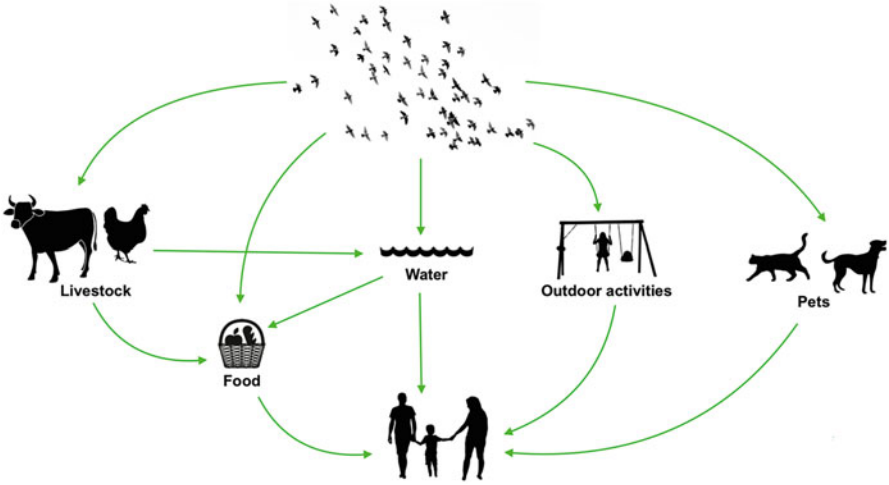

Fig. 1 Major known and suspected pathways for indirect feco-oral transmission of major bacterial pathogens (*Salmonella*, *Campylobacter*, *Escherichia coli*) from wild birds to humans

The following section will focus on zoonoses believed to have a major impact on human health (i.e., *Salmonella*, *Campylobacter*, *Escherichia coli*, *Chlamydophila psittaci*), for which wild birds are known reservoirs (i.e., West Nile virus, *Borrelia* spp.), or which may be acquired through direct contact with wild birds (i.e., mites, ticks). Bacterial, viral, fungal, and parasitic zoonoses with a minor, theoretical, or potential emerging risk of infection will also be briefly discussed (e.g., avian influenza, *Cryptococcus*, *Sarcocystis*). Although wild birds may contribute to the transmission and dissemination of antimicrobial resistance (Greig et al. 2015), this particular topic will not be addressed in this chapter.

33.4 The Usual Suspects

33.4.1 *Salmonella*

33.4.1.1 The Pathogen

Salmonella is a genus of Gram-negative rod-shaped bacteria belonging to the Enterobacteriaceae family of organisms. *Salmonella* are broadly divided into two major groups, referred to as typhoidal *Salmonella* (human-human transmission only) and non-typhoidal *Salmonella* (animal-human transmission). Over 2500 serovars of non-typhoidal *Salmonella* have been identified, which are determined based on the presence of H and O surface antigens. Certain serovars are host-adapted and rarely cause serious disease in their host species, but are likely to cause clinical illness in another species. Infection with non-typhoidal *Salmonella* typically results in mild gastrointestinal illness, but the immunocompromised, the elderly, and the young are

more likely to suffer serious, and potentially life-threatening, infections. Some individual animals and humans maintain a persistent carrier state and actively shed *Salmonella* in their feces, without suffering from any overt signs of clinical illness.

33.4.1.2 Epidemiology in Birds and Humans

The focus of this section will be on non-typhoidal *Salmonella* (i.e., *Salmonella enterica* subsp. *enterica*), henceforth *Salmonella*, and its relevance to humans and wild birds. *Salmonella* is responsible for a major global burden of illness in humans across the world. A recent global estimate suggests that *Salmonella* infections result in nearly 94 million cases and 155,000 deaths every year (Majowicz et al. 2010). *Salmonella* is considered an endemic disease that is predominantly acquired through the consumption of contaminated food or water, although outbreaks often occur as well. The serovars most commonly implicated in human illness are *S.* Typhimurium and *S.* Enteritidis, but a diversity of serovars have been implicated from a variety of food and animal sources (e.g., *S.* Heidelberg, *S.* Infantis, *S.* Enteritidis). The predominant serovars causing illness in humans vary by country and continent and can change over time. Most recently, *S.* Newport has emerged as a major cause of human illness worldwide (Elbediwi et al. 2020).

It appears that certain strains of *Salmonella* are specifically adapted to wild birds (most commonly *S.* Typhimurium) and rarely affect other animal species or humans. Mass mortality events related to *Salmonella* and wild birds (particularly songbirds, in association with garden bird feeders) have been recorded all over the world, beginning in the 1950s (Mather et al. 2016). The scale of these mortality events can be massive, with one study recording a total of 11,888 individual birds affected (Hall and Saito 2008). The overall burden of these mortality events on bird populations is unknown; although some suggest rapid recovery of populations following such events, there is some evidence that the frequency of mortality events is continuing to increase every year (12% annual increase in the United States over a 20-year period) (Hall and Saito 2008). Mortality events associated with bird feeders typically peak between January and April, reflecting the stress of cold winter months and low food availability, along with high densities of different bird species and poor hygiene at feeders that facilitate feco-oral transmission of *Salmonella*. Most commonly, pine siskins (*Spinus pinus*), American goldfinches (*Spinus tristis*), northern cardinals (*Cardinalis cardinalis*), house sparrows (*Passer domesticus*), and greenfinches (*Chloris chloris*) are affected, but other species from the orders Piciformes (e.g., woodpeckers) and Columbiformes (e.g., pigeons) have also been affected (Hall and Saito 2008). Despite widespread documentation of salmonellosis affecting birds at bird feeding stations, a recent study examining over 200 bird feeders during the winter months in both urban and rural settings in Poland failed to recover any *Salmonella* (Frątczak et al. 2021), highlighting the variability in the geographic occurrence of this organism in wild bird populations. Overall, the prevalence of *Salmonella* among different wild bird species ranging from waterfowl to passerines and gulls is typically less than 5% (Andrés et al. 2013; Dos Santos et al. 2020; Navarro-Gonzalez et al. 2020), but the prevalence is variable and can be as high as 20–60% (Feare et al. 1999; Martín-Maldonado et al. 2019), depending on factors

such as bird species and diet, geographic region, season, and proximity to anthropogenic sources (i.e., landfills, sewage) (Benskin et al. 2009; Vogt et al. 2020). One recent study comparing wild and captive Andean condors (*Vultur gryphus*) in Argentina only recovered *Salmonella* in the captive condors (with a prevalence of 2.8% ($n = 2/71$) and not in wild condors ($n = 0\%$, $n = 0/56$) (Wiemeyer et al. 2021).

Currently available evidence suggests that wild birds represent a reservoir of *Salmonella* for a small proportion of all human *Salmonella* cases (Lawson et al. 2014), with the majority being attributable to livestock sources and food sources such as raw or undercooked eggs (Thorns 2000). One study comparing the molecular epidemiology of wild bird-associated *Salmonella* strains using PFGE (pulsed-field gel electrophoresis) found that human cases with these strains mirrored the seasonal peak and spatial occurrence in wild birds in England between 1993 and 2012 (Lawson et al. 2014). Additional cases of human outbreaks of *Salmonella* linked to wild birds have been documented over the past several decades (Kapperud et al. 1998; Nesse et al. 2005; Penfold et al. 1979; Thornley et al. 2003). More recently, the use of highly discriminatory methods using whole-genome sequencing data and phylogenetics has revealed highly similar or indistinguishable *Salmonella* strains in wild birds and sporadic human cases or outbreaks of disease (Bloomfield et al. 2017; Collins et al. 2019; Ford et al. 2019; Fu et al. 2022; Mather et al. 2016; Söderlund et al. 2019). Case-control studies have also identified contact with wild birds as a potential risk factor for human *Salmonella* cases (Collins et al. 2019; Ford et al. 2019). Together, these findings provide compelling, but not definitive, evidence of wild birds as a potential source of *Salmonella* for humans, since the directionality of potential transmission has not yet been firmly established.

Wild birds are also thought to play a role in the dissemination and introduction of virulent strains of *Salmonella* into new geographic regions, due to their ability to travel and migrate long distances. Another major suspected route of indirect transmission of *Salmonella* from wild birds to humans is the contamination of water by aquatic bird species (Khalefa et al. 2021), and use of water for recreational purposes by humans, or irrigation of crops that are later consumed raw or lightly cooked (Fig. 1). There is evidence from Australia and Sweden that companion animals may also bridge the gap between wild birds and humans, particularly with outdoor cats that hunt weakened, ill songbirds that are infected with *Salmonella*, potentially causing salmonellosis in cats, colloquially known as "songbird fever" (Simpson et al. 2018; Söderlund et al. 2019). Based on an assessment of phage types and serotypes of almost 40,000 *Salmonella* isolates from livestock and nearly 800 wild bird carcasses in Great Britain, there appears to be minimal transmission (<1% overlap) between livestock and wild birds in Great Britain (Pennycott et al. 2006).

33.4.1.3 Disease in Birds

Most birds infected with *Salmonella* are asymptomatic carriers and do not become clinically ill. All bird species have the potential to become infected and clinically ill in the presence of predisposing factors (starvation, cold weather, high population densities), host susceptibility factors (stress, poor immunity, and young birds,

in particular), and *Salmonella* strain virulence. Affected birds may appear blind (due to neurological dysfunction), disoriented, and unable to fly, with ruffled feathers and increased respiratory rates. Birds that are clinically affected typically do not survive.

33.4.1.4 Disease in Humans

Salmonella infection in humans typically results in a mild gastroenteric illness (i.e., vomiting, diarrhea) lasting under 24 hours. Immunocompromised individuals and the young and elderly are particularly vulnerable to *Salmonella* and may suffer more serious consequences of infection, including systemic, multi-organ illness resulting from bloodborne infections (i.e., septicemia) that can result in hospitalization or fatality, depending on where the infection seeds in the body (Al Kaabi et al. 2021).

33.4.1.5 Public Health Importance

The overall burden of *Salmonella* in humans that is attributable to wild birds is currently unknown since the indirect transmission pathways are so difficult to characterize, namely, the interaction of wild birds with environmental reservoirs and sources, companion animals, and livestock (Fig. 1). As a result of these many possible routes of transmission and the considerable global burden of salmonellosis from foodborne sources (Fatica and Schneider 2011), it is plausible that *Salmonella* contributes to one of the greatest burdens of disease in people, among the various zoonoses transmitted from wild birds to humans. Evidence from case-control studies assessing indirect contact with wild birds as a risk factor for human *Salmonella* infections is not conclusive; one study found an increased odds of exposure among cases compared to controls (odds ratio: 6.9, 95% confidence interval: 2.3–21.0; Collins et al. 2019), but another failed to find an association (MacDonald et al. 2018).

33.4.1.6 Public Health Measures

The most common and likely route of direct and indirect transmission is feco-oral; thus, good hygiene is most useful in the prevention of salmonellosis. Although discouraging domestic cats from hunting wild birds by placement of a bell on their collar is likely to be of some benefit, the most effective preventive measure for this disease is to clean bird feeders frequently (every 2 weeks), using gloves, especially during the cold winter months. In general, it is recommended to avoid overfeeding wild birds and creating an unhealthy reliance on anthropogenic food sources, especially for food items that are not biologically appropriate, which may contribute to poor nutrition (e.g., bread). In warmer months, children and adults should avoid submerging their heads in shallow beach water that may be contaminated with feces from waterfowl and gulls. Those working in wildlife rehabilitation or conducting bird-banding activities should practice good hygiene (i.e., handwashing) after handling birds and before consuming food. The risk from handling wild birds is generally low, however, since mechanical transmission from feathers, feet, or the oral cavity does not appear to be a major mode of transmission (Navarro-Gonzalez et al. 2020).

33.4.2 *Campylobacter*

33.4.2.1 The Pathogen

Campylobacter is a genus of Gram-negative spiral organisms that require specific conditions in order to replicate; they are microaerophilic, meaning they require low oxygen (~5% O_2) conditions in order to grow. *Campylobacter* are also referred as "thermophilic," since they grow best at temperatures between 40 and 42 °C and will not replicate below temperatures of 30 °C; if they are exposed to temperatures below this, they may enter a state of senescence, or inactivation, where they do not replicate, but may still cause infections in humans and animals. Due to their fastidious nature and specific growth needs, *Campylobacter* can be difficult to culture, but there is no consensus about which culture method is optimal (Jokinen et al. 2012; Vaz et al. 2014). It is clear, however, that delays in processing of samples can result in the death of the organism and false-negative test results. *Campylobacter* undergo high rates of recombination and have high genetic diversity; thus, historical typing methods such as PFGE are being superseded by novel typing methods like the 40-locus binary typing scheme (i.e., comparative genomic fingerprinting) developed by Taboada et al. (2012), and high-resolution typing methods based on sequencing data (Llarena et al. 2017). *Campylobacter* is considered a commensal organism of the gastrointestinal tract of birds, since colonization is common (likely due to the higher body temperature of birds, as compared to mammals), and the vast majority of infected birds are clinically healthy. Infection with *Campylobacter* typically causes mild gastrointestinal illness in people. *Campylobacter jejuni* is by far the most common species identified in humans, with *Campylobacter coli*, *Campylobacter lari*, and other *Campylobacter* species occurring at a lower prevalence (Man 2011).

33.4.2.2 Epidemiology in Birds and Humans

Campylobacteriosis has been documented as the leading cause of foodborne illness in many countries around the world (Man 2011). *Campylobacter* infections in people typically occur as sporadic events, but outbreaks do occur, often in relation to untreated water and consumption of undercooked poultry products. It is believed that the human burden of illness due to *Campylobacter* is systematically underestimated due to the presentation of sporadic cases, typically self-limiting illnesses, and the nature of passive surveillance programs (Thomas et al. 2013). A seasonal pattern in the incidence of human *Campylobacter* cases has been widely documented, with the greatest number of cases occurring during the summer months. It has been hypothesized that this pattern is related to increased human exposures to potential sources of *Campylobacter* during warmer months and during outdoor activities (e.g., swimming) and picnics and the ability of the organism to replicate in warmer temperatures.

Campylobacter has been isolated from a wide variety of wild bird species. Prevalence estimates are highly variable, ranging from 0% to 93% (Kürekci et al. 2021; Lillehaug et al. 2005). The occurrence of *Campylobacter* is likely related to a host of factors, including the species of interest, feeding habitats and the ecological niche occupied, migration patterns, flock size, season, and geographic region of sampling

(Benskin et al. 2009; Vogt et al. 2020). Birds such as crows and gulls that frequent landfills and that live in close proximity to livestock and domestic animals are often found with a higher prevalence of microorganisms, including *Campylobacter* (Hald et al. 2016; Ito et al. 1988; Whelan et al. 1988). Even birds that exploit the same food resources (e.g., all bird species which feed on insects) may vary in their carriage of *Campylobacter*, as demonstrated by Waldenström et al. (2002), who demonstrated differences in the prevalence of this organism among ground-foraging insectivores (20.3%, $n = 14/69$), arboreal insectivores (0.6%, $n = 3/464$), and aerial insectivores (0%, $n = 0/42$). Seasonal peaks in the carriage of *Campylobacter* have been documented in spring and fall seasons, but the timing of peak prevalence is not always consistent and likely depends on a host of factors, such as bird species and behavior, health status, and feeding habits, among others (Broman et al. 2002; Taff et al. 2016; Vogt et al. 2020; Waldenström et al. 2002). Migration and larger flock sizes promoting inter- and intra-species mingling during colder seasons are thought to increase the risk of feco-oral transmission and contribute to *Campylobacter* infection, in addition to increased survival of *Campylobacter* in cooler temperatures (Moriarty et al. 2012).

There is some evidence suggesting that the carriage of less common *Campylobacter* species by wild birds is affected by exposures to this organism in their immediate environment. For instance, *Campylobacter coli* is rarely isolated from wild birds but is commonly isolated from pigs; two reports document an association with this species of *Campylobacter* and wild birds sampled near swine farms or swine manure (Hald et al. 2016; Vogt et al. 2019). Similarly, the occurrence of *Campylobacter lari* in predominantly marine bird species such as gulls might be related to the common occurrence of this species of *Campylobacter* in shellfish and aquatic environments (Motarjemi and Adams 2006; Waldenström et al. 2002). Further research is needed to clarify whether the presence of *Campylobacter lari* in aquatic environments is predominantly driven by marine birds or if it originates primarily from food sources and exposures in this environment.

Due to the fastidious nature of *Campylobacter*, which is prone to destruction by UV radiation and desiccation, the type of sample used is a fundamental consideration in the interpretation of prevalence estimates and risk assessments of wild birds. One study comparing the utility of fecal samples from Bonelli's eaglets (*Aquila fasciata*) with cloacal swabs did not recover any *Campylobacter* from dried fecal samples in the nest, but cloacal swabs from the same nestlings yielded a prevalence of 11% (95% CI: 4–24%) (Martín-Maldonado et al. 2019). Another comparison of different sampling techniques from a number of wild duck species found that the isolation of *Campylobacter jejuni* was significantly greater in cecal specimens compared to cloacal swabs (Luechtefeld et al. 1980).

Campylobacter is typically acquired through indirect feco-oral transmission of the organism from animal feces to humans, via contaminated food or water, or accidental exposure during outdoor activities (Fig. 1). Wild and domestic birds, livestock, and pets are considered major reservoirs of *Campylobacter*. The following have been identified as risk factors for human infection: consumption of raw or undercooked poultry, consumption of unpasteurized milk, exposure to untreated water, animal contact (livestock, pets), and outdoor activities (e.g., open water

swimming) (Cody et al. 2019; Lévesque et al. 2013; Rukambile et al. 2019). Not all human infections result in clinical illness. Children, due to their tendency of hand-to-mouth behavior, are thought to be at a higher risk of *Campylobacter* infection from environmental sources of animal fecal material, including playgrounds (French et al. 2009). The immunocompromised and the elderly are also at a greater risk of infection with *Campylobacter*. Several human outbreaks have specifically been linked to wild birds, including the consumption of milk from bottles with bird-pecked tops (Riordan et al. 1993) and raw peas that were contaminated with fecal material from sandhill cranes (Gardner et al. 2011; Kwan et al. 2014). Overall, these scenarios appear to be exceedingly rare, and the contribution of wild birds to the human burden of *Campylobacter* appears to be minor (<5% of human cases), based on a body of work, including a systematic review, comparing bird isolates to human clinical cases using multi-locus sequence typing (MLST) (Cody et al. 2015, 2019; Kovanen et al. 2019; Lévesque et al. 2013; Mäesaar et al. 2020; Marotta et al. 2020). A recent study comparing whole-genome sequencing data from wild bird isolates along with other animal and human sources in the Netherlands also demonstrated that wild birds contributed 0.4% (95% CI: 0.0–1.0%) to human cases (Mughini-Gras et al. 2021). Additional source attribution studies incorporating genomic data are needed to confirm these preliminary findings.

A major challenge with quantifying the contribution of wild birds to human *Campylobacter* cases, however, is the multitude of complex transmission pathways that exist (Fig. 1). The interaction of wild birds with poultry, livestock, and pets (other known reservoirs of *Campylobacter*) represents an unquantified pathway of transmission and amplification of the organism. Phylogenetic analyses of *Campylobacter* carried by wild birds have consistently demonstrated the presence of wild bird-specific strains that have rarely, or never, been recovered from humans or domestic animals (Aksomaitiene et al. 2019; Griekspoor et al. 2015; Kovanen et al. 2019; Weis et al. 2016). It is still unclear whether these strains have the potential to induce clinical illness in people or if they are generally nonpathogenic. These wild bird strains often make up the majority of *Campylobacter* strains found in waterways and rivers (Mughini-Gras et al. 2016; Mulder et al. 2020; Shrestha et al. 2019). In addition, wild birds have been shown to carry "generalist" *Campylobacter* strains found in a variety of animal sources and previously documented in human cases of clinical illness (Aksomaitiene et al. 2019; Hepworth et al. 2011; Weis et al. 2016). Based on these findings, it appears that wild birds represent a biological vector for transmission and dissemination of known pathogenic *Campylobacter* strains from other sources, but determination of the pathogenicity of host-adapted wild bird *Campylobacter* strains is needed.

33.4.2.3 Disease in Birds

Campylobacter is considered a commensal organism of all species of birds. Unlike *Salmonella*, *Campylobacter* has rarely been documented in association with clinical illness in wild birds. Although outbreaks of enteritis in domestic poultry have been attributed to *Campylobacter*, overt illness in wild birds has not been documented – only poor body condition – which could impact long-term survival (Taff and Townsend 2017; Waldenström et al. 2010). In one assessment, *Campylobacter*

infection did not affect the body condition, fledging success, or survival of American crow (*Corvus brachyrhynchos*) nestlings (Taff and Townsend 2017).

33.4.2.4 Disease in Humans

The majority of *Campylobacter* infections in people result in self-limiting gastrointestinal illness (e.g., vomiting, diarrhea), but a small proportion of cases (<1%) result in serious, long-term sequelae such as Guillain-Barré syndrome (Allos 2001). Additional medical conditions have been documented in association with *Campylobacter*, but a causal link has not yet been established for these conditions: esophageal cancer, colorectal cancer, irritable bowel syndrome, and Barrett's esophagus (Igwaran and Okoh 2019).

33.4.2.5 Public Health Importance

According to the World Health Organization, *Campylobacter* is the most common cause of bacterial enteritis across the world, and it is estimated to cause between 400 and 500 million infections every year (Igwaran and Okoh 2019). Unfortunately, despite a large body of evidence documenting the carriage of *Campylobacter* by a wide variety of wild bird species, their contribution to human cases remains unclear, since major research gaps regarding the pathogenicity of wild bird strains of *Campylobacter* remain, and assessment of potential spillover from wild birds to humans requires modeling of the entire transmission pathway and not only prevalence assessments (Smith et al. 2020).

33.4.2.6 Public Health Measures

Since consumption of poultry and poultry products accounts for the vast majority of *Campylobacter* infections in people, the most important measure to avoid infection is to ensure these products are properly cooked and stored and avoiding cross-contamination during meal preparation. Game meat (domestic and wild) is also considered a potential source of *Campylobacter* (Seguino et al. 2018); thus, hygienic meat handling measures also apply. Washing of fruits and vegetables prior to consumption, especially for those consumed raw, is important, since *Campylobacter jejuni* can survive up to 8 days on unwashed produce (Newell et al. 2016). Handwashing and good hygiene is recommended after handling of poultry, livestock, and pets and following outdoor activities in areas where wild birds are commonly found (e.g., parks). As with *Salmonella*, children should avoid submerging their heads underwater in shallow water that is also frequented by wild birds, and practice good hygiene after playing outdoors, including playground areas. Ensuring drinking water is treated is also recommended.

33.4.3 *Escherichia coli*

33.4.3.1 The Pathogen

Escherichia coli belong to a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria. They comprise the normal microflora of the gastrointestinal

tract of mammals and birds, and most strains are harmless. In immunocompromised people, or when normal gastrointestinal barriers have been breached, commensal *E. coli* are capable of producing disease in the host. A subset of *E. coli* strains are considered pathogenic, even in healthy individuals. Pathogenic *E. coli* are phylogenetically distinct from commensal *E. coli* and typically possess a variety of virulence genes and mechanisms that allow them to cause disease in the intestinal tract or survive in different niches outside of the gastrointestinal tract (i.e., extraintestinal pathogenic *E. coli*). Pathogenic *E. coli* are characterized by different pathotypes, depending where in the body they produce disease, and the typical clinical disease they produce: **e**ntero**p**athogenic ***E. coli*** (EPEC), **e**ntero**h**emorrhagic ***E. coli*** (EHEC), and **u**ro**p**athogenic ***E. coli*** (UPEC). Classification of these different pathotypes is complex, and details are covered elsewhere (Kaper et al. 2004), involving a combination of virulence traits, genetic differences, and serotyping (e.g., O, H, and K antigens). Birds, especially domestic poultry, are susceptible to clinical illness as a result of infection with **a**vian **p**athogenic ***E. coli*** (APEC), but most wild birds colonized or infected with these *E. coli* do not exhibit any signs of clinical illness. In addition to APEC, this section will focus on major groups of pathogenic *E. coli* affecting humans that have been well-researched in wild birds, including **S**higa **t**oxin-producing ***E. coli*** (STEC, also known as **v**ero**t**oxin-producing ***E. coli***; VTEC) and EPEC, as well as lesser-examined pathogenic *E. coli*, like UPEC.

33.4.3.2 Epidemiology in Birds and Humans

Worldwide, *E. coli* are the most common agent causing urinary tract infections; an estimated 130–175 million human urinary tract infections occur every year, with 80% caused by UPEC strains (Russo and Johnson 2003). Women are more frequently affected by UPEC, and commensal *E. coli* (non-UPEC strains) can also be the cause of these infections. Rarely, urinary tract infections progress to kidney infection (pyelonephritis) and result in kidney failure and possibly death. EPEC strains are among the leading causes of diarrhea across the world and are predominantly associated with children (Alonso et al. 2017). In developed countries, EPEC is occasionally responsible for outbreaks in pediatric wards and day care centers. A considerable proportion of childhood diarrhea in developing countries is attributed to EPEC, and in some countries, outbreaks have resulted in mortality rates as high as 30% (Senerwa et al. 1989).

Shiga toxin-producing *E. coli* (STEC, also known as VTEC) are the predominant cause of bloody diarrhea in humans, and serotype O157:H7 is the most important and well-known STEC strain in North America, United Kingdom, and Japan, but other serotypes have also been identified (e.g., O26, O111), and more than 400 O:H types of STEC are associated with human infections (Alonso et al. 2017; Kaper et al. 2004). Children are particularly vulnerable to STEC infections, and the infection can progress to hemolytic uremic syndrome and may result in acute kidney failure and possibly death. STEC infections are the leading cause of hemolytic uremic syndrome in humans. Many serogroups have been associated with HUS outbreaks in people, including O26, O45, O103, O111, O121, and O145 (Kuehne et al. 2016). The major pathogenicity factor of STEC is Shiga toxin, or Shiga-like toxin (verocytotoxin),

conferred by genes *stx*$_1$, *stx*$_2$, and others (Kobayashi et al. 2009). Humans can acquire STEC from other infected humans, domestic animals (including livestock and pets), contaminated drinking water, or foodborne exposures (e.g., undercooked meats, contaminated produce). Cattle are considered the main reservoir of STEC, but wildlife, including wild birds and wild mammals, have been shown to carry pathogenic *E. coli*, including STEC (Espinosa et al. 2018).

Avian pathogenic *E. coli* (APEC) are considered a bird-adapted pathotype of *E. coli* and frequently colonize and infect domestic and wild birds without causing any clinical illness. APEC is particularly known for causing outbreaks in domestic poultry worldwide, with considerable mortality rates (~20%) and significant economic losses for the poultry industry (Bélanger et al. 2011). Also known as avian colibacillosis, APEC causes a variety of infections in birds, including systemic illnesses (e.g., septicemia, air sacculitis, death) and local syndromes (e.g., cellulitis). Although not all APEC are zoonotic, a subset of these pathotypes has been documented to cause illness in people; worldwide, O_2 is one of the serogroups most associated with zoonotic APEC strains (Moulin-Schouleur et al. 2007). Less is known about the epidemiology of APEC in wild birds, since this pathotype is rarely associated with clinical illness in wild birds, and the zoonotic potential of most APEC strains is suspected, but not confirmed (Jørgensen et al. 2019).

Virulence-associated genes (VAGs) found in various *E. coli* pathotypes have consistently been identified among wild bird populations. Unfortunately, the presence of such virulence genes alone does not confirm pathogenicity of *E. coli* strains, as further testing is typically required, and, oftentimes, the identification of VAGs in wild birds represents a preliminary scan for potentially harmful *E. coli* (Knöbl et al. 2011). In some cases, the presence of certain VAGs is often used to imply pathogenicity, such as with Shiga toxin genes (e.g., *stx*$_1$, *stx*$_2$), but not all STEC strains possessing these genes are pathogenic to humans, and additional genes are often required for cellular invasion (e.g., *eae*). Nonetheless, the characterization of potentially pathogenic *E. coli* in wild birds provides an important first step in assessing potential zoonotic risk from these species.

The overall prevalence of STEC in wild birds likely varies due to factors discussed in the previous sections (e.g., bird species, geographic region), but prevalences between 0% and 1% are commonly found (Alonso et al. 2017; Bolton et al. 2012; Borges et al. 2017; Gaukler et al. 2009; Sanches et al. 2017). Several studies of feral pigeons (*Columba livia domestica*) and certain setting of wild birds, such as wildlife rehabilitation centers, have demonstrated higher prevalences of STEC, between 10% and 15% (Morabito et al. 2001; Russo et al. 2021; Schmidt et al. 2000). A meta-analysis of studies examining the prevalence of STEC among North American breeding bird species reported an overall prevalence of 20% (±6.3% standard error; $n = 9185$ individuals, 36 studies) (Smith et al. 2020). Studies characterizing the probable pathogenicity of *E. coli* from wild birds based on their phylogroup (i.e., A, B1, B2, D) have documented the presence of both pathogenic and nonpathogenic strains in wild birds, and their occurrence appears to be species-dependent (Borges et al. 2017; Kuczkowski et al. 2016; Oh et al. 2011; Rybak et al. 2022). There is some research to suggest that wild birds in captivity are more likely to carry pathogenic *E. coli* or virulence genes than free-living wild birds (Blyton

et al. 2015; Díaz-Sánchez et al. 2012). There is limited available literature regarding the prevalence of other *E. coli* pathotypes, but, like STEC, it appears that the prevalence of EPEC among wild birds is also highly variable (2–70%) (Borges et al. 2017; Oh et al. 2011; Sanches et al. 2017).

Transmission of pathogenic *E. coli* (VTEC/STEC) between wild birds and livestock in farm environments is likely, based on overlap in strains determined using molecular typing (e.g., PFGE, single-nucleotide polymorphisms) (Navarro-Gonzalez et al. 2020; Nielsen et al. 2004; Rapp et al. 2021), serotyping (Fahim et al. 2019), and studies of experimental infection and transmission (Kauffman and LeJeune 2011). Exchange between cattle and starlings is thought to be common, since these livestock species are often housed with access to the outdoors, and wild birds often congregate in large numbers in barns and defecate near or onto animal feed, facilitating feco-oral transmission of the organism to cattle. In contrast with wild birds, however, the prevalence of STEC in cattle has been shown to be several orders of magnitude larger; one study of wild birds and cattle in California assessed the prevalence in cattle to range from 4.5% to 65%, depending on the season, whereas the prevalence in birds in the same study was $<1\%$ for all STEC strains (both O157 and non-O157) (Navarro-Gonzalez et al. 2020). There is some evidence supporting zoonotic transmission of pathogenic *E. coli* between wild birds and humans for APEC strains, based on serotyping (Kobayashi et al. 2009) and sequence typing (Handrova and Kmet 2019; Johnson et al. 2008). Recent assessments using comparative genomics are suggestive of inter-species transmission of pathogenic *E. coli* between humans, wild birds, dogs, and environmental sources (e.g., water) for sequence type 131 (a UPEC strain) (Li et al. 2021) and sequence type 410 (Schaufler et al. 2016). Concerns about migration and long-distance dissemination of pathogenic *E. coli* clones by wild birds are substantiated in part by the identification of a sequence type 131 O25 of human origin in wild birds living on a remote Russian island lacking human presence (Hernandez et al. 2010). Conversely, it should not be assumed that wild birds necessarily are a major driver of human infections with certain pathogenic *E. coli*; recent work addressing gaps about whether feral pigeons are responsible for human STEC infections (specifically related to the stx_{2f} gene) using a whole-genome MLST scheme demonstrated no overlap between pigeon and human strains (van Hoek et al. 2019).

33.4.3.3 Disease in Birds

Outbreaks of mortality related to *E. coli* have rarely been documented in wild birds, but in some instances, these outbreaks been associated with bird feeders, similar to *Salmonella* (Pennycott et al. 2002). *E. coli* infections in wild birds have also been associated with enteritis and diarrhea, caused by pathotypes such as EPEC or ETEC. More commonly, *E. coli* are responsible for opportunistic infections in domestic birds, often resulting from host stress, poor hygiene (e.g., APEC in poultry), or hypovitaminosis A (especially in parrots). Affected birds may exhibit depression, fever, yellowish/greenish droppings, and localized (e.g., cellulitis) or generalized infections (e.g., septicemia, air sacculitis, death). Clinically healthy wild birds have been demonstrated to carry *E. coli* that are considered pathogenic to humans.

33.4.3.4 Disease in Humans

Depending on the pathotype, clinical signs of *E. coli* infection in people are variable. As discussed above, two general syndromes occur: intestinal disease and extra-intestinal disease (e.g., meningitis, urinary tract infections). The severity of these infections can vary depending on host factors (e.g., stress, immune status), and pathogenic *E. coli* cause illness in immunocompetent individuals. A subset of STEC cause bloody diarrhea (e.g., *E. coli* O157:H7) and are more likely to result in complications in children and the elderly, the most important of which is hemolytic uremic syndrome, which can result in acute renal failure and death.

33.4.3.5 Public Health Importance

The degree to which wild birds contribute to the human burden of *E. coli* illness is complicated by the difficulties in determining whether *E. coli* isolated from wild birds are pathogenic or nonpathogenic, since there is no single gold standard test for determination of pathogenicity status (Knöbl et al. 2011). In addition, *E. coli* are often considered secondary invaders; thus, the pathogenicity of an *E. coli* strain may depend on host factors (e.g., stress) and environmental factors (e.g., hygiene), as well as the location of infection. The bulk of existing research in wild birds currently assesses only a subset of *E. coli* known to be pathogenic to humans (predominantly STEC), but other pathotypes may also cause disease in people. In particular, the zoonotic potential of APEC found in wild birds merits further research. Nonetheless, the identification of similar or identical strains of *E. coli* in wild birds, human clinical cases, other animals, and environmental samples using comparative genomics offers some evidence of zoonotic transmission (Li et al. 2021; Schaufler et al. 2016).

33.4.3.6 Public Health Measures

Similar to other agents of foodborne illness discussed above, practicing good hygiene and careful preparation and cooking of food are important for the prevention of exposure to pathogenic *E. coli.* Similarly, treatment of drinking water is recommended. Due to its extremely low infectious dose (<100 cells) (Kaper et al. 2004) and ability to persist for several weeks in water and in outdoor environments (McGee et al. 2002), caution is recommended regarding recreational activities in water during the summertime. Avoiding ingestion of untreated water or underwater submersion in outdoor recreational areas close to agricultural regions following heavy precipitation is advised. Although *E. coli* have rarely been recovered from the exterior of wild birds (i.e., feet and feathers) (Navarro-Gonzalez et al. 2020), the route of transmission is generally feco-oral; thus, handling of wild birds is not considered a high-risk activity for transmission of this organism, but good hygiene (i.e., handwashing) should always be practiced.

33.4.4 *Chlamydophila psittaci*

33.4.4.1 The Pathogen

Chlamydophila psittaci (formerly *Chlamydia psittaci*) is a Gram-negative, obligate intracellular bacterium for which birds are considered a major reservoir, but this organism is also present in domestic and wild mammalian species (Perez-

Martinez and Storz 1985). This organism is the causative agent of the rare, but important, zoonotic disease known as psittacosis, or "parrot fever" in humans. It was originally believed that only parrots transmitted this organism, but it has since been determined that over 450 bird species can carry *C. psittaci* (Sukon et al. 2021), including domestic poultry (chickens and turkeys), and the preferred name for the disease in humans is now "ornithosis." Ornithosis results in an atypical pneumonia in some people, but most infected people remain asymptomatic (Harkinezhad et al. 2009). Similarly, birds can remain asymptomatic with *C. psittaci* infection, but they can also develop severe, systemic respiratory illness (avian chlamydiosis).

33.4.4.2 Epidemiology in Birds and Humans

Human cases of ornithosis are distributed worldwide and typically occur as sporadic cases, although outbreaks have been reported in association with poultry processing plants (Hinton et al. 1993). Additional outbreaks have been reported in communities (Williams et al. 1998) and in association with the handling of an injured wild bird in a veterinary clinic (Branley et al. 2008). This disease can be a difficult one to diagnose, due to a lack of sensitive and specific testing methods and limited awareness of this rare zoonotic disease (Chaber et al. 2021). Most human cases of ornithosis are self-limiting, while others require hospitalization, but infections rarely cause death. Ornithosis usually presents as an atypical pneumonia that is unresponsive to conventional treatments. Although ornithosis has typically been described as an occupational risk for those working with wild and domestic birds (e.g., pet store employees, bird-banders, zookeepers, poultry workers), the risk factors for infection with *C. psittaci* appear to differ from the risk factors for clinical illness due to *C. psittaci*. Contact with birds (particularly parrots and pigeons) is a well-recognized risk factor for exposure to *C. psittaci* (Harkinezhad et al. 2009), but this factor is poorly predictive of whether someone will develop ornithosis; thus, additional research is needed to clarify what additional factors need to be present for the development of clinical disease (Olsen et al. 1998; Williams et al. 1998).

Like other pathogenic bacterial agents, the prevalence of *C. psittaci* varies by geographic region and host species (Stokes et al. 2020). One study of the genetic variability of *C. psittaci* among pigeon in different regions of Switzerland based on MLST found distinct sequence types in different areas, suggesting limited exchange of the organism between different pigeon populations (Mattmann et al. 2019). Prevalences between 0% and 95% of *C. psittaci* have been recorded in a wide range of bird species (Prukner-Radovcić et al. 2005; Stokes et al. 2021). In addition to parrots, pigeons and other birds of the order Columbiformes are generally considered to be major reservoirs of this organism (Bracewell and Bevan 1986). Most infected wild birds are asymptomatic carriers, but some persistently infected individuals can shed the organism in their feces intermittently or develop clinical illness during periods of stress (e.g., breeding, migration) (European Commission 2002). Outbreaks of avian chlamydiosis have been documented in wild birds (Franson and Pearson 1995; Grimes et al. 1966), but the frequency of these events appears to be on the decline (Andersen and Vanrompay

2000), along with evidence of decreasing prevalence of *C. psittaci* infections among pigeons in Slovenia over a 13-year period (Dovč et al. 2004).

A total of nine genotypes of *C. psittaci* have been identified, which are considered host specific: genotype B in pigeons, genotype A in psittacines, genotype M56 in rodents (Liu et al. 2019). There is some evidence that certain *C. psittaci* strains are not zoonotic or do not readily infect non-host species; Johnson and Grimes (1983) demonstrated that *C. psittaci* originating from ruminants does not infect wild birds, and Olsen et al. (1998) found that strains carried by wild songbirds (passerines) are rarely infectious to humans. Further research is needed to clarify the significance of *C. psittaci* in wild birds and their zoonotic potential. Additional research is also needed to confirm findings of higher prevalences of *C. psittaci* infection among wild or domestic birds in captivity versus those that are free-living in natural environments (Amery-Gale et al. 2020; Liu et al. 2019; Soon et al. 2021). However, challenges with diagnosis of this disease/infection also apply to birds, due to a lack of sensitive and specific testing methods (Luján-Vega et al. 2018) and intermittent shedding of the organism by persistently infected birds. Thus, it is recommended to obtain multiple samples from birds (e.g., pharyngeal swabs, cloacal swabs, and fecal samples) to ensure maximum testing sensitivity.

33.4.4.3 Disease in Birds

The majority of birds remain asymptomatic with *C. psittaci* infection, but it is believed that periods of stress can result in individuals progressing to clinical disease. Signs of infection include respiratory signs, conjunctivitis, purulent rhinitis, yellow-green droppings, polyuria, and lethargy. Particularly among domestic fowl, outbreaks may result in high morbidity and mortality (Sachse et al. 2015), but outbreaks are rare among wild bird populations.

33.4.4.4 Disease in Humans

Ornithosis typically presents as a flu-like illness, often with the following clinical signs: fever, malaise, headache, myalgia, and chills. People may or may not experience a dry cough. Disease may progress to pneumonia. Ornithosis is rarely fatal.

33.4.4.5 Public Health Importance

Overall, ornithosis is considered a rare disease in humans, in spite of probable underreporting of mild cases and missed diagnoses. It has been estimated that 1% of community-acquired pneumonia is caused by *C. psittaci* (Hogerwerf et al. 2017). However, since severe cases require prompt antimicrobial treatment, obtaining an accurate diagnosis using multiple tests (e.g., culture, serology, PCR) is paramount to the reduction of morbidity and mortality in affected people (Vande Weygaerde et al. 2018).

33.4.4.6 Public Health Measures

Transmission of *C. psittaci* is typically through inhalation of dust containing the organism or via ingestion of contaminated material (fecal material, secretions). The organism has been shown to survive in feces and bedding for up to 30 days (Sachse et al. 2015); thus, cleaning and disinfection are very important to remove the

environmental burden of *C. psittaci* among birds in captivity (including wild birds). Ensuring good ventilation and lowering the humidity of indoor environments housing birds will also help to decrease the risk of transmission, as will the use of personal protective equipment while cleaning bird mews. People who often work in close contact with wild or domestic birds are at increased risk of infection, but not necessarily clinical illness (Olsen et al. 1998; Williams et al. 1998), but good hygiene measures and frequent handwashing for those handling and in close contact with birds are still recommended. Most importantly, an awareness of this disease is likely to be most useful for those in occupations with frequent contact with birds, should they become ill, so that they can flag this disease for their attending physician, if necessary. Human-to-human transmission is rare; thus, quarantine or isolation of affected individuals is not indicated.

33.5 Ectoparasites of Wild Birds with Zoonotic Risks

Wild birds may also be carriers of mites and ticks that possess zoonotic potential. Mites such as the poultry red mite (*Dermanyssus gallinae*) have been found on wild birds and are capable of causing a rash in humans, often on the legs (George et al. 2015). *Ixodes* spp. ticks (hard ticks) can sometimes be found parasitizing wild birds and are competent vectors of Lyme disease, caused by the *Borrelia burgdorferi s.l.* species complex. Wild mammals such as deer and mice are considered the major wildlife reservoirs of *Borrelia* spp., but wild birds represent another competent reservoir of these organisms. Following the bite of an infected tick, many people initially experience a flu-like illness with a bull's-eye rash (erythema migrans) at the site of the tick bite and later progress to arthritis with swelling of the joints, cardiac signs, nerve pain, headaches, and chronic fatigue (Steere et al. 1998). Diagnosis and treatment of Lyme disease in humans is challenging; thus, prevention of exposure to infected ticks is considered key. Tick checks and prompt removal (including the head and mouth parts of the tick) following contact with wild birds are recommended, since it generally takes >24 hours for transmission of *Borrelia* spp. to occur (Hojgaard et al. 2008).

33.6 Wild Birds as Reservoirs of Viral Diseases

A number of viral diseases affect wild birds, including reportable diseases such as Newcastle disease virus. The two major viruses highlighted in this section represent viruses for which wild birds are a significant reservoir and may or may not cause clinical disease in people, but they pose a real or theoretical risk due to their widespread, global distribution among wild bird populations.

33.6.1 West Nile Virus

West Nile virus is a member of the *Flavivirus* genus. Avian species represent a major reservoir of these viruses, for which mosquitoes (primarily *Culex* spp.) are required

for transmission to humans and horses, both of which are considered dead-end hosts (no viral replication occurs). Raptors (hawks, owls) and corvid species (crows, ravens, jays) are particularly susceptible to infection with West Nile virus (Nemeth et al. 2007). Following the initial introduction to the virus to various parts of the world in recent decades, widespread mortality was observed in wild bird populations (Habarugira et al. 2020). Characteristic neurological signs in affected birds include head tilt, tremors, loss of coordination, weakness, convulsions, and possibly death. Overall, there is evidence of population recovery in many, but not all, bird species (Kilpatrick and Wheeler 2019). Most people infected with West Nile virus experience a mild, flu-like illness that is self-limiting. Other people, especially immunocompromised individuals, are more likely to suffer serious effects, such as meningitis, encephalitis, optic neuritis, or paresis.

33.6.2 Avian Influenza Virus

Avian influenza belongs to the Orthomyxoviridae family of viruses. There are three classes of avian influenza viruses, A, B, and C; the most important type in the context of zoonoses are type A viruses. These viruses can infect both humans and birds, but they are considered a disease of birds, not humans, and the disease is thus colloquially referred to as the "bird flu." Wild birds are considered a reservoir of these viruses and have the potential to introduce new strains over long distances due to their migratory capabilities. Avian influenza viruses have a high propensity for rapid genetic mutation, and there is a concern that strains of these viruses can suddenly develop into highly pathogenic strains (Stephenson and Democratis 2006). Highly pathogenic avian influenza virus subtype H5N1 has caused outbreaks in both people and domestic fowl in many parts of the world since the zoonotic strain was first detected in 1997 (Stephenson and Democratis 2006). Evidence of transmission between poultry and humans exists, but the risk of transmission from wild birds to humans remains theoretical (Koopmans et al. 2004; Tsiodras et al. 2008). Clinical signs of infection in people and birds are typical of influenza viruses, including respiratory signs, keratoconjunctivitis, fever, and diarrhea. Some cases may progress to more serious, potentially life-threatening illness, such as encephalitis.

33.7 Zoonoses of Wild Birds with Minor, Theoretical, or Unknown Risks

A variety of bacterial, fungal, and parasitic pathogens have been isolated from wild birds, including, but not limited to, *Yersinia* spp., *Listeria monocytogenes*, *Cryptosporidium* spp., *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Mycobacterium avium* (avian tuberculosis) (Kozdrun et al. 2015; Tsiodras et al. 2008). For some of these pathogens, it appears that immunosuppression is a major risk factor for disease (*M. avium*, *C. neoformans*, *H. capsulatum*), along with exposure to soil contaminated with bird or bat droppings (*C. neoformans*, *H. capsulatum*). For other

organisms, it is unclear whether wild birds are drivers of disease transmission or if they primarily act as sentinels of environmental contamination from other primary sources (e.g., livestock); thus, further research using high-resolution molecular typing methods is needed to clarify what role birds play in the transmission of these pathogens to humans.

Botulism is another disease of wild birds that is responsible for die-offs that can number into the hundreds of individual birds (Lima et al. 2020). The bioaccumulation of the botulism toxin in the food chain, from algae to fish, and then to fish-eating birds, causes paralysis of respiratory muscles and, ultimately, death. The toxin produced by *Clostridium botulinum*, the causative bacterial agent, is also capable of causing serious illness in people and their pets (i.e., life-threatening paralysis), following ingestion of contaminated material (i.e., affected deceased birds or fish containing the botulinum toxin).

Hunters should be aware of *Sarcocystis* spp. parasitic organisms that appear as small grains of rice in the breast meat of affected birds (usually waterfowl); although cooking the meat destroys the parasite, rendering it inactive, it is recommended to discard meat containing large cysts (Costanzo 1990).

33.8 Cross-References

- ▶ *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control
- ▶ Cryptosporidium and Cryptosporidiosis: Trickle or Treat?
- ▶ Enterohemorrhagic *E. coli* (EHEC): Environmental-Vehicle-Human Interface
- ▶ The Zoonotic Agent Salmonella
- ▶ Vector-Borne Zoonoses
- ▶ West Nile Virus: From Africa to Europe, America, and Beyond
- ▶ Zoonoses Transmitted by Poultry
- ▶ Zoonotic Transmission of *Chlamydia* spp.: Known for 140 Years, but Still Underestimated

References

Aksomaitiene J, Ramonaite S, Tamuleviciene E, Novoslavskij A, Alter T, Malakauskas M (2019) Overlap of antibiotic resistant *Campylobacter jejuni* MLST genotypes isolated from humans, broiler products, dairy cattle and wild birds in Lithuania. Front Microbiol 10:1377

Al Kaabi S, Al Kaabi A, Al NH (2021) What is beyond *Salmonella* gastroenteritis? A case of acute pancreatitis complicating *Salmonella* infection in a child: a case report and literature review. BMC Pediatr 21:353

Alerstam T, Bäckman J, Grönroos J, Olofsson P, Strandberg R (2019) Hypotheses and tracking results about the longest migration: the case of the arctic tern. Ecol Evol 9:9511–9531

Alley MR, Connolly JH, Fenwick SG, Mackereth GF, Leyland MJ, Rogers LE, Haycock M, Nicol C, Reed CE (2002) An epidemic of salmonellosis caused by *Salmonella* Typhimurium DT160 in wild birds and humans in New Zealand. N Z Vet J 50:170–176

Allos BM (2001) *Campylobacter jejuni* infections: update on emerging issues and trends. Clin Infect Dis 32:1201–1206

Alonso CA, Mora A, Díaz D, Blanco M, González-Barrio D, Ruiz-Fons F, Simón C, Blanco J, Torres C (2017) Occurrence and characterization of stx and/or eae-positive *Escherichia coli* isolated from wildlife, including a typical EPEC strain from a wild boar. Vet Microbiol 207:69–73

Amery-Gale J, Legione AR, Marenda MS, Owens J, Eden PA, Konsak-Ilievski BM, Whiteley PL, Dobson EC, Browne EA, Slocombe RF, Devlin JM (2020) Surveilland for *Chlamydia* spp. with multilocus sequence typing analysis in wild and captive birds in Victoria, Australia. J Wildl Dis 56:16–26

Andersen AA, Vanrompay D (2000) Avian chlamydiosis. Rev Sci Tech 19:396–404

Andrés S, Vico JP, Garrido V, Grilló MJ, Samper S, Gavín P, Herrera-León S, Mainar-Jaime RC (2013) Epidemiology of subclinical salmonellosis in wild birds from an area of high prevalence of pig salmonellosis: phenotypic and genetic profiles of *Salmonella* isolates. Zoonoses Public Health 60:355–365

Bélanger L, Garenaux A, Harel J, Boulianne M, Nadeau E, Dozois CM (2011) *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. FEMS Immunol Med Microbiol 62:1–10

Bengis RG, Leighton FA, Fischer JR, Artois M, Mörner T, Tate CM (2004) The role of wildlife in emerging and re-emerging zoonoses. Rev Sci Tech 23:497–511

Benskin CM, Wilson K, Jones K, Hartley IR (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biol Rev Camb Philos Soc 84:349–373

Bloomfield SJ, Benschop J, Biggs PJ, Marshall JC, Hayman DTS, Carter PE, Midwinter AC, Mather AE, French NP (2017) Genomic analysis of *Salmonella enterica* serovar Typhimurium DT160 associated with a 14-year outbreak, New Zealand, 1998–2012. Emerg Infect Dis 23:906–913

Blyton MD, Pi H, Vangchhia B, Abraham S, Trott DJ, Johnson JR, Gordon DM (2015) Genetic structure and antimicrobial resistance of *Escherichia coli* and cryptic clades in birds with diverse human associations. Appl Environ Microbiol 81:5123–5133

Bolat A (2016) The Kazakh minority in Mongolia: falconry as a symbol of Kazakh identity. Senri Ethnological Studies 93:107–125

Bolton DJ, O'Neill CJ, Fanning S (2012) A preliminary study of *Salmonella*, verocytotoxigenic *Escherichia coli/Escherichia coli* O157 and *Campylobacter* on four mixed farms. Zoonoses Public Health 59:217–228

Borges CA, Cardozo MV, Beraldo LG, Oliveira ES, Maluta RP, Barboza KB, Werther K, Ávila FA (2017) Wild birds and urban pigeons as reservoirs for diarrheagenic *Escherichia coli* with zoonotic potential. J Microbiol 55:344–348

Bracewell CD, Bevan BJ (1986) Chlamydiosis in birds in Great Britain. 1. Serological reactions to chlamydia in birds sampled between 1974 and 1983. J Hyg (Lond) 96:447–451

Branley JM, Roy B, Dwyer DE, Sorrell TC (2008) Real-time PCR detection and quantitation of *Chlamydophila psittaci* in human and avian specimens from a veterinary clinic cluster. Eur J Clin Microbiol Infect Dis 27:269–273

Broman T, Palmgren H, Bergström S, Sellin M, Waldenström J, Danielsson-Tham ML, Olsen B (2002) *Campylobacter jejuni* in black-headed gulls (*Larus ridibundus*): prevalence, genotypes, and influence on *C. jejuni* epidemiology. J Clin Microbiol 40:4594–4602

Cabe PR (2021) European starlings (*Sturnus vulgaris*) as vectors and reservoirs of pathogens affecting humans and domestic livestock. Animals (Basel) 11:466

Caspermeyer J (2016) Evolutionary study of birds' sense of smell reveals important clues for behavior and adaptation. Mol Biol Evol 33:295–296

Chaber AL, Jelocnik M, Woolford L (2021) Undiagnosed cases of human pneumonia following exposure to *Chlamydia psittaci* from an infected rosella parrot. Pathogens 10:968

Chancey C, Grinev A, Volkova E, Rios M (2015) The global ecology and epidemiology of West Nile virus. Biomed Res Int 2015:376230

Chung O, Jin S, Cho YS, Lim J, Kim H, Jho S, Kim HM, Jun J, Lee H, Chon A, Ko J, Edwards J, Weber JA, Han K, O'Brien SJ, Manica A, Bhak J, Paek WK (2015) The first whole genome and transcriptome of the cinereous vulture reveals adaptation in the gastric and immune defense systems and possible convergent evolution between the Old and New World vultures. Genome Biol 16:215

Chung DM, Ferree E, Simon DM, Yeh PJ (2018) Patterns of bird-bacteria associations. Ecohealth 15:627–641

Clark L (2014) Disease risks posed by wild birds associated with agricultural landscapes. In: Matthews KR, Sapers GM, Gerba CP (eds) The produce contamination problem, Second edn. Academic Press, Elsevier Inc, Boston, pp 139–165

Cody AJ, McCarthy ND, Bray JE, Wimalarathna HM, Colles FM, Jansen van Rensburg MJ, Dingle KE, Waldenström J, Maiden MC (2015) Wild bird-associated *Campylobacter jejuni* isolates are a consistent source of human disease, in Oxfordshire, United Kingdom. Environ Microbiol Rep 7:782–788

Cody AJ, Maiden MC, Strachan NJ, McCarthy ND (2019) A systematic review of source attribution of human campylobacteriosis using multilocus sequence typing. Euro Surveill 24:1800696

Colbert-White EN, Covington MA, Fragaszy DM (2011) Social context influences the vocalizations of a home-raised African Grey parrot (*Psittacus erithacus erithacus*). J Comp Psychol 125: 175–184

Collins J, Simpson KMJ, Bell G, Durrheim DN, Hill-Cawthorne GA, Hope K, Howard P, Kohlenberg T, Lawrence K, Lilly K, Porigneaux P, Sintchenko V, Wang Q, Ward MP, Wiethoelter A, Mor SM, Flint J (2019) A one health investigation of *Salmonella enterica* serovar Wangata in North-Eastern New South Wales, Australia, 2016-2017. Epidemiol Infect 147:e150

Costanzo GR (1990) Sarcocystis in American black ducks wintering in New Jersey. J Wildl Dis 26: 387–389

Deepe GS Jr (2018) Outbreaks of histoplasmosis: the spores set sail. PLoS Pathog 14:e1007213

Díaz-Sánchez S, Sánchez S, Ewers C, Höfle U (2012) Occurrence of avian pathogenic *Escherichia coli* and antimicrobial-resistant *E. coli* in red-legged partridges (*Alectoris rufa*): sanitary concerns of farming. Avian Pathol 41:337–344

Dos Santos EJE, Azevedo RP, Lopes ATS, Rocha JM, Albuquerque GR, Wenceslau AA, Miranda FR, Rodrigues DDP, Maciel BM (2020) *Salmonella* spp. in wild free-living birds from atlantic forest fragments in southern Bahia, Brazil. Biomed Res Int 2020:7594136

Dovč A, Zorman-Rojs O, Vergles Rataj A, Bole-Hribovsek V, Krapez U, Dobeic M (2004) Health status of free-living pigeons (*Columba livia domestica*) in the city of Ljubljana. Acta Vet Hung 52:219–226

Elbediwi M, Pan H, Biswas S, Li Y, Yue M (2020) Emerging colistin resistance in *Salmonella enterica* serovar Newport isolates from human infections. Emerg Microbes Infect 9:535–538

Espín S, Sánchez-Virosta P (2021) A review of metal-induced effects on vitamins A, E and D3 in birds. Ecotoxicology 30:1–16

Espinosa L, Gray A, Duffy G, Fanning S, McMahon BJ (2018) A scoping review on the prevalence of Shiga-toxigenic *Escherichia coli* in wild animal species. Zoonoses Public Health 65:911–920

European Commission (2002) Avian chlamydiosis as a zoonotic disease and risk reduction strategies. Report of the European Commission's Scientific Committee on Animal Health and Animal Welfare https://ec.europa.eu/food/system/files/2020-12/sci-com_scah_out73_en.pdf

Fahim KM, Ismael E, Khalefa HS, Farag HS, Hamza DA (2019) Isolation and characterization of *E. coli* strains causing intramammary infections from dairy animals and wild birds. Int J Vet Sci Med 7:61–70

Fatica MK, Schneider KR (2011) *Salmonella* and produce: survival in the plant environment and implications in food safety. Virulence 2:573–579

Feare CJ, Sanders MF, Blasco R, Bishop JD (1999) Canada goose (*Branta canadensis*) droppings as a potential source of pathogenic bacteria. J R Soc Promot Heal 119:146–155

Fonseca JM, Ravishankar S, Sanchez CA, Park E, Nolte KD (2020) Assessing the food safety risk posed by birds entering leafy greens fields in the US southwest. Int J Environ Res Public Health 17:8711

Ford L, Ingle D, Glass K, Veitch M, Williamson DA, Harlock M, Gregory J, Stafford R, French N, Bloomfield S, Grange Z, Conway ML, Kirk MD (2019) Whole-genome sequencing of *Salmonella* Mississippi and Typhimurium Definitive Type 160, Australia and New Zealand. Emerg Infect Dis 25:1690–1697

Franson JC, Pearson JE (1995) Probable epizootic chlamydiosis in wild California (*Larus californicus*) and ring-billed (*Larus delawarensis*) gulls in North Dakota. J Wildl Dis 31:424–427

Frątczak M, Indykiewicz P, Dulisz B, Nowakowski JJ, Janiszewski T, Szeptycki J, Wilczyński J, Tryjanowski P (2021) Lack of evidence that bird feeders are a source of salmonellosis during winter in Poland. Animals (Basel) 11:1831

French NP, Midwinter A, Holland B, Collins-Emerson J, Pattison R, Colles F, Carter P (2009) Molecular epidemiology of *Campylobacter jejuni* isolates from wild-bird fecal material in children's playgrounds. Appl Environ Microbiol 75:779–783

Fu Y, M'ikanatha NM, Lorch JM, Blehert DS, Berlowski-Zier B, Whitehouse CA, Li S, Deng X, Smith JC, Shariat NW, Nawrocki EM, Dudley EG (2022) *Salmonella enterica* serovar Typhimurium from wild birds in the United States represent distinct lineages defined by bird type. Appl Environ Microbiol:AEM0197921. Epub ahead of print

Gardner TJ, Fitzgerald C, Xavier C, Klein R, Pruckler J, Stroika S, McLaughlin JB (2011) Outbreak of campylobacteriosis associated with consumption of raw peas. Clin Infect Dis 53:26–32. Erratum in: Clin Infect Dis. 2012;54:1040

Gaukler SM, Linz GM, Sherwood JS, Dyer NW, Bleier WJ, Wannemuehler YM, Nolan LK, Logue CM (2009) *Escherichia coli*, *Salmonella*, and *Mycobacterium avium* subsp. *paratuberculosis* in wild European starlings at a Kansas cattle feedlot. Avian Dis 53:544–551

George DR, Finn RD, Graham KM, Mul MF, Maurer V, Moro CV, Sparagano OA (2015) Should the poultry red mite *Dermanyssus gallinae* be of wider concern for veterinary and medical science? Parasit Vectors 8:178

Girdwood RW, Fricker CR, Munro D, Shedden CB, Monaghan P (1985) The incidence and significance of *Salmonella* carriage by gulls (*Larus* spp.) in Scotland. J Hyg (Lond) 95:229–241

Golden NH, Rattner BA (2003) Ranking terrestrial vertebrate species for utility in biomonitoring and vulnerability to environmental contaminants. Rev Environ Contam Toxicol 176:67–136

Greig J, Rajić A, Young I, Mascarenhas M, Waddell L, LeJeune J (2015) A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain. Zoonoses Public Health 62:269–284

Griekspoor P, Hansbro PM, Waldenström J, Olsen B (2015) *Campylobacter jejuni* sequence types show remarkable spatial and temporal stability in Blackbirds. Infect Ecol Epidemiol 5:28383

Grimes JE, Sullivan TD, Irons JV (1966) Recovery of ornithosis agent from naturally infected white-winged doves. J Wildlife Manag 30:594–598

Guzman-Pando A, Chacon-Murguia MI (2021) DeepFoveaNet: deep fovea eagle-eye bioinspired model to detect moving objects. IEEE Trans Image Process 30:7090–7100

Habarugira G, Suen WW, Hobson-Peters J, Hall RA, Bielefeldt-Ohmann H (2020) West Nile virus: an update on pathobiology, epidemiology, diagnostics, control and "One Health" implications. Pathogens 9:589

Hald B, Skov MN, Nielsen EM, Rahbek C, Madsen JJ, Wainø M, Chriél M, Nordentoft S, Baggesen DL, Madsen M (2016) *Campylobacter jejuni* and *Campylobacter coli* in wild birds on Danish livestock farms. Acta Vet Scand 58:11

Hall AJ, Saito EK (2008) Avian wildlife mortality events due to salmonellosis in the United States, 1985-2004. J Wildl Dis 44:585–593

Hamer SA, Lehrer E, Magle SB (2012) Wild birds as sentinels for multiple zoonotic pathogens along an urban to rural gradient in greater Chicago, Illinois. Zoonoses Public Health 59:355–364

Handrova L, Kmet V (2019) Antibiotic resistance and virulence factors of *Escherichia coli* from eagles and goshawks. J Environ Sci Health B 54:605–614

Harkinezhad T, Verminnen K, De Buyzere M, Rietzschel E, Bekaert S, Vanrompay D (2009) Prevalence of *Chlamydophila psittaci* infections in a human population in contact with domestic and companion birds. J Med Microbiol 58:1207–1212

Hedenström A (2008) Adaptations to migration in birds: behavioural strategies, morphology and scaling effects. Philos Trans R Soc Lond Ser B Biol Sci 363:287–299

Hepworth PJ, Ashelford KE, Hinds J, Gould KA, Witney AA, Williams NJ, Leatherbarrow H, French NP, Birtles RJ, Mendonca C, Dorrell N, Wren BW, Wigley P, Hall N, Winstanley C

(2011) Genomic variations define divergence of water/wildlife-associated *Campylobacter jejuni* niche specialists from common clonal complexes. Environ Microbiol 13:1549–1560

Hernandez J, Bonnedahl J, Eliasson I, Wallensten A, Comstedt P, Johansson A, Granholm S, Melhus A, Olsen B, Drobni M (2010) Globally disseminated human pathogenic *Escherichia coli* of O25b-ST131 clone, harbouring blaCTX-M-15, found in Glaucous-winged gull at remote Commander Islands, Russia. Environ Microbiol Rep 2:329–332

Hinton DG, Shipley A, Galvin JW, Harkin JT, Brunton RA (1993) Chlamydiosis in workers at a duck farm and processing plant. Aust Vet J 70:174–176

Hogerwerf L, de Gier B, Baan B, van der Hoek W (2017) *Chlamydia psittaci* (psittacosis) as a cause of community-acquired pneumonia: a systematic review and meta-analysis. Epidemiol Infect 145:3096–3105

Hojgaard A, Eisen RJ, Piesman J (2008) Transmission dynamics of *Borrelia burgdorferi s.s.* during the key third day of feeding by nymphal *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol 45: 732–736

Horns JJ, Şekercioğlu ÇH (2018) Conservation of migratory species. Curr Biol 28:R980–R983

Igwaran A, Okoh AI (2019) Human campylobacteriosis: a public health concern of global importance. Heliyon 5:e02814

Irestedt M, Jønsson KA, Fjeldså J, Christidis L, Ericson PG (2009) An unexpectedly long history of sexual selection in birds-of-paradise. BMC Evol Biol 9:235

Ito K, Kubokura Y, Kaneko K, Totake Y, Ogawa M (1988) Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. J Wildl Dis 24:467–470

Johnson MC, Grimes JE (1983) Resistance of wild birds to infection by *Chlamydia psittaci* of mammalian origin. J Infect Dis 147:162

Johnson TJ, Wannemuehler Y, Johnson SJ, Stell AL, Doetkott C, Johnson JR, Kim KS, Spanjaard L, Nolan LK (2008) Comparison of extraintestinal pathogenic *Escherichia coli* strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. Appl Environ Microbiol 74:7043–7050

Jokinen CC, Koot JM, Carrillo CD, Gannon VP, Jardine CM, Mutschall SK, Topp E, Taboada EN (2012) An enhanced technique combining pre-enrichment and passive filtration increases the isolation efficiency of *Campylobacter jejuni* and *Campylobacter coli* from water and animal fecal samples. J Microbiol Methods 91:506–513

Jørgensen SL, Stegger M, Kudirkiene E, Lilje B, Poulsen LL, Ronco T, Pires Dos Santos T, Kiil K, Bisgaard M, Pedersen K, Nolan LK, Price LB, Olsen RH, Andersen PS, Christensen H (2019) Diversity and population overlap between avian and human *Escherichia coli* belonging to sequence type 95. mSphere 4:e00333–e00318

Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic *Escherichia coli*. Nat Rev Microbiol 2:123–140

Kapperud G, Rosef O (1983) Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway. Appl Environ Microbiol 45:375–380

Kapperud G, Stenwig H, Lassen J (1998) Epidemiology of *Salmonella* Typhimurium O:4-12 infection in Norway–evidence of transmission from an avian wildlife reservoir. Am J Epidemiol 147:774–782

Kauffman MD, LeJeune J (2011) European starlings (*Sturnus vulgaris*) challenged with *Escherichia coli* O157 can carry and transmit the human pathogen to cattle. Lett Appl Microbiol 53:596–601

Khalefa HS, Ahmed ZS, Abdel-Kader F, Ismail EM, Elshafiee EA (2021) Sequencing and phylogenetic analysis of the *stn* gene of *Salmonella* species isolated from different environmental sources at Lake Qarun protectorate: the role of migratory birds and public health importance. Vet World 14:2764–2772

Kilpatrick AM, Wheeler SS (2019) Impact of West Nile Virus on bird populations: limited lasting effects, evidence for recovery, and gaps in our understanding of impacts on ecosystems. J Med Entomol 56:1491–1497

Kirk JH, Holmberg CA, Jeffrey JS (2002) Prevalence of *Salmonella* spp. in selected birds captured on California dairies. J Am Vet Med Assoc 220:359–362

Knöbl T, Saidenberg ABS, Moreno AM, Gomes TAT, Vieira MAM, Leite DS, Blanco JE, Ferreira JP (2011) Serogroups and virulence genes of *Escherichia coli* isolated from psittacine birds. Pesq Vet Bras 31:916–921

Kobayashi H, Kanazaki M, Hata E, Kubo M (2009) Prevalence and characteristics of *eae*- and *stx*-positive strains of *Escherichia coli* from wild birds in the immediate environment of Tokyo Bay. Appl Environ Microbiol 75:292–295

Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, Meijer A, van Steenbergen J, Fouchier R, Osterhaus A, Bosman A (2004) Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet 363:587–593

Kovanen S, Rossi M, Pohja-Mykrä M, Nieminen T, Raunio-Saarnisto M, Sauvala M, Fredriksson-Ahomaa M, Hänninen ML, Kivistö R (2019) Population genetics and characterization of *Campylobacter jejuni* isolates from western jackdaws and game birds in Finland. Appl Environ Microbiol 85:e02365–e02318

Kozdrun W, Czekaj, Stys N (2015) Avian zoonoses - a review. Bull Vet Inst Pulawy 59:171–178

Kuczkowski M, Krawiec M, Voslamber B, Książczyk M, Płoskońska-Bugla G, Wieliczko A (2016) Virulence genes and the antimicrobial susceptibility of *Escherichia coli*, isolated from wild waterbirds, in the Netherlands and Poland. Vector Borne Zoonotic Dis 16:528–536

Kuehne A, Bouwknegt M, Havelaar A, Gilsdorf A, Hoyer P, Stark K, Werber D (2016) HUS active surveillance network Germany. Estimating true incidence of O157 and non-O157 Shiga toxin-producing *Escherichia coli* illness in Germany based on notification data of haemolytic uraemic syndrome. Epidemiol Infect 144:3305–3315

Kürekci C, Sakin F, Epping L, Knüver MT, Semmler T, Stingl K (2021) Characterization of *Campylobacter* spp. strains isolated from wild birds in Turkey. Front Microbiol 12:712106

Kurihara T, Hirata A, Yamaguchi T, Okada H, Kameda M, Sakai H, Haridy M, Yanai T (2020) Avipoxvirus infection in two captive Japanese cormorants (*Phalacrocorax capillatus*). J Vet Med Sci 82:817–822

Kwan PS, Xavier C, Santovenia M, Pruckler J, Stroika S, Joyce K, Gardner T, Fields PI, McLaughlin J, Tauxe RV, Fitzgerald C (2014) Multilocus sequence typing confirms wild birds as the source of a *Campylobacter* outbreak associated with the consumption of raw peas. Appl Environ Microbiol 80:4540–4546

Laguë SL (2017) High-altitude champions: birds that live and migrate at altitude. J Appl Physiol (1985) 123:942–950

Lawson B, de Pinna E, Horton RA, Macgregor SK, John SK, Chantrey J, Duff JP, Kirkwood JK, Simpson VR, Robinson RA, Wain J, Cunningham AA (2014) Epidemiological evidence that garden birds are a source of human salmonellosis in England and Wales. PLoS One 9:e88968

Lévesque S, Fournier E, Carrier N, Frost E, Arbeit RD, Michaud S (2013) Campylobacteriosis in urban versus rural areas: a case-case study integrated with molecular typing to validate risk factors and to attribute sources of infection. PLoS One 8:e83731

Li L, Jiang Z (2014) International trade of CITES listed bird species in China. PLoS One 9:e85012

Li D, Wyrsch ER, Elankumaran P, Dolejska M, Marenda MS, Browning GF, Bushell RN, McKinnon J, Chowdhury PR, Hitchick N, Miller N, Donner E, Drigo B, Baker D, Charles IG, Kudinha T, Jarocki VM, Djordjevic SP (2021) Genomic comparisons of *Escherichia coli* ST131 from Australia. Microb Genom 7:000721

Lillehaug A, Monceyron Jonassen C, Bergsjø B, Hofshagen M, Tharaldsen J, Nesse LL, Handeland K (2005) Screening of feral pigeon (*Colomba livia*), mallard (*Anas platyrhynchos*) and graylag goose (*Anser anser*) populations for *Campylobacter* spp., *Salmonella* spp., avian influenza virus and avian paramyxovirus. Acta Vet Scand 46:193–202

Lima PC, Dutra IS, Araújo FAA, Lustosa R, Zeppelini CG, Franke CR (2020) First record of mass wild waterfowl mortality due to *Clostridium botulinum* in Brazilian semiarid. An Acad Bras Cienc 92:e20180370

Liu SY, Li KP, Hsieh MK, Chang PC, Shien JH, Ou SC (2019) Prevalence and genotyping of *Chlamydia psittaci* from domestic waterfowl, companion birds, and wild birds in Taiwan. Vector Borne Zoonotic Dis 19:666–673

Llarena AK, Taboada E, Rossi M (2017) Whole-genome sequencing in epidemiology of *Campylobacter jejuni* infections. J Clin Microbiol 55:1269–1275

Lu X, Ke D, Zeng X, Gong G, Ci R (2009) Status, ecology, and conservation of the Himalayan griffon *Gyps himalayensis* (Aves, Accipitridae) in the Tibetan plateau. Ambio 38:166–173

Luechtefeld NA, Blaser MJ, Reller LB, Wang WL (1980) Isolation of *Campylobacter fetus* subsp. *jejuni* from migratory waterfowl. J Clin Microbiol 12:406–408

Luján-Vega C, Hawkins MG, Johnson CK, Briggs C, Vennum C, Bloom PH, Hull JM, Cray C, Pesti D, Johnson L, Ciembor P, Ritchie BR (2018) Atypical *Chlamydiaceae* in wild populations of hawks (*Buteo* spp.) in California. J Zoo Wildl Med 49:108–115

Lynch MF, Tauxe RV, Hedberg CW (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137:307–315

MacDonald E, White R, Mexia R, Bruun T, Kapperud G, Brandal LT, Lange H, Nygård K, Vold L (2018) The role of domestic reservoirs in domestically acquired *Salmonella* infections in Norway: epidemiology of salmonellosis, 2000-2015, and results of a national prospective case-control study, 2010-2012. Epidemiol Infect 147:e43

Mäesaar M, Tedersoo T, Meremäe K, Roasto M (2020) The source attribution analysis revealed the prevalent role of poultry over cattle and wild birds in human campylobacteriosis cases in the Baltic States. PLoS One 15:e0235841

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM (2010) International collaboration on enteric disease 'burden of illness' studies. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50:882–889

Man SM (2011) The clinical importance of emerging *Campylobacter* species. Nat Rev Gastroenterol Hepatol 8:669–685

Marotta F, Janowicz A, Di Marcantonio L, Ercole C, Di Donato G, Garofolo G, Di Giannatale E (2020) Molecular characterization and antimicrobial susceptibility of *C. jejuni* isolates from Italian wild bird populations. Pathogens 9:304

Martín-Maldonado B, Montoro-Dasi L, Pérez-Gracia MT, Jordá J, Vega S, Marco-Jiménez F, Marin C (2019) Wild Bonelli's eagles (*Aquila fasciata*) as carrier of antimicrobial resistant *Salmonella* and *Campylobacter* in Eastern Spain. Comp Immunol Microbiol Infect Dis 67:101372

Mather AE, Lawson B, de Pinna E, Wigley P, Parkhill J, Thomson NR, Page AJ, Holmes MA, Paterson GK (2016) Genomic analysis of *Salmonella enterica* serovar Typhimurium from wild passerines in England and Wales. Appl Environ Microbiol 82:6728–6735

Mattmann P, Marti H, Borel N, Jelocnik M, Albini S, Vogler BR (2019) Chlamydiaceae in wild, feral and domestic pigeons in Switzerland and insight into population dynamics by *Chlamydia psittaci* multilocus sequence typing. PLoS One 14:e0226088

McGee P, Bolton DJ, Sheridan JJ, Earley B, Kelly G, Leonard N (2002) Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. J Appl Microbiol 93:706–713

Minette HP (1986) Salmonellosis in the marine environment. A review and commentary. Int J Zoonoses 13:71–75

Morabito S, Dell'Omo G, Agrimi U, Schmidt H, Karch H, Cheasty T, Caprioli A (2001) Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. Vet Microbiol 82:275–283

Moriarty EM, Weaver L, Sinton LW, Gilpin B (2012) Survival of *Escherichia coli*, enterococci and *Campylobacter jejuni* in Canada goose faeces on pasture. Zoonoses Public Health 59:490–497

Motarjemi Y, Adams M (2006) Emerging foodborne pathogens, 1st edn. Woodhead Publishing Ltd., Cambridge, England

Moulin-Schouleur M, Répérant M, Laurent S, Brée A, Mignon-Grasteau S, Germon P, Rasschaert D, Schouler C (2007) Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. J Clin Microbiol 45:3366–3376

Mughini-Gras L, Penny C, Ragimbeau C, Schets FM, Blaak H, Duim B, Wagenaar JA, de Boer A, Cauchie HM, Mossong J, van Pelt W (2016) Quantifying potential sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*. Water Res 101:36–45

Mughini-Gras L, Pijnacker R, Coipan C, Mulder AC, Fernandes Veludo A, de Rijk S, van Hoek AHAM, Buij R, Muskens G, Koene M, Veldman K, Duim B, van der Graaf-van BL, van der Weijden C, Kuiling S, Verbruggen A, van der Giessen J, Opsteegh M, van der Voort M, Castelijn GAA, Schets FM, Blaak H, Wagenaar JA, Zomer AL, Franz E (2021) Sources and transmission routes of campylobacteriosis: a combined analysis of genome and exposure data. J Infect 82: 216–226

Mulder AC, Franz E, de Rijk S, Versluis MAJ, Coipan C, Buij R, Müskens G, Koene M, Pijnacker R, Duim B, Bloois LVG, Veldman K, Wagenaar JA, Zomer AL, Schets FM, Blaak H, Mughini-Gras L (2020) Tracing the animal sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*. Water Res 187:116421

Navarro-Gonzalez N, Wright S, Aminabadi P, Gwinn A, Suslow TV, Jay-Russell MT (2020) Carriage and subtypes of foodborne pathogens identified in wild birds residing near agricultural lands in California: a repeated cross-sectional study. Appl Environ Microbiol 86:e01678–e01619

Nemeth NM, Beckett S, Edwards E, Klenk K, Komar N (2007) Avian mortality surveillance for West Nile virus in Colorado. Am J Trop Med Hyg 76:431–437

Nesse LL, Refsum T, Heir E, Nordby K, Vardund TV, Holstad G (2005) Molecular epidemiology of *Salmonella* spp. isolates from gulls, fishmeal factories, animals and humans in Norway based on pulsed-field gel electrophoresis. Epidemiol Infect 133:53–58

Newell DG, Mughini-Gras L, Kalupahana R, Wagenaar JA (2016) *Campylobacter* epidemiology–sources and routes of transmission for human infection. In: Klein G (ed) *Campylobacter*: features, detection, and prevention of foodborne disease. Academic Press, Long, UK; San Diego

Newman EA, Eisen L, Eisen RJ, Fedorova N, Hasty JM, Vaughn C, Lane RS (2015) *Borrelia burgdorferi sensu lato* spirochetes in wild birds in northwestern California: associations with ecological factors, bird behavior and tick infestation. PLoS One 10:e0118146

Nielsen EM, Skov MN, Madsen JJ, Lodal J, Jespersen JB, Baggesen DL (2004) Verocytotoxin-producing *Escherichia coli* in wild birds and rodents in close proximity to farms. Appl Environ Microbiol 70:6944–6947

Oh JY, Kang MS, Hwang HT, An BK, Kwon JH, Kwon YK (2011) Epidemiological investigation of *eaeA*-positive *Escherichia coli* and *Escherichia albertii* strains isolated from healthy wild birds. J Microbiol 49:747–752

Olsen B, Persson K, Broholm KA (1998) PCR detection of *Chlamydia psittaci* in faecal samples from passerine birds in Sweden. Epidemiol Infect 121:481–484

Penfold JB, Amery HC, Peet PJ (1979) Gastroenteritis associated with wild birds in a hospital kitchen. Br Med J 2:802

Pennycott TW, Cinderey RN, Park A, Mather HA, Foster G (2002) *Salmonella enterica* subspecies *enterica* serotype Typhimurium and *Escherichia coli* O86 in wild birds at two garden sites in south-west Scotland. Vet Rec 151:563–567

Pennycott TW, Park A, Mather HA (2006) Isolation of different serovars of *Salmonella enterica* from wild birds in Great Britain between 1995 and 2003. Vet Rec 158:817–820

Perez-Martinez JA, Storz J (1985) Antigenic diversity of *Chlamydia psittaci* of mammalian origin determined by microimmunofluorescence. Infect Immun 50:905–910

Ponitz B, Schmitz A, Fischer D, Bleckmann H, Brücker C (2014) Diving-flight aerodynamics of a peregrine falcon (*Falco peregrinus*). PLoS One 9:e86506

Potier S, Mitkus M, Kelber A (2020) Visual adaptations of diurnal and nocturnal raptors. Semin Cell Dev Biol 106:116–126

Prukner-Radovcić E, Horvatek D, Gottstein Z, Grozdanić IC, Mazija H (2005) Epidemiological investigation of *Chlamydophila psittaci* in pigeons and free-living birds in Croatia. Vet Res Commun 29:17–21

Rapp D, Ross CM, Maclean P, Cave VM, Brightwell G (2021) Investigation of on-farm transmission routes for contamination of dairy cows with top 7 *Escherichia coli* O-Serogroups. Microb Ecol 81:67–77

Riordan T, Humphrey TJ, Fowles A (1993) A point source outbreak of *Campylobacter* infection related to bird-pecked milk. Epidemiol Infect 110:261–265

Rodrigues GCJ, Nair HP, O'Kane C, Walker CA (2021) Prevalence of multidrug resistance in *Pseudomonas* spp. isolated from wild bird feces in an urban aquatic environment. Ecol Evol 11: 14303–14311

Rukambile E, Sintchenko V, Muscatello G, Kock R, Alders R (2019) Infection, colonization and shedding of *Campylobacter* and *Salmonella* in animals and their contribution to human disease: a review. Zoonoses Public Health 66:562–578

Russo TA, Johnson JR (2003) Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes Infect 5:449–456

Russo TP, Pace A, Varriale L, Borrelli L, Gargiulo A, Pompameo M, Fioretti A, Dipineto L (2021) Prevalence and antimicrobial resistance of enteropathogenic bacteria in yellow-legged gulls (*Larus michahellis*) in southern Italy. Animals (Basel) 11:275

Rybak B, Krawczyk B, Furmanek-Blaszk B, Wysocka M, Fordon M, Ziolkowski P, Meissner W, Stepniewska K, Sikorska K (2022) Antibiotic resistance, virulence, and phylogenetic analysis of *Escherichia coli* strains isolated from free-living birds in human habitats. PLoS One 17: e0262236

Sachse K, Laroucau K, Vanrompay D (2015) Avian chlamydiosis. Curr Clin Microbiol R 2:10–21

Sanches LA, Gomes MDS, Teixeira RHF, Cunha MPV, Oliveira MGX, Vieira MAM, Gomes TAT, Knobl T (2017) Captive wild birds as reservoirs of enteropathogenic *E. coli* (EPEC) and Shiga-toxin producing *E. coli* (STEC). Braz J Microbiol 48:760–763

Sauvala M, Woivalin E, Kivistö R, Laukkanen-Ninios R, Laaksonen S, Stephan R, Fredriksson-Ahomaa M (2021) Hunted game birds - carriers of foodborne pathogens. Food Microbiol 98: 103768

Schaufler K, Semmler T, Wieler LH, Wöhrmann M, Baddam R, Ahmed N, Müller K, Kola A, Fruth A, Ewers C, Guenther S (2016) Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410--another successful pandemic clone? FEMS Microbiol Ecol 92:fiv155

Schmidt H, Scheef J, Morabito S, Caprioli A, Wieler LH, Karch H (2000) A new Shiga toxin 2 variant (Stx_{2f}) from *Escherichia coli* isolated from pigeons. Appl Environ Microbiol 66:1205–1208

Seguino A, Chintoan-Uta C, Smith SH, Shaw DJ (2018) Public health significance of *Campylobacter* spp. colonisation of wild game pheasants (*Phasianus colchicus*) in Scotland. Food Microbiol 74:163–170

Senerwa D, Olsvik O, Mutanda LN, Lindqvist KJ, Gathuma JM, Fossum K, Wachsmuth K (1989) Enteropathogenic *Escherichia coli* serotype O111:HNT isolated from preterm neonates in Nairobi, Kenya. J Clin Microbiol 27:1307–1311

Sevilla E, Marín C, Delgado-Blas JF, González-Zorn B, Vega S, Kuijper E, Bolea R, Mainar-Jaime RC (2020) Wild griffon vultures (*Gyps fulvus*) fed at supplementary feeding stations: potential carriers of pig pathogens and pig-derived antimicrobial resistance? Transbound Emerg Dis 67: 1295–1305

Shrestha RD, Midwinter AC, Marshall JC, Collins-Emerson JM, Pleydell EJ, French NP (2019) *Campylobacter jejuni* strains associated with wild birds and those causing human disease in six high-use recreational waterways in New Zealand. Appl Environ Microbiol 85:e01228–e01219

Simpson KMJ, Hill-Cawthorne GA, Ward MP, Mor SM (2018) Diversity of *Salmonella* serotypes from humans, food, domestic animals and wildlife in New South Wales, Australia. BMC Infect Dis 18:623

Smith OM, Snyder WE, Owen JP (2020) Are we overestimating risk of enteric pathogen spillover from wild birds to humans? Biol Rev Camb Philos Soc 95:652–679

Söderlund R, Jernberg C, Trönnberg L, Pääjärvi A, Ågren E, Lahti E (2019) Linked seasonal outbreaks of *Salmonella* Typhimurium among passerine birds, domestic cats and humans, Sweden, 2009 to 2016. Euro Surveill 24:1900074

Soon XQ, Gartrell B, Gedye K (2021) Presence and shedding of *Chlamydia psittaci* in waterfowl in a rehabilitation facility and in the wild in New Zealand. N Z Vet J 69:240–246

Spottiswoode CN, Begg KS, Begg CM (2016) Reciprocal signaling in honeyguide-human mutualism. Science 353:387–389

Steere AC, Sikand VK, Meurice F, Parenti DL, Fikrig E, Schoen RT, Nowakowski J, Schmid CH, Laukamp S, Buscarino C, Krause DS (1998) Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. N Engl J Med 339:209–215

Stephenson I, Democratis J (2006) Influenza: current threat from avian influenza. Br Med Bull 75-76:63–80

Stokes HS, Martens JM, Walder K, Segal Y, Berg ML, Bennett ATD (2020) Species, sex and geographic variation in chlamydial prevalence in abundant wild Australian parrots. Sci Rep 10: 20478

Stokes HS, Berg ML, Bennett ATD (2021) A review of chlamydial infections in wild birds. Pathogens 10:948

Sukon P, Nam NH, Kittipreeya P, Sara-In A, Wawilai P, Inchuai R, Weerakhun S (2021) Global prevalence of chlamydial infections in birds: a systematic review and meta-analysis. Prev Vet Med 192:105370

Taboada EN, Ross SL, Mutschall SK, Mackinnon JM, Roberts MJ, Buchanan CJ, Kruczkiewicz P, Jokinen CC, Thomas JE, Nash JH, Gannon VP, Marshall B, Pollari F, Clark CG (2012) Development and validation of a comparative genomic fingerprinting method for high-resolution genotyping of *Campylobacter jejuni*. J Clin Microbiol 50:788–797

Taff CC, Townsend AK (2017) *Campylobacter jejuni* infection associated with relatively poor body condition and low survival in a wild bird. J Avian Biol 48:1071–1076

Taff CC, Weis AM, Wheeler S, Hinton MG, Weimer BC, Barker CM, Jones M, Logsdon R, Smith WA, Boyce WM, Townsend AK (2016) Influence of host ecology and behavior on *Campylobacter jejuni* prevalence and environmental contamination risk in a synanthropic wild bird species. Appl Environ Microbiol 82:4811–4820

Thomas MK, Murray R, Flockhart L, Pintar K, Pollari F, Fazil A, Nesbitt A, Marshall B (2013) Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. Foodborne Pathog Dis 10:639–648

Thornley CN, Simmons GC, Callaghan ML, Nicol CM, Baker MG, Gilmore KS, Garrett NK (2003) First incursion of *Salmonella enterica* serotype Typhimurium DT160 into New Zealand. Emerg Infect Dis 9:493–495

Thorns CJ (2000) Bacterial food-borne zoonoses. Rev Sci Tech 19:226–239

Tsiodras S, Kelesidis T, Kelesidis I, Bauchinger U, Falagas ME (2008) Human infections associated with wild birds. J Infect 56:83–98

Tucker VA (1998) Gliding flight: speed and acceleration of ideal falcons during diving and pull out. J Exp Biol 201:403–414

van Hoek AHAM, van Veldhuizen JNJ, Friesema I, Coipan C, Rossen JWA, Bergval IL, Franz E (2019) Comparative genomics reveals a lack of evidence for pigeons as a main source of stx_{2f}-carrying Escherichia coli causing disease in humans and the common existence of hybrid Shiga toxin-producing and enteropathogenic *E. coli* pathotypes. BMC Genomics 20:271

Vande Weygaerde Y, Versteele C, Thijs E, De Spiegeleer A, Boelens J, Vanrompay D, Van Braeckel E, Vermaelen K (2018) An unusual presentation of a case of human psittacosis. Respir Med Case Rep 23:138–142

Vaz CS, Voss-Rech D, Pozza JS, Coldebella A, Silva VS (2014) Isolation of *Campylobacter* from Brazilian broiler flocks using different culturing procedures. Poult Sci 93:2887–2892

Vogt NA, Pearl DL, Taboada EN, Reid-Smith RJ, Mulvey MR, Janecko N, Mutschall SK, Jardine CM (2019) A repeated cross-sectional study of the epidemiology of *Campylobacter* and antimicrobial resistant Enterobacteriaceae in free-living Canada geese in Guelph, Ontario, Canada. Zoonoses Public Health 66:60–72

Vogt NA, Stevens CPG, Pearl DL, Taboada EN, Jardine CM (2020) Generalizability and comparability of prevalence estimates in the wild bird literature: methodological and epidemiological considerations. Anim Health Res Rev 21:89–95

Waldenström J, Broman T, Carlsson I, Hasselquist D, Achterberg RP, Wagenaar JA, Olsen B (2002) Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Appl Environ Microbiol 68:5911–5917

Waldenström J, Axelsson-Olsson D, Olsen B, Hasselquist D, Griekspoor P, Jansson L, Teneberg S, Svensson L, Ellström P (2010) *Campylobacter jejuni* colonization in wild birds: results from an infection experiment. PLoS One 5:e9082

Weis AM, Storey DB, Taff CC, Townsend AK, Huang BC, Kong NT, Clothier KA, Spinner A, Byrne BA, Weimer BC (2016) Genomic comparison of *Campylobacter* spp. and their potential for zoonotic transmission between birds, primates, and livestock. Appl Environ Microbiol 82: 7165–7175

Whelan CD, Monaghan P, Girdwood RW, Fricker CR (1988) The significance of wild birds (*Larus* sp.) in the epidemiology of *Campylobacter* infections in humans. Epidemiol Infect 101:259–267

Wiemeyer GM, Plaza PI, Bustos CP, Muñoz AJ, Lambertucci SA (2021) Exposure to anthropogenic areas may influence colonization by zoonotic microorganisms in scavenging birds. Int J Environ Res Public Health 18:5231

Williams J, Tallis G, Dalton C, Ng S, Beaton S, Catton M, Elliott J, Carnie J (1998) Community outbreak of psittacosis in a rural Australian town. Lancet 351:1697–1699

Wobeser GA (2007) Disease in wild animals: investigation and management, 2nd edn. Springer, New York

Zepeda Mendoza ML, Roggenbuck M, Manzano Vargas K, Hansen LH, Brunak S, Gilbert MTP, Sicheritz-Pontén T (2018) Protective role of the vulture facial skin and gut microbiomes aid adaptation to scavenging. Acta Vet Scand 60:61

Bat-Related Zoonoses

34

Bruno B. Chomel, Henri-Jean Boulouis, Chao-chin Chang, Alvaro Aguilar Setién, and Matthew J. Stuckey

Contents

B. B. Chomel (✉)
Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, Davis, CA, USA
e-mail: bbchomel@ucdavis.edu

H.-J. Boulouis
Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, Cedex, France
e-mail: henri-jean.boulouis@vet-alfort.fr

C.-c. Chang
Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung, Taiwan
e-mail: changcc@dragon.nchu.edu.tw

A. A. Setién
Unidad de Investigación Médica en Inmunología, Coordinación de Investigación, Instituto Mexicano del Seguro Social (IMSS, Mexico), Mexico City, DF, Mexico
e-mail: estiviro@hotmail.com

M. J. Stuckey
Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, Davis, CA, USA

Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, Cedex, France

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_28

Abstract

Bats are increasingly implicated as hosts of zoonotic and potentially zoonotic pathogens. As a whole, chiropterans now represent the largest known reservoir of emerging viruses (Calisher et al. 2006; Wong et al. 2007). Among the 60 viral species currently associated with bats, 59 are RNA viruses of importance in the current generation of emerging and reemerging human infections (Wong et al. 2007). Lyssaviruses, paramyxoviruses, filoviruses, and coronaviruses are among viral those pathogens impacting the health and well-being of both people and nonhuman animals around the globe. In comparison to the studies conducted on viral infections, much less attention has been paid to the nonviral pathogens of zoonotic importance within bat populations (Frick et al. 2010; Reichard and Kunz 2009; Van Brussel and Holmes 2021). This is changing, however, as more research is now being conducted to detect and describe bacteria ranging from vector-borne to enteric pathogens, as well as protozoan parasites, and fungal agents in a variety of bat hosts. This chapter focuses on zoonotic pathogens that bats can harbor and potentially transmit to humans.

Keywords

Zoonoses · Bats · Chiropters

34.1 Introduction

Bats are increasingly implicated as hosts of zoonotic and potentially zoonotic pathogens. As a whole, chiropterans now represent the largest known reservoir of emerging viruses (Calisher et al. 2006; Wong et al. 2007). Among the 60 viral species currently associated with bats, 59 are RNA viruses of importance in the current generation of emerging and reemerging human infections (Wong et al. 2007). Lyssaviruses, paramyxoviruses, filoviruses, and coronaviruses are among those pathogens impacting the health and well-being of both people and nonhuman animals around the globe. In comparison to the studies conducted on viral infections, much less attention has been paid to the nonviral pathogens of zoonotic importance within bat populations (Frick et al. 2010; Reichard and Kunz 2009; Van Brussel and

Holmes 2021). This is changing, however, as more research is now being conducted to detect and describe bacteria ranging from vector-borne to enteric pathogens, as well as protozoan parasites, and fungal agents in a variety of bat hosts.

The many emerging infectious diseases associated with chiropteran species can have major impacts on both ecosystem and public health (Calisher et al. 2006; Mühldorfer 2013; Wibbelt et al. 2010; Wood et al. 2012). As such, the scope of this chapter is to provide an overview of those potential bat-related zoonoses and their clinical relevance to people. With increased disease surveillance and a trend toward more human contact with bat populations, it is likely that additional zoonotic diseases will continue to be identified. Bat infection dynamics are driven by a complex interplay of ecological, immunological, behavioral, and anthropogenic factors (Hayman et al. 2013). Interdisciplinary work will be needed in the future to better understand the drivers of disease emergence in bat populations and ultimately mitigate the threats that face both people and bats themselves.

34.2 Viral Zoonoses

34.2.1 Rhabdoviridae

34.2.1.1 Rabies and Rabies-Related Viruses

Among bat-associated viral zoonoses, rabies (RABV) is certainly one of the most widespread in a broad range of bat species and around the world, with several new lyssaviruses identified in recent years. In several countries considered to be free of terrestrial rabies, rabid bats and human cases of bat-associated rabies have been identified in the last decades, such as in Australia where three human cases have occurred and in the United Kingdom, with one human case in Scotland (Banyard et al. 2011; Fooks et al. 2003). In Latin America, more human rabies cases are now related to bat exposure (especially vampire bats) than to dog bites (Condori-Condori et al. 2013). There are 19 known Lyssaviruses identified, 17 of them being present in bats: Aravan, Australian, Bokeloh, Duvenhage, European bat 1 and bat 2, Gannoruwa, Irkut, Khujand, Kotalathi, Lagos bat, Lleida, Malto, rabies, Shimoni, Taiwan, and West Caucasian (Fooks et al. 2021; Van Brussel and Holmes 2021).

Although the opportunity for lyssavirus cross-species transmission seems rare, adaptation in a new host and the possibility of onward transmission to humans require continued investigation (Fooks et al. 2021). Host switching of RABV from bats appears to be more frequent in the Americas, while events involving other Old World (Africa, Europe, and Asia, or Afro-Eurasia) lyssaviruses appear to be rare (Fooks et al. 2021).

In Latin America, a review of the literature through 1990 reported 330 cases of bat-transmitted human rabies (Schneider et al. 2009). These cases, along with PAHO data to the end of 2006, revealed 637 reported cases of bat-transmitted human rabies in Latin America. Of 199 human cases transmitted by bats during the period 1996–2006, 146 (73%) were transmitted by vampire bats, 16 (8%) by nonvampire

bats, and 37 (19%) with no species reported (Schneider et al. 2009). Between 2009 and 2018, 70% (134) of the 192 human cases reported in South America were related to bat exposure, mainly vampire bats, especially in Peru (Meske et al. 2021). For instance, in Peru, during 2002–2007, 293 (77%) of the rabies cases diagnosed were associated with vampire bats, whereas 87 (23%) were related to dog rabies virus variants (Condori-Condori et al. 2013). It was also shown that vampire bat rabies variants spread gradually and involve different vampire bat subpopulations with different transmission cycles. Bovine paralysis caused by rabid vampire bat bites also has a major economic impact on cattle production in Mexico and several South American countries (Streicker et al. 2012). Emergence of rabies in insectivorous bats in several countries in Latin America (such as Argentina, Brazil, Chile, Peru, and Uruguay) has also been reported.

A study took advantage of recent outbreaks of vampire bat rabies among livestock in the Sao Paulo region of Brazil to test whether seroprevalence in *D. rotundus* reflects the incidence of rabies in nearby livestock populations (Megid et al. 2021). Sixty-four *D. rotundus* were captured during and after outbreaks from roost located in municipalities belonging to three regions with different incidences of rabies in herbivores. Sixteen seropositive bats were then kept in captivity for up to 120 days, and their antibodies and virus levels were quantified at different time points using the rapid fluorescent focus inhibition test (RFFIT). Antibody titers were associated with the occurrence of an ongoing outbreak, with a higher proportion of bats showing titer >0.5 IU/ml in the region with a recent outbreak. However, low antibody titers were still detected in bats from regions not reporting a rabies outbreak for the last 3 years prior to sampling. It clearly shows that serological surveillance of rabies in vampire bats can be used as a tool to evaluate the risk of outbreaks in cattle and human populations at risk.

Vaccination is the most effective measure to control rabies in cattle. Similarly, reduction of hematophagous bat populations, using anticoagulants, is a treatment that has also been used since the 1970s. However, this measure is temporary, and it destabilizes vampire populations, causing replacement of animals and consequently displacements of infectious foci (Benavides et al. 2020). Furthermore, using poisonous substances, such as anticoagulants, could affect beneficial animals such as scavengers or insectivores and frugivorous bats.

Since the 1990s, vaccination of bats against rabies has been considered as a useful tool by creating an immune barrier that prevents the spread of the virus, similar to what is done through oral rabies vaccination campaigns in foxes and raccoons (Aguilar-Sétien et al. 1998, 2002; Almeida et al. 2008). This concept has recently been reconsidered in several works (Cárdenas-Canales et al. 2020; Gilbert et al. 2020; Stading et al. 2017; Turmelle et al. 2010). This methodology would have the advantage of not using lethal procedures, while preventing the spread of virus.

In North America, rabies remains an important public health concern in the United States, with most human cases associated with bat rabies virus variants. Cases of rabies virus infection in bats are widely distributed across the continental United States (Patyk et al. 2012). Between 2001 and 2009, more than 205,439 bats were submitted for rabies virus diagnosis, and 6.7% of these bats were rabid. Increased odds of a submitted bat being rabid were associated with species that

exhibit inconspicuous roosting habits, bats originating in the Southwest, and bats submitted for diagnosis during the fall (Patyk et al. 2012). Between 2000 and 2020, 52 human rabies cases were reported in the USA, including 38 Indigenous cases of which 31 (82%) were bat related (Ma et al. 2021). Spillover from bat rabies variants to terrestrial mammals has also been reported (Leslie et al. 2006).

In Europe, bat rabies cases are principally attributed to two lyssaviruses, namely European bat lyssavirus-1 (EBLV-1) and European bat lyssavirus-2 (EBLV-2). Between 1977 and 2011, 961 cases of bat rabies were reported, with the vast majority (>97%) being attributed to EBLV-1, frequently isolated in The Netherlands, Northern Germany, Denmark, Poland, and also in parts of France and Spain (Schatz et al. 2013). At present (2021), 1338 cases have been reported (Rabies Bulletin Europe, accessed October 27, 2021). Most EBLV-2 isolates originated from the United Kingdom (UK) and The Netherlands, and EBLV-2 was also detected in Germany, Finland, and Switzerland. So far, there has been less than 50 cases of EBLV-2 detected in bats in Europe. Bat rabies due to EBLV-2 has been detected in Daubenton's bats (*Myotis daubentonii*) in Great Britain since 1996 (Folly et al. 2021). Across Europe, European bat lyssavirus 1 (EBLV-1) is commonly associated with serotine bats (*Eptesicus serotinus*) (Kohl et al. 2021). Despite the presence of serotine bats across large parts of southern England, EBLV-1 had not previously been detected in this population. However, in 2018, EBLV-1 was detected through passive surveillance in a serotine bat from Dorset, England. Subsequent EBLV-1-positive serotine bats have been identified in South-West England during 2018, 2019, and 2020 (Folly et al. 2021).

In addition, limited isolations of unique lyssaviruses from European insectivorous bats were reported in south-west Russia in 2002 (West Caucasian bat virus, WCBV), in Germany in 2010, and in France in 2012 (Bokeloh bat lyssavirus) (McElhinney et al. 2013; Picard-Meyer et al. 2013), and Lleida bat lyssavirus was identified in a bent-winged bat (*Miniopterus schreibersii*) in Spain (Aréchiga Ceballos et al. 2013). In June 2020, a cat from Arezzo (Italy) that died from a neurological disease was diagnosed with WCBV. The virus retained high identity across the whole-genome with the reference isolate found in 2002 from a Russian bent-winged bat (Leopardi et al. 2021). In a tunnel located near the cat's house, Leopardi et al. (2021) identified a group of bent-winged bats that showed virus-neutralizing antibodies to WCBV across four sampling occasions, but no virus in salivary swabs.

A few human cases related to bat exposure have also been reported from Europe. In Russia in 1985, only one other case of human encephalitis caused by this strain was confirmed, and two more cases of rabies were described in Finland in 1985 and in Scotland in 2002 caused by the EBLV-2 which killed two scientists specializing in bats research. Another bat-related case was also reported in 1977 from Ukraine, but the variant was not determined. The first human case of bat origin in France was reported in 2008 in French Guyana (Meynard et al. 2012). An autochthonous case was also reported in continental France in 2019. A man died of rabies in Limoges, in southwest central France, most probably after being bitten or scratched by a bat. The 59 year-old patient without specific past medical history died from encephalitis in August 2019. A colony of bats lived in an outbuilding of his house. No diagnosis

was made using standard procedures. When trying to identify the cause of this undocumented encephalitis, genetic analysis of postmortem samples showed that he had contracted a lyssavirus, European Bat Lyssavirus type 1 (EBLV-1) (Regnault et al. 2021). A human case caused by the Irkut virus was reported in 2007 from Far East Russia (Leonova et al. 2010).

In Africa, new lyssaviruses have been identified in bats, beside Lagos bat virus first isolated in Nigeria on Lagos Island in 1956 from African straw-colored fruit bat (*Eidolon helvum*) and Duvenhage virus first reported in 1970 in a human case in South Africa. Two more human cases of Duvenhage virus have occurred since then, one in 2006 when a man was scratched on the face by a bat and the last one in a Dutch woman who had visited a cave and had received two superficial wounds on the face from a bat in 2007 (Markotter et al. 1987). No human case caused by Lagos bat virus has been reported so far, but a few cases in cats ($n = 3$) and dogs ($n = 2$) have been reported from Africa (Coertse et al. 2021).

In Asia, limited reports on identification of lyssaviruses or antibodies to lyssaviruses have been published (Liu et al. 2013b). It is certainly a part of the world where new variants are likely to be identified in the near future, when better wildlife rabies surveillance will be set in this part of the world. Many aspects of the ecology of lyssaviruses in bats need still to be investigated, such as low prevalence of infection, potential survival to infection, and effective shedding of the virus. Recently a new Lyssavirus strain was identified as Taiwan bat Lyssavirus identifies in two Japanese pipistrelle bats (*Pipistrellus abramus*) in 2016 and 2017 (Hu et al. 2018). In Australia, three human cases of Australian bat lyssavirus have been identified, two in women rescuing flying foxes (1996, 1998) and one in a young boy who was infected in southern Australia (2013).

The incubation period of rabies in humans is typically 2–8 weeks but can be as short as 10 days and as long as 6 years. Initial signs include headache, slight fever, malaise, and pain at the bite wound. The disease, which lasts from 2 to 6 days without medical support, progresses to paralysis of the muscles of deglutition, hyperesthesia, and generalized convulsions. Death ensues shortly thereafter (Hoar et al. 1998). In bats, infection rate and mortality are usually low, although this has been studied in few species. Experimental studies in vampire bats indicate that a high viral load is necessary to induce mortality, with either no observable clinical signs or squeaking, tremor, paralysis, and loss of appetite (Aguilar-Setien et al. 2005). In cattle infected by vampire bats, rabies is mainly expressed by paralysis with a rather long incubation period (25 to 150 days or more) and lasts for 2 to 5 days before causing death (Hoar et al. 1998).

34.2.2 Paramyxoviridae: Henipaviruses

34.2.2.1 Hendra, Nipah, and Menangle Viruses

Several important zoonotic paramyxoviruses have been associated with animal and human deaths in Australasia since the end of the twentieth century. The henipaviruses are naturally harbored by Pteropid fruit bats (flying foxes) and some microbat species.

Hendra virus: In Australia, Hendra virus was first recognized in 1994 when 21 horses and two humans were infected, leading to the death of 13 horses and one human. As of December 2012, a total of 45 outbreaks of Hendra virus have occurred in north-eastern Australia, all involving infection of horses (Aljofan 2013). As a result of these events, more than 100 animals (105 horses and two dogs) in 63 natural spillover events have died or been euthanized by March 2021 (Annand et al. 2021). These cases have all occurred in Queensland and in northeast New South Wales. Case fatality rate in humans is 60% (4 of 7 recorded cases) and in horses 75%. Human infections with Hendra virus range from mild influenza-like illness to fatal respiratory or neurological disease. Infected people initially develop fever, headaches, myalgia (muscle pain), sore throat, and a dry cough. They could also have enlarged lymph nodes, lethargy, and vertigo. The incubation period ranges from 5 to 14 days. Hendra virus is transmitted to people through close contact with infected horses or their body fluids. To date, no human-to-human transmission of Hendra virus has been documented. No specific treatment is available, but a vaccine has been developed for immunization of horses and is available since the end of 2012. The following signs have all been associated with Hendra virus cases in horses, but not all these signs will be found in any one infected horse: rapid onset of illness, increased body temperature/fever and heart rate, discomfort/weight shifting between legs, depression, and rapid deterioration with either respiratory and/or nervous signs. Respiratory signs include respiratory distress, increased respiratory rates, and nasal discharge at death that can be initially clear until progressing to stable white froth and/or stable blood-stained froth. Nervous signs include wobbly gait, apparent loss of vision in one or both eyes, aimless walking in a dazed state, head tilting and circling, muscle twitching, urinary incontinence, and inability to rise. Horses get infected when very high concentrations of virus material are deposited directly under trees in what is call the "drip zone," and almost no virus is deposited once the horses leave the perimeter of the trees. This area of the trees where the spats and the urine of feeding flying foxes will be dropped potentially poses an extremely high risk for horses (Australian Veterinary Association: http://www.ava.com.au/hendra-virus). The natural reservoirs are the black flying fox (*Pteropus alecto*), the grey headed flying fox (*P. poliocephalus*), the spectacled flying fox (*P. conspicillatus*), and the little red flying fox (*P. scapulatus*) present in the urine and birthing fluids of these bats (Van Brussel and Holmes 2021). A new virus variant was recently identified (Annand et al. 2021; Wang et al. 2021). Since 2013, 98 flying foxes were tested and 11 were positive for the new HeV variant (Wang et al. 2021). No samples were positive for the original HeV. Ten of the positive samples were from grey-headed flying foxes (*Pteropus poliocephalus*); however, this species was overrepresented in the opportunistic sampling, as 83% of bats tested were *P. poliocephalus*.

Nipah virus (NiV): In Malaysia and Singapore, in late 1998 and early 1999 an outbreak of human disease characterized by febrile encephalitis among pig farmers, which appeared to be linked to cases of respiratory and neurological disease in commercially farmed pigs, was described as well as in 11 employees at a slaughter plant in Singapore (Aljofan 2013; Clayton et al. 2013). There were 265 patients, of

whom 105 died, reported as having NiV-induced viral encephalitis, mostly among adult males who were involved in pig farming or pork production activities. However, the reported number of patients who survived the acute NiV encephalitis was 160 with 7.5% prevalence of relapsed encephalitis (12/160 patients) more than 24 months after the outbreak. Of the 89 patients previously known to have non-encephalitic or asymptomatic Nipah virus infection, 3 (3.4%) developed late-onset encephalitis. Most patients presented with a severe acute encephalitic syndrome, but some also had significant pulmonary manifestations. The Malaysian outbreak was controlled by the culling of over one million pigs and strict quarantine measures on pig movements.

Nipah virus reemerged in 2001 in outbreaks of human disease in India and Bangladesh. Since 2001, outbreaks of NiV infection have occurred almost annually in Bangladesh, with many outbreaks featuring smaller clusters of cases (Clayton et al. 2013). A second outbreak in India, close to the Bangladesh border, was reported in 2007. Sequencing and genetic characterization of these isolates revealed that they were closely related to, but distinguishable from, the causative agent of disease in Malaysia. Since the emergence of NiV in Bangladesh and India, and an outbreak in the Philippines (meat consumption from sick horses) due to a virus closely related to the Nipah virus, at least 646 human cases have been identified, with an overall case fatality of (386/646) 59.7% (Hauser et al. 2021). Most cases are related to consumption of unwashed fruits or palm juice contaminated by fruit bats secretions (saliva, urine, and fecal materials). Outbreaks in Bangladesh and India were characterized by bat (*Pteropus medius*, the Indian flying fox)-to-human and human-to-human transmissions. *Pteropus* spp. serve as the wildlife reservoir for NiV across a wide area of South-east Asia, including countries from which no known outbreaks have emerged such as Cambodia, Thailand, Indonesia, and Papua New Guinea (Van Brussel and Holmes 2021). Seropositive bats for henipaviruses were also detected in Madagascar, Ghana, and a henipavirus, or henipa-like virus, also appears to circulate in both fruit bats and microbats in China (Clayton et al. 2013).

Menangle virus: The Menangle virus, another paramyxovirus, was first identified in 1997 after a piggery in Menangle (New South Wales) experienced a high number of stillbirths (Aljofan 2013; Hoar et al. 1998). Two workers at the piggery became ill with unexplained, flu-like symptoms but subsequently recovered. Investigations later found that the virus was transmitted from a nearby population of flying foxes, through pigs which act as a carrier of the virus. Bats appear to be an asymptomatic host, with infection caused through contact with body fluids of infected animals. Like for Hendra virus, the grey headed flying fox, the black flying fox, and the spectacled flying fox were identified as the likely reservoirs (Van Brussel and Holmes 2021).

New potentially zoonotic paramyxovirus: A novel morbillivirus from a vespertilionid bat species (*Myotis riparius*) in Brazil named myotis bat morbillivirus (MBaMV) was identified by metagenomics. MBaMV used *Myotis* spp. CD150 much better than human and dog CD150 in fusion assays, using live MBaMV that was rescued by reverse genetics. However, MBaMV replicated efficiently in primary human myeloid but not lymphoid cells, suggesting a potential zoonotic availability (Lee et al. 2021).

34.2.3 Filoviridae

34.2.3.1 Marburg and Ebola Viruses

Marburg virus: Marburg virus causes sporadic outbreaks of severe hemorrhagic disease in sub-Saharan Africa. Bats have been implicated as likely natural reservoir hosts based most recently on an investigation of cases among miners infected in 2007 at the Kitaka mine, Uganda, which contained a large population of Marburg virus-infected *Rousettus aegyptiacus* fruit bats (Amman et al. 2012).

In July and September 2007, miners working in Kitaka Cave, Uganda, were diagnosed with Marburg hemorrhagic fever. The likely source of infection in the cave was Egyptian fruit bats (*Rousettus aegyptiacus*) based on detection of Marburg virus RNA in 31/611 (5.1%) bats, virus-specific antibody in bat sera, and isolation of genetically diverse virus from bat tissues (Towner et al. 2009). The virus isolates were collected 9 months apart, demonstrating long-term virus circulation. The bat colony was estimated to be over 100,000 animals using mark and recapture methods, predicting the presence of over 5000 virus-infected bats. The genetically diverse virus genome sequences from bats and miners closely matched. These data indicate common Egyptian fruit bats can represent a major natural reservoir and source of Marburg virus with potential for spillover into humans.

A study conducted at Python Cave in Uganda, where an American and a Dutch tourist acquired Marburg virus infection in December 2007 and July 2008, found that about 2.5% of more than 1600 bats captured between August 2008 and November 2009 were actively infected with the virus, 7 of which yielded Marburg virus isolates (Amman et al. 2012). Moreover, Q-RT-PCR-positive lung, kidney, colon, and reproductive tissues were found, consistent with potential for oral, urine, fecal, or sexual transmission. The combined data for *R. aegyptiacus* tested from Python Cave and Kitaka mine indicate low-level horizontal transmission throughout the year. However, Q-RT-PCR data showed distinct pulses of virus infection in older juvenile bats (~6 months of age) that temporarily coincide with the peak twice-yearly birthing seasons. Retrospective analysis of historical human infections suspected to have been the result of discrete spillover events directly from nature found 83% (54/65) events occurred during these seasonal pulses in virus circulation, perhaps demonstrating periods of increased risk of human infection.

The incubation period ranges from 2 to 21 days, and the clinical outcome can be divided into three phases: initial generalized phase (day 1–4), early organ phase (day 5–13), and either a late organ or convalescence phase (day 13 onward). Pigott et al. (2015) reported ten natural outbreaks in Africa between 1975 and 2014, with three of them affecting more than ten people in Democratic Republic of Congo (Durba): 128 deaths out of 154 cases between October 1998 and August 2000; in Angola (Uige): 227 deaths out of 252 cases between October 2004 and July 2005; and in Uganda (Ibanda): 14 deaths out of 15 cases between August and October 2012.

Ebola virus: Evidence of Ebola virus antibodies was reported in various bat species in Africa (Pourrut et al. 2009) and of Ebola-Reston virus in *Rousettus amplexicaudatus* bats from the Philippines (Taniguchi et al. 2011). In Africa, 1030

animals were captured in Gabon and the Republic of Congo, including 679 bats, 222 birds, and 129 small terrestrial vertebrates, and were tested for evidence of infection by Ebola virus (Leroy et al. 2005). Of the infected animals identified during these field collections, immunoglobulin G (IgG) specific for Ebola virus was detected in serum from three different bat species (4/17 *Hypsignathus monstrosus*, 8/117 *Epomops franqueti,* and 4/58 *Myonycteris torquata*). Viral nucleotide sequences were detected in livers and spleens in other bats from the same populations (4/21, 5/117, and 4/141, respectively). No viral RNA was detected in kidney, heart, or lung in these animals after amplification by polymerase chain reaction (PCR), and no viral nucleotide sequences were revealed in any of the other animal species tested.

Twelve years after the Kikwit Ebola outbreak in 1995, Ebola virus reemerged in the Occidental Kasaï province of the Democratic Republic of Congo (DRC) between May and November 2007, affecting more than 260 humans and causing 186 deaths (Leroy et al. 2009). During the latter outbreak, several epidemiological investigations were conducted to identify the underlying ecological conditions and animal sources. Qualitative social and environmental data were collected through interviews with villagers and by direct observation (Leroy et al. 2009). The local populations reported no unusual morbidity or mortality among wild or domestic animals, but they described a massive annual fruit bat migration toward the southeast, up the Lulua River. Migrating bats settled in the outbreak area for several weeks, between April and May, nestling in the numerous fruit trees in Ndongo and Koumelele islands as well as in palm trees of a largely abandoned plantation. They were massively hunted by villagers, for whom they represented a major source of protein. By tracing back the initial human-to-human transmission events, it was shown that in May the putative first human victim bought freshly killed bats from hunters to eat. This study provided the most likely sequence of events linking a human Ebola outbreak to exposure to fruit bats, a putative virus reservoir. Such findings support the suspected role of bats in the natural cycle of Ebola virus and indicate that the massive seasonal fruit bat migrations should be considered in operational Ebola risk maps and seasonal alerts in the DRC (Leroy et al. 2009).

34.2.4 Coronaviridae

34.2.4.1 SARS- COVID19 and MERS-Coronaviruses

Severe acute respiratory syndrome (SARS) was first reported in February 2003 in China. When the World Health Organization declared the outbreak over on 5 July 2003, more than 8000 cases (and almost 800 fatal) had been reported in 32 countries worldwide (Field 2009; Wang et al. 2006). Initial symptoms are flu-like and may include fever, myalgia, lethargy symptoms, cough, sore throat, and other nonspecific symptoms, leading to sever pneumonia. The only symptom common to all patients appears to be a fever above 38 °C (100 °F). Shortness of breath may occur later.

A succession of phylogenetic and epidemiological findings suggested that SARS had a wildlife origin, and that “wet markets” in southern China were the origin of the

outbreak. Subsequently, two groups independently identified SARS-like coronaviruses (SARS-CoV) in species of bats in China. Li et al. (2005) reported serological and molecular evidence of a cluster of SARS-like coronaviruses in several species of free-living horseshoe bats (*Rhinolophus* spp., especially *R. sinicus*) in southern China. They contend that the virus responsible for the SARS outbreak in humans in 2003 emerged from this cluster of viruses, and that bats are the origin of the SARS coronavirus. *Rhinolophus* species are more likely to foster host shifts of coronaviruses than other bat species; this propensity, when combined with the potential for close contact between bats, civets, and humans in the wildlife trade in southern China, supports SARS-like coronaviruses as being the source of the SARS coronavirus (Field 2009; Van Brussel and Holmes 2021). The majority of the coronaviruses originated from African, Asian, and European bats (Corman et al. 2013). In addition to SARS-CoV, four human coronaviruses (HCoVs), termed HCoV-OC43, −229E, -NL63, and -HKU1, are known. Recently, a sixth HCoV was described, the MERS-CoV, which can cause coughing, fever, and pneumonia. This virus emerged in Saudi Arabia in 2012 and has been reported in some other Gulf States, France, Germany, Italy, Tunisia, Korea, and Britain [all cases to date can be epidemiologically linked to Saudi Arabia, Qatar, United Arab Emirates, and Jordan]. Since the disease was first identified in Saudi Arabia in April 2012, 2594 cases of Middle East respiratory syndrome coronavirus (MERS-CoV) have been detected in 27 countries of which 942 (36.3%) have died (ECD, as of October 4, 2024, consulted on October 27, 2021). Close relatives of this betacoronavirus termed MERS-CoV and of HCoV-229E exist in Old World bats, especially of the genus *Pipistrellus,* and HCoV-NL63 could be grown in immortalized bat cells, demonstrating the zoonotic potential of previously reservoir-bound bat CoVs (Van Brussel and Holmes 2021). The recent description of a bat CoV related to MERS-CoV in Mexican bats (Anthony et al. 2013) and in bats from Saudi Arabia (Memish et al. 2013) emphasized the relevance of investigating neotropical bats for CoVs.

SARS-CoV-2: This virus emerged in late 2019 in the city of Wuhan, Popular Republic of China. Several of the early cases of SARS-CoV-2 have been linked to the Huanan market in Wuhan, China. Given the SARS-CoV pandemic and the resulting increased interest in bat CoV, a bat CoV (RaTG13, 96.2% id) detected in *Rhinolophus affinis* in the Yunnan province was quickly identified as the closest relative. SARS-CoV and SARS-CoV-2 share 79.6% sequence identity only, although both viruses are using the ACE2 receptor for cell entry. The origin of this pandemic is still not clear, as suspicion of a bat origin is likely, but how it reached humans has not been validated. The lack of clarity from Chinese authorities about the first human cases cannot rule out a possible laboratory leak, as emergence from the Wuhan wet market cannot be proven nor confirmed. Five SARS-CoV-2-related coronaviruses have been identified in three *Rhinolophus* species (*R. malayanus*, *R. pusillus,* and *R. marshalli*) in Laos, with three of them that can bind to the human ACE2 receptor (Van Brussel and Holmes 2021).

It is so far the largest pandemic in recent history leading to several millions of deaths worldwide within less than 24 months. Several outbreaks were also reported in wild captive felids, in mink farms, and even in domestic carnivores (mainly cats and dogs) owned by people who got infected by the virus and likely were the source

of their pet infection (Haake et al. 2020). Experimentally, it has been demonstrated that SARS-CoV-2 replicates more efficiently in cats than in dogs and that cats can transmit the virus through aerosols. With approximately 470 million pet dogs and 370 million pet cats cohabitating with their human owners worldwide, the finding of natural SARS-CoV-2 infection in these household pets has important implications for potential zoonotic transmission events during the COVID-19 pandemic as well as future SARS-related outbreaks (Murphy and Ly 2021).

Identification of sequences of a group C betacoronavirus (β)-CoV in bat guano was recently reported (Wacharapluesadee et al. 2013). The detection of nucleic acid of this group C (β)-CoV and the previous isolation of viruses from bat feces and urine warrant some concerns that guano miners might be exposed to bat pathogens in fresh excreta as well as in soil substances. Therefore, bat guano miners should use preventive measures of personal hygiene and improved barrier protection to reduce the possibility of exposure to zoonotic pathogens.

34.2.5 Other Viral Pathogens

Many other viruses have been isolated or detected by molecular methods or by the presence of specific antibodies in bats, such as Hantaan virus in various bat species in Asia and Africa (Hance et al. 2006; Hoar et al. 1998; Wong et al. 2007), Japanese encephalitis virus in China (Liu et al. 2013a), Venezuelan equine encephalitis virus in vampire bats, and antibodies in bats from Guatemala (Hoar et al. 1998). In their review, Hoar et al. (1998) reported also detection of Chikungunya virus in African bats (*Scotophilus* sp.), Rio Bravo virus in Mexican free-tailed bats, and Rift valley fever virus in bats from the Republic of Guinea. In Europe, most viruses were found in Myotis spp., Pipistrellusspp., and Eptesicus spp. (Kohl et al. 2021).

In Uganda, four human cases of Kasokero virus isolated from *Rousettus aegyptiacus* bats living in the Kasokero cave occurred in laboratory workers (Kalunda et al. 1986). Infected laboratory workers had fever, headache, abdominal pain and diarrhea, sever myalgia, and arthralgia. Signs lasted 7 to 10 days and were followed by complete recovery. It was demonstrated that 67% of 74 bats from that cave were seropositive for Kasokero virus. Kyasanur virus has been isolated from bats in India, and the Vesicular Stomatitis Virus (New Jersey type), which causes flu-like symptoms in infected humans, has been isolated from bats in Panama and Guatemala (Hoar et al. 1998).

34.2.5.1 HepaDNAviruses, Hepatitis C-like and Hepatitis D (ex-Delta virus), and Bats

In recent years, several viruses related to human hepatitis viruses (Hepatitis A, Hepatitis B and D, Hepatitis C, and Hepatitis E) have been identified in bats as well as domestic animals (cats, dogs, and horses) (Bergner et al. 2021a; Drexler et al. 2012, 2013, 2015; Quan et al. 2013).

Hepatitis E virus (HEV) is one of the most common causes of acute hepatitis in tropical and temperate climates. Tropical genotypes 1 and 2 are associated with

foodborne and waterborne transmission. Zoonotic reservoirs (mainly pigs, wild boar, and deer) are considered for genotypes 3 and 4, which exist in temperate climates. In view of the association of several zoonotic viruses with bats, 3869 bat specimens from 85 different species and from five continents were analyzed for hepevirus RNA (Drexler et al. 2012). HEVs were detected in African, Central American, and European bats, forming a novel phylogenetic clade in the family Hepeviridae. Bat hepeviruses were highly diversified and comparable to human HEV in sequence variation. No evidence for the transmission of bat hepeviruses to humans was found in over 90,000 human blood donations and individual patient sera.

The hepatitis B virus (HBV), family Hepadnaviridae, is one of most relevant human pathogens. HBV origins are enigmatic, and no zoonotic reservoirs are known. Total 3080 specimens from 54 bat species representing 11 bat families were screened for hepadnaviral DNA (Drexel et al. 2013). Ten specimens (0.3%) from Panama and Gabon yielded unique hepadnaviruses in coancestral relation to HBV. Hepatic tropism in bats was shown by quantitative PCR and in situ hybridization. Infected livers showed histopathologic changes compatible with hepatitis. Human hepatocytes transfected with all three bat viruses cross-reacted with sera against the HBV core protein, concordant with the phylogenetic relatedness of these hepadnaviruses and HBV. Phylogenetic analyses carried out with generated virus sequences suggested that bat HBV was more closely related to primate HBV than to those of other mammalian orders (Bonvicino et al. 2014). These data suggest that bats may have been ancestral sources of primate hepadnaviruses.

Hepatitis delta virus (HDV) is an unusual RNA agent that replicates using host machinery but exploits hepatitis B virus (HBV) to mobilize its spread within and between hosts (Bergner et al. 2021a). In doing so, HDV enhances the virulence of HBV. Among 96,695 RNA sequence datasets, delta viruses were shown to infect bats, rodents, and an artiodactyl from the Americas but were absent from geographically overrepresented Old-World representatives of each mammalian order, suggesting a relatively recent diversification within the Americas (Bergner et al. 2021b). A field study also showed that common vampire bats (*Desmodus rotundus*) in Peru were infected by two divergent delta viruses, indicating multiple introductions into a single host species. One vampire bat-associated delta virus was detected in the saliva of up to 35% of individuals, formed phylogeographically compartmentalized clades, and infected a sympatric bat, illustrating horizontal transmission within and between species on ecological timescales (Bergner et al. 2021b). Consistent absence of HBV-like viruses in two delta virus-infected bat species indicated acquisitions of novel viral associations during the divergence of bat- and human-infecting delta viruses. Such data are supportive of an American zoonotic origin of HDV and reveal prospects for future cross-species emergence of delta viruses.

Hepatitis A: The hepatitis A virus (HAV) is a leading cause of acute viral hepatitis worldwide. It is transmitted mainly by direct contact with patients who have been infected or by ingesting contaminated water or food. The virus is endemic in low-income countries where sanitary and sociodemographic conditions are poor. Paradoxically, improving sanitary conditions in these countries, which reduces the incidence of HAV infections, can lead to more severe disease in susceptible adults.

The populations of developed countries are highly susceptible to HAV, and large outbreaks can occur when the virus is spread by globalization and by increased travel and movement of foodstuffs (Migueres et al. 2021). The existence of evolutionarily ancestral hepatoviruses in bats and shrews compared with the presence of more closely related viruses in rodents and primates is reminiscent of hantavirus host associations (Drexler et al. 2015). Of eight HAV-positive sera (7.3%), six were from West African *Eidolon helvum*, and one each from Central African *Rousettus aegyptiacus* and *Micropteropus pusillus* (Drexler et al. 2015).

34.3 Dengue (DENV) and Zika (ZIKV)

Bats are important natural reservoir hosts of a diverse range of viruses that can be transmitted to humans and have been suggested to be involved in the transmission cycle of Dengue (DEN) and Zika (ZIK) viruses. However, the exact role of bats in the epidemiology of Flaviviruses is still controversial.

34.3.1 Dengue Virus

The presence of antibodies against dengue virus (DENV) and/or viral RNA have led to the hypothesis that neotropical bats could have a potential role in the transmission cycles of DENV (Aguilar-Setién et al. 2008; de Thoisy et al. 2009; Machain-Williams et al. 2013; Sotomayor-Bonilla et al. 2014; Calderón et al. 2019, 2021). Considering some previous reports about the susceptibility of bats to DENV (Reagan and Brueckner 1952; Platt et al. 2000), the Aguilar-Setién group carried out a sampling of neotropical bats from DENV-endemic areas of the Pacific and Gulf coasts of Mexico, finding that out of 162 sampled bats of several species, 19 (12%) presented DENV antibodies (Aguilar-Setién et al. 2008). In this study, DENV serotype 2 was also detected by RT–PCR in four samples from three bat species: two frugivorous, *Artibeus jamaicensis* (2/9) and *Carollia brevicauda* (1/2), and one insectivorous, *Myotis nigricans* (1/11) (Aguilar-Setién et al. 2008). Supporting this finding, DENV RNA was reported in 14 species of neotropical bats sampled between 2001 and 2007 in French Guyana (de Thoisy et al. 2009). Other authors have also reported the presence of antibodies and/or RNA of DENV in bats from different countries in the Americas (Calderón et al. 2019, 2021; Machain-Williams et al. 2013; Sotomayor-Bonilla et al. 2014). Furthermore, an experimental infection of *Artibeus* bats with different viral loads of DENV-2 was performed (Perea-Martínez et al. 2013). Unexpectedly, a high percentage (43%) of bats developed macroscopic lesions consisting of bruises (hemorrhage) on the chest and/or on the wings (Fig. 1). Histological analyses showed structural alterations in the spleen and bleeding in the liver and intestine, but the virus was not detected by RT-PCR in any of the analyzed tissues except for one infected bat kidney, by seminested RT-PCR. In sera, the viral RNA was detected by seminested RT-PCR in 39% of bats, but only 8% of bats seroconverted. Overall, these data indicate that DENV-2 replicates poorly in these

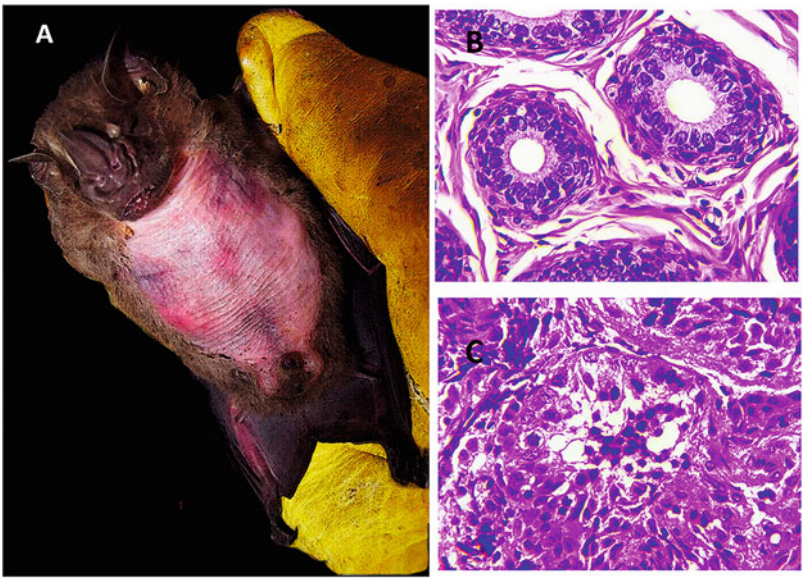

Fig. 1 Some of the macroscopic and microscopic changes founded in experimentally infected bats with DENV or ZIKV. (**a**) Hemorrhages in a bat experimentally infected with DENV B and C: Epididymis H&E 600 X . (**b**) Control *Artibeus* bat non infected; (**c**) *Artibeus* bat inoculated with ZIKV

bats, suggesting they are not suitable hosts to this virus (Perea-Martínez et al. 2013). It is possible that the observed macroscopic lesions (hemorrhage) and histopathological alterations could not be due directly to DENV as experiments were made using bats captured in the wild and other factors as other microbiological and/or physical agents could intervene in such pathologic manifestations.

Supporting the poor replication of DENV in experimentally infected *Artibeus* bats, no evidence of sustained replication of DENV was documented in experimentally inoculated *Artibeus jamaicensis* bats with DENV serotypes 1 or 4 (Cabrera-Romo et al. 2014).

Additionally, in vitro studies using several neotropical bat cell lines and experimental infection of *Artibeus* bats with DENV confirmed that these species of bats are inadequate hosts and likely do not play an important role in DENV transmission (Bittar et al. 2018; Cabrera-Romo et al. 2014; Moreira-Soto et al. 2017).

34.3.2 ZIKA Virus

For ZIKV, anecdotal experimental infections and field studies performed in the 1950s–1960s have documented the susceptibility and presence of clinical signs in African and American bat species (Reagan et al. 1955; Shepherd and Williams 1964; Simpson et al. 1968). Based on these previous studies and considering the

neotropical bat species richness as well as limited information of ZIKV hosts to date, 1872 bats were sampled (blood for ZIKV detection by qRT-PCR) within the ZikAlliance consortium, belonging to different neotropical species and countries (Peru, French Guyana, and Costa Rica) between 2010 after the emergence of Zika virus into the Americas (2015) and 2019 before the arrival of the disease. Sampling included 33 genera from 6 bat families. None of these 1872 bats was positive for either Zika antibodies or RNA. This lack of detection was consistent with another serosurvey of Brazilian bats (Bittar et al. 2018). However, a sampling bias cannot be excluded, due to the diversity of bat species in Latin America.

In 2019, ZIKV experimental infection was performed in a breeding colony *of Artibeus jamaicensis* bats. ZIKV antigens were detected in several organs (salivary glands, testes, SNC, and lung), and antibodies identified by ELISA were observed in less than half of the nine studied animals (Malmlov et al. 2019). ZIKV antigens were detected by PCR in only three samples (two urine samples and one nervous tissue) (Malmlov et al. 2019). Some ZIKV inoculated animals presented histopathological alterations in testes and CNS. These results raised the possibility that bats may have a role in ZIKV ecology which could even endanger bat populations. Another ZIKV experimental infection of *Artibeus* bats captured in the wild was conducted in Mexico (Aguilar-Setién et al., unpublished data). The results were consistent with Malmlov et al. 2019, findings, as ZIKV RNA was detected by rtPCR in only two urine samples, and none of the experimentally infected animals presented antibodies against ZIKV, measured by the more specific virus neutralization test (L'Huillier et al. 2017). As in the first study, in the present also histopathological alterations were found in infected animals mainly in testicles, ovaries, and SNC of some infected animals. Additionally, as it was reported for DENV, we observed that some infected animals showed hemorrhages on the chests, wings, and one animal presented on the bladder, as with DENV, without this being shown to directly attribute to ZIKV (Fig. 1).

Despite the difficulties in detecting ZIKV RNA in free-living bats, a recent Mexican study has detected ZIKV RNA in 9% (2/22) of *Artibeus* bats in Merida, Yucatan (Torres-Castro et al. 2021). Similarly, in our ZIKV experimental infection study, we had to exclude a few individuals that tested positive prior to inoculation for ZIKV RNA in urine samples (Aguilar-Setién et al., unpublished results).

As with the DENV, the set of obtained results with ZIKV seems to indicate that the bats studied are inadequate ZIKV hosts and maybe do not play an important role in ZIKV transmission. Nevertheless, a consistent fact in the two studies of experimental infection of bats with ZIKV carried out to date is the appearance of histopathological alterations mainly in the reproductive tract which opens the possibility that this virus could affect the reproduction of these animals.

34.3.3 Bats as Potential Reservoirs or Vectors of DENV and ZIKV

Some reports that inform concerning the presence of antibodies and/or DENV or ZIKV RNA speculate about how Chiropters could be contaminated with these Flaviviruses. The most logical explanations are that DENV Or ZIKV vectors feed on bats or that bats were contaminated consuming DENV or ZIKV vectors. Vicente-Santos et al.

(2017) in a peri-domestic study found only limited exposure of bats to a DENV likely due to closeness to humans and consumption of DENV vectors (Vicente-Santos et al. 2017). Nevertheless, information on vectors of arbovirus feeding on bats and consumption of these vectors by bats is scarce. *Aedes* mosquitoes are the main vectors of ZIKV and DENV. Some studies demonstrated that *Aedes funereus* mosquitoes fed on flying foxes (*Pteropus* sp.) (Ryan et al. 1997). In an experiment, *Artibeus* bats were bitten by *Aedes aegypti* mosquitoes in an attempt to infect these mammals with DENV (Cabrera-Romo et al. 2014), suggesting that *Aedes* mosquitoes could feed on bats. However, it is not known how often this happens and in which bat species. DENV and ZIKV have been isolated from mosquitoes other than *Aedes* such as *Culex* and *Mansonia* (Musso and Gubler 2016). DENV serotype 2 (DENV-2) was reported for the first time in Sao Paulo, Brazil, in *Culex spp*. and *Culex vaxus* pools, while ZIKV was identified in *Anopheles cruzii*, *Limatus durhamii,* and *Wyeomyia confusa* pools suggesting the possibility of a sylvatic enzootic cycle in these areas (Barrio-Nuevo et al. 2020). However, it is not known if these mosquito species feed on bats or are consumed by bats (Barrio-Nuevo et al. 2020).

Regarding the relationship of mosquitoes other than *Aedes* and bats, Tiawsirisup et al. (2012) collected mosquitoes from five genera inside a bat cave in Thailand to investigate the sylvatic circulation of Japanese Encephalitis Virus (JBEV). These collections included *Culex quinquefasciatus*, which was shown to mainly feed on Leschnault's rousette (*Rousettus leschenaulti*) bats. In the State of New York, *Culiseta morsitans* mosquitoes, the known vector of Eastern Equine Encephalitis Virus (EEEV), were found to have unfrequently fed on Eastern pipistrelle bats (*Pipistrellus subflavus*), as these blood meals comprised only 1% of the total blood meals identified from this mosquito species (Molaei et al. 2006). In Africa, blood of Egyptian roussette (*Rousettus aegyptiacus*) bat was detected in *Coquillettidia fuscopennata* mosquitoes in the Congo basin region (Crabtree et al. 2013). Insectivorous bats play an important role to reduce harmful insect populations and may constitute an appropriate biological control system for many mosquito-borne zoonotic viruses with a direct impact on human health (Hutson et al. 2001; Puig-Montserrat et al. 2020). In Canada, *Myotis lucifugus* was shown to feed predominately on mosquitoes suggesting that this species could play an important role in biological control of these important viral vectors; in Australia, foraging ranges of insectivorous bats *Vespadelus vulturnus* were shown to shift relative to changes in mosquito *Aedes vigilax* abundance (Facione et al. 1991). It is not known if bats could be contaminated by eating mosquitoes harboring arboviruses. Logically, in frugivorous bats, this source of contamination is less likely; however, it has been shown that frugivorous species could shift their diets to some degree of insect consumption to meet their protein requirements (Dinerstein 1986; Herrera et al. 2002).

The susceptibility of bat-adapted ectoparasitic diptera to DENV or ZIKV is not well known. In Hidalgo Mexico, Flavivirus sequences in 38 pools of specific ectoparasites (Diptera: Streblidae, *Strebla wiedemanni,* and *Trichobius parasiticus*) of common vampire bats (*Desmodus rotundus*) were detected by RT-PCR using primers specifically directed against the NS5 gene, a gene highly conserved among Flaviviruses. Phylogenetic inference analysis performed using the maximum likelihood algorithm showed that six sequences clustered with DENV (Abundes-Gallegos

et al. 2018). In order to verify the susceptibility of the bat ectoparasitic fly *Strebla wiedemanni* to DENV infection, we performed an experimental infection of organ explants of *S. wiedemanni* using as control *Melophagus ovinus* (a sheep ectoparasitic fly) organ explants, and C6/36 cells culture was performed. Viral titers (UFP/mL) were determined at 0, 48, and 96 hours post infection (PI). Infected organs were observed by electron microscopy and under the confocal microscopy indirect immunofluorescence (IIF) using specific conjugates against DENV. The infected organs of both species of ectoparasites replicated DENV at similar titers to those obtained with the C6/36 cell cultures ($\geq 10^6$ UFP/mL). Electron microscopy and IIF showed DENV replication in the digestive tract, tracheoles, and milk glands (MG) of both fly species. Areas with a high affinity for the DENV were observed in the fatty bodies of the MG of *M. ovinus* (Aguilar-Setién et al. 2017). The replication of DENV in organs of *S. wiedemanni* and *M. ovinus* was thus demonstrated in this work. Such data could suggest parallel cycles with maintenance of DENV in other nonclassical vectors and mammal hosts but will need to be confirmed (Table 1).

The results obtained from sampling different species of bats show a low and infrequent presence of DENV and ZIKV antibodies and/or viral RNA ranging from negative results (Bittar et al. 2018; Cabrera-Romo et al. 2016) to less than 15% (Aguilar-Setién et al. 2008; Calderón et al. 2019, 2021; de Thoisy et al. 2009; Irving et al. 2020; Sotomayor-Bonilla et al. 2014), confirming bat exposure to DENV and ZIKV in different geographic areas, where bats may be either accidental hosts or dead-end hosts.

Concerning experimental infections of bats with DENV and ZIKV, results indicate that bat species studied replicated poorly these Flaviviruses suggesting that they are not suitable hosts and do not play an important role in the epidemiology of these zoonotic viruses. Nevertheless, some histopathological alterations were observed mainly in the reproductive tract of bats infected with ZIKV suggesting a clinical impact on infected bats.

Most field or experimental studies were conducted using either blood samples (sera for serological tests) or different tissues such as spleen, heart, kidney, or liver for viral RNA detection. However, urine samples were rarely analyzed. Some studies in humans showed that ZIKV RNA can be detected in urine samples at higher levels and for a longer time period after the onset of infection compared to blood and other fluids (Duarte et al. 2017; Lamb et al. 2016; Van den Bossche et al. 2015). In the two experimental infections of *Artibeus* bats with ZIKV, viral RNA was only detected in four urine samples and in one nervous tissue sample, but not in other organs, despite the fact that ZIKV antigens were detected by immunohistochemical tests in testes or lungs of these bats. It suggests that urine samples may be most appropriate to seek for flaviviruses in bats. Fagre et al. (2021) recently demonstrated that subgenomic flavivirus RNA (sfRNA) of the 3′ untranslated region (UTR), which persists in tissues due to XRN-1 stalling during RNA decay, was more effective to detect ZIKV RNA in bats than the more commonly used amplification of NS5 gen sequences; as in experimentally infected bats with ZIKV, viral RNA was detected in most tissues when primers directed against the 3′ untranslated region (UTR) (sfRNA) were used, while none was positive when using primers directed against NS5 gen (Fagre et al. 2021).

Table 1 Family and genera of neotropical bats sampled for ZIKV anitibodies and viral RNA by the ZikAlliance Consortium*. All animals resulted negative

Family	Genera
Phyllostomidae	*Anoura*
	Artibeus
	Carollia
	Chiroderma
	Dermanura
	Desmodus
	Glossophaga
	Linchonycteris
	Lionycteris
	Lonchaphylla
	Lonchorhina
	Lophostoma
	Mimon
	Mycronycteris
	Phylloderma
	Phyllostomus
	Platyrrhinus
	Rhinophylla
	Sturnira
	Tonatia
	Trachops
	Uroderma
Molosidae	*Cynomops*
	Eumops
	Molossus
Vespertilonidae	*Eptesicus*
	Rhogeessa
	Myotis
Emballonuridae	*Peropteryx*
	Rhynchonycteris
	Saccopteryx
Noctilionidae	*Noctilio*
Mormoopidae	*Pteronotus*

ZikaAlliance Consortum: Blood sampled were taken in Costa Rica French Guyana, México and Peru by: Aguilar-Setién A, Salas Rojas M, Gálvez Romero G, Almazán Marín C, Moreira Soto A, Alfonso-Toledo J, Obregón Morales C, García Flores M, García Baltazar A, Serra-Cobo J, López-Roig M, Reyes Puma N, Piche-Ovares M, Romero-Vega M, Barrantes-Murillo D, Soto-Garita C, Alfaro-Alarcón A, Corrales-Aguilar E and Drexler JF

Japanese encephalitis: A recent study in Indonesia suggests a reservoir role for Japanese encephalitis virus in bats from western Kalimantan (Diptyanusa et al. 2021). As the presence of pig holdings is uncommon in West Kalimantan, another reservoir host might have played a role in the local transmission of JE virus in this

area. A total of 373 blood samples from bats were tested for JE virus, among which 21 samples (5.6%) showed positive results, mainly from *Cynopterus brachyotis* (lesser short-nosed fruit bat) found in residential areas. In Chinese bats, a high-genetic homogeneity among the bat JEVs isolated in different geographical areas from various bat species at different time periods was observed. All eight bat JEV isolates belonged to genotype III, indicating that bats might be involved in the natural cycle of JEV (Liu et al. 2013a).

34.4 Bacterial Zoonoses

34.4.1 Enteropathogenic Bacteria

34.4.1.1 *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter*

Enteric pathogens such as *Salmonella*, *Shigella, Yersinia,* and *Campylobacter* species have occasionally been found in bats (Mühldorfer 2013). A variety of different *Salmonella* serotypes have been isolated from apparently healthy and diseased bats. Almost all of them are serotypes with a broad host-range. *Salmonella* Enteritidis and *Salmonella* Typhimurium have been frequently identified, which belong to a small group of *Salmonella* serotypes mainly associated with disease in humans and animals. Both serotypes have been isolated from organ tissues of three individual bats of the family *Vespertilionidae* that were found dead or severely injured near human habitations (Mühldorfer 2013). It was also reported in vampire bats (Hoar et al. 1998). In Trinidad, of 377 tested bats, representing 12 species, 4 bats (1.1%) were positive for *Salmonella* spp., 49 (13.0%) were positive for *E. coli*, and no bats were positive for *E. coli* O157 or *Campylobacter* spp. (Adesiyun et al. 2009). Isolated serotypes of *Salmonella* included Rubislaw and Molade, both from *Noctilio leporinus*, a fish-eating bat, Caracas recovered from *Molossus major*, and *Salmonella* Group I from *Molossus ater*, both insect-eating bats. Of the 49 isolates of *E. coli* tested, 40 (82%) exhibited resistance to one or more antimicrobial agents. The presence of *Campylobacter* and *Salmonella* was examined in 631 fresh fecal samples of wild insectivorous bats from the Netherlands, using a specially developed method for the simultaneous isolation of low numbers of these pathogens in small-sized fecal samples ($\leq$ 0.1 g) (Hazeleger et al. 2018). Salmonella was not detected, but thermotolerant *Campylobacter* species were confirmed in 3% ($n = 17$) of the bats belonging to six different bat species, at different sites, in different ecosystems during the whole bat flying season. Molecular typing of these 17 isolated strains indicated *C. jejuni* ($n = 9$), *C. coli* ($n = 7$), and *C. lari* ($n = 1$), including genotypes also found in humans, wildlife, environmental samples, and poultry.

Shigella, causing a dysenteric infection in humans, was isolated from a *Molossus bondae* bat in Colombia (Arata et al. 1968). *Shigella* strains of serogroups B to D have been isolated from mega- and microbats of diverse feeding habitats (Mühldorfer 2013). *Shigella flexneri* in particular was detected in more than 3% of bats investigated.

A high prevalence of different *Yersinia* species (~35%) was detected in the feces of 70 insectivorous *Myotis myotis* collected from natural populations in Poland (Mühldorfer 2013). Most of the *Yersinia* species isolated from bats are widely distributed in the environment and rarely associated with disease in mammals and birds. Cases of systemic *Y. pseudotuberculosis* infection have been described once in an adult insectivorous bat (*M. myotis*) found dead in Germany after hibernation (Mühldorfer 2013) and a bat in England (Hoar et al. 1998), respectively. More recently, *Yersinia enterocolitica* was also isolated from the intestines of 3 out of 11 dead *Miniopterus schreibersii* bats in the Republic of Georgia after a massive die off in a cave (Imnadze et al. 2020).

In the same country, several zoonotic pathogens were detected in bats (Bai et al. 2017), as 218 bats belonging to eight species collected from four regions of Georgia were examined for *Bartonella* (see below), *Brucella*, *Leptospira*, and *Yersinia* using molecular approaches. *Brucella* DNA was detected in two *Miniopterus schreibersii* bats and in two *Myotis blythii* bats, all of which were from Imereti (west-central region). *Leptospira* DNA was detected in 25 (13%) bats that included 4 *M. schreibersii* bats and 21 *M. blythii* bats collected from two regions. The *Leptospira* sequences represented five genetic variants with one of them being closely related to the zoonotic pathogen *L. interrogans* (98.6% genetic identity). No *Yersinia* DNA was detected in the bats. Mixed infections were observed in several cases. One *M. blythii* bat and one *M. schreibersii* bat were coinfected with *Bartonella, Brucella*, and *Leptospira*; one *M. blythii* bat and one *M. schreibersii* bat were coinfected with *Bartonella* and *Brucella*; and 15 *M. blythii* bats and 3 *M. schreibersii* bats were coinfected with *Bartonella* and *Leptospira* (Bai et al. 2017).

34.4.2 Vector-Borne Bacteria

34.4.2.1 *Borrelia*, *Bartonella*, and *Neorickettsia*

Several *Borrelia* and *Bartonella* species and the causative agent of Potomac horse fever disease *Neorickettsia risticii* have been detected in blood and organ tissues of bats (Mühldorfer 2013). The majority of infected animals appear to be healthy; only two vespertilionid bats (*Pipistrellus* sp. and *Natalus tumidirostris*) revealed severe borrelial spirochetemia.

In recent years, many new *Bartonella* species have been isolated or detected from bats around the world, including the United Kingdom, Kenya, Guatemala, Peru, the Republic of Georgia (Bai et al. 2012, 2017), Taiwan (Lin et al. 2012), France, and Mexico (Stuckey et al. 2017a, c). In the Republic of Georgia, *Bartonella* DNA was detected in 77 (35%) bats from all eight species and was distributed in all four regions. The prevalence ranged 6–50% per bat species. The *Bartonella* DNA represented 25 unique genetic variants that clustered into 21 lineages (Bai et al. 2017). Phylogenetic analyses of *Bartonella* strains derived from bats identified several distinct phylogroups indicating the presence of a variety of novel *Bartonella* species in bats. It is notable that bats of the same species as well as bats of the same geographic origin and ecological niche (i.e., *Desmodus rotundus*, members of the

family Vespertilionidae) shared closely related strains of *Bartonella* (Stuckey et al. 2017b). It is not known if these *Bartonella* species are zoonotic. Furthermore, soft ticks (family Argasidae) and other ectoparasites commonly found on bats or in bat habitats are infected with *Bartonella*, *Borrelia,* and *Rickettsia* species, posing a potential risk of intra- and interspecies transmission cycles between bats, humans, and domestic animals (Mühldorfer 2013).

In a European study, 221 bat fecal and 118 bird pellet samples were collected from Hungary and the Netherlands and screened for a broad range of vector-borne bacteria using PCR-based methods (Hornok et al. 2018). *Rickettsia* DNA was detected in 13 bat-fecal DNA extracts, including the sequence of a rickettsial insect endosymbiont, a novel *Rickettsia* genotype, and *Rickettsia helvetica*. Fecal samples of the pond bat (*Myotis dasycneme*) were positive for a *Neorickettsia* sp. (*N. risticii*) and for hemoplasmas of the *Mycoplasma haemofelis* group. Therefore, bats can pass rickettsia and hemoplasma DNA in their feces (Hornok et al. 2018).

34.4.3 Other Bacterial Pathogens

A variety of pathogenic *Leptospira* species have been identified in bats in Asia, Europe, Australia, and the Americas (Bessa et al. 2010; Cox et al. 2005; Hoar et al. 1998; Mühldorfer 2013; Vashi et al. 2010). To date, *Leptospira* infection has been evidenced in over 50 bat species belonging to eight of the nine investigated bat families, encompassing various geographical regions in the tropics and subtropics, as well as Europe, although to a limited extent (Dietrich et al. 2015). The prevalence of leptospiral infections in bats varied from almost 2% to 35% depending on the sample size of the respective study. The family Phyllostomidae comprised the majority of microbats infected with *Leptospira*, whereas in obligate insectivorous species (i.e., families Vespertilionidae and Molossidae) leptospiral infection with pathogenic strains has occasionally been found. In Australia, native flying fox populations (genus *Pteropus*) were suggested as possible carriers of pathogenic *Leptospira* responsible for infections in humans and other animals because of high bacterial detection rates in kidney (11%) and urine samples (39%) and high seroprevalences (18%, 28%) (Mühldorfer 2013). Similarly, bats from Madagascar and Comoros islands harbor a notable diversity of *Leptospira* spp., a finding similar to the diversity found in a comparable investigation of bats in the Amazon region (Lagadec et al. 2012; Matthias et al. 2005). Leptospirosis incubation is 1–32 days (median 9 days) and median duration is 14 days. Most symptomatic patients develop a mild illness consisting of fever, chills, headache, and myalgia. Severe forms of the disease may manifest in acute renal failure, hepatitis, jaundice, myocarditis and meningoencephalitis, and outbreaks of severe pulmonary hemorrhagic leptospirosis have occurred resulting in high morbidity and mortality (Leshem et al. 2011). More recent data of *Leptospira* infection in bats have been reported (Cilia et al. 2021; Dietrich et al. 2015). Direct transmission of bat-borne *Leptospira* to humans has already been suggested, but never evidenced, following a case of serologically confirmed human leptospirosis after bat exposure (Dietrich et al. 2015).

A few other zoonotic agents, such as *Coxiella burnetii*, the agent of Q fever, or *Mycobacterium bovis,* were isolated from bats in Morocco and southern USSR and from captive Indian fruit bats in England, respectively (Hoar et al. 1998). Agglutinin antibodies against *Brucella* were detected in 5 of 53 vampire bats captured in areas of Brazil where incidence of brucellosis in cattle was high (Ricciardi et al. 1976). Similarly, *Coxiella burnetii* DNA was detected in bats, as four samples (3.4%, 95% CI, 0.9–8.3) out of 119 bats were positive for the htpAB gene of *C. burnetii* [spleen (2), liver (1), and heart (1)] (Ferreira et al. 2018). Several *Pasteurella* species (i.e., *P. multocida*, *P. pneumotropica,* and *Pasteurella* species B) have been identified as primary pathogens in bats responsible for a variety of localized and systemic infections in European bat species; most *Pasteurella* strains isolated from organ tissues of 29 vespertilionid bats represented *P. multocida* ssp. *septica* (85%) and capsular type A (75%) (Mühldorfer 2013).

34.5 Protozoan Parasites

34.5.1 *Trypanosoma*, *Toxoplasma*, *Coccidia*, and *Leishmania*

Few parasites of bats are known to be pathogenic to humans and are usually transmitted mechanically via an intermediate vector (Hoar et al. 1998). Many species of trypanosomes can infect bats, but one of main concern is *Trypanosoma cruzi*, the agent of Chagas disease. Bats have long been associated with blood-borne protozoal trypanosomes of the *Schizotrypanum* subgenus, which includes the zoonotic parasite *Trypanosoma cruzi*, agent of Chagas disease (Hodo et al. 2016). Another member of the subgenus, *Trypanosoma dionisii*, infects bats of Europe and South America, and genetic similarities between strains from the two continents suggest transcontinental movement of this parasite via bats. Hearts and blood from eight species of insectivorous bats from 30 counties across Texas were collected (Hodo et al. 2016). Using PCR and DNA sequencing, 593 bats for trypanosomes were tested, with 1 bat positive for *T. cruzi* (0.17%), 9 for *T. dionisii* (1.5%), and 5 for *Blastocrithidia* spp. (0.8%), a group of insect trypanosomes. The *T. cruzi*-infected bat was carrying TcI, the strain type associated with human disease in the USA. In the T. dionisii-infected bats, three unique variants associated with the three infected bat species were detected. A new genotype of *T. cruzi*, associated with bats from anthropic areas and which could be a potential source of infection to humans, has been described (Marcili et al. 2009). Chagas disease is commonly transmitted by reduviid bed bugs. In humans, the disease is characterized by high fever, adenitis, anemia, and facial edema in the acute form and myocarditis in the chronic form. Pathogenicity of bat trypanosomes for humans is not clearly established. *T. cruzi* has been detected in vampire bats, *Desmodus rotundus*, which can be of concern in terms of zoonotic transmission, as these bats feed on mammals, including humans (Ramírez et al. 2013).

Infection of bats with *Toxoplasma gondii* has been reported based on serological studies and more recently on its isolation from bats in Brazil (Cabral et al. 2013; Sun et al. 2013). Therefore, consumption of undercooked bats could be a source of

human infection. In bats, systemic toxoplasmosis caused by *T. gondii* was diagnosed in two juvenile, captive flying-foxes (*Pteropus conspicillatus* and *P. scapulatus*), which died following respiratory distress. One animal displayed clinical signs suggestive of neurological disease (Sangster et al. 2012).

Coccidia of the genus *Eimeria* have been isolated from several species of bats in many parts of the world (Hoar et al. 1998). Many new *Eimeria* species have been reported (McAllister et al. 2012). Prevalence in bats is usually low (<1 to 5%), and it is not known if they are pathogens for humans (Hoar et al. 1998).

Leishmaniasis is a zoonotic disease caused by parasites of the genus *Leishmania*. It has expanded beyond its natural range and is becoming increasingly urban (Shapiro et al. 2013). Using PCR and PCR-RFLP, *Leishmania (Viannia) braziliensis* was detected in two bats (Chiroptera) in Mato Grosso do Sul, Brazil, an endemic area. The animals testing positive were found in both a rural site and an urban site. These results indicate the need for further research into the viability of *Leishmania* in bats. It could have implications for public health in that part of Brazil, given the large populations of urban bats, their mobility, and their ability to roost at close proximity to humans within residences and other buildings (Shapiro et al. 2013). In Spain, samples from spleen, hair, and blood were analyzed to detect *L. infantum* DNA in bats from the Community of Madrid (Azami-Conesa et al. 2020). Infection by *L. infantum* was detected in 59.2% of the bats studied ($n = 16/27$), with the spleen being selected as the site for detection, yielding 14/16 positive results (87.5% sensitivity), followed by hair ($n = 7/16$) and blood ($n = 6/16$), the first report of *L. infantum* detection in the common urban bat (*Pipistrellus pipistrellus*) in Europe.

34.6 Fungal Pathogens

34.6.1 *Histoplasma*, *Coccidioides*, and Other Fungal Infections

Despite the emergence of white nose syndrome caused by *Pseudogymnoascus destructans,* which destroyed an estimated 6 to 7 million bats in North America in recent years (first reported in 2007 in some New York state caves), the main zoonotic fungal diseases related to bats is histoplasmosis and to a lesser extent coccidioidomycosis and a few other fungal infections also identified in bats (Hoar et al. 1998).

Histoplasmosis: Histoplasmosis is caused by *Histoplasma capsulatum*, a dimorphic fungus that is endemic in the Americas and parts of Asia and Africa (Hoar et al. 1998). There are two varieties that are pathogenic to humans, var. *duboisii* and var. *capsulatum*. The former exists only in Africa, while var. *capsulatum* is most prevalent in regions of North, Central, and South America, but has also been reported from parts of Africa, Southern and Eastern Europe, Eastern Asia, and Australia (Cottle et al. 2013). It grows as a mold in soil enriched with bird or bat guano; human infection occurs after inhalation of the dust generated when such soil is disturbed. Visiting caves, collecting or being exposed to bat guano, are the main sources of human contamination from bats (CDC 2012; Cottle et al. 2013; Hoar et al. 1998; Jülg et al. 2008; Kajfasz and Basiak 2012; Schwarz and Kauffman 1977). The

threat of *Histoplasma capsulatum* infection in bat-inhabited caves should be emphasized to travelers and also to physicians, as 3 of 4 travelers were hospitalized after returning from visiting bat-infested caves in Ecuador and having contact with bat guano (Kajfasz and Basiak 2012). Bats usually are healthy carriers and shed the fungus in their feces. In humans, clinical manifestations in humans vary according to host immunity and exposure intensity, ranging from asymptomatic infection (in most healthy persons exposed to a low inoculum; about 80% of the time) to life-threatening pneumonia with respiratory failure (Cottle et al. 2013; Hoar et al. 1998). Between these extremes, clinical presentations include acute or subacute pulmonary disease, pericarditis, rheumatological syndromes with erythema nodosum, progressive disseminated disease, and mediastinal complications. Acute pulmonary histoplasmosis in returning travelers typically presents as a flu-like illness with high-grade fever, chills, headache, nonproductive cough, pleuritic chest pain, and fatigue. Chest radiographs often show diffuse reticulonodular infiltrates and mediastinal lymphadenopathy. Symptom onset is usually 1 to 3 weeks following exposure, and most individuals recover spontaneously within 3 weeks. Disseminated disease is a rare complication, more likely to occur in persons with severely impaired cellular immunity (Cottle et al. 2013). The African species, *H. capsulatum* var. *duboisii,* is associated with cutaneous lesions and occasionally infection of long bones (Hoar et al. 1998).

Coccidioidomycosis: *Coccidioides immitis*, causing coccidioidomycosis, also known as valley fever in California, has been isolated from bat guano (Krutzsch and Watson 1978). Coccidioidomycosis is a systemic disease caused by *Coccidioides immitis* and *C. posadasii* spp., which are predominant in arid zones of the American continent, mainly in the Southwestern United States and the northern states of Mexico, as well as other regions with different environmental conditions (Welsh et al. 2012). Some countries of Central and South America are also endemic zones. Most infected patients are asymptomatic. Disseminated disease develops in less than 5% of clinically affected individuals. Culture, biopsy, and DNA probes are used for fungus identification. Prognosis is related to low antibody detection and a positive intradermic skin reaction to coccidioidin. Immunosuppressed patients and pregnant women require special attention in diagnosis, therapy, and prognosis. Amphotericin B in its different forms, itraconazole, and fluconazole, is the most frequently used treatment. Both fungi have been detected in bats and bat guano (Krutzsch and Watson 1978; de Cordeiro et al. 2012). In Brazil, *Coccidioides posadasii* was recovered from *Carollia perspicillata* bat lungs (de Cordeiro et al. 2012). Immunologic studies detected coccidioidal antibodies and antigens in *Glossophaga soricina* and *Desmodus rotundus* bats.

Candidiasis: Candida albicans, which causes mucocutaneous candidiasis ("thrush" or oropharyngeal candidiasis) in the mouth or throat of humans, was isolated from liver, kidney, spleen, and intestinal content of several bats captured in Nigeria (Oyeka 1994). The most common symptoms of oral thrush in humans are white patches or plaques on the tongue and other oral mucous membranes. It was indicated that bat consumption is common in that country and people could get infected by improper handling of bats or consumption of raw or undercooked bat meat (Oyeka 1994). In a recent study conducted in Brazil, 7 (12.3%) of 57 bats

showed yeasts in their feces. Five species of the genus *Candida* were isolated: *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. pelliculos* (Botelho et al. 2012).

Other fungal infections: Other fungal infections have been described in bats, some of which could potentially be transmitted to humans. Bats are susceptible hosts and reservoirs for *Histoplasma capsulatum, Paracoccidioides brasiliensis,* and *Sporotrichum schenckii* (Raymond et al. 1997). *Sporothrix schenckii, Scopulariopsis* sp., and *Cryptococcus neoformans* have been isolated from bats or bat guano in the Americas (Hoar et al. 1998; Kajihiro 1965). Blastomycosis is a granulomatous disease of mucous membranes. *Blastomyces dermatitidis* has been isolated from the lungs of an asymptomatic insectivorous bat (*Rhinopoma hardwickei hardwickei*) from India, and insectivorous bats orally inoculated with *B. dermatitidis* transiently shed viable organisms in their feces. Mexican free-tailed bats (*Tadarida brasiliensis*) intraperitoneally injected with *B. dermatitidis* developed systemic blastomycosis and excreted viable fungi in their feces. Apparently, bats can serve as both hosts and vectors for *B. dermatitidis* and may be potential sources for human infection (Raymond et al. 1997).

34.7 Conclusion

Bats themselves have an undeniable impact on our planet; with over 1200 chiropteran species identified to date, bats comprise one-fifth of all mammalian species globally and provide critical ecosystem services ranging from pollination to insect control (Wibbelt et al. 2010). Their vast numbers, capability of flight, and a variety of ecological, immunological, and socioeconomic factors also enable bats to transmit an increasingly recognized spectrum of pathogens (Calisher et al. 2006; Mühldorfer 2013; Wibbelt et al. 2010; Wood et al. 2012). The potential for the emergence of zoonoses will continue to increase, as demonstrated by the COVID19 pandemic, as human development encroaches on bat populations. As such, future research will be needed to monitor infection and better understand those underlying drivers of disease.

34.8 Cross-References

► A Review of Hendra Virus and Nipah Virus Infections in Man and Other Animals
► Elimination of Rabies: A Missed Opportunity

References

Abundes-Gallegos J, Salas-Rojas M, Galvez-Romero G, Perea-Martínez L, Obregón-Morales CY, Morales-Malacara JB, Chomel BB, Stuckey MJ, Moreno-Sandoval H, García-Baltazar A, Nogueda-Torres B, Zuñiga G, Aguilar-Setién A (2018) Detection of dengue virus in bat flies (*Diptera: Streblidae*) of common vampire bats, *Desmodus rotundus*, in Progreso, Hidalgo, Mexico. Vector Borne Zoonotic Dis 18(1):70–73

Adesiyun AA, Stewart-Johnson A, Thompson NN (2009) Isolation of enteric pathogens from bats in Trinidad. J Wildl Dis 45:952–961

Aguilar-Sétien A, Brochier B, Tordo N, De Paz O, Desmettre P, Péharpré D, Pastoret PP (1998) Experimental rabies infection and oral vaccination in vampire bats (*Desmodus rotundus*). Vaccine 16(11–12):1122–1126. https://doi.org/10.1016/s0264-410x(98)80108-4

Aguilar-Setién A, Leon YC, Tesoro EC, Kretschmer R, Brochier B, Pastoret PP (2002) Vaccination of vampire bats using recombinant vaccinia-rabies virus. J Wildl Dis 38(3):539–544

Aguilar-Setien A, Loza-Rubio E, Salas-Rojas M, Brisseau N, Cliquet F, Pastoret PP, Rojas-Dotor S, Tesoro E, Kretschmer R (2005) Salivary excretion of rabies virus by healthy vampire bats. Epidemiol Infect 133:517–522

Aguilar-Setién A, Romero-Almaraz ML, Sánchez-Hernández C, Figueroa R, Juárez-Palma LP, García-Flores MM, Vázquez-Salinas C, Salas-Rojas M, Hidalgo-Martínez AC, Pierlé SA, García-Estrada C, Ramos C (2008) Dengue virus in Mexican bats. Epidemiol Infect 136(12):1678–1683

Aguilar-Setién A, Baltazar AG, Leyva IO, Rojas MS, Koldenkova VP, García MP, Ceballos NA, Romero GG, Villegas EOL, Malacara JBM, Marín CA (2017) Ectoparasitic hematophagous dipters: potential reservoirs of dengue virus? Gac Med Mex 153:S82–S90

Aljofan M (2013) Hendra and Nipah infection: emerging paramyxoviruses. Virus Res 117(2):119–126. S0168-1702(13)00265-7

Almeida MF, Martorelli LF, Aires CC, Barros RF, Massad E (2008) Vaccinating the vampire bat *Desmodus rotundus* against rabies. Virus Res 137(2):275–277. https://doi.org/10.1016/j.virusres.2008.07.024. Epub 2008 Sep 17

Amman BR, Carroll SA, Reed ZD, Sealy TK, Balinandi S, Swanepoel R, Kemp A, Erickson BR, Comer JA, Campbell S, Cannon DL, Khristova ML, Atimnedi P, Paddock CD, Crockett RJ, Flietstra TD, Warfield KL, Unfer R, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Rollin PE, Ksiazek TG, Nichol ST, Towner JS (2012) Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. PLoS Pathog 8(10):e1002877

Annand EJ et al (2021) Novel Hendra virus variant detected by sentinel surveillance of Australian horses. Emerg Infect Dis 21:1245. https://doi.org/10.1101/2021.07.16.452724. (preprint)

Anthony S, Ojeda-Flores R, Rico-Chávez O, Navarrete-Macias I, Zambrana-Torrelio C, Rostal MK, Epstein JH, Tipps T, Liang E, Sanchez-Leon M, Sotomayor-Bonilla J, Aguirre AA, Avila R, Medellín RA, Goldstein T, Suzán G, Daszak P, Lipkin WI (2013) Coronaviruses in bats from Mexico. J Gen Virol 94(Pt 5):1028–1038

Arata AA, Vaughn JB, Newell KW, Barth RA, Gracian M (1968) *Salmonella* and *Shigella* infections in bats in selected areas of Colombia. Am J Trop Med Hyg 17:92–95

Aréchiga Ceballos N, Vázquez Morón S, Berciano JM, Nicolás O, Aznar López C, Juste J, Rodríguez Nevado C, Aguilar Setién A, Echevarría JE (2013) Novel lyssavirus in bat, Spain. Emerg Infect Dis 19:793–795

Azami-Conesa I, Martínez-Díaz RA, González F, Gómez-Muñoz MT (2020) First detection of *Leishmania infantum* in common urban bats *Pipistrellus pipistrellus* in Europe. Res Vet Sci 132:172–176. https://doi.org/10.1016/j.rvsc.2020.06.019. Epub 2020 Jun 19

Bai Y, Recuenco S, Gilbert AT, Osikowicz LM, Gómez J, Rupprecht C, Kosoy MY (2012) Prevalence and diversity of *Bartonella* spp. in bats in Peru. Am J Trop Med Hyg 87:518–523

Bai Y, Urushadze L, Osikowicz L, McKee C, Kuzmin I, Kandaurov A, Babuadze G, Natradze I, Imnadze P, Kosoy M (2017) Molecular survey of bacterial zoonotic agents in bats from the country of Georgia (Caucasus). PLoS One 12(1):e0171175. https://doi.org/10.1371/journal.pone.0171175. PMID: 28129398; PMCID: PMC5271587

Banyard AC, Hayman D, Johnson N, McElhinney L, Fooks AR (2011) Bats and lyssaviruses. Adv Virus Res 79:239–289

Barrio-Nuevo KM, Cunha MS, Luchs A, Fernandes A, Rocco IM, Mucci LF, de Souza RP, Medeiros-Sousa AR, Ceretti-Junior W, Marrelli MT (2020) Detection of Zika and dengue viruses in wild-caught mosquitoes collected during field surveillance in an environmental protection area in São Paulo, Brazil. PLoS One 15(10):e0227239

Benavides JA, Valderrama W, Recuenco S, Uieda W, Suzán G, Avila-Flores R, Velasco-Villa A, Almeida M, Andrade FAG, Molina-Flores B, Vigilato MAN, Pompei JCA, Tizzani P, Carrera

JE, Ibanez D, Streicker DG (2020) Defining new pathways to manage the ongoing emergence of bat rabies in Latin America. Viruses 12(9):1002. https://doi.org/10.3390/v12091002. PMID: 32911766; PMCID: PMC7551776

Bergner LM, Becker DJ, Tello C, Carrera JE, Streicker DG (2021a) Detection of *Trypanosoma cruzi* in the saliva of diverse neotropical bats. Zoonoses Public Health 68(3):271–276. https://doi.org/10.1111/zph.12808. Epub 2021 Jan 23

Bergner LM, Orton RJ, Broos A, Tello C, Becker DJ, Carrera JE, Patel AH, Biek R, Streicker DG (2021b) Diversification of mammalian deltaviruses by host shifting. Proc Natl Acad Sci U S A 118(3):e2019907118. https://doi.org/10.1073/pnas.2019907118. PMID: 33397804; PMCID: PMC7826387

Bessa TA, Spichler A, Chapola EG, Husch AC, de Almeida MF, Sodré MM, Savani ES, Sacramento DR, Vinetz JM (2010) The contribution of bats to leptospirosis transmission in Sao Paulo City, Brazil. Am J Trop Med Hyg 82:315–317

Bittar C, Machado RRG, Comelis MT, Bueno LM, Morielle-Versute E, Beguelini MR, de Souza RP, Nogueira ML, Rahal P (2018) Lack of serological and molecular evidence of arbovirus infections in bats from Brazil. PLoS One 13(11):e0207010

Bonvicino CR, Moreira MA, Soares MA (2014) Hepatitis B virus lineages in mammalian hosts: potential for bidirectional cross-species transmission. World J Gastroenterol 20(24):7665–7674. https://doi.org/10.3748/wjg.v20.i24.7665. PMID: 24976704; PMCID: PMC4069295

Botelho NS, de Paula SB, Panagio LA, Pinge-Filho P, Yamauchi LM, Yamada-Ogatta SF (2012) *Candida* species isolated from urban bats of Londrina-Paraná, Brazil and their potential virulence. Zoonoses Public Health 59:16–22

Cabral AD, Gama AR, Sodré MM, Savani ES, Galvão-Dias MA, Jordão LR, Maeda MM, Yai LE, Gennari SM, Pena HF (2013) First isolation and genotyping of *Toxoplasma gondii* from bats (Mammalia: Chiroptera). Vet Parasitol 193:100–104

Cabrera-Romo S, Recio-Tótoro B, Alcalá AC, Lanz H, del Ángel RM, Sánchez-Cordero V, Rodríguez-Moreno Á, Ludert JE (2014) Experimental inoculation of *Artibeus jamaicensis* bats with dengue virus serotypes 1 or 4 showed no evidence of sustained replication. Am J Trop Med Hyg 91(6):1227–1234

Cabrera-Romo S, Max Ramirez C, Recio-Tótoro B, Tolentino-Chi J, Lanz H, Del Ángel RM, Sánchez-Cordero V, Rodríguez-Moreno Á, Ludert JE (2016) No evidence of dengue virus infections in several species of bats captured in central and southern Mexico. Zoonoses Public Health 63(8):579–583

Calderón A, Guzmán C, Mattar S, Rodriguez V, Martínez C, Violet L, Martínez J, Figueiredo LTM (2019) Dengue virus in bats from Córdoba and Sucre, Colombia. Vector Borne Zoonotic Dis 19 (10):747–751

Calderón A, Guzmán C, Oviedo-Socarras T, Mattar S, Rodríguez V, Castañeda V, Moraes Figueiredo LT (2021) Two cases of natural infection of Dengue-2 virus in bats in the Colombian Caribbean. Trop Med Infect Dis 6(1):35

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T (2006) Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev 19:531–545

Cárdenas-Canales EM, Gigante CM, Greenberg L, Velasco-Villa A, Ellison JA, Satheshkumar PS, Medina-Magües LG, Griesser R, Falendysz E, Amezcua I, Osorio JE, Rocke TE (2020) Clinical presentation and serologic response during a rabies epizootic in captive common vampire bats *(Desmodus rotundus)*. Trop Med Infect Dis 5(1):34. https://doi.org/10.3390/tropicalmed5010034. PMID: 32121499; PMCID: PMC7157733

Centers for Disease Control and Prevention-CDC (2012) Histoplasmosis outbreak among day camp attendees–Nebraska, June 2012. MMWR Morb Mortal Wkly Rep 61:747–748

Cilia G, Bertelloni F, Albini S, Fratini F (2021) Insight into the epidemiology of leptospirosis: a review of *Leptospira* isolations from "unconventional" hosts. Animals (Basel) 11(1):191. https://doi.org/10.3390/ani11010191. PMID: 33466962; PMCID: PMC7830643

Clayton BA, Wang LF, Marsh GA (2013) Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. Zoonoses Public Health 60:69–83

Coertse J, Geldenhuys M, le Roux K, Markotter W (2021) Lagos bat virus, an under-reported rabies-related lyssavirus. Viruses 13(4):576. https://doi.org/10.3390/v13040576. PMID: 33805487; PMCID: PMC8067007

Condori-Condori RE, Streicker DG, Cabezas-Sanchez C, Velasco-Villa A (2013) Enzootic and epizootic rabies associated with vampire bats, Peru. Emerg Infect Dis 19:1463–1469

Corman VM, Rasche A, Diallo TD, Cottontail VM, Stöcker A, Souza BF, Corrêa JI, Carneiro AJ, Franke CR, Nagy M, Metz M, Knörnschild M, Kalko EK, Ghanem SJ, Morales KD, Salsamendi E, Spínola M, Herrler G, Voigt CC, Tschapka M, Drosten C, Drexler JF (2013) Highly diversified coronaviruses in neotropical bats. J Gen Virol 94:1984–1994

Cottle LE, Gkrania-Klotsas E, Williams HJ, Brindle HE, Carmichael AJ, Fry G, Beeching NJ (2013) A multinational outbreak of histoplasmosis following a biology field trip in the Ugandan rainforest. J Travel Med 20:83–87

Cox TE, Smythe LD, Leung LK (2005) Flying foxes as carriers of pathogenic *Leptospira* species. J Wildl Dis 41:753–757

Crabtree MB, Kading RC, Mutebi JP, Lutwama JJ, Miller BR (2013) Identification of host blood from engorged mosquitoes collected in western Uganda using cytochrome oxidase I gene sequences. J Wildl Dis 49(3):611–626

de Cordeiro AR, de Silva KR, Brilhante RS, Moura FB, Duarte NF, Marques FJ, de Cordeiro RA, Filho RE, de Araújo RW, de Bandeira TJ, Rocha MF, Sidrim JJ (2012) *Coccidioides posadasii* infection in bats, Brazil. Emerg Infect Dis 18:668–670

de Thoisy B, Lacoste V, Germain A, Muñoz-Jordán J, Colón C, Mauffrey JF, Delaval M, Catzeflis F, Kazanji M, Matheus S, Dussart P, Morvan J, Setién AA, Deparis X, Lavergne A (2009) Dengue infection in neotropical forest mammals. Vector Borne Zoonotic Dis 9(2):157–170

Dietrich M, Mühldorfer K, Tortosa P, Markotter W (2015) Leptospira and bats: story of an emerging friendship. PLoS Pathog 11(11):e1005176. https://doi.org/10.1371/journal.ppat.1005176. PMID: 26562435; PMCID: PMC4643053

Dinerstein E (1986) Reproductive ecology of fruit bats and the seasonality of fruit production in a Costa Rican Cloud Forest. Biotropica 18:313–318

Diptyanusa A, Herini ES, Indarjulianto S, Satoto TBT (2021) The detection of Japanese encephalitis virus in Megachiropteran bats in West Kalimantan, Indonesia: a potential enzootic transmission pattern in the absence of pig holdings. Int J Parasitol Parasites Wildl 14:280–286. https://doi.org/10.1016/j.ijppaw.2021.03.009. PMID: 33898229; PMCID: PMC8056122

Drexler JF, Seelen A, Corman VM, Fumie Tateno A, Cottontail V, Melim Zerbinati R, Gloza-Rausch F, Klose SM, Adu-Sarkodie Y, Oppong SK, Kalko EK, Osterman A, Rasche A, Adam A, Müller MA, Ulrich RG, Leroy EM, Lukashev AN, Drosten C (2012) Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. J Virol 86(17):9134–9147. https://doi.org/10.1128/JVI.00800-12. Epub 2012 Jun 13. PMID: 22696648; PMCID: PMC3416139

Drexler JF, Geipel A, König A, Corman VM, van Riel D, Leijten LM, Bremer CM, Rasche A, Cottontail VM, Maganga GD, Schlegel M, Müller MA, Adam A, Klose SM, Carneiro AJ, Stöcker A, Franke CR, Gloza-Rausch F, Geyer J, Annan A, Adu-Sarkodie Y, Oppong S, Binger T, Vallo P, Tschapka M, Ulrich RG, Gerlich WH, Leroy E, Kuiken T, Glebe D, Drosten C (2013) Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes. Proc Natl Acad Sci U S A 110(40):16151–16156. https://doi.org/10.1073/pnas.1308049110. Epub 2013 Sep 16. PMID: 24043818; PMCID: PMC3791787

Drexler JF, Corman VM, Lukashev AN, van den Brand JM, Gmyl AP, Brünink S, Rasche A, Seggewi βN, Feng H, Leijten LM, Vallo P, Kuiken T, Dotzauer A, Ulrich RG, Lemon SM, Drosten C, Hepatovirus Ecology Consortium (2015) Evolutionary origins of hepatitis A virus in small mammals. Proc Natl Acad Sci U S A 112(49):15190–15195. https://doi.org/10.1073/pnas.1516992112. Epub 2015

Duarte G, Moron AF, Timerman A, Fernandes CE, Mariani Neto C, Almeida Filho GL, Werner Junior H, Espírito Santo HFBD, Steibel JAP, Bortoletti Filho J, Andrade JBB, Burlá M, Silva de

Sá MF, Busso NE, Giraldo PC, Moreira de Sá RA, Passini Junior R, Mattar R, Francisco RPV (2017) Zika virus infection in pregnant women and microcephaly. Rev Bras Ginecol Obstet 39 (5):235–248

Facione N, Marceron T, Fenton MB (1991) Evidence of mosquito consumption in *Myotis lucifigus*. Bat Res News 32:2–3

Fagre AC, Lewis J, Miller MR, Mossel EC, Lutwama JJ, Nyakarahuka L, Nakayiki T, Kityo R, Nalikka B, Towner JS, Amman BR, Sealy TK, Foy B, Schountz T, Anderson J, Kading RC (2021) Subgenomic flavivirus RNA (sfRNA) associated with Asian lineage Zika virus identified in three species of Ugandan bats (family Pteropodidae). Sci Rep 11(1):8370

Ferreira MS, Guterres A, Rozental T, Novaes RLM, Vilar EM, Oliveira RC, Fernandes J, Forneas D, Junior AA, Brandão ML, Cordeiro JLP, Del Valle Alvarez MR, Althoff SL, Moratelli R, Cordeiro-Estrela P, Silva RCD, Lemos ERS (2018) Coxiella and Bartonella spp. in bats (Chiroptera) captured in the Brazilian Atlantic Forest biome. BMC Vet Res 14(1):279. https://doi.org/10.1186/s12917-018-1603-0. PMID: 30200947; PMCID: PMC6131887

Field HE (2009) Bats and emerging zoonoses: henipaviruses and SARS. Zoonoses Public Health 56:278–284

Folly AJ, Marston DA, Golding M, Shukla S, Wilkie R, Lean FZX, Núñez A, Worledge L, Aegerter J, Banyard AC, Fooks AR, Johnson N, McElhinney LM (2021) Incursion of European Bat Lyssavirus 1 (EBLV-1) in serotine bats in the United Kingdom. Viruses 13(10): 1979. https://doi.org/10.3390/v13101979

Fooks AR, McElhinney LM, Pounder DJ, Finnegan CJ, Mansfield K, Johnson N, Brookes SM, Parsons G, White K, McIntyre PG, Nathwani D (2003) Case report: isolation of a European bat lyssavirus type 2a from a fatal human case of rabies encephalitis. J Med Virol 71:281–289

Fooks AR, Shipley R, Markotter W, Tordo N, Freuling CM, Müller T, McElhinney LM, Banyard AC, Rupprecht CE (2021) Renewed public health threat from emerging lyssaviruses. Viruses 13 (9):1769. https://doi.org/10.3390/v13091769. PMID: 34578350; PMCID: PMC8472001

Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH (2010) An emerging disease causes regional population collapse of a common North American bat species. Science 329(5992):679–682

Gilbert AT, Wu X, Jackson FR, Franka R, McCracken GF, Rupprecht CE (2020) Safety, immunogenicity, and efficacy of intramuscular and oral delivery of ERA-G333 recombinant rabies virus vaccine to big brown bats (*Eptesicus fuscus*). J Wildl Dis 56:620–630

Haake C, Cook S, Pusterla N, Murphy B (2020) Coronavirus infections in companion animals: virology, epidemiology, clinical and pathologic features. Viruses 12(9):1023. https://doi.org/10.3390/v12091023. PMID: 32933150; PMCID: PMC7551689

Hance P, Garnotel E, Morillon M (2006) *Chiroptera* and zoonoses: an emerging problem on all five continents. Med Trop (Mars) 66:119–124

Hauser N, Gushiken AC, Narayanan S, Kottilil S, Chua JV (2021) Evolution of Nipah virus infection: past, present, and future considerations. Trop Med Infect Dis 6(1):24. https://doi.org/10.3390/tropicalmed6010024. PMID: 33672796; PMCID: PMC8005932

Hayman DT, Bowen RA, Cryan PM, McCracken GF, O'Shea TJ, Peel AJ, Gilbert A, Webb CT, Wood JL (2013) Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. Zoonoses Public Health 60:2–21

Hazeleger WC, Jacobs-Reitsma WF, Lina PHC, de Boer AG, Bosch T, van Hoek AHAM, Beumer RR (2018) Wild, insectivorous bats might be carriers of *Campylobacter* spp. PLoS One 13(1): e0190647. https://doi.org/10.1371/journal.pone.0190647. PMID: 29324839; PMCID: PMC5764278

Herrera GL, Gutierrez E, Hobson KA, Altube B, Díaz WG, Sánchez-Cordero V (2002) Sources of assimilated protein in five species of New World frugivorous bats. Oecologia 133(3):280–287

Hoar BR, Chomel BB, Argazz Rodriguez FJ, Colley PA (1998) Zoonoses and potential zoonoses transmitted by bats. J Am Vet Med Assoc 212:1714–1720

Hodo CL, Goodwin CC, Mayes BC, Mariscal JA, Waldrup KA, Hamer SA (2016) Trypanosome species, including *Trypanosoma cruzi*, in sylvatic and peridomestic bats of Texas, USA. Acta Trop 164:259–266. https://doi.org/10.1016/j.actatropica.2016.09.013. Epub 2016 Sep 16. PMID: 27647574; PMCID: PMC5107337

Hornok S, Szőke K, Estók P, Krawczyk A, Haarsma AJ, Kováts D, Boldogh SA, Morandini P, Szekeres S, Takács N, Kontschán J, Meli ML, Fernández de Mera IG, de la Fuente J, Gyuranecz M, Sulyok KM, Weibel B, Gönczi E, de Bruin A, Sprong H, Hofmann-Lehmann R (2018) Assessing bat droppings and predatory bird pellets for vector-borne bacteria: molecular evidence of bat-associated *Neorickettsia* sp. in Europe. Antonie Van Leeuwenhoek 111(9): 1707–1717. https://doi.org/10.1007/s10482-018-1043-7. Epub 2018 Feb 28

Hu SC, Hsu CL, Lee MS, Tu YC, Chang JC, Wu CH, Lee SH, Ting LJ, Tsai KR, Cheng MC, Tu WJ, Hsu WC (2018) Lyssavirus in Japanese Pipistrelle, Taiwan. Emerg Infect Dis 24(4):782–785. https://doi.org/10.3201/eid2404.171696. PMID: 29553328; PMCID: PMC5875257

Hutson AM, Mickleburgh SP, Racey PA (2001) Microchiropteran bats: global status survey and conservation action plan. Information Press/IUCN, Oxford, UK/Gland/Cambridge, UK

Imnadze T, Natradze I, Zhgenti E, Malania L, Abazashvili N, Sidamonidze K, Khmaladze E, Zakalashvili M, Imnadze P, Arner RJ, Motin V, Kosoy M (2020) Identification of a novel *Yersinia enterocolitica* strain from bats in association with a bat die-off that occurred in Georgia (Caucasus). Microorganisms 8(7):1000. https://doi.org/10.3390/microorganisms8071000. PMID: 32635480; PMCID: PMC7409352

Irving AT, Rozario P, Kong PS, Luko K, Gorman JJ, Hastie ML, Chia WN, Mani S, Lee BP, Smith GJD, Mendenhall IH, Larman HB, Elledge SJ, Wang LF (2020) Robust dengue virus infection in bat cells and limited innate immune responses coupled with positive serology from bats in Indo-Malaya and Australasia. Cell Mol Life Sci 77(8):1607–1622

Jülg B, Elias J, Zahn A, Köppen S, Becker-Gaab C, Bogner JR (2008) Bat-associated histoplasmosis can be transmitted at entrances of bat caves and not only inside the caves. J Travel Med 15:133–136

Kajfasz P, Basiak W (2012) Outbreak of pulmonary histoplasmosis involving a group of four Polish travellers returning from Ecuador. Int Marit Health 63:59–62

Kajihiro ES (1965) Occurrence of dermatophytes in fresh bat guano. Appl Microbiol 13:720–724

Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J (1986) Kasokero virus: a new human pathogen from bats (*Rousettus aegyptiacus*) in Uganda. Am J Trop Med Hyg 35:387–392

Kohl C, Nitsche A, Kurth A (2021) Update on potentially zoonotic viruses of European bats. Vaccines (Basel) 9(7):690. https://doi.org/10.3390/vaccines9070690. PMID: 34201666; PMCID: PMC8310327

Krutzsch PH, Watson RH (1978) Isolation of *Coccidioides immitis* from bat guano and preliminary findings on laboratory infectivity of bats with *Coccidioides immitis*. Life Sci 22:679–684

Lagadec E, Gomard Y, Guernier V, Dietrich M, Pascalis H, Temmam S, Ramasindrazana B, Goodman SM, Tortosa P, Dellagi K (2012) Pathogenic *Leptospira* spp. in bats, Madagascar and Union of the Comoros. Emerg Infect Dis 18:1696–1698

Lamb LE, Bartolone SN, Kutluay SB, Robledo D, Porras A, Plata M, Chancellor MB (2016) Advantage of urine based molecular diagnosis of Zika virus. Int Urol Nephrol 48(12):1961–1966

Lee B, Ikegame S, Carmichael J, Wells H, Furler R, Acklin J, Chiu HP, Oguntuyo K, Cox R, Patel A, Kowdle S, Stevens C, Eckley M, Zhan S, Lim J, Hashiguchi T, Durigon EL, Schountz T, Epstein J, Plemper R, Daszak P, Anthony S (2021) Zoonotic potential of a novel bat morbillivirus. Res Sq [Preprint]. https://doi.org/10.21203/rs.3.rs-926789/v1. PMID: 34611656; PMCID: PMC8491849

Leonova GN, Belikov SI, Kondratov IG, Krylova NV, Pavlenko EV, Romanova EV et al (2010) A fatal case of bat lyssavirus infection in Primorye Territory of the Russian Far East. Rabies Bull Eur 33(4):58

Leopardi S, Barneschi E, Manna G, Zecchin B, Priori P, Drzewnioková P, Festa F, Lombardo A, Parca F, Scaravelli D, Maroni Ponti A, De Benedictis P (2021) Spillover of West Caucasian Bat Lyssavirus (WCBV) in a domestic cat and westward expansion in the Palearctic region. Viruses 13(10):2064. https://doi.org/10.3390/v13102064

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Délicat A, Paweska JT, Gonzalez JP, Swanepoel R (2005) Fruit bats as reservoirs of Ebola virus. Nature 438(7068): 575–576

Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, Formenty P (2009) Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis 9:723–728

Leshem E, Meltzer E, Schwartz E (2011) Travel-associated zoonotic bacterial diseases. Curr Opin Infect Dis 24:457–463

Leslie MJ, Messenger S, Rohde RE, Smith J, Cheshier R, Hanlon C, Rupprecht CE (2006) Bat-associated rabies virus in skunks. Emerg Infect Dis 12:1274–1277

L'Huillier AG, Hamid-Allie A, Kristjanson E, Papageorgiou L, Hung S, Wong CF, Stein DR, Olsha R, Goneau LW, Dimitrova K, Drebot M, Safronetz D, Gubbay JB (2017) Evaluation of Euroimmun Anti-Zika Virus IgM and IgG enzyme linked immunosorbent assays for Zika Virus serologic testing. J Clin Microbiol 55(8):2462–2471

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310(5748):676–679

Lin JW, Hsu YM, Chomel BB, Lin LK, Pei JC, Wu SH, Chang CC (2012) Identification of novel *Bartonella* spp. in bats and evidence of Asian gray shrew as a new potential reservoir of *Bartonella*. Vet Microbiol 156(1–2):119–126

Liu S, Li X, Chen Z, Chen Y, Zhang Q, Liao Y, Zhou J, Ke X, Ma L, Xiao J, Wu Y, Chen Z, Zhou J, Zheng X, Li J, Chen Q (2013a) Comparison of genomic and amino acid sequences of eight Japanese encephalitis virus isolates from bats. Arch Virol 158(12):2543–2552. https://doi.org/10.1007/s00705-013-1777-5

Liu Y, Zhang S, Zhao J, Zhang F, Hu R (2013b) Isolation of Irkut virus from a *Murina leucogaster* bat in China. PLoS Negl Trop Dis 7(3):e2097

Ma X, Monroe BP, Wallace RM, Orciari LA, Gigante CM, Kirby JD, Chipman RB, Fehlner-Gardiner C, Cedillo VG, Petersen BW, Olson V, Bonwitt J (2021) Rabies surveillance in the United States during 2019. J Am Vet Med Assoc 258(11):1205–1220. https://doi.org/10.2460/javma.258.11.1205

Machain-Williams C, López-Uribe M, Talavera-Aguilar L, Carrillo-Navarrete J, Vera-Escalante L, Puerto-Manzano F, Ulloa A, Farfán-Ale JA, Garcia-Rejon J, Blitvich BJ, Loroño-Pino MA (2013) Serologic evidence of flavivirus infection in bats in the Yucatan Peninsula of Mexico. J Wildl Dis 49(3):684–689

Malmlov A, Bantle C, Aboellail T, Wagner K, Campbell CL, Eckley M, Chotiwan N, Gullberg RC, Perera R, Tjalkens R, Schountz T (2019) Experimental Zika virus infection of Jamaican fruit bats (*Artibeus jamaicensis*) and possible entry of virus into brain via activated microglial cells. PLoS Negl Trop Dis 13(2):e0007071

Marcili A, Lima L, Cavazzana M, Junqueira AC, Veludo HH, Maia Da Silva F, Campaner M, Paiva F, Nunes VL, Teixeira MM (2009) A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and Histone H2B genes and genotyping based on ITS1 rDNA. Parasitology 136:641–655

Markotter W, Coertse J, De Vries L, Geldenhuys M, Mortlock M (1987) Bat-borne viruses in Africa: a critical review. J Zool 311(2):77–98. https://doi.org/10.1111/jzo.12769. Epub ahead of print. PMID: 32427175; PMCID: PMC7228346

Matthias MA, Díaz MM, Campos KJ, Calderon M, Willig MR, Pacheco V (2005) Diversity of bat associated *Leptospira* in the Peruvian Amazon inferred by Bayesian phylogenetic analysis of 16S ribosomal DNA sequences. Am J Trop Med Hyg 73:964–974

McAllister CT, Seville RS, Roehrs ZP (2012) A new species of *Eimeria* (*Apicomplexa: Eimeriidae*) from the northern myotis, *Myotis septentrionalis* (*Chiroptera: Vespertilionidae*), in Oklahoma. J Parasitol 98:1003–1005

McElhinney LM, Marston DA, Leech S, Freuling CM, van der Poel WH, Echevarria J, Vázquez-Moron S, Horton DL, Müller T, Fooks AR (2013) Molecular epidemiology of bat lyssaviruses in Europe. Zoonoses Public Health 60(1):35–45

Megid J, Benavides Tala JA, Belaz Silva LD, Castro Castro FF, Ribeiro BLD, Appolinário CM, Katz ISS, Scheffer KC, Silva SR, Rosa AR, Barone GT, Alves Martorelli LF, de Almeida MF

(2021) Serological surveillance of rabies in free-range and captive common vampire bats *Desmodus rotundus*. Front Vet Sci (8):681423. https://doi.org/10.3389/fvets.2021.681423. PMID: 34660750; PMCID: PMC8511519

Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH et al (2013) Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis [Internet] 19(11): 1819–1823. https://doi.org/10.3201/eid1911.131172

Meske M, Fanelli A, Rocha F, Awada L, Soto PC, Mapitse N, Tizzani P (2021) Evolution of rabies in South America and inter-species dynamics (2009–2018). Trop Med Infect Dis 6(2):98. https://doi.org/10.3390/tropicalmed6020098. PMID: 34207822; PMCID: PMC8293400

Meynard JB, Flamand C, Dupuy C, Mahamat A, Eltges F, Queuche F, Renner J, Fontanella JM, Hommel D, Dussart P, Grangier C, Djossou F, Dacheux L, Goudal M, Berger F, Ardillon V, Krieger N, Bourhy H, Spiegel A (2012) First human rabies case in French Guiana, 2008: epidemiological investigation and control. PLoS Negl Trop Dis 6(2):e1537. https://doi.org/10.1371/journal.pntd.0001537. Epub 2012 Feb 21. PMID: 22363830; PMCID: PMC3283561

Migueres M, Lhomme S, Izopet J (2021) Hepatitis A: epidemiology, high-risk groups, prevention and research on antiviral treatment. Viruses 13(10):1900. https://doi.org/10.3390/v13101900. PMID: 34696330; PMCID: PMC8540458

Molaei G, Oliver J, Andreadis TG, Armstrong PM, Howard JJ (2006) Molecular identification of blood-meal sources in *Culiseta melanura* and *Culiseta morsitans* from an endemic focus of eastern equine encephalitis virus in New York. Am J Trop Med Hyg 75(6):1140–1147

Moreira-Soto A, Soto-Garita C, Corrales-Aguilar E (2017) Neotropical primary bat cell lines show restricted dengue virus replication. Comp Immunol Microbiol Infect Dis 50:101–105

Mühldorfer K (2013) Bats and bacterial pathogens: a review. Zoonoses Public Health 60:93–103

Murphy HL, Ly H (2021) Understanding the prevalence of SARS-CoV-2 (COVID-19) exposure in companion, captive, wild, and farmed animals. Virulence 12(1):2777–2786. https://doi.org/10.1080/21505594.2021.1996519. Epub ahead of print

Musso D, Gubler DJ (2016) Zika virus. Clin Microbiol Rev 29(3):487–524

Oyeka CA (1994) Isolation of *Candida* species from bats in Nigeria. Mycoses 37:353–355

Patyk K, Turmelle A, Blanton JD, Rupprecht CE (2012) Trends in national surveillance data for bat rabies in the United States: 2001–2009. Vector Borne Zoonotic Dis 12(8):666–673

Perea-Martínez L, Moreno-Sandoval HN, Moreno-Altamirano MM, Salas-Rojas M, García-Flores MM, Aréchiga-Ceballos N, Tordo N, Marianneau P, Aguilar-Setién A (2013) Experimental infection of *Artibeus intermedius* bats with serotype-2 dengue virus. Comp Immunol Microbiol Infect Dis 36(2):193–198

Picard-Meyer E, Servat A, Robardet E, Moinet M, Borel C, Cliquet F (2013) Isolation of Bokeloh bat lyssavirus in *Myotis nattereri* in France. Arch Virol 158(11):2333–2340. https://doi.org/10.1007/s00705-013-1747-y

Pigott DM, Golding N, Mylne A, Huang Z, Weiss DJ, Brady OJ, Kraemer MU, Hay SI (2015) Mapping the zoonotic niche of Marburg virus disease in Africa. Trans R Soc Trop Med Hyg 109 (6):366–378. https://doi.org/10.1093/trstmh/trv024. Epub 2015 Mar 27. PMID: 25820266; PMCID: PMC4447827

Platt KB, Mangiafico JA, Rocha OJ, Zaldivar ME, Mora J, Trueba G, Rowley WA (2000) Detection of dengue virus neutralizing antibodies in bats from Costa Rica and Ecuador. J Med Entomol 37 (6):965–967

Pourrut X, Souris M, Towner JS, Rollin PE, Nichol ST, Gonzalez JP, Leroy E (2009) Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus*. BMC Infect Dis 9:159

Puig-Montserrat X, Flaquer C, Gómez-Aguilera N, Burgas A, Mas M, Tuneu C, Marquès E, López-Baucells A (2020) Bats actively prey on mosquitoes and other deleterious insects in rice paddies: potential impact on human health and agriculture. Pest Manag Sci 76(11):3759–3769

Quan PL, Firth C, Conte JM, Williams SH, Zambrana-Torrelio CM, Anthony SJ, Ellison JA, Gilbert AT, Kuzmin IV, Niezgoda M, Osinubi MO, Recuenco S, Markotter W, Breiman RF, Kalemba L,

Malekani J, Lindblade KA, Rostal MK, Ojeda-Flores R, Suzan G, Davis LB, Blau DM, Ogunkoya AB, Alvarez Castillo DA, Moran D, Ngam S, Akaibe D, Agwanda B, Briese T, Epstein JH, Daszak P, Rupprecht CE, Holmes EC, Lipkin WI (2013) Bats are a major natural reservoir for hepaciviruses and pegiviruses. Proc Natl Acad Sci U S A 110(20):8194–8199. https://doi.org/10.1073/pnas.1303037110. Epub 2013 Apr 22. PMID: 23610427; PMCID: PMC3657805

Ramírez JD, Tapia-Calle G, Muñoz-Cruz G, Poveda C, Rendón LM, Hincapié E, Guhl F (2014) Trypanosome species in neo-tropical bats: biological, evolutionary and epidemiological implications. Infect Genet Evol 22:250–256. https://doi.org/10.1016/j.meegid.2013.06.022. Epub 2013 Jul 2. PMID: 23831017; PMCID: PMC7106241

Raymond JT, White MR, Kilbane TP, Janovitz EB (1997) Pulmonary blastomycosis in an Indian fruit bat (*Pteropus giganteus*). J Vet Diagn Investig 9(1):85–87

Reagan R, Brueckner AL (1952) Studies of dengue fever virus in the cave bat (*Myotus lucifugus*). J Infect Dis 91(2):145–146

Reagan RL, Rumbaugh H, Nelson H, Brueckner AL (1955) Effect of zika virus and bwamba virus in the cave bat (*Myotus lucifugus*). Trans Am Microsc Soc 74:77–79

Regnault B, Evrard B, Plu I, Dacheux L, Troadec E, Cozette P, Chrétien D, Duchesne M, Jean-Michel V, Jamet A, Leruez M, Pérot P, Bourhy H, Eloit M, Seilhean D (2021) First case of lethal encephalitis in Western Europe due to European bat lyssavirus type 1. Clin Infect Dis 15: ciab443. https://doi.org/10.1093/cid/ciab443. Epub ahead of print

Reichard JD, Kunz T (2009) White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropterologica 11:457–464

Ricciardi ID, Nunes MP, Andrade CM, Da Silva AG (1976) Anti-*Brucella* agglutinins in bats and "*Callithrix*" monkeys. J Wildl Dis 12:52–54

Ryan PA, Martin L, Mackenzie JS, Kay BH (1997) Investigation of gray-headed flying foxes (*Pteropus poliocephalus*) (Megachiroptera: Pteropodidae) and mosquitoes in the ecology of Ross River virus in Australia. Am J Trop Med Hyg 57(4):476–482

Sangster CR, Gordon AN, Hayes D (2012) Systemic toxoplasmosis in captive flying-foxes. Aust Vet J 90:140–142

Schatz J, Fooks AR, McElhinney L, Horton D, Echevarria J, Vázquez-Moron S, Kooi EA, Rasmussen TB, Müller T, Freuling CM (2013) Bat rabies surveillance in Europe. Zoonoses Public Health 60:22–34

Schneider MC, Romijn PC, Uieda W, Tamayo H, da Silva DF, Belotto A, da Silva JB, Leanes LF (2009) Rabies transmitted by vampire bats to humans: an emerging zoonotic disease in Latin America? Rev Panam Salud Publica 25:260–269

Schwarz J, Kauffman CA (1977) Occupational hazards from deep mycoses. Arch Dermatol 113: 1270–1275

Shapiro JT, da Costa Lima Junior MS, Dorval ME, de Oliveira França A, Cepa Matos MD, Bordignon MO (2013) First record of *Leishmania braziliensis* presence detected in bats, Mato Grosso do Sul, southwest Brazil. Acta Trop 128(1):171–174. https://doi.org/10.1016/j.actatropica.2013.07.004. pii: S0001-706X(13)00184-8

Shepherd RC, Williams MC (1964) Studies on viruses in East African bats (Chiroptera). 1. Haemagglutination inhibition and circulation of arboviruses. Zoonoses Res 3(3):125–139

Simpson DI, Williams MC, O'Sullivan JP, Cunningham JC, Mutere FA (1968) Studies on arboviruses and bats (*Chiroptera*) in East Africa. II. Isolation and haemagglutination-inhibition studies on bats collected in Kenya and throughout Uganda. Ann Trop Med Parasitol 62(4):432–440

Sotomayor-Bonilla J, Chaves A, Rico-Chávez O, Rostal MK, Ojeda-Flores R, Salas-Rojas M, Aguilar-Setien Á, Ibáñez-Bernal S, Barbachano-Guerrero A, Gutiérrez-Espeleta G, Aguilar-Faisal JL, Aguirre AA, Daszak P, Suzán G (2014) Dengue virus in bats from southeastern Mexico. Am J Trop Med Hyg 91(1):129–131

Stading B, Ellison JA, Carson WC, Satheshkumar PS, Rocke TE, Osorio JE (2017) Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11(10):e0005958. https://doi.org/10.1371/journal.pntd.0005958. PMID: 28976983; PMCID: PMC5643138

Streicker DG, Recuenco S, Valderrama W, Gomez Benavides J, Vargas I, Pacheco V, Condori Condori RE, Montgomery J, Rupprecht CE, Rohani P, Altizer S (2012) Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. Proc Biol Sci 279(1742):3384–3392

Stuckey MJ, Boulouis HJ, Cliquet F, Picard-Meyer E, Servat A, Aréchiga-Ceballos N, Echevarría JE, Chomel BB (2017a) Potentially zoonotic *Bartonella* in bats from France and Spain. Emerg Infect Dis 23(3):539–541. https://doi.org/10.3201/eid2303.160934. PMID: 28221109; PMCID: PMC5382759

Stuckey MJ, Chomel BB, de Fleurieu EC, Aguilar-Setién A, Boulouis HJ, Chang CC (2017b) *Bartonella*, bats and bugs: a review. Comp Immunol Microbiol Infect Dis 55:20–29. https://doi.org/10.1016/j.cimid.2017.09.001. Epub 2017 Sep 18

Stuckey MJ, Chomel BB, Galvez-Romero G, Olave-Leyva JI, Obregón-Morales C, Moreno-Sandoval H, Aréchiga-Ceballos N, Salas-Rojas M, Aguilar-Setién A (2017c) *Bartonella* infection in hematophagous, insectivorous, and phytophagous bat populations of Central Mexico and the Yucatan Peninsula. Am J Trop Med Hyg 97(2):413–422. https://doi.org/10.4269/ajtmh.16-0680. Epub 2017 Jul 19. PMID: 28722567; PMCID: PMC5544074

Sun H, Wang Y, Zhang Y, Ge W, Zhang F, He B, Li Z, Fan Q, Wang W, Tu C, Li J, Liu Q (2013) Prevalence and genetic characterization of *Toxoplasma gondii* in bats in Myanmar. Appl Environ Microbiol 79:3526–3528

Taniguchi S, Watanabe S, Masangkay JS, Omatsu T, Ikegami T, Alviola P, Ueda N, Iha K, Fujii H, Ishii Y, Mizutani T, Fukushi S, Saijo M, Kurane I, Kyuwa S, Akashi H, Yoshikawa Y, Morikawa S (2011) Reston Ebolavirus antibodies in bats, the Philippines. Emerg Infect Dis 17:1559–1560

Tiawsirisup S, Junpee A, Nuchprayoon S (2012) Mosquito distribution and Japanese encephalitis virus infection in a bat cave and its surrounding area in Lopburi province, Central Thailand. Thai J Vet Med 42:43

Torres-Castro M, Noh-Pech H, Hernández-Betancourt S, Peláez-Sánchez R, Lugo-Caballero C, Puerto FI (2021) West Nile and Zika viruses in bats from a suburban area of Merida, Yucatan, Mexico. Zoonoses Public Health 68(7):834–841

Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, Formenty PB, Albarino CG, Miller DM, Reed ZD, Kayiwa JT, Mills JN, Cannon DL, Greer PW, Byaruhanga E, Farnon EC, Atimnedi P, Okware S, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Ksiazek TG, Nichol ST, Rollin PE (2009) Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog 5: e1000536

Turmelle AS, Allen LC, Schmidt-French BA, Jackson FR, Kunz TH, McCracken GF, Rupprecht CE (2010) Response to vaccination with a commercial inactivated rabies vaccine in a captive colony of Brazilian free-tailed bats (*Tadarida brasiliensis*). J Zoo Wildl Med 41(1):140–143. https://doi.org/10.1638/2008-0161.1

Van Brussel K, Holmes EC (2021) Zoonotic disease and virome diversity in bats. Curr Opin Virol 52:192–202. https://doi.org/10.1016/j.coviro.2021.12.008

Van den Bossche D, Cnops L, Van Esbroeck M (2015) Recovery of dengue virus from urine samples by real-time RT-PCR. Eur J Clin Microbiol Infect Dis 34(7):1361–1367

Vashi NA, Reddy P, Wayne DB, Sabin B (2010) Bat-associated leptospirosis. J Gen Intern Med 25: 162–164

Vicente-Santos A, Moreira-Soto A, Soto-Garita C, Chaverri LG, Chaves A, Drexler JF, Morales JA, Alfaro-Alarcón A, Rodríguez-Herrera B, Corrales-Aguilar E (2017) Neotropical bats, that co-habit with humans, function as dead-end hosts for dengue virus. PLoS Negl Trop Dis 11(5):e0005537

Wacharapluesadee S, Sintunawa C, Kaewpom T, Khongnomnan K, Olival KJ, Epstein JH, Rodpan A, Sangsri P, Intarut N, Chindamporn A, Suksawa K, Hemachudha T (2013) Group C betacoronavirus in bat guano fertilizer, Thailand. Emerg Infect Dis 19:1349–1351

Wang LF, Shi Z, Zhang S, Field H, Daszak P, Eaton BT (2006) Review of bats and SARS. Emerg Infect Dis 12:1834–1840

Wang J, Anderson DE, Halpin K, Hong X, Chen H, Walker S, Valdeter S, van der Heide B, Neave MJ, Bingham J, O'Brien D, Eagles D, Wang LF, Williams DT (2021) A new Hendra virus

genotype found in Australian flying foxes. Virol J 18(1):197. https://doi.org/10.1186/s12985-021-01652-7. PMID: 34641882; PMCID: PMC8510678

Welsh O, Vera-Cabrera L, Rendon A, Gonzalez G, Bonifaz A (2012) Coccidioidomycosis. Clin Dermatol 30:573–591

Wibbelt G, Moore MS, Schountz T, Voigt CC (2010) Emerging diseases in *Chiroptera*: why bats? Biol Lett 6:438–440

Wong S, Lau S, Woo P, Yuen KY (2007) Bats as a continuing source of emerging infections in humans. Rev Med Virol 17:67–91

Wood JL, Leach M, Waldman L, Macgregor H, Fooks AR, Jones KE, Restif O, Dechmann D, Hayman DT, Baker KS, Peel AJ, Kamins AO, Fahr J, Ntiamoa-Baidu Y, Suu-Ire R, Breiman RF, Epstein JH, Field HE, Cunningham AA (2012) A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. Philos Trans R Soc Lond Ser B Biol Sci 367(1604):2881–2892

Vector-Borne Zoonoses

35

Filipe Dantas-Torres and Domenico Otranto

Contents

Abstract

Vector-borne zoonoses (VBZ) are diseases caused by a range of pathogens that affect animals and humans. A plethora of vectors, such as mosquitoes, ticks, fleas, phlebotomine sand flies, lice, and kissing bugs, may transmit numerous bacteria, protozoa, helminths, and viruses to animals and humans. The burden of VBZ is still considerable in poor rural areas in tropical and subtropical regions. Indeed, some of these diseases represent a current public health concern in low- and middle-income countries as well as in wealthy ones. A number of factors, such as increases in travel and trade, climate and land-use changes, and socioeconomic and political upheavals, may drive or alter the dynamics of VBZ in animals and humans. In this chapter, we review selected aspects of VBZ affecting animal and human populations worldwide. Unresolved issues regarding the epidemiology and control of this group of zoonoses are also discussed.

F. Dantas-Torres (✉)
Department of Immunology, Aggeu Magalhães Institute, Fiocruz, Recife, PE, Brazil
e-mail: filipe.torres@fiocruz.br

D. Otranto
Department of Veterinary Medicine, University of Bari, Valenzano, BA, Italy

Faculty of Veterinary Sciences, Bu-Ali Sina University, Hamedan, Iran
e-mail: domenico.otranto@uniba.it

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_27

Keywords

West Nile Virus · Visceral Leishmaniasis · Cutaneous Leishmaniasis · Lyme Borreliosis · Spotted Fever · Dengue · Zika

35.1 Introduction

Vector-borne zoonoses (VBZ) constitute a group of diseases caused by a wide range of pathogenic organisms, including bacteria (e.g., *Rickettsia conorii*, *Rickettsia rickettsii*, and *Borrelia burgdorferi*), protozoa (e.g., *Babesia divergens*, *Babesia microti, Plasmodium knowlesi*, and *Trypanosoma cruzi*), helminths (e.g., *Dirofilaria immitis, Dirofilaria repens, Onchocerca lupi*, and *Thelazia callipaeda*), and viruses (e.g., Crimean-Congo hemorrhagic fever virus, West Nile virus, and tick-borne encephalitis virus) (Colwell et al. 2011; Dantas-Torres et al. 2012b; Kilpatrick and Randolph 2012; Otranto et al. 2013; Bezerra-Santos et al. 2021; Mendoza-Roldan et al. 2021a). These pathogenic agents may be transmitted to animals and humans through the bite (during blood feeding) of a variety of arthropods, such as mosquitoes (family Culicidae), fleas (order Siphonaptera), lice (order Phthiraptera), phlebotomine sand flies (subfamily *Phlebotominae*), black flies (family Simulidae), biting midges (family Ceratopogonidae), kissing bugs (subfamily Triatominae), fruit flies (subfamily Steganinae), and ticks (order Ixodida). Some diseases such as plague, a flea-borne disease that claimed the lives of thousands of people since the Byzantine Empire (Gage and Kosoy 2005; Raoult et al. 2013; Bezerra et al. 2022), have been part of humankind for long time and have changed the course of our history. In recent years, a number of new VBZ have also been described (Dantas-Torres et al. 2012b), with direct implications for the diagnosis of this group of zoonoses, whose clinical features may overlap (Paddock et al. 2008).

The epidemiology and distribution of VBZ are influenced by several factors, but the presence of animal hosts and arthropod vectors in a given area is a *sine qua non* condition for the enzootic and zoonotic cycles to occur. For instance, small mammals and reptiles may act as hosts for a number of pathogens (e.g., *Babesia microti*, *Borrellia burgdorferi*, *Rickettsia* spp.) potentially causing disease in humans and may also serve as hosts for arthropod vectors, such as ticks (Dantas-Torres et al. 2012a; Mendoza-Roldan et al. 2021b). Importantly, the complex interactions between animal hosts, arthropod vectors, and people occurring in the enzootic and zoonotic transmission cycles of vector-borne pathogens partly explain the difficulties faced by public health authorities trying to control this group of diseases.

The burden of VBZ is still heavier in poor rural areas in tropical and subtropical regions, where arthropod vectors find a more suitable environment for their perpetuation and where access to health care services is often limited. For example, leishmaniasis and Chagas disease are still one of the leading causes of disability worldwide and responsible for thousands of deaths, mainly in rural and peri-urban areas of several low-income countries (Alvar et al. 2006; Bern et al. 2008; Christou 2011; Lozano et al. 2012). Furthermore, climatic, socioeconomic, and political

changes have caused a profound impact on the epidemiology and distribution of VBZ, some of which currently represent a public health concern in industrialized countries as well (Vorou et al. 2007; Otranto et al. 2013). In the present chapter, we provide an overview on selected aspects of VBZ affecting animal and human populations around the world. Unresolved issues regarding the epidemiology and control of this group of zoonoses are also addressed.

35.2 Morbidity and Mortality

From a global perspective, VBZ such as leishmaniasis, Chagas disease, and African trypanosomiasis are still causing a considerable burden in terms of morbidity and mortality in a number of countries. Particularly, the burden of such VBZ is still heavier in low- and middle-income countries as compared with high-income countries. On the other hand, several VBZ such as tick-borne encephalitis and Lyme disease are also increasing in many industrialized countries (Bhate and Schwartz 2011). Nonetheless, it is difficult to estimate the actual burden of VBZ, mainly in developing countries due to the absence of surveillance and/or deficiencies in the national case notification system. Furthermore, many cases of VBZ remain without a definitive diagnosis, particularly in remote rural areas where the access to basic health care services is still incipient.

Human leishmaniasis is a group of phlebotomine sand fly-borne diseases caused by several species of *Leishmania*, which are prevalent in at least 98 countries and three territories in all continents, except Oceania (Alvar et al. 2012); the *Leishmania* species circulating presently in Australia is apparently restricted to kangaroos. The global burden of leishmaniasis in terms of morbidity and mortality has recently been reassessed. It has been estimated that approximately 0.2–0.4 million visceral leishmaniasis cases and 0.7–1.2 million cutaneous leishmaniasis cases occur each year in countries where these diseases are endemic (Alvar et al. 2012). Remarkably, more than 90% of all visceral leishmaniasis cases reported worldwide occur in six countries: India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia. In a similar manner, Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru are responsible for 70–75% of the cutaneous leishmaniasis cases estimated to occur annually. It has also been estimated that 20,000–40,000 leishmaniasis deaths occur each year worldwide. Nevertheless, the number of cases and deaths that remain underreported is probably higher than currently estimated.

Trypanosomiases are neglected tropical diseases caused by *Trypanosoma* species, which might be transmitted by different arthropod vectors. American trypanosomiasis (Chagas disease) is caused by *Trypanosoma cruzi*, which is primarily transmitted by kissing bugs. In spite of the efforts toward the elimination of the vectorial transmission of *T. cruzi* in endemic areas, Chagas disease is still a leading cause of morbidity and mortality worldwide. Indeed, it is estimated that 10–20 million people are infected with *T. cruzi*, mostly in Latin America, causing between 20,000 and 50,000 deaths per year (Tarleton and Curran 2012; Leony et al. 2019). Differently, African trypanosomiasis (sleeping sickness) is caused by species of *Trypanosoma* (*Trypanosoma brucei*

rhodesiense and *Trypanosoma brucei gambiense*), which are transmitted by tsetse flies (*Glossina* genus). The disease is restricted to sub-Saharan Africa, where 70 million people are estimated to be at risk of infection. Importantly, the number of cases of sleeping sickness in Africa decreased about 82% in recent years (e.g., 37,991 cases in 1998 and 6743 cases in 2011), which is the result of the control efforts toward the elimination of the disease from this continent (Simarro et al. 2012).

Tick-borne bacterial diseases such as Lyme disease, Rocky Mountain spotted fever, Mediterranean spotted fever, granulocytic anaplasmosis, monocytic ehrlichiosis, and Q fever constitute emerging public health concerns worldwide (Christou 2011; Dantas-Torres et al. 2012b). For instance, Lyme borreliosis is on the rise in Europe, where more than 50,000 cases are reported each year. Similarly, over 250,000 cases of Lyme borreliosis were reported between 2000 and 2010 in the United States (Dantas-Torres et al. 2012b). Furthermore, human granulocytic anaplasmosis has been increasing in incidence and expanding its distribution in the United States in recent years (Christou 2011; Matos et al. 2022).

Arboviruses (i.e., arthropod-borne viruses) such as yellow fever virus, dengue fever virus, Japanese encephalitis virus, West Nile virus, tick-borne encephalitis, Chikungunya virus, Zika virus, Rift Valley fever virus, and Crimean-Congo haemorrhagic fever virus are responsible for considerable morbidity and mortality worldwide (LaBeaud et al. 2011). For instance, the World Health Organization official estimates indicate that 50–100 million dengue infections occur annually around the globe, but a more recent estimate increased this figure to 390 million dengue infections per year (Bhatt et al. 2013). Moreover, around 200,000 cases and 30,000 deaths by yellow fever are estimated to occur each year in tropical areas of Africa and Latin America, respectively. Similarly, more than 67,000 cases of Japanese encephalitis are estimated to occur each year throughout most Asia and parts of western Pacific, with a case-fatality rate ranging from 10–30% (Hu et al. 2012). Furthermore, more than 5300 West Nile virus disease cases occurred in 2012 in the United States, representing the highest number of cases reported to the Centres for Disease Control and Prevention since 2003 (http://www.cdc.gov/ncidod/dvbid/westnile/index.htm).

The recent introduction and subsequent emergence of several exotic VBZ (e.g., West Nile virus, Chikungunya virus, and Zika virus) worldwide appears to be influenced by several biotic and abiotic factors, including increased travel and trade, which ultimately favoured the introduction and/or the expansion of arthropod vectors. Nonetheless, the emergence or re-emergence of endemic VBZ (e.g., Lyme disease, tick-borne encephalitis, leishmaniasis, and malaria) has been attributed to climate changes, land-use, and social changes, which have greatly impacted on the ecology of vector species (Colwell et al. 2011; Kilpatrick and Randolph 2012; Dantas-Torres 2015).

35.3 Drivers and Dynamics of VBZ

At the local level, the probability (or the risk) of being exposed to and, thus, becoming infected by a vector-borne pathogen depends on the contact with competent vectors, which in turn may be influenced by several factors (e.g., living

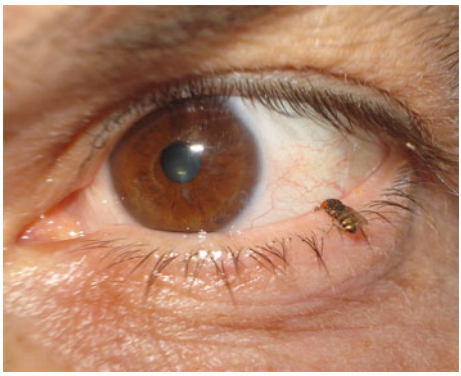

Fig. 1 *Phortica variegata*, the vector of *Thelazia callipaeda*, feeding on a human eye

conditions, labor and leisure activities, knowledge of the disease transmission and control). From a broader perspective, the dynamics of VBZ in a given area are driven by several biotic (e.g., reservoir and vector population densities) and abiotic factors (e.g., land-use and social changes, increased travel and trade), which may vary in time and space.

In the past century, a number of exotic VBZ were introduced and established into new areas. For instance, canine thelaziasis is a VBZ caused by the spirurid nematode *Thelazia callipaeda*, which is transmitted by the fruit fly *Phortica variegata* (Fig. 1) (Otranto et al. 2013). The disease was initially thought to be restricted to the former Soviet Union and to countries in the Far East (the People's Republic of China, South Korea, Japan, Indonesia, Thailand, Taiwan, and India), but nowadays it is widespread in Europe (Otranto et al. 2013), and it has also been reported in the USA (Schwartz et al. 2021). Cases have been reported in almost all European countries (e.g., Italy, Belgium, France, Greece, Portugal, Spain, and Switzerland) as well as in the Balkans (Caron et al. 2013; Otranto et al. 2013, 2021). The emergence of thelaziosis in several European countries has been attributed to several factors, such as movement of infected animals, local changes in vector ecology and distribution, as well as to increased awareness of medical physicians, veterinary practitioners, and parasitologists. Indeed, the increasing in trade and travel is considered as a major driver of exotic vector-borne pathogen introductions into non-endemic areas (Kilpatrick and Randolph 2012; Dantas-Torres 2015). For instance, infected livestock and people as well as migratory or dispersing birds are potential carriers for the introduction of exotic pathogens (e.g., Crimean-Congo haemorrhagic fever, Chikungunya virus, and West Nile virus) into new regions (Kilpatrick and Randolph 2012).

Upon arrival in a new area, an exotic pathogen needs to find suitable reservoir hosts and arthropod vectors to establish locally. For example, the immigration waves of Europeans from leishmaniasis endemic countries (e.g., Portugal, Spain, and Italy) into the New World, since the arrival of the Conquistadores, resulted in multiple introductions of *Leishmania infantum*, the causative agent of zoonotic visceral leishmaniasis, into this region (Kuhls et al. 2011; Dantas-Torres et al. 2012c). The establishment of *L. infantum* in the New World was likely facilitated by the presence

of highly susceptible human populations and competent animal hosts, such as foxes, domestic dogs, and other wild animals co-habiting the same environment (Desjeux 2004; Dantas-Torres et al. 2012c). In the same way, the existence of a ubiquitous competent vector (*Lutzomyia longipalpis sensu lato*), migration waves from rural to urban areas, and increased trade have probably favored the rapid spread of zoonotic visceral leishmaniasis in some Latin American countries such as Brazil (Desjeux 2004; Kuhls et al. 2011; Dantas-Torres et al. 2012c).

The presence of an established vector population and of susceptible hosts that are immunologically naïve to the introduced pathogen might result in explosive epidemics of VBZ (Kilpatrick and Randolph 2012). Indeed, the emergence of endemic VBZ might be driven by several factors, which may bring large contingents of non-immune people into contact with vectors and associated pathogens. Road building, oil prospecting, mining, farming, irrigation, forestry development, tourism, as well as political upheavals, military conflicts, and natural disasters (e.g., the hurricane Katherine) have been associated with the emergence of several VBZ such as dirofilarioses, leishmaniasis, malaria, yellow fever, and tick-borne encephalitis (Desjeux 2004; Colwell et al. 2011; Kilpatrick and Randolph 2012; Antinori et al. 2013). For example, the largest outbreak of cutaneous leishmaniasis recorded in Colombia occurred during 2005–2009 in soldiers of the Colombian Army, when roughly 40,000 cases were detected (Vélez et al. 2012). In this outbreak, military incursions into the jungle, with the mission to combat illicit crops and the guerrilla, resulted in exposure of soldiers and military dogs to infected phlebotomine sand flies. Similarly, the emergence of malaria by *Plasmodium knowlesi* in south-eastern Asia has been associated with males who had a history of visiting or staying for some days in jungle areas, where wild macaques and anopheline mosquitoes maintain the natural cycle of the parasite (Singh et al. 2004; Lee et al. 2011; Antinori et al. 2013).

While deforestation (for road building, establishment of grazing areas, crop plantations, etc.) may be associated with the emergence of VBZ, reforestation has also been linked with changes in local abundance of animal hosts and arthropod vectors of certain pathogens. For instance, the emergence of Lyme disease in north-eastern United States in the mid-twentieth century has been partly attributed to the rise in deer (*Odocoileus virginianus*) and tick (*Ixodes scapularis*) populations as a result of reforestation occurring during the twentieth century (Barbour and Fish 1993). On the other hand, forest fragmentation in eastern regions of Canada and the United States and changes in predators' communities have been associated with increases in the relative abundance of wildlife hosts of *Borrelia burgdorferi* and in the infection prevalence in nymphal ticks (Logiudice et al. 2008; Levi et al. 2012). Certainly, forest fragmentation and/or reforestation may exert profound changes in wildlife host communities, with potential effects on arthropod vectors and associated pathogens. The effects of these changes in the dynamics of several VBZ may be unpredictable (Dantas-Torres 2015).

Socioeconomic and political changes may also increase the risk of VBZ (Otranto et al. 2017). For example, the upsurge of tick-borne encephalitis in central and eastern European states has been correlated with poverty and household expenditure

on food, after the collapse of the Soviet Union (Sumilo et al. 2008). Indeed, changes in land-use, reduced use of pesticides, increased unemployment, and poverty might have resulted in increased interactions between people and infected ticks. The linkage between poverty and VBZ is well documented for some neglected diseases, such as leishmaniasis, African trypanosomiasis and Chagas disease. For example, the cracked walls and damp earth floors, together with an absence of sanitation and inadequate garbage collection in impoverished urban and peri-urban settings create phlebotomine sand fly breeding sites and increase the risk of leishmaniasis (Alvar et al. 2006). In the same way, people living in poor rural communities in Latin America, in proximity to forest areas and under precarious housing conditions and underlying poverty are at risk of VBZ, such as Chagas disease (Briceno-Leon 1987).

35.4 Clinical and Diagnostic Considerations

Patients (whether animal or human) suffering from a given VBZ may present with a suit of clinical signs and symptoms, ranging from a single, localized skin ulcer to severe, life-threatening systemic disease. For instance, canine and human patients affected by American cutaneous leishmaniasis caused by *Leishmania braziliensis* may present localized skin ulcers or mucocutaneous lesions. In turn, those suffering from zoonotic visceral leishmaniasis by *L. infantum* may exhibit systemic clinical signs (e.g., weight loss, fever, lymph node enlargement, and hepatosplenomegaly) with a potential fatal outcome (Lainson and Shaw 2005; Dantas-Torres 2009). Other leishmanial species such as *Leishmania amazonensis* may induce a range of clinical signs in dogs and humans and eventually produce a visceral form of leishmaniasis that may be confounded with *L. infantum* infections (Lainson and Shaw 2005; Dantas-Torres 2009),

Human patients affected by spotted fever rickettsioses may present with unspecific clinical signs and symptoms (e.g., flu-like illness) in the early stages of the disease (Dantas-Torres 2007), potentially leading to misdiagnosis, delays in treatment initiation, and death. Certainly, the variety of clinical signs and symptoms animals and humans may present when suffering from VBZ make the clinical diagnosis a challenging task for physicians and veterinary practitioners working in both endemic and non-endemic areas. In endemic areas, they may be more used to typical clinical presentations of endemic VBZ, but atypical cases in immunosuppressed individuals and co-infections may further complicate the clinical diagnosis.

Vector-borne nematodes may cause ocular infestations in animals and humans (Otranto and Eberhard 2011; Otranto et al. 2013). For instance, thelaziosis is a VBZ of animals (e.g., dogs and cats) and humans caused by *T. callipaeda*, whose adults live primarily under the nictitating membrane of the eye and may induce lacrimation, epiphora, conjunctivitis, keratitis, and even corneal ulcers (Otranto and Eberhard 2011). However, several other vector-borne nematode species may invade the eyes of animals and humans and must be included in the differential diagnosis. As examples, a rare nematode (*Pelicitus* genus) was retrieved from the eye of a human patient from the Amazon region in Brazil (Bain et al. 2011) and an enigmatic

Dirofilaria sp. molecularly close to *Dirofilaria immitis* was characterized from a human patient from northern Brazil (Otranto et al. 2011a). In addition, cases of *Onchocerca lupi* ocular infestations was originally diagnosed in a patient from Turkey (Otranto et al. 2011b) and afterwards in Germany, Tunisia, and Iran (Otranto et al. 2012a; Rojas et al. 2021). Remarkably, *O. lupi* is a little known, but emerging parasite of dogs in parts of the United States and Europe (Labelle et al. 2013; Otranto et al. 2012b), whose zoonotic potential was underestimated until recently. Finally, human infestations by *Dirofilaria* spp. often result in pulmonary nodule formations, which may be erroneously diagnosed as malignant neoplasm, hence representing a further challenge to physicians (Genchi et al. 2011).

A number of rickettsial organisms have been described in recent years and some of them have been implicated in human disease (Dantas-Torres et al. 2012b; Oteo and Portillo 2012). For example, *Rickettsia massiliae* was originally described from ticks in 1994 from France and recently found to be a ubiquitous emerging pathogen of humans (Vitale et al. 2006; Parola et al. 2008; García-García et al. 2010). All published cases of *R. massiliae* infections were clinically similar to Mediterranean spotted fever suggesting that many cases of *R. massiliae* infections are likely to be misdiagnosed as by *Rickettsia conorii* infections (Parola et al. 2008). The same is true for *Rickettsia parkeri*, which has been described as a human pathogen in the United States and South America, where the disease is likely to be misdiagnosed as other infectious illnesses, including Rocky Mountain spotted fever, dengue fever, and leptospirosis (Romer et al. 2011).

The above-mentioned examples illustrate how difficult the clinical diagnosis of VBZ may be and underline that medical physicians and veterinary practitioners, as well as public health authorities should be prepared to react promptly in face of atypical cases in endemic and non-endemic areas. It is vital to avoid delays in the treatment initiation, which may eventually result in the death of the patient (Dantas-Torres et al. 2012b).

35.5 Advancements in VBZ Diagnosis

There have been several advancements in the field of VBZ diagnosis in the past few decades. The refinement of serological tests and the development of molecular biology tools, along with the decipherment of the genomes for several pathogens, have improved considerably our capacity to diagnose VBZ in animals and humans, allowing the detection and characterization of new, emerging pathogens (Otranto 2015). Moreover, the adoption of a holistic diagnostic approach has culminated in the discovery of new human pathogens, including several bacterial species. For instance, *R. rickettsii*, the causative agent of Rocky Mountain spotted fever, was for many years the only rickettsial agent to be definitely associated with human diseases in the United States. In 2004, the use of a comprehensive diagnostic approach (including serological testing, immunohistochemical staining, cell culture isolation, and molecular methods) led to the description of the first cases of rickettsiosis by *R. parkeri*, a spotted fever group first identified 65 years ago in Gulf Coast ticks (*Amblyomma maculatum*) collected from the southern United States (Paddock et al. 2004).

Remarkably, *R. parkeri* has been detected in *A. maculatum* and *A. triste* in several South America countries, which suggests that the distribution of this *Rickettsia* in the western hemisphere is probably wider than currently known. Furthermore, a case report from Argentina suggested that *R. parkeri* infections in this region are likely to be misdiagnosed as other infectious diseases, including Rocky Mountain spotted fever and dengue fever (Romer et al. 2011). The use of molecular tools has been proven essential for the correct aetiological diagnosis of tick-borne spotted fever rickettsioses in the United States and other countries of the western hemisphere.

Human malaria has traditionally been associated to four human-adapted *Plasmodium* species: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*. Recently, a fifth species (namely, *P. knowlesi*) was implicated in several cases of human malaria across south-eastern Asia (Antinori et al. 2013). These cases were initially misidentified as *P. malariae* infections by microscopy, but refined molecular study revealed a large focus of naturally acquired human infections by *P. knowlesi* in Malaysian Borneo (Singh et al. 2004). Importantly, the initial suspicion that another *Plasmodium* species was involved in human malaria in Malaysian Borneo was based on clinical (e.g., fever with chills and rigor, headache, cough, and vomiting), epidemiological (e.g., mostly adults), and laboratory data (e.g., high parasitaemia, over 5000 parasites per μl) (Singh et al. 2004). Once again, the "*P. knowlesi* example" underlines the importance of using a comprehensive approach, coupling clinical and epidemiological data with modern diagnostic test results toward a correct diagnosis of VBZ.

The use of molecular biology in epidemiological studies has also advanced our understanding of the transmission dynamics and origin of several pathogens causing VBZ. For long time, *Leishmania chagasi* was considered to be the agent of zoonotic visceral leishmaniasis in the New World and referred to as a distinct species from *L. infantum*. However, molecular analyses confirmed the Old World origin of the so-called *L. chagasi*, which is now widely accepted as a synonym with *L. infantum* (Kuhls et al. 2011). Another example of the utility of molecular tools in the diagnosis of VBZ is represented by the first ever described case of zoonotic ocular infestation by *O. lupi* in a patient from Turkey (Otranto et al. 2011b). The nematode detected in a subconjunctival mass was detected on the superonasal quadrant of bulbar conjunctiva, but cut during the surgical removal. The nematode was initially identified as belonging to the genus *Onchocerca* and later on molecularly characterized as *O. lupi*. After this first occurrence, additional cases were detected in human patients as well as in dogs and cats worldwide (Rojas et al. 2021).

The development of rapid tests including point-of-care assays is also an important advancement in terms of diagnosis of VBZ such as leishmaniasis and Chagas disease (Barfield et al. 2011; Grimaldi et al. 2012; Leony et al. 2019; Rodrigues et al. 2022). For instance, a new rapid test (PATH-Lemos rapid test) for the point-of-care diagnosis of Chagas disease was compared with a commercially available rapid test (Chagas STAT-PAK, Chembio). As compared to the reference test (the Ortho *T. cruzi* ELISA, Johnson & Johnson), the PATH-Lemos rapid test demonstrated an optimal sensitivity of 99.5% and specificity of 96.8%, respectively, while the Chagas STAT-PAK showed a sensitivity of 95.3% and specificity of 99.5% (Barfield et al. 2011). More recently, a study assessed the performance of chimeric proteins for the detection of anti-*T. cruzi*

IgG antibodies in dogs, with promising results in terms of sensitivity and specificity (Leony et al. 2019). Some of these chimeric proteins were used to develop an immunochromatographic rapid test for the serological diagnosis of *T. cruzi* infection in wild and domestic canids (Rodrigues et al. 2022). These results show that both rapid tests present high levels of sensitivity and specificity, representing reliable tools for screening and diagnosis of Chagas disease. As another example, a new rapid test for plague detection in humans and other mammalian hosts has recently been developed, with high sensitivity and specificity (Bezerra et al. 2022).

Undoubtedly, these advancements will influence the clinical practice of medical physicians and veterinary practitioners. Hopefully, these tools will also be made available for point-of care use in poor rural settings in VBZ-endemic countries in order to improve the diagnostic standard for VBZ in these areas.

35.6 Unresolved Issues

The epidemiology and distribution of VBZ is changing due to several factors, such as unplanned urbanization, illegal deforestation, changing demographics, economic crisis, increased global movement of animals and people, changes in human behavior, land use and practices (Colwell et al. 2011; Kilpatrick and Randolph 2012; Dantas-Torres 2015). As a consequence, our understanding regarding several aspects (from aetiology to control) of VBZ has also changed in recent years. For example, the refinement and widespread use of genetic tools has greatly improved our capacity of detecting and identifying microorganisms in animals, humans, and arthropods. Indeed, a number of microorganisms have recently been detected in arthropods (e.g., ticks) and some of them have been implicated in human disease (Dantas-Torres et al. 2012b). On the other hand, the pathogenic potential of several recently described organisms remain largely unknown and further research is fundamental to predict the emergence of novel VBZ in animals and humans.

The diagnosis of VBZ in animals and humans has advanced considerably in the past decades. For example, rapid immunochromatographic diagnostic tests and PCR-based tools have been developed (Dantas-Torres et al. 2012c), even if most of these tools are still largely restricted to research institutes and reference diagnostic centers. Indeed, the effective implementation of these new diagnostic tools in the field may still be far from reality in some VBZ-endemic areas. Considering the diversity of pathogenic agents that may potentially infect animals and humans in tropical and subtropical regions, the use of accurate diagnostic methods is fundamental to improve clinical practice. Similarly, a precise and rapid etiological diagnosis is pivotal to expedite treatment decisions.

It is acknowledged that the control of VBZ in animals and humans is a difficult task due to the inherent complexities involved in the transmission cycles of these diseases. When a VBZ is established in a given area, it is very difficult to eradicate the pathogen and/or the vector or even to reduce the burden of disease. An appropriate example is the case of zoonotic visceral leishmaniasis, which is still causing considerable morbidity and mortality in endemic areas. In Brazil, for example, a control program against zoonotic

visceral leishmaniasis has been in place since more than 50 years ago with limited or no impact on the incidence of the disease in dogs and humans (Romero and Boelaert 2010; Dantas-Torres et al. 2019). For any VBZ to be controlled, it is fundamental to reduce the contact between susceptible hosts (animals and people) and vectors. A number of tools for reducing the contact of animals with arthropods (e.g., phlebotomine sand flies, mosquitoes, and ticks) have been developed in recent years, such as collars and spot-on pipettes containing active compounds with insecticide and repellent activity (Dantas-Torres et al. 2012c). Insecticide impregnated bed nets may reduce the exposure of humans to arthropod vectors and thus contribute to VBZ control (Kroeger et al. 2003). Unfortunately, most people living in poor rural communities, tropical and subtropical regions of the world cannot handle the costs of preventive tools. In such cases, public health authorities should elaborate and implement governmental programs for the control of VBZ in order to reduce the burden of these diseases in animals and humans living in poor rural communities.

Continuous education for medical physicians and veterinary practitioners is pivotal for controlling VBZ, as they are in the front line and should be up-to-date regarding recent advances in VBZ and base their clinical practice on the highest quality scientific evidence available. Improving VBZ detection and reporting in developing countries is central to have more precise burden estimates in these countries, which in turn is important to define priority in terms of control and management. In this context, the adoption of a One Health approach toward the management of VBZ has also been emphasized in recent years (Day 2011; Dantas-Torres et al. 2012b; Bezerra-Santos et al. 2021; Mendoza-Roldan et al. 2021a) and will be a critical step toward the control of these diseases. Indeed, it is imperative to reduce the gap of communication between, medical physicians, veterinarians, and public health authorities dealing with VBZ worldwide, particularly (but not exclusively) in low- and middle-income countries. Certainly, this is a simple but important step toward reducing the burden of VBZ in animals and humans around the world.

35.7 Cross-References

- ▶ Crimean-Congo Hemorrhagic Fever Virus: An Emerging and Re-emerging Pathogen of Public Health Concern
- ▶ Human African Trypanosomiasis: The Smoldering Scourge of Africa
- ▶ Q Fever (*Coxiella burnetii*)
- ▶ West Nile Virus: From Africa to Europe, America, and Beyond

References

Alvar J, Yactayo S, Bern C (2006) Leishmaniasis and poverty. Trends Parasitol 22:552–557

Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, WHO Leishmaniasis Control Team (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS One 7:e35671

Antinori S, Galimberti L, Milazzo L, Corbellino M (2013) *Plasmodium knowlesi*: the emerging zoonotic malaria parasite. Acta Trop 125:191–201

Bain O, Otranto D, Diniz DG, dos Santos JN, de Oliveira NP, Frota de Almeida IN, Frota de Almeida RN, Frota de Almeida LN, Dantas-Torres F, de Almeida Sobrinho EF (2011) Human intraocular filariasis caused by *Pelecitus* sp. nematode, Brazil. Emerg Infect Dis 17(5):867–869

Barbour AG, Fish D (1993) The biological and social phenomenon of Lyme disease. Science 260: 1610–1616

Barfield CA, Barney RS, Crudder CH, Wilmoth JL, Stevens DS, Mora-Garcia S, Yanovsky MJ, Weigl BH, Yanovsky J (2011) A highly sensitive rapid diagnostic test for Chagas disease that utilizes a recombinant *Trypanosoma cruzi* antigen. IEEE Trans Biomed Eng 58:814–817

Bern C, Maguire JH, Alvar J (2008) Complexities of assessing the disease burden attributable to leishmaniasis. PLoS Negl Trop Dis 2:e313

Bezerra MF, Dos Santos WJT, Rocha IV, Nadaes NR, Dantas-Torres F, da Silva Sales KG, de Melo Neto OP, da Silva MSB, Silva ED, de Almeida AMP, de Souza Reis CR (2022) Performance assessment of a new indirect rapid diagnostic test for plague detection in humans and other mammalian hosts. Acta Trop 231:106–427

Bezerra-Santos MA, Ramos RAN, Campos AK, Dantas-Torres F, Otranto D (2021) *Didelphis* spp. opossums and their parasites in the Americas: a one health perspective. Parasitol Res 120: 4091–4111

Bhate C, Schwartz RA (2011) Lyme disease: part I. Advances and perspectives. J Am Acad Dermatol 64:619–636

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI (2013) The global distribution and burden of dengue. Nature 496:504–507

Briceno-Leon R (1987) Rural housing for control of Chagas disease in Venezuela. Parasitol Today 3:384–387

Caron Y, Premont J, Losson B, Grauwels M (2013) *Thelazia callipaeda* ocular infection in two dogs in Belgium. J Small Anim Pract 54(4):205–208

Christou L (2011) The global burden of bacterial and viral zoonotic infections. Clin Microbiol Infect 17:326–330

Colwell DD, Dantas-Torres F, Otranto D (2011) Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet Parasitol 182:14–21

Dantas-Torres F (2007) Rocky Mountain spotted fever. Lancet Infect Dis 7:724–732

Dantas-Torres F (2009) Canine leishmaniosis in South America. Parasit Vectors 2(Suppl 1):S1

Dantas-Torres F (2015) Climate change, biodiversity, ticks and tick-borne diseases: the butterfly effect. Int J Parasitol Parasites Wildl 4:452–461

Dantas-Torres F, Aléssio FM, Siqueira DB, Mauffrey JF, Marvulo MF, Martins TF, Moraes-Filho J, Camargo MC, D'Auria SR, Labruna MB, Silva JC (2012a) Exposure of small mammals to ticks and rickettsiae in Atlantic Forest patches in the metropolitan area of Recife, North-Eastern Brazil. Parasitology 139:83–91

Dantas-Torres F, Chomel BB, Otranto D (2012b) Ticks and tick-borne diseases: a one health perspective. Trends Parasitol 28:437–446

Dantas-Torres F, Solano-Gallego L, Baneth G, Ribeiro VM, de Paiva-Cavalcanti M, Otranto D (2012c) Canine leishmaniosis in the old and new worlds: unveiled similarities and differences. Trends Parasitol 28:531–538

Dantas-Torres F, Miró G, Bowman DD, Gradoni L, Otranto D (2019) Culling dogs for zoonotic visceral leishmaniasis control: the wind of change. Trends Parasitol 35:97–101

Day MJ (2011) One health: the importance of companion animal vector-borne diseases. Parasit Vectors 4:49

Desjeux P (2004) Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 27:305–318

Gage KL, Kosoy MY (2005) Natural history of plague: perspectives from more than a century of research. Annu Rev Entomol 50:505–528

García-García JC, Portillo A, Núñez MJ, Santibáñez S, Castro B, Oteo JA (2010) A patient from Argentina infected with *Rickettsia massiliae*. Am J Trop Med Hyg 82:691–692

Genchi C, Kramer LH, Rivasi F (2011) Dirofilarial infections in Europe. Vector Borne Zoonotic Dis 11:1307–1317

Grimaldi G Jr, Teva A, Ferreira AL, dos Santos CB, Pinto I, de-Azevedo CT, Falqueto A (2012) Evaluation of a novel chromatographic immunoassay based on dual-Path platform technology (DPP® CVL rapid test) for the serodiagnosis of canine visceral leishmaniasis. Trans R Soc Trop Med Hyg 106:54–59

Hu Q, Chen B, Zhu Z, Tian J, Zhou Y, Zhang X, Zheng X (2012) Recurrence of Japanese encephalitis epidemic in Wuhan, China, 2009–2010. PLoS One 8:e52687

Kilpatrick AM, Randolph SE (2012) Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet 380:1946–1955

Kroeger A, Villegas E, Ordoñez-González J, Pabon E, Scorza JV (2003) Prevention of the transmission of Chagas' disease with pyrethroid-impregnated materials. Am J Trop Med Hyg 68:307–311

Kuhls K, Alam MZ, Cupolillo E, Ferreira GE, Mauricio IL, Oddone R, Feliciangeli MD, Wirth T, Miles MA, Schönian G (2011) Comparative microsatellite typing of new world *Leishmania infantum* reveals low heterogeneity among populations and its recent Old World origin. PLoS Negl Trop Dis 5:e1155

Labeaud AD, Bashir F, King CH (2011) Measuring the burden of arboviral diseases: the spectrum of morbidity and mortality from four prevalent infections. Popul Health Metrics 9:1

Labelle AL, Maddox CW, Daniels JB, Lanka S, Eggett TE, Dubielzig RR, Labelle P (2013) Canine ocular onchocercosis in the United States is associated with *Onchocerca lupi*. Vet Parasitol 193(1–3):297–301

Lainson R, Shaw JJ (2005) New World leishmaniasis. In: Cox FEG, Kreier JP, Wakelin D (eds) Topley and Wilson's microbiology and microbial infections, parasitology. Arnold, London, pp 313–349

Lee KS, Divis PC, Zakaria SK, Matusop A, Julin RA, Conway DJ, Cox-Singh J, Singh B (2011) *Plasmodium knowlesi*: reservoir hosts and tracking the emergence in humans and macaques. PLoS Pathog 7:e1002015

Leony LM, Freitas NEM, Del-Rei RP, Carneiro CM, Reis AB, Jansen AM, Xavier SCC, Gomes YM, Silva ED, Reis MG, Fraga DBM, Celedon PAF, Zanchin NIT, Dantas-Torres F, Santos FLN (2019) Performance of recombinant chimeric proteins in the serological diagnosis of *Trypanosoma cruzi* infection in dogs. PLoS Negl Trop Dis 13:e0007545

Levi T, Kilpatrick AM, Mangel M, Wilmers CC (2012) Deer, predators, and the emergence of Lyme disease. Proc Natl Acad Sci U S A 109:10942–10947

Logiudice K, Duerr ST, Newhouse MJ, Schmidt KA, Killilea ME, Ostfeld RS (2008) Impact of host community composition on Lyme disease risk. Ecology 89:2841–2849

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY et al (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. Lancet 380:2095–2128

Matos AL, Curto P, Simões I (2022) Moonlighting in Rickettsiales: expanding virulence landscape. Trop Med Infect Dis 7:32

Mendoza-Roldan JA, Ravindran Santhakumari Manoj R, Latrofa MS, Iatta R, Annoscia G, Lovreglio P, Stufano A, Dantas-Torres F, Davoust B, Laidoudi Y et al (2021a) Role of reptiles and associated arthropods in the epidemiology of rickettsioses: a one health paradigm. PLoS Negl Trop Dis 15:e0009090

Mendoza-Roldan JA, Mendoza-Roldan MA, Otranto D (2021b) Reptile vector-borne diseases of zoonotic concern. Int J Parasitol Parasites Wildl 15:132–142

Oteo JA, Portillo A (2012) Tick-borne rickettsioses in Europe. Ticks Tick Borne Dis 3:271–278

Otranto D (2015) Diagnostic challenges and the unwritten stories of dog and cat parasites. Vet Parasitol 212:54–61

Otranto D, Eberhard ML (2011) Zoonotic helminths affecting the human eye. Parasit Vectors 4:41
Otranto D, Diniz DG, Dantas-Torres F, Casiraghi M, de Almeida IN, de Almeida LN, dos Santos JN, Furtado AP, de Almeida Sobrinho EF, Bain O (2011a) Human intraocular filariasis caused by *Dirofilaria* sp. nematode, Brazil. Emerg Infect Dis 17:863–866
Otranto D, Sakru N, Testini G, Gürlü VP, Yakar K, Lia RP, Dantas-Torres F, Bain O (2011b) Case report: first evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae). Am J Trop Med Hyg 84:55–58
Otranto D, Dantas-Torres F, Cebeci Z, Yeniad B, Buyukbabani N, Boral OB, Gustinelli A, Mounir T, Mutafchiev Y, Bain O (2012a) Human ocular filariasis: further evidence on the zoonotic role of *Onchocerca lupi*. Parasit Vectors 5:84
Otranto D, Dantas-Torres F, Papadopoulos E, Petrić D, Ćupina AI, Bain O (2012b) Tracking the vector of *Onchocerca lupi* in a rural area of Greece. Emerg Infect Dis 18:1196–1200
Otranto D, Dantas-Torres F, Brianti E, Traversa D, Petri D, Genchi C, Capelli G (2013) Vector-borne helminths of dogs and humans in Europe. Parasit Vectors 6:16
Otranto D, Dantas-Torres F, Mihalca AD, Traub RJ, Lappin M, Baneth G (2017) Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis. Trends Parasitol 33:813–825
Otranto D, Mendoza-Roldan JA, Dantas-Torres F (2021) Thelazia callipaeda. Trends Parasitol 37: 263–264
Paddock CD, Sumner JW, Comer JA, Zaki SR, Goldsmith CS, Goddard J, McLellan SL, Tamminga CL, Ohl CA (2004) *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. Clin Infect Dis 38:805–811
Paddock CD, Finley RW, Wright CS, Robinson HN, Schrodt BJ, Lane CC, Ekenna O, Blass MA, Tamminga CL, Ohl CA, McLellan SL, Goddard J, Holman RC, Openshaw JJ, Sumner JW, Zaki SR, Eremeeva ME (2008) *Rickettsia parkeri* rickettsiosis and its clinical distinction from Rocky Mountain spotted fever. Clin Infect Dis 47:1188–1196
Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, Labauge P, Raoult D (2008) Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS Negl Trop Dis 2:e338
Raoult D, Mouffok N, Bitam I, Piarroux R, Drancourt M (2013) Plague: history and contemporary analysis. J Infect 66:18–26
Rodrigues ES, Santos GQ, da Silva MV, Barros JHS, Bernardo AR, Diniz RL, Rubim NM, Roque ALR, Jansen AM, Silva ED, Xavier SCC (2022) Chagas immunochromatographic rapid test in the serological diagnosis of *Trypanosoma cruzi* infection in wild and domestic canids. Front Cell Infect Microbiol 12:835383
Rojas A, Morales-Calvo F, Salant H, Otranto D, Baneth G (2021) Zoonotic ocular onchocercosis by *Onchocerca lupi*. Yale J Biol Med 94:331–341
Romer Y, Seijo AC, Crudo F, Nicholson WL, Varela-Stokes A, Lash RR, Paddock CD (2011) *Rickettsia parkeri* Rickettsiosis, Argentina. Emerg Infect Dis 17:1169–1173
Romero GA, Boelaert M (2010) Control of visceral leishmaniasis in Latin America – a systematic review. PLoS Negl Trop Dis 4:e584
Schwartz AB, Lejeune M, Verocai GG, Young R, Schwartz PH (2021) Autochthonous *Thelazia callipaeda* infection in dog, New York, USA, 2020. Emerg Infect Dis 27:1923–1926
Simarro PP, Cecchi G, Franco JR, Paone M, Diarra A, Ruiz-Postigo JA, Fèvre EM, Mattioli RC, Jannin JG (2012) Estimating and mapping the population at risk of sleeping sickness. PLoS Negl Trop Dis 6:e1859
Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, Thomas A, Conway DJ (2004) A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet 363:1017–1024
Sumilo D, Bormane A, Asokliene L, Vasilenko V, Golovljova I, Avsic-Zupanc T, Hubalek Z, Randolph SE (2008) Socio-economic factors in the differential upsurge of tick-borne encephalitis in central and Eastern Europe. Rev Med Virol 18:81–95

Tarleton RL, Curran JW (2012) Is Chagas disease really the "new HIV/AIDS of the Americas"? PLoS Negl Trop Dis 6:e1861

Vélez ID, Carrillo LM, López L, Rodríguez E, Robledo SM (2012) An epidemic outbreak of canine cutaneous leishmaniasis in Colombia caused by *Leishmania braziliensis* and *Leishmania panamensis*. Am J Trop Med Hyg 86:807–811

Vitale G, Mansuelo S, Rolain JM, Raoult D (2006) *Rickettsia massiliae* human isolation. Emerg Infect Dis 12:174–175

Vorou RM, Papavassiliou VG, Tsiodras S (2007) Emerging zoonoses and vector-borne infections affecting humans in Europe. Epidemiol Infect 135:1231–1247

Borrelia Ecology, Evolution, and Human Disease: A Mosaic of Life

36

Gabriele Margos, Anna J. Henningsson, Sabrina Hepner, Mateusz Markowicz, Andreas Sing, and Volker Fingerle

Contents

All authors are members of ESGBOR, European Society for Clinical Microbiology and Infectious Diseases Study Group for Lyme borreliosis.

G. Margos · S. Hepner · V. Fingerle (✉)
National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
e-mail: Gabriele.Margos@lgl.bayern.de; Sabrina.Hepner@lgl.bayern.de; Volker.Fingerle@lgl.bayern.de

A. J. Henningsson
Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linköping, Sweden

National Reference Laboratory for Borrelia and Other Tick-Borne Bacteria, Department of Laboratory Medicine, Division of Clinical Microbiology, Region Jönköping County, Jönköping, Sweden
e-mail: anna.jonsson.henningsson@rjl.se

M. Markowicz
AGES - Austrian Agency for Health and Food Safety, Vienna, Austria
e-mail: mateusz.markowicz@ages.at

A. Sing
Dept. of Public Health Microbiology, Bavarian Health and Food Safety Authority (LGL), National Consiliary Laboratory on Diphtheria and National Reference Center for Borrelia, Oberschleißheim, Germany

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_49

Abstract

Lyme borreliosis (LB) and relapsing fever (RF) are zoonotic diseases that are caused by spirochetal bacteria belonging to the genus *Borrelia*. The agents are generally maintained in natural transmission cycles by vector ticks (exception: body louse) and reservoir hosts. Lyme borreliosis (synonym in North America: Lyme disease, LD) is the most frequently reported tick-borne disease in Europe and North America. It mainly affects skin, large joints, nervous system or heart and is considered a multi-system disorder. Relapsing fever manifests as recurrent febrile attacks accompanied by headaches, muscle and joint aches, interrupted by afebrile intervals. It mainly occurs in tropical and subtropical regions including North and South America, Africa, Asia, and South European countries. In this chapter we describe the genus Borrelia, the huge diversity that has become apparent in recent years, the geographical distribution of its species, and the complex genome that is reflected in the complex ecology and disease symptoms. We also give information on diagnostics and its challenges, therapy, and prophylactic measures.

Keywords

Lyme borreliosis · Relapsing fever · Taxonomy · Diagnosis · Vector ticks

36.1 Introduction

Lyme borreliosis (LB) and relapsing fever (RF) are zoonotic diseases that are caused by spirochetal bacteria belonging to the genus *Borrelia*. The agents are generally maintained in natural transmission cycles by vector ticks (exception: body louse) and reservoir hosts. Lyme borreliosis (synonym in North America: Lyme disease, LD) was named after the town Old Lyme in Connecticut, United States, where a cluster of

juvenile arthritis cases (Steere et al. 1976, 1978, 2016) led ultimately to the discovery of the spirochetal agent of LB, *Borrelia burgdorferi* (Burgdorfer et al. 1982). The bacterium was described as species in 1984 (Johnson et al. 1984). In Europe, it had been suspected since the turn of the last century that a tick-borne agent(s) was the cause of medical conditions that are now recognized as LB (reviewed by Stanek et al. 2002).

Descriptions of conditions reminiscent of relapsing fever go back to antiquity and Hippocrates' times, but only in 1843 was the name "relapsing fever" coined by David Craigie during an epidemic in Edinburgh. The first microscopic observation of the etiological agent of louse-borne relapsing fever, now known as *Borrelia recurrentis*, was in 1868 by Otto Obermeier in the blood of febrile patients and reported to the Berlin Medical Society ("Berliner medicinischen Gesellschaft") in 1873 (reviewed by Warrell 2019, see references therein). The spirochetal agent of louse-borne relapsing fever was originally named *Protomycetum recurrentis*, *Spirochaeta obermeieri*, or *Spirochaeta recurrentis* (Lebert 1876; Cutler 2010) and then *Borrelia recurrentis*, when the genus was renamed after the French microbiologist Amédée Borrel by Swellengrebel in 1907 (Bergey 1925; Skerman et al. 1980). "Tick fever" – now known as tick-borne relapsing fever – was initially described in East Africa in 1904 and later documented in other parts in Africa (Elbir et al. 2013; Trape et al. 2013). New species are still being discovered (Fingerle et al. 2016), and the taxonomy of soft tick vectors is still under investigation (Trape et al. 2013). Louse-borne RF had probably the most severe impact on humankind of all *Borrelia* species causing huge epidemics during times of war, migration, poverty, and poor hygiene (reviewed by Warrell 2019, for a historical report see Lebert 1876).

Lyme borreliosis is the most frequently reported tick-borne disease in Europe and North America. It can affect tissues and organs and is considered a multi-system disorder. It may affect the skin, joints, nervous system, and other internal organs (Steere 2001; Stanek et al. 2011). A typical manifestation of LB is erythema migrans (also called bull's-eye rash), which is a skin manifestation that appears as a roundish red lesion (often around a central clearing) that slowly grows in diameter. Other manifestations are lymphocytoma (lymphadenosis benigna cutis), meningopolyneuritis (also known as Garin-Bujadoux-Bannwarth syndrome), unilateral or bilateral facial palsy, meningitis, cranial neuropathies, acrodermatitis chronica atrophicans, Lyme arthritis, and Lyme carditis. Lyme neuroborreliosis is a condition that appears to be more frequent in Europe than in North America but with a more similar clinical picture than often assumed (Stanek et al. 2012; Koedel et al. 2015).

Relapsing fever manifests as recurrent febrile attacks accompanied by headaches, muscle and joint aches interrupted by afebrile intervals (Cutler 2010; Warrell 2019). It mainly occurs in tropical and subtropical regions including North and South America, Africa, Asia, and South European countries (Rebaudet and Parola 2006). *Borrelia* species that have been reported to cause relapsing fever in humans include *B. duttonii*, *B. crocidurae*, *B. hispanica*, *Ca.* B. kalaharica in Africa, *B. persica* in Asia, *B. hermsii*, and to a lesser extent *B. turicatae* and *B. parkeri* in Western North America (Hinnebusch et al. 1998; Rebaudet and Parola 2006; Schwan et al. 2007; Elbir et al. 2013; Fingerle et al. 2016). *Borrelia recurrentis* causes louse-borne relapsing fever, which is often more severe than tick-borne relapsing fever (Warrell 2019). Upon antibiotic treatment, a condition called Jarisch-Herxheimer reaction can

be provoked (less often and less severe also in other RF species) and trigger organ failure that may cause death of the patient. Nowadays, *B. recurrentis* is mainly found in the Horn of Africa (Hoch et al. 2015).

One tick-borne RF species is an exception to the rule: *Borrelia miyamotoi* is vectored by ticks of the genus *Ixodes*, occurs in the temperate zones of the Northern Hemisphere, and can cause untypical RF illness (Fukunaga et al. 1995; Scott et al. 2010; Platonov et al. 2011; Hovius et al. 2013; Telford et al. 2015; Boden et al. 2016).

In this chapter, we describe the genus *Borrelia,* the huge diversity that has become apparent in recent years, the geographical distribution of its species, and the complex genome that is reflected in the complex ecology and disease symptoms. We also give information on diagnostics and its challenges, therapy, and prophylactic measures.

36.2 The Genus *Borrelia*

The genus *Borrelia* (emended Margos et al. 2018) was first described by Swellengrebel 1907, with *Borrelia anserina* as the chosen type species (Skerman et al. 1989). The species is an avian pathogen that can cause clinical disease in fowl and has a worldwide distribution (CAB International 2002). The genus currently comprises 60 proposed and named species (Table 1), several of which have "Candidatus" status, which means that cultured isolates are not available (Stackebrandt et al. 2002) (see also List of Prokaryotic Names with Standing in Nomenclature (LPSN), https://www.bacterio.net/). There are 22 species with validly published names under the International Code of Nomenclature of Prokaryotes (ICNP) within the RF group of spirochetes, 20 species with validly published names within the LB group of spirochetes (also termed *Borrelia burgdorferi* sensu lato (s.l.) species complex), and one species (*B. turcica* Güner et al. 2004) within a third group, sometimes called reptile-associated group (Margos et al. 2018). Apart from these species, there is a large diversity of species that have not yet been named officially or do not have validly published names and their pathogenic potential is largely unknown (Mitani et al. 2004; Lin et al. 2005; Takano et al. 2011; Fedorova et al. 2014; Ivanova et al. 2014; Ehounoud et al. 2016; Fingerle et al. 2016; Loh et al. 2017; Kumagai et al. 2018; Qiu et al. 2019; Binetruy et al. 2020; Muñoz-Leal et al. 2020; Norte et al. 2020; Bermúdez et al. 2021; Weck et al. 2022). A phylogeny based on 16S rRNA sequences shows (Fig. 1 from Margos et al. 2020c) that some of these *Borrelia* lineages cluster within clades of previously characterized *Borrelia* species or form their own clades: for example, *Candidatus* B. kalaharica, *Ca.* B. texasensis, and *Borrelia* sp. from Tanzania fall in the argasid-transmitted RF clade; *Ca*. B. aligera (not shown), *B. chilensis,* and *Ca*. B. ibitipoquensis (not shown) fall in the *Ixodes*-transmitted LB clade; while *Ca*. B. africana and *Ca*. B. ivorensis form their own clade (Mitani et al. 2004; Lin et al. 2005; Ivanova et al. 2014; Ehounoud et al. 2016; Fingerle et al. 2016; Muñoz-Leal et al. 2020; Norte et al. 2020). A significant diversity exists in borreliae described from metastriate ticks that form unique, deeply branching lineages within the genus; these include *Ca*. Borrelia tachyglossi from echidnas (Loh et al. 2017), several novel species from snakes (Takano et al. 2010), Testudines (Takano

Table 1 Overview of currently known *Borrelia* species with type strains and other characteristics

Name	Type strain	Pathogenic to; disease	Reservoir hosts	Vector species	Nomenclatural status	Geographic distribution	Reference	Association
Borrelia afzelii	VS461	Human; LB	Rodents, insectivores	*I. ricinus, I. persulcatus, I. hexagonus*	Validly published under the ICNP, correct name	Europe, Asia	Canica et al. 1993; Validation list no. 48. Int J Syst Bacteriol 1994; 44:182–183	LB
Borrelia americana	SCW-41	Unknown	Birds, rodents	*I. minor, I. pacificus*	Validly published under the ICNP, correct name	North America	Postic et al. (2007), Rudenko et al. (2009)	LB
Borrelia bavariensis	PBi	Human; LB	Rodents	*I. ricinus, I. persulcatus*	Validly published under the ICNP, correct name	Europe, Asia	Margos et al. (2009, 2013)	LB
Borrelia bissettiae	DN-127	Potentially human; LB	Rodents	*I. ricinus, I. spinipalpis, I. pacificus*	Validly published under the ICNP, correct name	North America	Postic et al. (1998), Margos et al. (2016)	LB
Borrelia burgdorferi sensu stricto	B31	Human; LB	Rodents, birds, insectivores	*I. ricinus, I. hexagonus, I. scapularis, I. pacificus, I. minor, I. affinis*	Validly published under the ICNP, correct name	Europe, North America	Johnson et al. (1984)	LB

(continued)

Table 1 (continued)

Name	Type strain	Pathogenic to; disease	Reservoir hosts	Vector species	Nomenclatural status	Geographic distribution	Reference	Association
Borrelia californiensis	CA446	Unknown	Rodents	*I. pacificus, I. spinipalpis, I. jellisoni*	Validly published under the ICNP, correct name	North America	Postic et al. (2007), Margos et al. (2016)	LB
Borrelia carolinensis	SCW-22	Unknown	Rodents	*I. minor*	Validly published under the ICNP, correct name	North America	Rudenko et al. (2011)	LB
Borrelia garinii	20047	Human; LB	Birds	*I. ricinus, I. persulcatus, I. uriae*	Validly published under the ICNP, correct name	Europe, Asia, seabird colonies Canada	Baranton et al. (1992)	LB
Borrelia japonica	HO14	Unknown	Rodents	*I. ovatus*	Validly published under the ICNP, correct name	Asia	Kawabata et al. 1993, Validation list no. 50. Int J Syst Bacteriol 1994; 44:595	LB
Borrelia kurtenbachii	25015	Unknown	Rodents	Unknown	Validly published under the ICNP, correct name	North America	Margos et al. (2010, 2014)	LB

Borrelia lanei	CA28-91	Unknown	Lagomorphs?	*I. spinipalpis, I. pacificus*	Validly published under the ICNP, correct name	North America	Postic et al. (2007), Margos et al. (2017c)	LB
Borrelia lusitaniae	PoTiB2	Potentially human; LB	Lizards	*I. ricinus*	Validly published under the ICNP, correct name	Europe, North Africa	Le Fleche et al. (1997)	LB
Borrelia maritima	CA690	Unknown	Unknown	Unknown	Validly published under the ICNP, correct name	Western United States	Fedorova et al. (2014), Margos et al. (2020a)	LB
Borrelia mayonii	M14-1420	Human; borreliosis	Rodents?	*I. scapularis*	Validly published under the ICNP, correct name	North America	Pritt et al. (2016)	LB
Borrelia sinica	CMN3	Unknown	Rodents	*I. ovatus*	Validly published under the ICNP, correct name	Asia	Masuzawa et al. (2001)	LB
Borrelia spielmanii	PC-Eq17	Human; LB	Rodents (dormouse)	*I. ricinus, I. hexagonus*	Validly published under the ICNP, correct name	Europe	Richter et al. (2004, 2006)	LB

(continued)

Table 1 (continued)

Name	Type strain	Pathogenic to; disease	Reservoir hosts	Vector species	Nomenclatural status	Geographic distribution	Reference	Association
Borrelia tanukii	Hk501	Unknown	Rodents	*I. tanuki*	Validly published under the ICNP, correct name	Aisa	Fukunaga et al. (1996)	LB
Borrelia turdi	Ya501	Unknown	Birds	*I. turdus, I. frontalis*	Validly published under the ICNP, correct name	Asia, Europe	Fukunaga et al. (1996)	LB
Borrelia valaisiana	VS116	Nonpathogenic	Birds	*I. ricinus*	Validly published under the ICNP, correct name	Europe	Wang et al. (1997)	LB
Borrelia yangtzensis	Okinawa CW62	Potentially human; LB	Rodents	*I. granulatus*	Validly published under the ICNP, correct name	Asia	Margos et al. (2015b)	LB
Borrelia chilensis	VA1	Unknown	Rodents	*I. stilesi*	Not validly published	South America	Ivanova et al. (2014)	LB
Candidatus Borrelia aligera	ND	Unknown	Birds?	*Unknown*	Not validly published	Europe?	Norte et al. (2020)	LB
Candidatus Borrelia andersonii	ND	Unknown	Birds, rabbits	*I. dentatus*	Not validly published	North America	Marconi et al. (1995)	LB

Candidatus Borrelia ibitipoquensis	ND	Unknown	Birds?/Bbsl	*I. paranaensis*	Not validly published	South America	Muñoz-Leal et al. (2020)	LB
Borrelia turcica	IST7	Unknown	Tortoises, birds, rodents	*H. aegyptium*	Validly published under the ICNP, correct name	Turkey, Greece, Iberian Peninsula, Italy, Morocco	Güner et al. (2004)	REP
Candidatus Borrelia tachyglossi	ND	Unknown	Echidnas?	*Bothriocroton concolor*	Not validly published	Australia	Loh et al. (2017)	REP
Borrelia anserina (Sakharoff 1891)	ND	Birds, avian borreliosis	Fowl	*Argas sp.*	Validly published under the ICNP, correct name	Worldwide	Bergey et al. (1925), Skerman et al. Approved Lists (1980)	avian borreliosis
Borrelia baltazardii	B.“x”	Human; RF		*O. tholozani*	Validly published under the ICNP, correct name	Iran	Karimi et al. (1979), Validation list no. 10. Int J Syst Bacteriol 1983; 33:438-440	RF
Borrelia brasiliensis	ND	Human?; RF	Dogs, armadillos, rodents?	*O. brasiliensis*	Validly published under the ICNP, correct name	Southern Brazil	Davis (1952), Skerman et al. Approved Lists 1980	RF
Borrelia caucasica	ND	Human, RF	Rodents?	*O. verrucosus*	Validly published under the ICNP, correct name	Ukraine, Caucasus, southeast Europe	Davis (1957) (Approved Lists 1980)	RF

(continued)

Table 1 (continued)

Name	Type strain	Pathogenic to; disease	Reservoir hosts	Vector species	Nomenclatural status	Geographic distribution	Reference	Association
Borrelia coriaceae	ND		Deer?, cattle?	*O. coriaceus*	Validly published under the ICNP, correct name	Western North America, Northwest California	Johnson et al. (1987)	RF
Borrelia crocidurae	ND	Human; RF	Rodents, shrews	*O. erraticus, O. sonrai*	Validly published under the ICNP, correct name	North and West Africa	Davis 1957 (Approved Lists 1980)	RF
Borrelia dugesii	ND		Rodents, *Neotoma micropus*	*O. dugesii*	Validly published under the ICNP, correct name	Mexico	Davis 1957 (Approved Lists 1980)	RF
Borrelia duttonii	ND	Human; RF	Chicken, pigs?	*O. moubata, O. porcinus*	Validly published under the ICNP, correct name	Central, Eastern, and Southern Africa	Bergey et al. (1925) (Approved Lists 1980)	RF
Borrelia graingeri	ND	Human	Unknown	*O. graingeri*	Validly published under the ICNP, correct name	Kenya	Davis 1957 (Approved Lists 1980)	RF

Borrelia harveyi	ND	Monkeys; bacteremia		Unknown	Validly published under the ICNP, correct name	East Africa	Davis 1948 (Approved Lists 1980), Trevisan et al. (2021)	RF
Borrelia hermsii	ND	Human; RF	Rodents, deer	*O. hermsi*	Validly published under the ICNP, correct name	Western North America, British Columbia (Canada)	Steinhaus 1946 (Approved Lists 1980)	RF
Borrelia hispanica	ND	Human; RF, infectious for dogs, cats	Cattle, dogs, cats, pigs, rodents, sheep	*O. erraticus*	Validly published under the ICNP, correct name	Iberian Peninsula, North Africa	Steinhaus 1946 (Approved Lists 1980)	RF
Borrelia latyschewii	ND	Human; RF		*O. tartakovsky*	Validly published under the ICNP, correct name	Iran, Middle East	Davis 1948 (Approved Lists 1980)	RF
Borrelia mazzotti	ND	Human; RF		*O. talaje*	Validly published under the ICNP, correct name	Central America, Western North America	Davis 1956 (Approved Lists 1980)	RF
Borrelia miyamotoi	HT31	Human; borreliosis	Rodents	*I. ricinus, I. persulcatus, I. scapularis, I. pacificus*	Validly published under the ICNP, correct name	Europe, Asia, North America	Fukunaga et al. (1995)	RF

(continued)

Table 1 (continued)

Name	Type strain	Pathogenic to; disease	Reservoir hosts	Vector species	Nomenclatural status	Geographic distribution	Reference	Association
Borrelia parkeri	ND	Human; RF		*O. parkeri*	Validly published under the ICNP, correct name	Western North America	Steinhaus 1946 (Approved Lists 1980)	RF
Borrelia persica	ND	Human; RF, infectious for dogs, cats		*O. tholozani*	Validly published under the ICNP, correct name	Central Asia, Middle East, Egypt, India	Steinhaus 1946 (Approved Lists 1980)	RF
Borrelia recurrentis		Human; louse-borne RF	Human	*P. humanus*	Validly published under the ICNP, correct name	Ethiopia, xxx	Lebert (1874), Bergey et al. (1925) (Approved Lists 1980)	RF
Borrelia theileri		Bovine spirochetosis	Cattle, sheep, goats, horses, bats	*Rhipicephalus sp., Margaropus australis*	Validly published under the ICNP, correct name	Africa, Australia, Brazil, Northern South America	Bergey et al. (1925) (Approved Lists 1980)	Bovine spirochetosis
Borrelia tillae	ND	Unknown	Rodents	*O. zumpti*	Validly published under the ICNP, correct name	Southern Africa	Zumpt and Organ (1961) (Approved Lists 1980)	RF

Borrelia turicatae	ND	Human; RF		*O. turicata*	Validly published under the ICNP, correct name	Canada (British Columbia), Southcentral, Southwestern United States, Mexico	Steinhaus 1946 (Approved Lists 1980)	RF
Borrelia venezuelensis	ND	Human; RF		*O. rudis*	Validly published under the ICNP, correct name	Central America, northern South America	Brumpt 1922 (Approved Lists 1980)	RF
"Borrelia puertoricensis"	ND	Unknown	Rodents?	*O. puertoricensis*	Not validly published	Central Panama	Bermúdez et al. (2021)	RF
Candidatus Borrelia africana	ND	Unknown	Unknown	*Am. variegatum*	Not validly published	Africa (Cote d'Ivoire)	Ehounoud et al. (2016)	RF
Candidatus Borrelia algerica	ND	Human; RF	Unknown	*Unknown*	Not validly published	Africa (Algeria)	Fotso Fotso et al. (2015)	RF
Candidatus Borrelia fainii	ND	Human; RF	Bats		Not validly published	Asia (China)	Qiu et al. (2019), Han et al. (2020), Li et al. (2021)	RF
Candidatus Borrelia ivorensis	ND	Human; RF		*Rhipicephalus sp.*	not validly published	Africa (Cote d'Ivoire)	Ehounoud et al. 2016	RF
Candidatus Borrelia javanense	ND	Unknown	Pangolins?	*Am. javanense*	not validly published	Unknown	Jiang et al. (2021)	RF
Candidatus Borrelia johnsonii	ND	Unknown	Bats	*C. kelley*	not validly published	United States (Iowa)	Schwan et al. (2009)	RF

(continued)

Table 1 (continued)

Name	Type strain	Pathogenic to; disease	Reservoir hosts	Vector species	Nomenclatural status	Geographic distribution	Reference	Association
Candidatus Borrelia kalaharica	ND	Human; RF	Unknown	*O. savigni*	not validly published	Africa	Fingerle et al. (2016)	RF
Candidatus Borrelia mahuryensis	A-FGy1	Unknown	Birds?	*Am. longirostre, Am. geayi*	not validly published	Americas	Binetruy et al. (2020)	RF
Candidatus Borrelia queenslandica	ND	Unknown	*R. villosissimus*	*Unknown*	not validly published	Australia (Queensland)	Carley and Pope (1962)	RF
Candidatus	ND / TXW-1	Unknown	Coyotes?	*D. variabilis*	not validly published	North America	Lin et al. (2005)	RF
Borrelia lonestari	ND	Southern tick-associated rash illness (STARI)	Deer, birds	*Am. americanum*	not validly published	North America	Barbour et al. (1996)	STARI
B. merionesi[a]	ND	Unknown	Unknown	Unknown	not validly published		Trape et al. (2013)	RF
B. microti[a]	ND	Unknown	Hedgehogs, rodents	*O. erraticus*	not validly published		Naddaf et al (2012)	RF
B. neotropicalis[a]	ND	Unknown	Unknown		not validly published		Jakab et al. (2022)	RF
B. osphepa[a]	ND	Unknown	Unknown	*O. spheniscus*	not validly published		Jakab et al. (2022)	RF
B. sogdiana[a]	ND	Unknown	Rodents	*O. papillipes*	not validly published		Jakab et al. (2022)	RF
B. lonestari-like[a]	ND	Deer	Unknown	*Ha.* spp.	not validly published		Jakab et al. (2022)	

[a]Species marked with 'a' are putative species that have been mentioned in the literature (see, e.g., reviews by Jakab et al. (2022), Trevisan et al. (2021)) but have not been properly described nor do they appear in the LPSN list; *ND* not designated

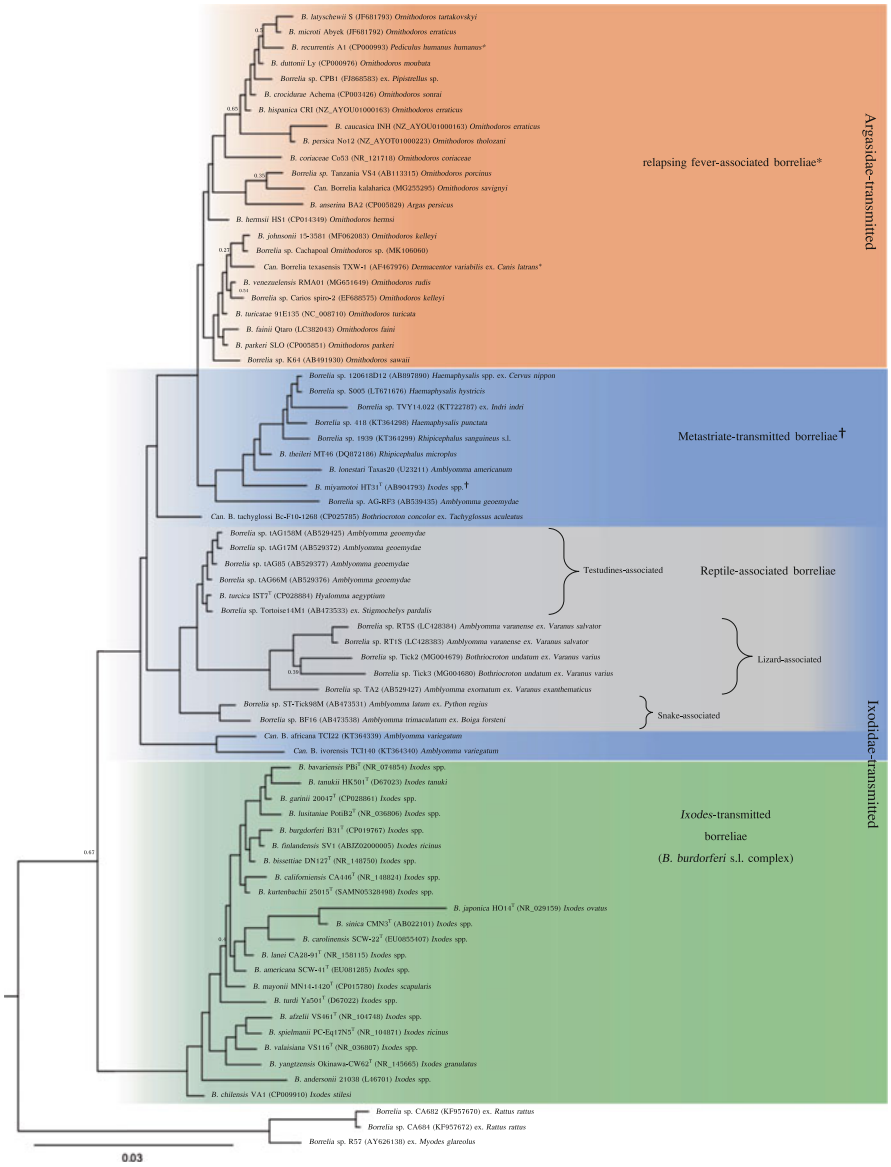

Fig. 1 Phylogenetic trees of *Borrelia* species based on the 16S rRNA locus. (Figure taken from (Margos et al. 2020c) with permission)

et al. 2011), lizards (Panetta et al. 2017; Kaenkan et al. 2020; Supriyono et al. 2019), and two putative species associated with *Haemaphysalis* spp. and Asian deer (Kumagai et al. 2018). Given the growing diversity in the genus *Borrelia* illustrated in Fig. 1, it would not be surprising if more species were found in the future (Elbir et al. 2013; Fingerle et al. 2016; Stete et al. 2018).

Historically, bacterial systematics relied on morphological and biochemical differences for differentiating bacterial taxa (Gajdacs 2019). Later on, bacterial taxonomy was complemented with DNA-DNA hybridization, melting temperature differences and 16S rRNA sequence analyses for characterization of species/strains. In the late 1990s and early 2000s, multilocus sequence typing (MLST), multilocus sequence analysis (MLSA), and genome comparison were accepted for taxonomic purposes (Stackebrandt et al. 2002; Gevers et al. 2006; Stackebrandt and Ebers 2006). For *Borrelia*, in 2008 an MLST/MLSA system has been deployed, which uses sequence fragments of eight chromosomally located housekeeping genes (Margos et al. 2008, 2009). The MLST database is maintained at the University of Oxford, Department of Zoology, and is part of the bacterial isolate genome sequence database (BIGSdb) (Jolley and Maiden 2010).

36.3 *Borrelia* Ecology: Hosts and Vectors

Borrelia are parasitic bacteria that depend on transmission between tick vectors and vertebrate hosts for survival; there are no free-living forms. As shown in Table 1, for *B. burgdorferi* s.l., a large array of vertebrates, including rodents, insectivores, lizards, and birds, can serve as reservoir hosts. However, the breadth of the ecological niches varies between species (Margos et al. 2019b). Some species, such as *B. burgdorferi* sensu stricto (s.s.), can use a wide variety of reservoir hosts (e.g., rodents, insectivores, birds), while others, such as *B. spielmanii*, have a narrow reservoir host spectrum, including dormice or potentially hedgehogs (reviewed by Wolcott et al. 2021). The same is true for vector association; while some species are adapted to use a wide range of vectors (e.g., *B. garinii* can utilize *Ixodes ricinus*, *I. persulcatus*, *I. pavlovskyi*, *I. uriae*), others are restricted to one vector species (e.g., *B. valaisiana* is mainly adapted to *I. ricinus*) (Margos et al. 2012a; Masuzawa 2004).

The genus *Borrelia* has increased considerably in diversity in recent years, and in addition to the classic division in relapsing fever group and *B. burgdorferi* s.l., there are lineages that take an intermediate position in phylogenies. These "intermediate" forms encompass several species that are associated with hard ticks and species that are associated with reptiles (snakes, tortoises, lizards). Transovarial transmission was suggested a regular feature in the ecology of RF spirochetes (at least for the soft tick-associated *Borrelia* species) but has not been proven for all species and not for intermediate, reptile-associated species (Kalmar et al. 2015). Given this ecology, i.e., transovarial transmission, it has been suggested that ticks not only function as vector but also as reservoir host for RF spirochetes (Piesman and Schwan 2010; Schwan and Raffel 2021; Schwan 2021). Thus, adaptation to vector tick species was thought to be an ecological driver, and several species are named after the tick species they were discovered in, e.g., *Borrelia hermsii*, *Ornithodoros hermsi*; *Borrelia coriaceae*, *O. coriaceus* (Trevisan et al. 2021; Jakab et al. 2022). As shown in Table 1 and reported by some researchers (Assous and Wilamowski 2009), this is not always the case, and some relapsing species do not match the name of its vector (e.g., *B. duttonii* – *O. moubata*) or may be able to use several tick species as vector (e.g., *B. crocidurae* – *O. erraticus, O. sonrai*). A notable exception in the RF group is

Borrelia recurrentis, which is transmitted by the human body louse *Pediculus humanus humanus* (Warrell 2019); there is no transovarial transmission in the louse, and humans are the only known host for this species. Although reservoir hosts may not be required for maintaining RF spirochetes in natural transmission cycles, as all ticks must to take blood meals from hosts for developing into the next stage or for egg production, spirochetes of the RF and the intermediate groups can also infect hosts, which may then function as reservoirs (Piesman and Schwan 2010).

Adaptation to different reservoir hosts or vectors are considered main drivers for diversification of species of the genus *Borrelia* and for determining the spatial range and structure of populations (Piesman 2002; Kurtenbach et al. 2006; Ogden et al. 2008; Hoen et al. 2009; Vollmer et al. 2011; Margos et al. 2012a, b; Elbir et al. 2013; Medlock et al. 2013; Mechai et al. 2015; Newman et al. 2015; Estrada-Peña et al. 2016; Talagrand-Reboul et al. 2018; Norte et al. 2020). As borreliae can be found in ticks that are not vector species and in animals that are not reservoir hosts, definitions for the terms **vector** and **reservoir host** have been established (Kahl et al. 2002): vector species and reservoir hosts have to fulfill the criteria that (i) they can become infected with borreliae, (ii) borreliae are maintained and can amplify, and (iii) transmission to the next host/vector must be successful. That means that not every tick or vertebrate that harbors *Borrelia* is also a "true" vector or reservoir host for that particular species. Thus, ideally, vector competence and reservoir host competence need to be experimentally confirmed (Margos et al. 2019b; Eisen 2020; Wolcott et al. 2021). The most reliable method for proving reservoir competence of a host and vector competence for a tick species is to perform transmission experiments, although this is laborious and logistically challenging. In xenodiagnosis, a common method in parasitology (Schenone 1999), naïve tick larvae feed on a host that has been challenged with *Borrelia* either by tick bite or needle inoculation. The viability of *Borrelia* in the resulting nymphs is evaluated; only reservoir hosts will transmit the bacteria to ticks (Ginsberg et al. 2005). If those nymphs are experimentally shown to infect a new host, then the vector competence of the tick is proven.

Although life history traits of ticks may vary, what all tick species have in common is that during their life cycle they produce eggs, larvae, nymphs, and adults. Larvae, nymphs, and adult female ticks require a blood meal to molt into the next developmental stage or to produce an egg batch, respectively (Sonenshine and Roe 2014; Gray et al. 2016). In some species, adult males may attach to hosts to facilitate feeding of females, but they do not require a blood meal (Wang et al. 1998). The number of nymphal stages may vary between taxa; often soft ticks have several sequential nymphal stages before molting into adult ticks. Soft ticks take fast blood meals within minutes, while *Ixodes* ticks require days to finish their blood meal (Vial 2009; Sonenshine and Roe 2014). Phenotypically, ticks can be nidicolous (endophilic) or non-nidicolous (exophilic). Between blood meals nidicolous ticks remain in close proximity to burrows or nests of their hosts; often they are more host specific than non-nidicolous ticks. Non-nidicolous ticks occupy open habitats and quest (i.e., search or wait for hosts) in the open (Apanaskevich and Oliver 2014). Ticks may vector a large variety of pathogens, including protozoa, bacteria, and viruses, and the differences in vector biology may significantly impact the epidemiology of transmitted microorganisms.

In the life cycle of *Borrelia*, ticks that have a generalist feeding behavior attach to a range of animals (including rodents and birds) and can serve as vectors for more than one *Borrelia* species. A good example for a hard tick species in Europe is *Ixodes ricinus* Linnaeus, 1758, which is known as vector for *B. afzelii* Canica et al 1993, *B. bavariensis* Margos et al. 2013, *B. burgdorferi s.s.* Johnson et al. 1984, *B. garinii* Baranton et al. 1992, *B. lusitaniae* Le Fleche et al. 1997, *B. miyamotoi* Fukunaga et al. 1996 (a RF species), *B. spielmanii* Richter et al. 2006, and *B. valaisiana* Wang et al. 1997 (Comstedt et al. 2011; Eisen 2020; Margos et al. 2012a; Masuzawa 2004). Transovarial transmission, which is well-known to occur in RF spirochetes, is infrequently reported in *B. burgdorferi* s.l. and may depend on the *Borrelia* sp.-*Ixodes* sp. combinations involved (Nefedova et al. 2004; Rollend et al. 2013; van Duijvendijk et al. 2016). Therefore, since experimental transmission of tick-borne agents in the laboratory is time-consuming and complex, researchers have (under the assumption of absence of transovarial transmission in *B. burgdorferi* s.l.) used infections in field-captured larvae collected feeding on hosts as a surrogate indicator of reservoir host competence.

The majority of RF spirochetes can be maintained in vector populations by transovarial transmission for prolonged periods of time because of the long life span of some soft tick species (Piesman and Schwan 2010; Schwan and Raffel 2021). Thus, ticks may be considered as infected hosts (Barbour and Hayes 1986). Certainly, the ticks need vertebrate animals for their blood meals and transmit *Borrelia* bacteria they carry to animals, which may then serve as reservoirs (Felsenfeld 1965; McCall et al. 2007; Cutler 2010; Elbir et al. 2013). Often, soft ticks are indiscriminate in their host choice and take blood meals from whatever hosts are available (Vial 2009).

As apparent in Fig. 1, the different clades of *Borrelia* use different tick genera as vectors. For the LB group of spirochetes ticks of the genus *Ixodes* serve as vectors, and it may appear in Fig. 1 that there is only one vector species per *Borrelia* species, but this is not always the case; see Table 1 (Comstedt et al. 2011; Margos et al. 2012a; Masuzawa 2004). Of the genus *Ixodes*, the most important vectors for human pathogenic *Borrelia* species (sometimes also termed bridge vectors) are the generalist species *I. ricinus* in Europe, *I. persulcatus* Schulze 1930 in Eastern Europe and Asia, and *I. scapularis* Say 1821 and *I. pacificus* Cooley & Kohls 1943 in North America (Swanson et al. 2006). Many of the RF species are transmitted by soft ticks of the genus *Ornithodoros;* one species, *B. recurrentis*, is adapted to the body louse; the type species, *B. anserina*, is associated with *Argas* spp., and one species, *B. miyamotoi*, is transmitted by *Ixodes* species that also transmit LB species, i.e. *I. pacificus*, *I. scapularis*, *I. ricinus*, and *I. persulcatus* (Fukunaga et al. 1995; Scoles et al. 2001; Richter et al. 2003; Warrell 2019; Trevisan et al. 2021; Jakab et al. 2022).

There can be a substantial fluctuation from year to year in tick and host abundance and *Borrelia* infection prevalence (Randolph et al. 2002; Ostfeld et al. 2006; Bregnard et al. 2020), and long-term data are required to address questions of increase/decrease of populations (Coipan et al. 2013; Bregnard et al. 2020; Okeyo et al. 2020).

36.4 *Borrelia* species: Geographic Distribution and Disease-Causing Species

Generally, the LB group of spirochetes is roughly distributed in a belt-like fashion between latitude 40 and 60, matching the presence of reservoir hosts and vector species (Fig. 2). However, *B. garinii* also occurs in seabird transmission cycles in the Southern and Northern Hemispheres, and in recent years, several species have been described to occur in South America (e.g., *B. chilensis*, *Ca*. B. ibitipoquensis, *Ca*. B. paulista) (Ivanova et al. 2014; Muñoz-Leal et al. 2020; Weck et al. 2022). Specifically, the adaptation of *Borrelia* species to reservoir host and vector species is reflected in the geographic distribution of individual species or populations (Margos et al. 2019b). In the *B. burgdorferi* s.l. complex, there are (i) species that are found in Europe only, including *B. spielmanii* and *B. valaisiana;* (ii) one species, *B. lusitaniae*, is found in Europe and Northern Africa; (iii) species that are found in Europe and Asia, e.g., *B. afzelii*, *B. bavariensis, B. garinii,* and *B. turdi*; (iv) species that are restricted to Asia, e.g., *B. japonica, B. sinica, B. tanukii,* and *B. yantzensis;* (v) species that have been found in North America, including *B. americana, B. andersonii, B. californiensis, B. carolinensis, B. kurtenbachii, B. lanei, B. maritima,* and *B. mayonii;* and (vi) species that are found in North America and Europe, e.g., *B. burgdorferi* s.s. and *B. bissettiae*.

Not all species that belong to the *B. burgdorferi* s.l. complex cause disease in humans. Six of the currently known 22 species are assured human pathogenic. Five of the species pathogenic to humans occur in Europe, including *B. afzelii*, *B. bavariensis*, *B. burgdorferi* s.s., *B. garinii*, and *B. spielmanii* (Fingerle et al. 2008; Stanek et al. 2011). Two species are the cause of human LD in North America,

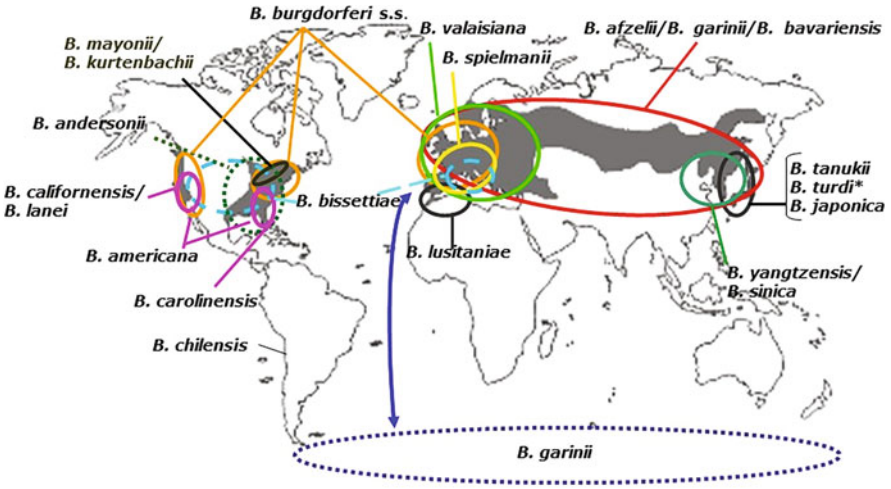

Fig. 2 Geographic distribution of Lyme borreliosis spirochetes. Image taken with permission from (Margos et al. 2019b), Frontiers in Ecology and Evolution

B. burgdorferi s.s. and *B. mayonii* (Spielman 1994; Steere et al. 2004; Pritt et al. 2016). The latter species was only discovered in 2016 in patients visiting the Mayo Clinic in Wisconsin (Pritt et al. 2016). Subsequently, additional symptomatic *B. mayonii*-infected patients have been discovered (Kingry et al. 2018).

In Europe, two additional species have been discussed as human pathogens; these are *B. lusitaniae* and *B. bissettiae*. *Borrelia lusitaniae* is commonly found in questing ticks in Mediterranean countries (Zhioua et al. 1999; De Michelis et al. 2000; Younsi et al. 2005; Dsouli et al. 2006; Amore et al. 2007; Norte et al. 2021). So far, two cases have been described suspecting *B. lusitaniae* as a human pathogen (Collares-Pereira et al. 2004; de Carvalho et al. 2008). One patient presented with vasculitis-like syndrome, the other with chronic skin lesion. *Borrelia bissettiae* has rarely been found in questing ticks in Europe (Hanincova et al. 2003; Coipan et al. 2016; Blazejak et al. 2018). However, one human isolate of *B. bissettiae* was obtained from a patient with symptoms resembling mild neuroborreliosis (Fingerle et al. 2008; Margos et al. 2016). In North America, *B. bissettiae* is commonly found in questing ticks at a regional scale and in certain habitat types (Postic et al. 1998; Picken and Picken 2000; Brown et al. 2006; Eisen et al. 2009; Fedorova et al. 2014), but no patient isolates have been obtained from humans (Girard et al. 2011).

Borrelia valaisiana has been reported to be nonpathogenic for humans (Margos et al. 2017b). Reservoir hosts are bird species, such as thrushes (*Turdus* spp.); its vector is *I. ricinus*; and it is being found as frequently as *B. garinii* in certain regions (Rauter and Hartung 2005). Although commonly found in questing ticks, to date not a single human isolate of *B. valaisiana* has been acquired (Margos et al. 2017b). For the remaining species (Table 1), the human pathogenic potential is unknown. Reasons may be that:

(i) Many of these species are transmitted by ticks that do not bite humans, and therefore, the species come not into contact with humans.
(ii) Or their genetic make-up may explain their lack of human pathogenicity.

Human disease-causing RF spirochetes often occur in subtropical and tropical regions (Fig. 3). Relapsing fever spirochetes have been divided into old and new world species (see Table 1). In North America, human disease-causing soft tick-associated species are mainly *B. hermsii* and *B. turicatae,* to a lesser extent *B. parkeri* (Piesman and Schwan 2010). Information on RF spirochetes in Central or South America is scarce and patchy (Lopez et al. 2016). In Africa, human disease is caused mainly by *B. duttonii*, *B. crocidurae*, and *B. recurrentis* (Elbir et al. 2013; Trape et al. 2013). For the louse-borne-associated species, *B. recurrentis*, foci of disease had been described in Peru and Africa, but nowadays, it seems to persist mainly in countries of the Horn of Africa (Warrell 2019). *Borrelia persica*, *B. latyschevii*, and *B. caucasica* cause human disease in the Middle and Central East, and *B. hispanica* is known to cause human and animal disease in Southern Europe and North Africa (Rebaudet and Parola 2006; Margos et al. 2020b). *Borrelia theileri* and *B. anserina* have a worldwide distribution and can cause disease in bovine and avian species, respectively (Trevisan et al. 2021; Jakab et al. 2022). *Borrelia miyamotoi* is a hard tick-associated relapsing fever species that uses the

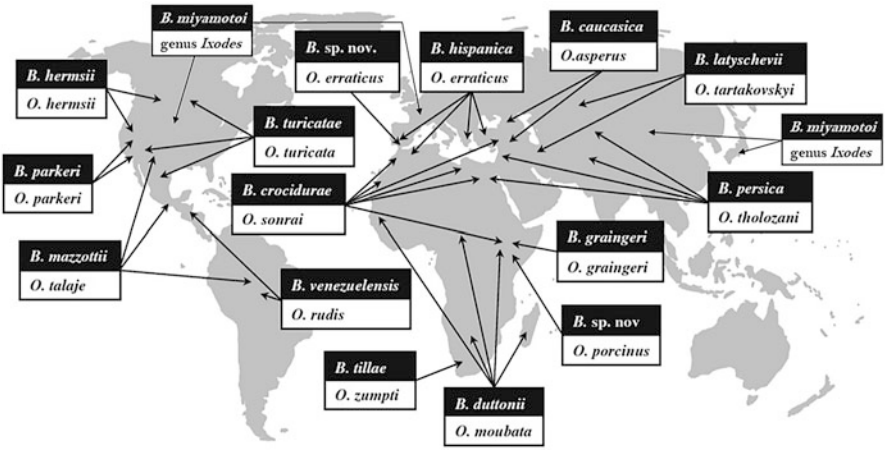

Fig. 3 Geographic distribution of relapsing fever spirochetes and their respective vectors. (Image with modifications taken with permission from Rebaudet and Parola 2006)

same vectors as LB species in North America, Europe, and Asia (Fukunaga et al. 1995; Scoles et al. 2001; Richter et al. 2003; Mun et al. 2006). It can cause human disease that neither represents typical relapsing fever nor typical LB (Platonov et al. 2011; Krause et al. 2013; Telford et al. 2015; Boden et al. 2016).

36.5 Cell Biology

Borreliae are helical bacteria. The cell body measures 0.2–0.3 μm in width and 10–30 μm in length (Barbour and Hayes 1986). *Borrelia* cell surface membranes differ in lipopolysaccharide (LPS) and protein richness from that of typical gram-negative bacteria (Takayama et al. 1987; Radolf et al. 1994). *Borrelia* possess a diderm cell envelope consisting of an outer-surface membrane and a cytoplasmic membrane, which are separated by a periplasmic space. The cytoplasmic membrane is covered by a peptidoglycan layer. Depending on the species, 7–30 flagella are inserted near the end of the protoplasmic cylinder of the cell extending into the periplasmic space (Hovind-Hougen 1974, 1995; Karimi et al. 1979; Cutler et al. 1997; Rosa et al. 2005; Guyard et al. 2013). These endoflagella give the bacteria a unique form of motility, permitting them to move in viscous media. They can flex and bend, propel forward and backward, and rotate (non-translational mode of motility) (Barbour and Hayes 1986; Charon et al. 2012), and this motility is crucial for host/vector infection (Sultan et al. 2013) (Fig. 4).

Borreliae possess outer membrane proteins (OMPs), which are integral membrane proteins that function as transporters for nutrients or other essential molecules (Kenedy et al. 2016). It was shown by freeze fracture electron microscopy that the outer membrane contains relatively few transmembrane proteins (Radolf et al. 1994; Shang et al. 1998). Such studies provided evidence that blebs, which are shed from *Borrelia*

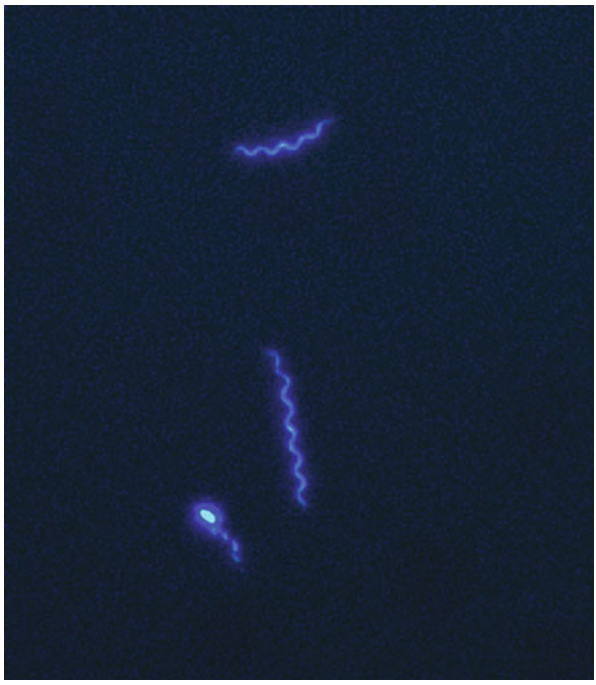

Fig. 4 *Candidatus* Borrelia kalaharica isolated from blood, DAPI stain (courtesy Dr. Volker Fingerle, German National Reference Centre for Borrelia, Oberschleißheim, Germany)

cells, are surrounded by a membrane(s), resembling the outer membrane, and/or the cytoplasmic membrane, suggesting that blebs are pinched-off sections of the cells.

Inserted in the outer-surface membrane via lipid moieties are outer-surface proteins (Osp's) (Berström and Zückert 2010); >150 potential Osp's have been identified in *B. burgdorferi* s.s. (Fraser et al. 1997). They have been named alphabetically in order of their identification, e.g., OspA, OspB, OspC, etc. Many of these proteins have functions in the interaction of the bacteria with their environment (host or vector).

Many OMP and Osp's are important for the interaction with host and vector in the life cycle of *Borrelia*, and intensive research efforts are being made to understand their function (e.g., Berström and Zückert 2010 ; Lin et al. 2012, 2014; Petzke and Schwartz 2015; Kuleshov et al. 2020; Rosa and Jewett 2021). Particularly interesting are the variable major surface protein (Vmp): the variable large and small surface proteins (Vlp and Vsp, respectively) of RF spirochetes and variable major surface protein-like proteins (vls) of LB spirochetes, which allow the bacteria to switch their surface antigens in a programmed manner, also known as antigenic variation, and escape from the hosts' immune response. In this system(s), an expression cassette lies adjacent to silent cassettes that contain variable sequences that can be imported (or parts of it) into the expression cassette changing the dominant antigen epitope of the protein (Barbour et al. 2006; Dai et al. 2006; Norris 2006; Chaconas et al. 2020; Kuleshov et al. 2020). The Vlp and Vsp's of RF spirochetes are similar to VlsE (expressed variant) and OspC of LB spirochetes, respectively (Lescot et al. 2008).

Vmp encoding regions have been located on a variety of plasmids, including lp23, lp24, lp29, and lp41 (Lescot et al. 2008; Kuleshov et al. 2020).

The in vitro cultivation of *Borrelia* has facilitated or permitted progress on topics, such as biology, genetics, genomics, and genetic manipulation of the bacteria. Other than many pathogenic bacteria, *Borrelia* are fastidious bacteria that do not grow well on plates but require a very rich liquid culture medium (Barbour and Hayes 1986). As microaerophilic bacteria, they require either growth in closed glass vials or under an atmosphere of 6% CO_2 (Margos et al. 2015a). For LB spirochetes, some laboratories use in-house prepared MKP medium (Preac-Mursic et al. 1986; Ruzic-Sabljic et al. 2006; Ruzic-Sabljic et al. 2014), but often a commercially available medium (BSK-H) is used that may be supplemented already with rabbit serum (BSK-H complete). For relapsing fever spirochetes, the use of BSK medium in various modifications has also been reported (Cutler et al. 1994; Replogle et al. 2021), while other reported the growth of *B. recurrentis*, *B. miyamotoi*, and *B. hispanica* in modifications of MKP medium (Margos et al. 2015a; Marosevic et al. 2017; Margos et al. 2020b). Growth in vitro varies for LB, reptile associated, and RF species; the former grow slower, and generation times may be as long as 8–12 h (Barbour and Hayes 1986; Güner et al. 2003).

36.6 Genomics

The genome of *B. burgdorferi* s.s. isolate B31 was the first to be completely sequenced within the genus *Borrelia* (Fraser et al. 1997). The genome turned out to be relatively small with a genome size of 1.5 Mbp. In addition, it is surprisingly fragmented (Fraser et al. 1997): it consists of a linear chromosome of about 910 kbp and of 12 linear and 9 circular plasmids, contributing another 600 kbp of DNA sequence (Fraser et al. 1997; Casjens et al. 2010, 2012). The structure of the genome, i.e., comprising of a linear chromosome as well as circular and linear plasmids, was found in all species investigated so far, although the number of plasmids may differ within and between species (Barbour 2016; Becker et al. 2016; Kingry et al. 2016, 2017a, b; Margos et al. 2017a, 2019a, b, 2020a; Casjens et al. 2018; Becker et al. 2020; Kuleshov et al. 2020). The main chromosome of B31 contains 820 open reading frames (803 protein coding sequences, 17 pseudogenes; 5 rRNA, 32 tRNA, 3 ncRNA), 10% of which match hypothetical proteins and 29% have no match in a database. The G+C content of the chromosome is around 28% (Fraser et al. 1997; Mongodin et al. 2013). The plasmids in B31 range in size from 5 to 60 kbp, containing additional 700 coding sequences, of which >90% have no convincing database match outside the genus *Borrelia* (Fraser et al. 1997; Casjens et al. 2000). The chromosomes of various LB species are very similar in size to B31 and show a high degree of synteny, although some chromosomes may have right end extensions (Casjens et al. 2012; Margos et al. 2019a). Not all *Borrelia* species/strains have as many plasmids as B31 (Margos et al. 2017a, 2019a; Casjens et al. 2018), so far the smallest genomes, with 7 plasmids, have been found for *B. garinii* isolate Far04 and *B. maritima* CA690 (Margos et al. 2020a).

Genomes of relapsing fever spirochetes are similar in size as LB spirochetes and range between 1.2 Mbp and 1.6 Mbp (Lescot et al. 2008; Elbir et al. 2014; Marosevic et al. 2017; Kuleshov et al. 2020). Chromosome size ranges between 906 kbp and 930 kbp and plasmid sizes from 6 kbp to 165 kbp. The number of protein coding genes on the chromosome ranges between 800 (20 pseudogenes) and 850 (22 pseudogenes); numbers of RNAs are 3 rRNA and 32 tRNA; G+C content is around 27.5%.

The linear components of the genomes, main chromosome and linear plasmids, are terminated by covalently closed hairpin structures, called telomeres (Barbour and Garon 1987; Hinnebusch et al. 1990; Casjens et al. 1997). These are created involving a telomere resolvase, ResT, an enzyme encoded on plasmid cp26 in LB spirochetes (Chaconas and Kobryn 2010; Kobryn and Chaconas 2014) and on plasmid lp23 in *B. recurrentis* and *B. duttonii* (Lescot et al. 2008). Plasmid-encoded genes are essential for the completion of the complex transmission cycle of *Borrelia* in nature (Lin et al. 2014; Iyer et al. 2015) but may be lost under in vitro culture conditions (Schwan et al. 1988; Norris et al. 1995; Labandeira-Rey and Skare 2001; Biskup et al. 2011). Some plasmids that encode genes for proteins that are essential for bacterial growth (e.g., cp26 containing the gene for the telomere resolvase ResT, an enzyme important for resolving the telomeres) are not lost in culture.

Initially plasmids have been named according to whether they are linear or circular and according to size, e.g., lp54 for a 54 kbp linear plasmids and cp26 for a 26 kbp circular plasmid (Casjens et al. 2000). However, since several cp32 or lp28 plasmids of similar size may be found in a single isolate and size differences of plasmids from the same plasmid family have been detected in different isolates, nowadays plasmids are typed according to their PFam32 locus. This locus was suggested to be homologous to plasmid partitioning protein (ParA) encoding genes in other bacteria (Casjens et al. 2012). However, the function of the PFam32 protein and related proteins (PFam49, PFam52, PFam57/60) in autonomous plasmid replication and maintenance needs to be confirmed in *Borrelia* (Chaconas and Kobryn 2010; Schwartz et al. 2021). Although this system of plasmid designation is well established and consistently used in LB spirochetes, it has not been fully integrated into genome analyses of RF spirochetes (Kuleshov et al. 2020; Kingry 2021).

Perhaps resulting from the parasitic life style, *Borrelia* have few genes for biosynthesis of cell components (Fraser et al. 1997; Lescot et al. 2008). The majority of chromosomal genes encode proteins for housekeeping and metabolic functions. Many of the plasmid-located genes encode outer-surface proteins (Osp's) that are required for interaction with host or vector. In *B. burgdorferi* s.l., analyses of plasmid sequences showed that extensive sequence rearrangements have taken place, and plasmid numbers and structures vary not only between species but also between strains of a single species (Casjens et al. 2012, 2017, 2018; Schwartz et al. 2021). Plasmids of the cp32 family have been shown to contain sequences that resemble prophages, and these may perhaps facilitate rearrangements and/or exchange of genetic material (Eggers and Samuels 1999; Eggers et al. 2001; Schwartz et al. 2021). Information on content and structure of the *B. burgdorferi* s.l. genome has been largely gained from strains of the species *B. burgdorferi* s.s. (Casjens et al. 2010, 2012, 2017). Although for other *Borrelia* species genomes have been sequenced, due to the complexity of the genomes and sequence similarity in some

plasmids, the complete set of plasmids has not been finalized for all of them (Becker et al. 2016, 2020; Elbir et al. 2017; Kingry et al. 2017a; b; Marosevic et al. 2017; Casjens et al. 2018; Kuleshov et al. 2018, 2020; Tyler et al. 2018; Kingry 2021; Schwartz et al. 2021; Kneubehl et al. 2022). However, the use of new sequencing technologies gives hope that this may be achieved in the near future (Margos et al. 2017a, 2020a; Kuleshov et al. 2020; Hepner, personal communication).

36.7 Epidemiology, Burden of Disease

The risk to become infected with *Borrelia* largely depends on contact of humans with vectors infected with human-pathogenic *Borrelia* species. This in turn directly correlates with the distribution of vector ticks, as well as the distribution, increase, and spread of the human population.

Almost all cases of LB are reported from the Northern Hemisphere focusing on the United States (USA) and Europe. Cases are also reported from Asia. There is considerable epidemiological uncertainty regarding LB incidence and prevalence (Lindgren and Jaenson 2006; Hofmann et al. 2017; Rauer et al. 2020). Not only varies the yearly incidence of reported LB cases greatly across Europe and within individual countries, the same is true for the United States: the basis of reported cases differs between reports, some taking into consideration clinically diagnosed cases, while others also report probable or suspected cases of LB (Lindgren and Jaenson 2006; Mueller et al. 2012; Wilking et al. 2015; Wilking and Stark 2014; Schwartz et al. 2017; Enkelmann et al. 2018; Woudenberg et al. 2020; Kugeler et al. 2021). In Europe, the reported incidence of LB cases for 100,000 inhabitants ranged from 0.6 in Ireland to 69 in Sweden and to 300 in Austria (Lindgren and Jaenson 2006). In Germany, incident cases reported to the Robert-Koch Institute lead to an estimation of about 85,000 cases of LB annually, while numbers extracted from a German health insurance company amounted >200,000 cases each year in Germany (Mueller et al. 2012). However, incidences based on health insurance data include false diagnoses, consultation of several health practitioners or misassignment of codes, etc. (Mueller et al. 2012; Kugeler et al. 2021). Thus, the lower numbers may be underreporting, while higher numbers are overestimations, and the true number of LB cases lies probably somewhere in between.

In the United States, a similar situation is reported. LB is a nationwide notifiable disease in the United States since 1991, with 25,000–30,000 confirmed cases annually reported to public health systems (Schwartz et al. 2017). In 1996 and 2008, marked increases of case numbers were noticed, which was likely due to the changes in diagnostic procedures (1996) and the introduction of modified case definition (2008) (Schwartz et al. 2017). A more recent analysis based on case numbers in health insurance data, Kugeler et al. estimated that during 2010–2018 approximately 476,000 persons were diagnosed with LB annually (which also includes false-positive diagnoses) in the United Stated (Kugeler et al. 2021).

Why is it problematic to get accurate numbers for LB cases? The answer lies partly in the complexity of the biological system: the longevity of ticks and some *Borrelia* reservoir hosts, the resulting long-term duration of transmission cycles that may

fluctuate between years, the infrequency of long-term studies on vector populations and *Borrelia* incidence, partly in inaccuracies with the official reporting systems, and, even with clear clinical case definitions, uncertainties in diagnostic methods (Hofmann et al. 2017; Kugeler and Eisen 2020; Rauer et al. 2020). In microbiological diagnostics, the gold standard usually is the cultivation of the causative agents. *Borrelia* are fastidious bacteria that need very rich culture medium and grow very slowly under in vitro conditions (generation time 8–12 h) (Barbour 1984; Preac-Mursic et al. 1986; Margos et al. 2015a). Compared with other bacterial infections, the numbers of spirochetes in human biopsies or tissues are often very low making adaptation of the bacteria to culture conditions difficult, because adaptation to artificial medium is a strong selection process (Norris et al. 1997). Clinical and laboratory data (often based on the detection of anti-*Borrelia* antibodies and borrelial DNA by PCR) as well as the history of a tick bite need to be taken into consideration. Importantly, the interpretation of test results requires ample diagnostic experience (Aguero-Rosenfeld et al. 2005; Stanek et al. 2011; Stanek et al. 2012). As already mentioned above, guidelines for diagnosis of LB (Mygland et al. 2010; Stanek et al. 2011; Hofmann et al. 2017; Rauer et al. 2020) and the reporting of LB cases are inconsistent in European countries; LB is a mandatory notifiable disease only in a few EU countries, and case definitions may differ in various countries (Sykes and Makiello 2017).

For RF spirochetes, disease burden is difficult to estimate for other reasons. Diagnostic procedures are comparatively easy as the bacteria can be detected in Giemsa- or DAPI-stained blood smears or be detected by PCR (see section "Diagnostics"). What can be problematic in malaria endemic regions is a lack of diagnostic routine, and therefore, RF may be misdiagnosed as malaria or other febrile diseases (Nordstrand et al. 2007). In North America, tick-borne RF cases caused by *B. hermsii*, *B. turicatae,* or *B. parkeri* are rare; between 1999 and 2011 483 cases have been reported mainly in California, Colorado, and Washington (https://www.cdc.gov/relapsing-fever/distribution/index.html). Few cases of *B. miyamotoi* infection have been reported in geographic regions, where the pathogen is present (Krause et al. 2015). There is only patchy data on RF spirochetes and its vectors from Central and South America, making it difficult to estimate disease burden (Lopez et al. 2016).

While climate change and its consequences as well as anthropogenic land use changes will most certainly have an effect on the habitats of vector and reservoir hosts, encroachment of humans into natural habitats is another factor that increases the risk of infection. As most soft ticks (vectors of RF spirochetes) have a nidicolous lifestyle, changing environmental conditions that will affect their hosts may have an impact on these vectors (Vial 2009). For LB spirochetes, the vector-pathogen-host life cycle is a highly complex ecological system, in which ixodid ticks, *Borrelia*, and vertebrate hosts interact at various environmental conditions. Consequently, each change in this system, driven by natural phenomena or by human intervention, affects the ecological balance and may alter the risk of human infection (Hartemink et al. 2008; Tsao 2009; Estrada-Peña et al. 2016). Anthropogenic impacts such as climate change, land cover changes, and geographical expansion of hosts (also incidental or dead-end hosts such as humans) are considered to be the main reasons for the increase in tick abundance, distribution of ticks in new habitats, and therefore the risk of infection (Bregnard et al.

2020). However, in contrast to reports of increasing tick populations as well as *Borrelia* prevalence and incidence (https://www.euro.who.int/data/assets/pdf_file/0008/246167/Fact-sheet-Lyme-borreliosis-Eng.pdf), two meta-analysis conducted in Europe for the periods 1984–2003 and 2010–2016, respectively, showed an increase in *Borrelia* prevalence in ticks from Western to Eastern Europe, but the overall mean prevalence remained the same 13.7% for the first period and 12.3% for the second period. Interestingly, the prevalence in adult ticks was higher (18.6%) in the first period than in the second period (14.9%), while in nymph, it remained stable (Rauter and Hartung 2005; Strnad et al. 2017). Long-term studies (6–10 years) on *Borrelia* prevalence in questing *I. ricinus* in Holland and Latvia showed that tick infection rates with *Borrelia burgdorferi* s.l. remained stable or even decreased over the study period (Coipan et al. 2013; Okeyo et al. 2020). Furthermore, reports based on seroprevalence studies (note: seroprevalence is an indication of exposure of the population to *Borrelia*) did not find an increase in incidence (Semenza and Menne 2009; Vanthomme et al. 2012; Cuellar et al. 2020; Woudenberg et al. 2020). In fact, a retrospective study conducted in Finland on the *Borrelia* seroprevalence of the population from 1962 to 1972 revealed a seroprevalence of 20%, much higher than that reported for 2011 (3.9%) (Cuellar et al. 2020).

Several studies on the transmission of *Borrelia* to humans after a tick bite suggest a low risk of developing LB even after being bitten by a *Borrelia*-infected tick (Fryland et al. 2011; Huegli et al. 2011; Wilhelmsson et al. 2016; Markowicz et al. 2021b). In these studies, around 5% of people being bitten by a tick received an infection (established by analyzing seroconversion); participants that developed symptoms ranged between 2% and 3%. Perhaps the situation in Europe is similar to that in the United States, where from 2008 to 2015 annual cases of LB (LD) have been stable in high incidence regions, where *Borrelia*-infected *I. scapularis* ticks have been endemic, while cases have increased in neighboring regions (Schwartz et al. 2017). Thus, published increases in incidence or annual cases may be due to different data sources; changes in reporting procedures; invasion of ticks, hosts, or *Borrelia* into low incidence regions; or increased awareness of general practitioners or the lay public (Kugeler and Eisen 2020).

As *Borrelia* are strictly dependent on their reservoir hosts and vector ticks, it is important to understand their ecology to discern the risk of humans to acquire the agent and develop LB or RF. There is clearly a need to study these complex ecological networks, over long periods of time, especially in the frame of climate change, as tick life cycles can take several years to be completed and most *Borrelia* reservoir hosts are long-lived.

36.8 Clinical Manifestations in Humans

36.8.1 Lyme Borreliosis

Lyme borreliosis (Lyme disease) typically affects skin, joints, heart, or nervous system and has traditionally been divided into different stages (Steere 1989):

Stage I: early, localized infection
Stage II: early, disseminated infection
Stage III: late infection

In clinical practice, the staging system may be used as a support to guide the choice of treatment regimen; however, over the years, it has become evident that most patients do not develop all stages, and overlaps between stages are not uncommon (Evans 2000).

The clinical manifestations and their relative frequencies differ between Europe and North America, probably depending on the geographical distribution and organotropism of the various human pathogenic *B. burgdorferi* sensu lato (s.l.) species (van Dam et al. 1993; Balmelli and Piffaretti 1995; Piesman and Gern 2004). The early, localized skin lesion called erythema migrans (EM) is the most common manifestation on both continents but tends to be associated with more systemic symptoms and earlier dissemination in North American patients, where *B. burgdorferi* sensu stricto (s.s.) generally is the causative species (Radolf et al. 2021). Since *B. burgdorferi* s.s. is prone to disseminate to joints and heart, Lyme arthritis and carditis are seen more frequently in North America. On the other hand, neurologic and late skin manifestations are more frequently seen in Europe, where the neurotropic species *B. garinii* and *B. bavariensis*, along with the dermatotropic *B. afzelii*, are the predominating species. Furthermore, asymptomatic seroconversion or subclinical infection appears to be at least as common as development of clinical disease in Europe (Wilhelmsson et al. 2016; Carlsson et al. 2018), whereas in North America, only a minor proportion of infected persons are asymptomatic (Steere et al. 2003).

36.8.1.1 Dermatoborreliosis

EM, the most frequently occurring manifestation of Lyme borreliosis, constitutes approximately 77–83.4% of all clinical cases (Berglund et al. 1995; Steere and Sikand 2003; Stanek and Strle 2018). It appears several days to weeks after the tick bite and presents as a maculopapular rash at the site of the bite. However, many patients do not recall the preceding tick bite (Strle et al. 2002; Wormser 2006), since these are not painful and therefore frequently pass unnoticed. As the red or bluish-red rash slowly enlarges, it may be associated with local symptoms, such as itching, burning, or pain, and with systemic symptoms, such as fatigue, headache, and migrating myalgia (Strle et al. 1996). Typically, the EM adopts an annular shape with a central clearing but can also be more homogenous. The diagnosis is based on the clinical appearance. Serology is positive in less than 50% of patients presenting with single EM (Strle et al. 1996) and is therefore not needed or recommended. For a reliable diagnosis, the EM should be at least 5 cm in diameter. Untreated EM may disappear spontaneously but can also persist and expand over weeks to months and reach a considerable size, involving several body parts like, for example, the trunk and extremities. Multiple EM lesions occur and are a sign of spirochetal dissemination (Stanek and Strle 2003) (Fig. 5).

Borrelial lymphocytoma (BL) and acrodermatitis chronica atrophicans (ACA) are other skin manifestations of European Lyme borreliosis, which are rarely, if ever, seen in North American patients. BL is a solitary bluish-red swelling appearing in the vicinity of the tick bite after weeks to months (Stanek and Strle 2003). It is typically located at the ear lobe or the nipple and is more common in children than in

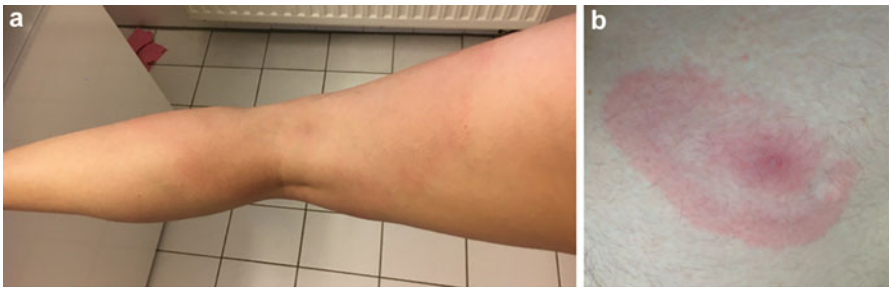

Fig. 5 Erythema migrans. (**a**) shows the leg of an infected person. The EM is pale and covers a substantial area of the leg. (**b**) shows the arm of an infected person. The EM is of typical annular shape (courtesy Dr. Volker Fingerle, German National Reference Centre for Borrelia, Oberschleißheim, Germany)

adults. For diagnosis, serology is essential (Stanek et al. 2011). In addition, PCR and histological examination of a skin punch biopsy can be used in ambiguous cases. ACA is a late dermatologic manifestation that develops slowly over months to years and is almost exclusively associated with *B. afzelii* infection (Ohlenbusch et al. 1996; Stanek and Strle 2018). The onset is insidious with a slight bluish-red discoloration and edema, most often located at the extensor sides of the hands, feet, elbows, or knees (Stanek and Strle 2003). Polyneuropathy and arthritis may occur in the same area as the affected skin. Gradually, the edema disappears and skin atrophy is developed. ACA is more often diagnosed in women than in men, and the patients are usually >40 years of age (Asbrink and Hovmark 1988). The condition is easily misinterpreted as a sign of vascular insufficiency, especially when located at the lower extremities. Unlike EM and BL, ACA does not resolve without antibiotic treatment (Asbrink and Hovmark 1988). Practically all patients presenting with ACA have high levels of specific IgG antibodies in serum, but PCR and histology of a skin biopsy may be used as complementary diagnostic methods.

36.8.1.2 Neuroborreliosis

Neuroborreliosis (NB) is typically characterized by subacute meningitis and involvement of cranial and peripheral nerves (Pachner and Steere 1985). In Europe, NB is the second most common manifestation of Lyme borreliosis, constituting approximately 16% of all cases (Berglund et al. 1995). The neurologic symptoms usually occur 1–12 (mostly 4–6) weeks after the tick bite (Mygland et al. 2010). Borrelial meningitis, in contrast to most other bacterial meningitis, usually causes relatively mild or intermittent headache and moderate neck pain, although in some patients, these symptoms may be more intense (Kristoferitsch 1991). Any cranial nerve can be involved, but the facial nerve is by far the most frequently affected, resulting in unilateral or sometimes bilateral peripheral facial palsy. If present, radiculoneuritis usually causes severe, radiating pain that typically exacerbates at night. Fever, nausea, and vomiting may occur in children but are usually absent in adults (Stanek and Strle 2003). Other manifestations involving the central nervous system, such as encephalitis and myelitis, are rare (Kristoferitsch 1991; Stanek et al. 2011). Occasional cases of NB presenting

with confusion, cerebellar ataxia, hemiparesis, stroke-like symptoms, and cerebral vasculitis have also been reported (Mygland et al. 2010; Topakian et al. 2008). NB is typically a subacute illness, with 95% of cases diagnosed as early NB (duration of symptoms <6 months at the time of diagnosis). Less than 5% of NB patients present with a symptom duration exceeding 6 months (classified as late NB) (Mygland et al. 2010). The diagnosis is based on the patient's medical history, clinical signs, and symptoms, along with simultaneous laboratory analysis of serum and cerebrospinal fluid (CSF). The inflammatory parameters in serum are usually normal or only slightly elevated, whereas the CSF shows moderate to prominent inflammation mirrored by mononuclear pleocytosis, intrathecal production of *Borrelia*-specific antibodies, and elevated levels of pro-inflammatory cytokines and chemokines (Mygland et al. 2010; Pietikäinen et al. 2016; Lepennetier et al. 2019).

36.8.1.3 Articular Borreliosis

Borrelial arthritis (Lyme arthritis) is the most common clinical sign of disseminated borreliosis in North America, constituting about 10% of all cases, but appears to be less common in Europe, where only 3–7% of borreliosis cases present with arthritis (Berglund et al. 1995; Steere 1989; Stanek and Strle 2018). Onset of borrelial arthritis occurs weeks to months, most commonly 2–3 months, after the tick bite (Steere et al. 1987; Herzer 1991). The condition is characterized by acute mono- or oligoarticular inflammation of large joints, most commonly in one knee. Sometimes an elbow, shoulder, ankle, or hip may be affected. The joints become swollen and warm but are generally only moderately painful and not erythematous (Steere et al. 1987). Joint involvement is usually asymmetric and intermittent with inflammatory attacks, lasting from a few days to several weeks, or sometimes several months. The clinical course is variable, usually recurrent, and may continue for several years (Stanek and Strle 2003). Arthritis may be associated with other manifestations of borreliosis, such as EM, ACA, or NB (Berglund et al. 1995).

The diagnosis is based on the medical history, clinical features, and laboratory analyses, most importantly serology, but detection of *Borrelia* DNA in synovial tissue or synovial fluid is also recommended in ambiguous cases and in areas with high seroprevalence. C-reactive protein is usually within the normal range, while the white blood cell count in synovial fluid is elevated with a dominance of polymorphonuclear leukocytes (Stanek and Strle 2003; Nocton et al. 1994).

36.8.1.4 Carditis

Carditis has a reported relative frequency of 4–10% in North American Lyme borreliosis patients and 0.5% in European patients (Berglund et al. 1995; Wormser 2006; Strle and Stanek 2009). The condition often occurs within 2 months after the tick bite and typically presents with acute onset of changing atrioventricular blocks grade I–II as a result of conduction disturbances (Steere et al. 1980). There may be signs of perimyocarditis, and it is often accompanied by other manifestations of borreliosis, such as EM, NB, or arthritis. The diagnosis is based on the patient's medical history, clinical signs, and symptoms together with serology, electrocardiography, and cardiac imaging. Other evident explanations for cardiac disease should be investigated and excluded (Strle and Stanek 2009).

36.8.1.5 Rare Manifestations

Eye manifestations are rare and are either a result of inflammation in various eye tissues (e.g., conjunctivitis, keratitis, iridiocyclitis, retinal vasculitis, optic neuritis) or of extraocular involvement (such as paresis of cranial nerves and orbital myositis) (Mikkilä et al. 2000). Diagnosis is challenging and should include medical history, other clinical signs and symptoms indicative of borrelia infection, and serology.

Sporadic case reports of patients with, for example, myositis, osteomyelitis, nodular fasciitis, scleroderma, and symptoms from other organ systems, such as the liver, urinary tract, or respiratory tract, have been interpreted as manifestations of borreliosis, although these associations still remain to be firmly established (Stanek and Strle 2003) (Table 2).

36.9 Relapsing Fever

Louse-borne relapsing fever (LBRF), caused by *B. recurrentis*, and tick-borne relapsing fever (TBRF), caused by several different *Borrelia* species, present with similar clinical symptoms, of which recurrent episodes of high fever are the most characteristic (Cutler 2015). However, LBRF generally has an epidemic occurrence, causing outbreaks in vulnerable populations during poor sanitary conditions, for example, in refugee camps due to wars or natural disasters, while TBRF causes sporadic human cases in geographical areas, where the disease is endemic (Jakab et al. 2022). The incubation time is 4–18 days (mean 7 days), mostly somewhat shorter for LBRF (Jakab et al. 2022; Kahlig et al. 2021; Radolf and Samuels 2021). Thereafter, a varying number of fever episodes, typically lasting for 2–7 days, are separated by afebrile periods of up to 10 days (Jakab et al. 2022). The fever is usually accompanied by chills, headache, back pain, myalgia, arthralgia, and abdominal pain. Clinical examination may also reveal jaundice or a petechial skin rash. Subconjunctival hemorrhages and epistaxis are common in LBRF (25% of cases), and there may also be signs of gastrointestinal, respiratory, or intracranial bleeding or even disseminated intravascular coagulopathy (Warrell 2019). White blood cell count is usually within the normal range or slightly elevated. During a crisis episode, leukopenia and thrombocytopenia may occur, and aminotransferase levels can be elevated. Especially the TBRF *Borreliae* show a certain neurotropism and may cause neurologic symptoms, such as facial palsy, radiculopathy, meningoencephalitis, and confusion. Moderately elevated levels of mononuclear leukocytes and albumin are found in the cerebrospinal fluid, while glucose levels typically remain normal.

The relapsing fever spirochete *B. miyamotoi* has rather recently been demonstrated to be pathogenic to humans (Platonov et al. 2011). Unlike other TBRF, the causative agent is transmitted by hard ticks within the Ixodidae family. *Borrelia miyamotoi* disease (BMD) generally presents as a systemic illness with fever, although recurrent fever episodes may not be quite as distinct as in other TBRF. Associated symptoms may be headache, fatigue, chills myalgia, and arthralgia. Skin rashes occur but are uncommon (Platonov et al. 2011; Molloy et al. 2015). Biochemical analyses often reveal leukopenia, thrombocytopenia, and elevated aminotransferases. BMD may also present with meningoencephalitis, including neurologic

Table 2 Lyme borreliosis: clinical manifestations and diagnostic approach

Organ system	Clinical manifestation	Case definition	Antibody detection	Puncture or biopsy for PCR/culture/histology
Early phase – Incubation period days to months				
General symptoms	Flu-like, subfebrile, headache, muscle/joint pain, lymphadenitis	No respiratory/gastrointestinal symptoms. Rarely transient arthritis. Diagnostically unclear	Unclear	No
Skin	Erythema migrans (EM)	Erythema centrifugal spread, partly central fading, at skin level, not overheated, >5 cm[a] in largest diameter. Consult dermatologist if atypical. Often seronegative. Multiple EM possible	Only unclear cases: if necessary immediately (baseline) and control[b]; sensitivity 20–>50	Only if atypical: skin biopsy
	Borrelia lymphocytoma	Bluish-red painless nodule or plaque, children on earlobe or scrotum, adults mainly mammillary region. Rarely multiple	Obligatory. If necessary, follow-up[a] Sensitivity 70–90%	Skin biopsy; histology (B and T lymphocytic infiltrates), PCR
Nervous system	Meningopolyneuritis (Bannwarth syndrome), meningitis, rarely myelitis, encephalitis	Bannwarth: radicular pain especially at night (fluctuating, drilling, burning), cranial nerve paresis (especially N. facialis). Children mainly show meningitis and facial palsy. Rarely encephalitis, myelitis, or cerebral vasculitis	Obligat. CSF/serum – pair. Demonstration of *Borrelia*-specific intrathecal antibody production and lymphocytic pleocytosis. If necessary, follow-up[b]; sensitivity 70–>90%	CSF. If necessary, detection of CXCL13 from CSF
Heart	AV block, myocarditis, pancarditis	Rare. Typically acute onset of atrioventricular block of varying degrees	Obligatory. If necessary, follow-up[b]	Unclear

Other	Conjunctivitis, anterior and posterior uveitis, papillitis, episcleritis, keratitis	Rare	Obligatory. If necessary, follow-up[b]	Unclear
Late phase – Incubation period months to years				
General symptoms	As in acute phase	Mostly less pronounced	No	No
Skin	Acrodermatitis chronica atrophicans	Initial swelling, gradual livid discoloration, later skin atrophy (perivascular plasma cell-rich infiltrates); ulnar striations, juxta-articular dermal fibroid nodules, possibly peripheral polyneuropathy and deformities in the area of affected skin	Obligatory. High IgG antibody levels, broad band spectrum in IgG blot. IgM not diagnostic; sensitivity 95–100%	Skin biopsy; histology, culture, PCR
Nervous system	Meningitis, myelitis, encephalitis, encephalomyelitis, peripheral neuropathy	Very rare, chronic course. Mostly myelitis with spasticity and ataxia; encephalopathy with cognitive impairment and chronic encephalomyelitis Peripheral polyneuropathy only in ACA	Obligatory. CSF/serum pair for detection of intrathecal antibody formation including signs of inflammation. IgM not diagnostic; sensitivity 95–100%	CSF. If necessary, detection of CXCL13 from CSF
Musculoskeletal system	Arthritis, bursitis, myositis	Mono- to oligoarthritis of large joints, mostly knee joint; effusion voluminous, recurrent or persistent. No axial skeletal involvement. Myositis very rare	Obligatory. High IgG antibodies, broad band spectrum in IgG blot; IgM not diagnostic; sensitivity 90–100%	Synovia/synovial fluid, culture rarely positive, PCR. Punctate diagnostics (granulocytic pleocytosis)
Vessels	Vasculitis	Rare. May lead to ischemia	Obligatory	Unclear

[a]For diameter <5 cm: history of tick bite, at least 2 days between tick detachment and appearance of an expanding rash
[b]Question: seroconversion, significant increase in titer. *CSF* cerebrospinal fluid

and cognitive deficits, especially in immunosuppressed individuals (Gugliotta et al. 2013; Hovius et al. 2013), although there are indications that also immunocompetent persons may be affected (Henningsson et al. 2019).

The mortality of untreated TBRF has been reported to be 2–10%, and with antibiotic treatment, mortality is less than 2% (Jakab et al. 2022). The mortality of untreated LBRF appears to be higher, up to 47.1% according to evidence-based data; however, reported case fatality rates may have been affected by other factors, such as malnutrition and insufficient healthcare (Kahlig et al. 2021). Mortality is highest during a crisis episode or within 24 hours after initiation of antibiotic treatment, when up to 80–90% of LBRF and 1–39% of TBRF patients may develop a Jarisch-Herxheimer reaction (Kahlig et al. 2021; Butler 2017; Radolf and Samuels 2021).

36.10 Animals and Borreliosis

As indicated in Table 1, there are very few *Borrelia* species known to cause disease in wild and domestic animals, and juvenile animals may be more at risk of developing symptoms than adult animals (Elelu 2018). *Borrelia* species known or suspected to cause clinical symptoms in animals include *B. anserina*, causing avian borreliosis in poultry, and *B. hispanica* and *B. persica*, which can cause various disease symptoms in domestic cats and dogs, including lethargy, pale mucosa, anorexia, cachexia, or mild abdominal respiration (Margos et al. 2020b; Baneth et al. 2016, 2022). *Borrelia anserina* was an important disease in poultry breeding early in the last century (Ataliba et al. 2007; McNeil et al. 1949; Hoffman and Jackson 1946; Hoffman et al. 1946). Symptoms described include fever, depression, ruffled feathers, anemia, and greenish diarrhea (Cooper and Bickford 1993; McNeil et al. 1949). Improvement of poultry houses and sanitation does prevent establishment of tick populations, and this has diminished the threat of avian spirochetosis in industrial breeding (Ataliba et al. 2007). It may, however, still be present and reemerge in small flocks or free-range husbandry systems.

Borrelia theileri can infect cattle, sheep deer, and other ruminants (McCoy et al. 2014; Qiu et al. 2021) and has been suspected to cause bovine borreliosis, although more recent research questions whether this condition is caused by *B. theileri* or coinfecting *Babesia* spp. (reviewed by Elelu 2018). *Borrelia coriaceae* has been hypothesized to cause epizootic bovine abortion, a condition widely distributed in Western United States. However, other research suggests that an intracellular bacterium (class δ-proteobacteria, order *Myxococcales*) that is also transmitted by *Ornithodoros coriaceus* is the etiological agent of epizootic bovine abortion (King et al. 2005; Teglas et al. 2011).

Although it is often considered that *B. burgdorferi* s.l. are not pathogenic for wild mammal hosts (Wright and Nielsen 1990), juvenile *Peromyscus leucopus* or dusky-footed woodrats (*Neotoma fuscipes)* may develop symptoms associated with *B. burgdorferi* s.l. infection, including joint, cardiac, and muscle lesions (Moody et al. 1994; Brown and Lane 1994). There is conflicting information of dogs developing disease symptoms following Bbsl infection; the main symptom appears to be arthritis and fever 2 to 5 months after exposure (Krupka and Straubinger 2010;

Pantchev et al. 2015; Krämer et al. 2020). The association of *B. burgdorferi* infection in dogs with so-called Lyme nephropathy (an immune-mediated glomerulonephritis) is controversially discussed (Littman et al. 2006). Antibodies to *B. burgdorferi* have been detected in cats, horses, and cattle, but no or rare associations with disease have been reported (Hahn et al. 1996; Shaw et al. 2001; Butler et al. 2005).

36.11 Diagnosis

36.11.1 Hard Facts and Fake News

Laboratory testing is only supportive in the presence of clinical manifestations of Lyme borreliosis (Stanek et al. 2011). Therefore, the decision for laboratory testing requires a thorough clinical evaluation of the symptoms, and it should not be performed in case of uncharacteristic symptoms. As for other infectious diseases, two different approaches can be used to confirm *Borrelia* infection: the direct identification of the pathogen, like culture or PCR, or indirect methods, reflecting the immune response of the host. The latter includes the measurement of specific antibodies or detection of chemokines or cytokines, reflecting the host's immune response after stimulation with pathogen-specific antigens. Each of these methods has their advantages and limitations for particular infectious agents. *Borrelia burgdorferi* s.l. is a fastidious microorganism, which requires special conditions for cultivation, and it has a relatively long generation time. Moreover, low concentration of the spirochetes in clinical samples hampers the sensitivity of this method, and this also refers to PCR-testing. The performance of both methods is at highest when analyzing skin samples from erythema migrans ranging from 35% to 81% (van Dam 2011); however, the diagnostic utility is limited, as the diagnosis of this manifestation is clinical in the majority of cases, and no laboratory confirmation is required. Regarding Lyme neuroborreliosis and Lyme arthritis, the sensitivities are not satisfactory from cerebrospinal fluid (CSF) and synovial fluid or synovial tissue in the diagnostic routine. Direct detection of *B. burgdorferi* s.l. in blood or serum is not recommended for any manifestation of Lyme borreliosis, because the concentration of the spirochetes in blood is low, and if spirochetemia occurs, it is limited to a short duration of time. This is in contrast to tick-borne relapsing fever borreliae, like *Borrelia miyamotoi*, *B. duttonii,* and *B. recurrentis*, which are visible in a blood smear during the febrile phase of the infection (Hoch et al. 2015).They are readily visible in Giemsa- and/or DAPI-stained smears. In countries where malaria is endemic, febrile conditions may be misdiagnosed when blood smears are not taken on time. Recently, it was demonstrated that among patients who were regularly tested by PCR after a tick bite in a course of a research study, 16 developed an erythema migrans and only one of them showed a positive blood PCR result (Markowicz et al. 2021b). In conclusion, direct detection of *B. burgdorferi* s.l. plays a minor role for diagnosing Lyme borreliosis, and it is rather used for research studies. There is promising evidence that other methods like metagenomics sequencing (Branda et al. 2021) may be superior to traditional PCR testing in the future, and these findings require further research.

The most common method for indirect identification of *B. burgdorferi* s.l. in clinical samples is serologic testing. Detection of *Borrelia*-specific IgG and IgM consists of a screening test, e.g., ELISA, and a confirmatory test for borderline and positive results of the screening test. Immunoblot can be used as the second-tier test, and it has the advantage of demonstrating reactivity to various *Borrelia*-specific proteins, whereas the first-tier test usually reacts with one or two proteins only. The number of reactive bands determines the final interpretation of the immunoblot result. Based on European guidelines, at least two positive bands lead to the positive interpretation for IgG, whereas for IgM, the reactivity to the outer protein C (OspC) is sufficient for a positive result (Dessau et al. 2018). Serology may be negative in the early stage of infection; therefore, it is not recommended for erythema migrans. The detection of IgG plays an important role for the confirmation of late, disseminated manifestations of Lyme borreliosis (Stanek et al. 2011), and it is characterized by a high negative predictive value. Therefore, lack of specific IgG rules out a late disseminated disease. Specific *Borrelia* antibodies can be found in healthy persons frequently exposed to ticks during occupational or recreational activities (Cetin et al. 2006) and in subjects after a successful treatment of different manifestations of Lyme borreliosis. Therefore, it is essential to discriminate between persons in whom positive serology points at an acute or chronic infection fulfilling the clinical criteria and persons with background seroprevalence, which does not require treatment with antibiotics. Although IgM antibodies occur in the early stage of the infection, the test results must be also put in the clinical context (Hillerdal and Henningsson 2021). Like IgG, IgM can persist after the infection and treatment, and the dynamics of decline is dependent on the individual immune reaction. Possible explanation for the persistence of IgM in healthy subjects are cross-reactive antigens of human or environmental origin sharing the same epitope with OspC of *B. burgdorferi* s.l. (Markowicz et al. 2021a), which is the key antigen in the early stage of *Borrelia* infection. Other possible explanation for nonspecific IgM is polyclonal stimulation of B cells (Goossens et al. 1999; Tuuminen et al. 2011). In conclusion, *Borrelia* IgM is only relevant in the presence of clinical signs of an early *Borrelia* infection, and unspecific test reactivities for IgM must be interpreted with caution.

36.11.2 Laboratory Diagnosis of Particular Manifestations of Lyme Borreliosis

36.11.2.1 Early Skin Manifestations

Erythema migrans is the most frequent manifestation of Lyme borreliosis, and it is caused by a local affection of the skin by *B. burgdorferi* s.l. Its typical appearance does not require laboratory testing, and the diagnosis is made upon clinical examination. PCR or culture from skin biopsy is possible in case of atypical skin lesions with a suspicion of Lyme borreliosis. The same recommendations apply to multiple erythema migrans. Borrelial lymphocytoma requires laboratory confirmation by serologic testing. Positive serology or seroconversion observed in a paired sample is mandatory. The recommended time interval between the two tests is 2–4 weeks.

Histologic examination of the skin and PCR or culture from the skin biopsy can be considered as additional diagnostic procedures.

36.11.2.2 Lyme Neuroborreliosis

The most common manifestations of Lyme neuroborreliosis include meningoradiculoneuritis, lymphocytic meningitis, and cranial neuritis, and the pathophysiological processes are localized in the CSF and the meninges (Ogrinc et al. 2022). Thus, the analysis of the CSF is essential for the laboratory diagnosis (Mygland et al. 2010). First, demonstration of CSF inflammation is mandatory. Lymphoplasmacellular pleocytosis occurs in the first days after onset of symptoms, with an expected cell count of 10–1000 leucocytes/mm^3. Since the inflammation in the CSF is not specific for Lyme neuroborreliosis, the etiology of the infection should be confirmed by the intrathecal synthesis of *Borrelia*-specific antibodies. With this method, the fractions of Borrelia-specific antibodies in blood and in the CSF are compared, and their relation is expressed as the antibody index (AI). Value >1,5 indicates intrathecal synthesis (Tumani et al. 1995). Noteworthy, the synthesis of such antibodies is delayed in comparison to the pleocytosis, and – in contrast to the total cell count – the AI can be still elevated even for years after successful treatment. Therefore, this parameter is not suitable for monitoring the efficacy of treatment. Further laboratory findings in the CSF can be high protein concentration, whereas the glucose ratio is usually normal (Hansen et al. 2013).

In recent years, it became evident that the B cell attracting chemokine CXCL13 can be used for the laboratory diagnosis of Lyme neuroborreliosis (Rupprecht et al. 2014; Henningsson et al. 2018). High concentrations of CXCL13 in the CSF can be measured in the early phase of the infection, and they occur before the AI becomes positive. Moreover, the concentration decreases more rapidly than the cell count after treatment. High concentration of the chemokine in the CSF is often able to distinguish between Lyme neuroborreliosis and other inflammatory conditions of the central nervous system. However, CXCL13 is not specific for Lyme neuroborreliosis, and it can occur in other diseases like lymphoma, neurosyphilis, or multiple sclerosis (Dersch et al. 2015; Fischer et al. 2009). There are numerous studies demonstrating that the cutoff values are dependent on the test system and on the selection of study groups. Given these circumstances, CXCL13 can be used as a complementary method for diagnosing Lyme neuroborreliosis, but it should not replace the methods mentioned above.

36.11.2.3 Late, Disseminated Manifestations

Clinical suspicion of late disseminated manifestations of Lyme borreliosis like Lyme arthritis and acrodermatitis chronica atrophicans requires confirmation by serologic testing. High specific IgG levels are expected in these cases. Typically, a broad range of antigens is reactive in the immunoblot. A PCR from tissue – skin or synovial fluid or synovial tissue – may be helpful as an additional test, but negative test results do not exclude the infection due to limited sensitivity (Hofmann et al. 2017; Jaulhac et al. 2019; Rauer et al. 2020).

36.11.2.4 Rare Manifestations

Ocular and cardiac manifestations require confirmation of specific serum antibodies. Possible ophthalmic affections like conjunctivitis, uveitis papillitis, keratitis, and episcleritis are diagnosed during routine ophthalmologic examinations. Lyme carditis usually presents with varying degrees of atrioventricular block, rarely with pericarditis and myocarditis. Routine cardiologic examinations including electrocardiogram should be performed. In case of rare manifestations of Lyme neuroborreliosis, e.g., chronic meningitis, encephalomyelitis, the same diagnostic approach is recommended as was described for Lyme neuroborreliosis (Hansen et al. 2013; Kristoferitsch et al. 2018).

36.12 Methods with Insufficient Evidence to Support the Diagnosis of Lyme Borreliosis

There are numerous test methods that are not useful for the diagnosis of *Borrelia* infection. One reason are misconceptions – e.g., for microscopic examinations of blood in LB, despite known biological evidence that *B. burgdorferi* s.l. does not cause high levels of spirochetemia (Aase et al. 2016). An exception is *B. mayonii*, a novel *Borrelia* species restricted geographically to the Midwestern United States (Pritt et al. 2016). With regard to some diagnostic approaches, which are used for other diseases like interferon gamma release assay or lymphocyte transformation assay, there is increasing evidence questioning their utility for clinical practice (Baarsma et al. 2022; Dessau et al. 2014; van Gorkom et al. 2018). A recent case control study involving 271 patients did not show that the performance of these methods was higher compared to serologic testing, and the results highlighted their low specificity (Baarsma et al. 2022). Therefore, such methods require additional optimalisation and validation by independent experts using well-characterized human specimens before being fit for conventional, clinical use. Finally, testing of ticks removed from the skin for presence of *B. burgdorferi* s.l. is not recommended. Although it was shown, that the risk for infection with *Borrelia* is higher when the spirochete was detected by PCR in such ticks (Markowicz et al. 2021b), there is no evidence to recommend antibiotic prophylaxis after a bite of an infected tick, since only a small fraction of individuals bitten by *Borrelia*-positive ticks will develop symptoms of Lyme borreliosis (Wilhelmsson et al. 2016).

36.13 Diagnosis of Tick-Borne Relapsing Fever

In contrast to Lyme borreliae, the agents of tick-borne relapsing fever cause spirochetemia, making the microscopic identification in blood smears diagnostically useful. Giemsa- or DAPI-stained spirochaetes (Fig. 4) confirm the diagnosis in patients with corresponding clinical picture in endemic regions (Hoch et al. 2015). The examination is essential for travelers returning from regions, where malaria is

endemic, because the symptoms of both diseases are similar. Serologic tests for tick-borne relapsing fever are based on the detection of antibodies against glycerophosphodiester phosphodiesterase (glpQ), which is a protein present in relapsing fever spirochaetes but not in *B. burgdorferi* s.l. (Schwan et al. 1996). However, the assay is not able to discriminate between particular relapsing fever *Borrelia* species due to cross-reactivity.

36.14 Conclusions for Laboratory Diagnostics

Due to substantial limitations of direct detection of *B. burgdorferi* s.l. from clinical samples, the serologic testing remains the main method for the laboratory diagnosis of Lyme borreliosis. Importantly, serology should be only used in the presence of clinical symptoms, suggestive of Lyme borreliosis. Erythema migrans is the only manifestation, which is diagnosed clinically. For other manifestations, the presence of specific antibodies should by confirmed by two-tier testing. Examining serum and CSF is required to diagnose Lyme neuroborreliosis, by demonstrating pleocytosis and the intrathecal synthesis of specific antibodies. CXCL-13 is elevated in the CSF and can be helpful for the diagnosis. Since antibodies to *Borrelia* can be detected in healthy individuals, reflecting the background seroprevalence after tick exposure, and since antibodies are common after successful treatment, there is still an urgent need for identifying a marker of an active *Borrelia* infection from blood. This would help to reduce unnecessary treatment and overuse of antibiotics and to avoid missing other underlying diseases. Before new diagnostic approaches can be introduced to the laboratory routine, profound validation studies by independent experts are mandatory. Results of such investigations need to be reproduced on various well-characterized samples and patient populations in order to demonstrate which groups of patients can benefit from them.

36.15 Notes on Antibiotic Therapy

Lyme borreliosis is principally considered to be an efficiently treatable disease with a good prognosis, especially the early forms of the disease. Therefore, every clinical manifestation of Lyme borreliosis should be treated with antibiotics. In this context, it is worthwhile mentioning that even without antibiotic therapy, early manifestations of Lyme borreliosis do not lead inevitably to a late manifestation but can heal spontaneously (Hofmann et al. 2017; Mygland et al. 2010; Cerar et al. 2010; Lantos et al. 2021; Pfister et al. 1989; Koedel et al. 2015). Post-therapeutic complaints without tangible signs of a still ongoing infection (like signs of inflammation in cerebrospinal fluid) may be experienced due to a defective healing process (especially following longer diseases with organ damage) or in the sense of a postinfectious syndrome. In these cases, further antibiotic interventions are not indicated, but a symptomatic therapy would be required.

The main goals of antibiotic therapy include to shorten the clinical course of disease, to prevent complications including defective healing, and to prevent the development of later forms of the disease (Hofmann et al. 2017; Lantos et al. 2021; Rauer et al. 2020; Jaulhac et al. 2019).

36.16 Antibiotic Selection

Many in vitro studies have shown that the different *B. burgdorferi* s.l. species are susceptible to some second- and third-generation cephalosporins, tetracyclines, penicillins, and macrolides, while they are intrinsically resistant to fluoroquinolones, rifampicin, trimethoprim-sulfamethoxazole, and first-generation cephalosporins (Preac Mursic et al. 1996; Hunfeld et al. 2003, 2004, 2005; Morgenstern et al. 2009; Veinović et al. 2013, 2021; Baradaran-Dilmaghani and Stanek 1996). So far, there is no evidence for emergence of secondary antibiotic resistance in *B. burgdorferi* s.l. to the antibiotics recommended.

The choice of antibiotics as well as the mode and duration of administration depend on the clinical manifestation, the age of the patient, the severity of the disease, drug-allergy and side-effect profile, and possible coinfections with other tick-borne pathogens. Notably, although there are differences with respect to *B. burgdorferi* s.l. species and clinical manifestations between Europe and North America, therapy recommendations and therapy outcome are remarkably similar (Hofmann et al. 2017; Lantos et al. 2021; Rauer et al. 2020; Jaulhac et al. 2019).

The preferred medications for therapy of early manifestations – except Lyme neuroborreliosis (LNB) – are doxycycline, amoxicillin, cefuroxime axetil, and phenoxymethylpenicillin, while macrolides, e.g., azithromycin, seem to be less effective in vivo and therefore are recommended as second-line treatment. Late forms can be treated orally with doxycycline or amoxicillin and intravenously with ceftriaxone, cefotaxime, or penicillin G. (Hofmann et al. 2017; Lantos et al. 2021; Jaulhac et al. 2019; Luft et al. 1996). LNB is usually treated intravenously with ceftriaxone, cefotaxime, or penicillin G but can also be treated orally with doxycycline (Rauer et al. 2020; Arnason and Skogman 2022; Stupica et al. 2021; Bremell and Dotevall 2014; Borg et al. 2005; Ljøstad et al. 2008).

The duration of treatment is between 5 and 30 days, depending on the clinical manifestation and the antibiotic used. A significant prolongation is normally not advisable as the risk of severe side effects, such as pseudomembranous colitis, increases disproportionately (Nguala et al. 2021).

The minimum duration necessary for successful antibiotic treatment for the different antimicrobial agents has never been assessed in detail. For erythema migrans, it has been shown that a 10-day course of oral doxycycline was not inferior compared to 2 weeks (Stupica et al. 2012; Wormser et al. 2003). However, relevant studies regarding a shorter treatment for immunocompromised patients or for antibiotics other than doxycycline are not available.

Actually, most guidelines recommend antibiotic therapy for 10–21 days in case of early localized disease and a 2–4-week course for disseminated and late-stage

manifestations (Hofmann et al. 2017; Lantos et al. 2021; Rauer et al. 2020; Jaulhac et al. 2019; Pancewicz et al. 2015).

The success of therapy can only be assessed weeks to months after the end of therapy, especially in the case of long-standing disease manifestations (Table 3).

36.17 Therapy During Pregnancy and Lactation

In principle, pregnant and nursing women are treated with the same antibiotics and same duration as nonpregnant women. However, doxycycline should not be administered in the third trimester and macrolides not during the first trimester. In pregnancy and lactation, oral therapy with amoxicillin is the first choice. Alternatively, penicillin G and ceftriaxone can be used, the latter especially in cases of neurological manifestations (Hofmann et al. 2017; Rauer et al. 2020; Jaulhac et al. 2019; Lakos and Solymosi 2010; Maraspin et al. 2011). In cases of proven penicillin allergy, azithromycin or cefuroxime axetil may be prescribed according to strict indications. Under clinical supervision, ceftriaxone can also be given intravenously, since the risk of cross-allergy with third generation cephalosporins is only about 1% (Buonomo et al. 2014).

36.18 Therapy of Children

Children from the age of 8 years, after enamel formation is complete, can be treated with doxycycline. In children under 8 years of age, the therapy of choice is amoxicillin (Table). This antibiotic is required to be given in three daily doses, which may be difficult in kindergarten and school children. Alternatively, cefuroxime axetil, azithromycin, or clarithromycin can be used (Nizič et al. 2012).

36.19 Therapy of Immunocompromised Individuals

Although only limited data on treatment of LB are available for immunocompromised patients, the recommended antibiotic treatment approach – same antibiotics, dosage and duration – appears to be effective also in this group of individuals (Merkac et al. 2015; Fürst et al. 2006; Maraspin et al. 1999, 2006). One study on patients receiving tumor necrosis factor-alpha (TNF-α) inhibitors reported treatment failure in 25% (4/16); however, re-treatment resulted in an overall favorable outcome also for these patients (Maraspin et al. 2019).

36.20 Long-Term Antibiotic Therapy

Several randomized clinical trials did not provide relevant support for prolonged antibiotic treatment in patients who suffer from persistent symptoms after recommended antibiotic treatment attributed to “persistent” Lyme borreliosis

(Berende et al. 2016; Klempner et al. 2001; Krupp et al. 2003; Fallon et al. 2008). Treatment beyond 30 days for late manifestations is therefore not recommended (Hofmann et al. 2017; Lantos et al. 2021; Rauer et al. 2020; Jaulhac et al. 2019).

36.21 Not Recommended Therapeutic Interventions

Therapeutic interventions that cannot be recommended include, but are not limited to, fluconazole, vancomycin, gyrase inhibitors, metronidazole, hydroxychloroquine, long-term-, combination-, or pulsed-antibiotic therapy, photon therapy, electrotherapy, intravenous bismuth or H_2O_2, colloidal silver, hyperthermia, hypothermia, or stem cell transplantation (Hofmann et al. 2017; Koedel et al. 2015; Rauer et al. 2020; Figoni et al. 2019).

36.22 Treatment of Tick-Borne Relapsing Fever

Relapsing-fever *Borrelia* can be treated very effectively using appropriate medication, although well-designed studies regarding optimal length of therapy, dosing, or efficacy of different antimicrobial agents with regard to the different RF species are scarce to nonexistent. There are no reports of acquired antibiotic resistance, but rifampicin, sulfonamides, fluoroquinolones, aminoglycosides, and metronidazole are intrinsically poor or not effective. Tetracyclines, penicillins, and other ß-lactam antibiotics are most suitable for therapy, the latter especially in pediatric patients, where the use of tetracyclines is usually not recommended for children under 8 years. The efficacy of macrolides is less well-established, but they are an alternative in the second and third trimesters of pregnancy or intolerance for ß-lactams and tetracyclines. Chloramphenicol is also effective but is rarely used outside developing countries, mainly because of the well-tolerated alternatives. In general, the use of tetracyclines is favored because they are the most effective, and the lowest recurrence rate has been observed (Guerrier and Doherty 2011; Jakab et al. 2022). Oral tetracycline (4 × 500 mg per day) or doxycycline (2 × 100 mg per day) or intravenous doxycycline (2 × 100 mg per day) is usually used. Alternatively, intravenous or intramuscular penicillin G (600.000 IU daily) is available as standard therapy, meanwhile mostly replaced by ceftriaxone (1 × 2 g per day) especially in patients with central nervous system involvement. Erythromycin (4 × 500 mg per day) given orally or intravenously is a further alternative.

In louse-borne, RF therapy can be administered as a single dose, while in cases of TBRF, a single dose is usually not sufficient. This is at least partly due to the stronger CNS affinity of TBRF *Borrelia*. Particularly in compartments with limited antibiotic penetration capacity, such as the CNS, there are often niches in which the pathogens persist in spite of antibiotic treatment resulting in relapses. Relapse rates of up to 20% (despite antibiotic therapy) are observed in TBRF (Jakab et al. 2022).

Table 3 Lyme borreliosis: therapy

Manifestation	Drug/administration	Dosage per day		Duration in days
		Adults	Children	
Erythema migrans, Borrelial Lymphocytoma	Doxycycline/p.o.[a]	1 × 200 mg or 2 × 100 mg	4 mg/kg (max. 200 mg) in 1–2 doses	10–21
	Amoxicillin/p.o.	3 × 500–1000 mg	50 mg/kg (max. 2 g), in 3 doses	14–21
	Cefuroxime axetil/ p.o.	2 × 500 mg	30 mg/kg, in 2 doses	14–21
	Azithromycin/p.o.[b]	500 mg	5–10 mg/kg	5–10
Neuroborreliosis[d]	Doxycycline/p.o.[a,c]	1 × 200 mg or 2 × 100 mg	4 mg/kg (max. 200 mg) in 1–2 doses	14–21
	Ceftriaxone/i.v.	1 × 2 g	50–100 mg/kg (max. 2 g) in 1 dose	14–21
	Cefotaxime/i.v.	3 × 2 g	150 mg/kg in 3 doses	14–21
	Penicillin G/i.v.	4 × 5 Mio IU	0.25–0.5 Mio IU /kg in 4 doses (max. 4 × 5 Mio IU)	14–21
Carditis[e]	Doxycycline/p.o.[a]	1 × 200 mg or 2 × 100 mg	4 mg/kg (max. 200 mg)	14–21
	Amoxicillin/p.o.	3 × 500–1000 mg	50 mg/kg (max. 2 g), in 3 doses	14–21
	Ceftriaxone/i.v.	1 × 2 g	50–100 mg/kg (max. 2 g) in 1 dose	14–21
	Cefotaxime/i.v.	3 × 2 g	150 mg/kg in 3 doses	14–21
Lyme Arthritis, Acrodermatitis Chronica Atrophicans	Doxycycline/p.o.[a]	1 × 200 mg or 2 × 100 mg	4 mg/kg (max. 200 mg)	21–30
	Amoxicillin/p.o.	3 × 500–1000 mg	50 mg/kg (max. 2 g), in 3 doses	21–30
	Ceftriaxone/i.v.	1 × 2 g	50–100 mg/kg (max. 2 g) in 1 dose	14–28
	Cefotaxime/i.v.	3 × 2 g	150 mg/kg in 3 doses	14–28

i.v. intravenous; *p.o.* oral; *IU* international units; *NA* not available

[a]Relative contraindication for age <8 years. Not for adolescents or adults under 50 kg body weight; absorption of doxycycline may be affected by 2- or 3-valent cations (dairy products, calcium-containing fruit juices), magnesium in antacids, iron preparations, medicinal activated charcoal, and cholestyramine. Keep a time interval of 2–3 h between intake and meals

[b]Only for patients intolerant to doxycycline, amoxicillin, and cefuroxime

[c]Only in uncomplicated cases; possibly 300 mg/day needed

[d]For late neuroborreliosis therapy for 14–28 days

[e]In first-degree atrioventricular block and PR <30 ms oral therapy for 14–21 days

According to a recent review, the recommended treatment duration is usually 7–10 days, in the case of central nervous system involvement 10–14 days.

36.23 Jarisch-Herxheimer Reaction

During therapy, within a few hours after taking the first dose of antibiotics, a sometimes life-threatening Jarisch-Herxheimer reaction (JHR) can occur as a result of cytokine release (especially TNF-alpha). An initial chill phase with restlessness, intense rigors, sharp rise in temperature and blood pressure, tachycardia, tachypnea, delirium, gastrointestinal symptoms, cough, and limb pains is followed by a flush phase with massive sweating, drop in blood pressure, and a slow decline in temperature. Treatment with intravenous tetracycline carries the highest risk for a JHR; in children JHR is less common (Bryceson et al. 1970; Warrell 2019). In louse-borne RF, a JHR is observed in around 50% (up to 100%) of patients and in TBRF in about 20% (up to 40%) of all cases (Jakab et al. 2022; Butler 2017). Therapy is only symptomatic.

TBRF-related case fatality rate is around 6.5%, range of 2–10% (Goddard 2018). Mortality in TBRF appears to be primarily due to neurologic complications and ARDS.

36.24 Prophylaxis

Lyme borreliosis (Hofmann et al. 2017; Lantos et al. 2021; Figoni et al. 2019; Eisen and Dolan 2016; Pages et al. 2014; Due et al. 2013): Since no vaccine for humans is available, prophylaxis is limited to measures to reduce the risk of tick bites and to remove already attached ticks as early as possible. The risk of being bitten can be reduced by wearing light-colored clothing that covers the body, avoiding tick-infested areas and using repellents on the skin and clothing. After spending time outdoors, the body should be searched promptly and the tick removed as early and gently as possible, as the risk of transmission increases with the duration of the ticks' blood meal. To do this – if necessary with the aid of a magnifying glass – grasp the tick, e.g., with pointed, sturdy tweezers, as close as possible to the skin and slowly pull it out. Commercially available tools, such as a tick card or tick loop, can also be used according to the manufacturer's instructions; if no tools are at hand, simply use your fingernails. Finally, the wound must be thoroughly disinfected. If tick parts remain are still visible in the wound during the final inspection, it is merely the tick's proboscis, which is an intracutaneous foreign body with no specific risk of infection. A visit to the doctor is necessary if typical symptoms occur. Prophylactic antibiotic administration cannot be recommended in Europe at present. In the United States, a single dose of oral doxycycline within 72 h of tick removal for adults and children is recommended but only if the tick bite was from an identified tick vector species and occurred in a highly endemic area and the tick was attached for $\geq$36 h. In general, serological monitoring, self-performed point-of-care (POC) tests, and an examination of the tick

for *Borrelia* are not indicated. During the following 6 weeks, the tick-bite area should be checked for development of an erythema migrans. In that case, medical advice should be sought and the tick-bite should be mentioned.

Repellents for the prevention of tick bites for direct application on the skin include DEET (N,N-Diethyl-meta-toluamide), icaridin, IR3535 (ethyl-3-(N-n-butyl-N-acetyl) aminopropionate), or *Eucalyptus citriodora* oil hydrated/cyclized (EC oil (H/C))(p-menthane-3,8-idol). They should only be applied to uncovered areas of the skin strictly according to the manufacturer's instructions and should not be applied together with sunscreen (Schalka et al. 2014). Permethrin that kills ticks on contact provide highly effective protection and can be used to impregnate clothing. However, its tolerability in case of regular long-term use has never been assessed (Faulde et al. 2015; Vaughn et al. 2014).

Soft Tick-Borne Relapsing Fever (CDC; ECDC): Measures in countries where TBRF is endemic should mainly focus on preventing human-tick contact. Recommended measures include avoiding tick-infested areas, avoiding sleeping in rodent-infested areas, wearing light-colored clothing that covers the body and tucking trouser legs into socks, using tick repellents (see above) for the skin and permethrin-impregnated clothing, and using permethrin-impregnated sleeping bags or bed nets tucked under the mattress when sleeping on the ground or camping. Furthermore, rodenticides and acaricides to treat "cracks and crevices" or natural predators, like domestic cats, are recommended, as well as reducing rodent-friendly environments inside and around buildings.

Due to the short feeding period and the immediate transmission of the pathogen, recommending early removal of soft ticks is inefficient.

36.25 Cross-References

► Vector-Borne Zoonoses
► Wild Birds and Zoonotic Pathogens

References

Aase A, Hajdusek O, Øines Ø, Quarsten H, Wilhelmsson P, Herstad TK, Kjelland V, Sima R, Jalovecka M, Lindgren PE, Aaberge IS (2016) Validate or falsify: Lessons learned from a microscopy method claimed to be useful for detecting *Borrelia* and *Babesia* organisms in human blood. Infect Dis (Lond) 48:411–419

Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP (2005) Diagnosis of Lyme borreliosis. Clin Microbiol Rev 18:484–509

Amore G, Tomassone L, Grego E, Ragagli C, Bertolotti L, Nebbia P, Rosati S, Mannelli A (2007) *Borrelia lusitaniae* in immature *Ixodes ricinus* (Acari: Ixodidae) feeding on common wall lizards in Tuscany, central Italy. J Med Entomol 44:303–307

Apanaskevich D, Oliver J (2014) Life cycles and natural history of ticks. In: Sonenshine DE, Roe M (eds) Biology of ticks. Oxford University Press, Oxford

Arnason S, Skogman BH (2022) Effectiveness of antibiotic treatment in children with Lyme neuroborreliosis - a retrospective study. BMC Pediatr 22:332

Asbrink E, Hovmark A (1988) Early and late cutaneous manifestations in Ixodes-borne borreliosis (erythema migrans borreliosis, Lyme borreliosis). Ann N Y Acad Sci 539:4–15

Assous MV, Wilamowski A (2009) Relapsing fever borreliosis in Eurasia--forgotten, but certainly not gone! Clin Microbiol Infect 15:407–414

Ataliba AC, Resende JS, Yoshinari N, Labruna MB (2007) Isolation and molecular characterization of a Brazilian strain of *Borrelia anserina*, the agent of fowl spirochaetosis. Res Vet Sci 83:145–149

Baarsma ME, van de Schoor FR, Gauw SA, Vrijmoeth HD, Ursinus J, Goudriaan N, Popa CD, Ter Hofstede HJ, Leeflang MM, Kremer K, van den Wijngaard CC, Kullberg BJ, Joosten LA, Hovius JW (2022) Diagnostic parameters of cellular tests for Lyme borreliosis in Europe (VICTORY study): a case-control study. Lancet Infect Dis 22:1388–1396

Balmelli T, Piffaretti JC (1995) Association between different clinical manifestations of Lyme disease and different species of *Borrelia burgdorferi* sensu lato. Res Microbiol 146:329–340

Baneth G, Nachum-Biala Y, Halperin T, Hershko Y, Kleinerman G, Anug Y, Abdeen Z, Lavy E, Aroch I, Straubinger RK (2016) *Borrelia persica* infection in dogs and cats: clinical manifestations, clinicopathological findings and genetic characterization. Parasit Vectors 9:244

Baneth G, Dvorkin A, Ben-Shitrit B, Kleinerman G, Salant H, Straubinger RK, Nachum-Biala Y (2022) Infection and seroprevalence of *Borrelia persica* in domestic cats and dogs in Israel. Parasit Vectors 15:102

Baradaran-Dilmaghani R, Stanek G (1996) In vitro susceptibility of thirty *Borrelia* strains from various sources against eight antimicrobial chemotherapeutics. Infection 24:60–63

Baranton G, Postic D, Saint Girons I, Boerlin P, Piffaretti JC, Assous M, Grimont PA (1992) Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol 42:378–383

Barbour AG (1984) Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 57:521–525

Barbour AG (2016) Chromosome and plasmids of the tick-borne relapsing fever agent *Borrelia hermsii*. Genome Announc 4:e00528

Barbour AG, Garon CF (1987) Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. Science 237:409–411

Barbour AG, Hayes SF (1986) Biology of *Borrelia* species. Microbiol Rev 50:381–400

Barbour AG, Maupin GO, Teltow GJ, Carter CJ, Piesman J (1996) Identification of an uncultivable Borrelia species in the hard tick *Amblyomma americanum*: possible agent of a Lyme disease-like illness. J Infect Dis 173(2):403–409. https://doi.org/10.1093/infdis/173.2.403. PMID: 8568302

Barbour AG, Dai Q, Restrepo BI, Stoenner HG, Frank SA (2006) Pathogen escape from host immunity by a genome program for antigenic variation. Proc Natl Acad Sci U S A 103:18290–18295

Becker NS, Margos G, Blum H, Krebs S, Graf A, Lane RS, Castillo-Ramirez S, Sing A, Fingerle V (2016) Recurrent evolution of host and vector association in bacteria of the *Borrelia burgdorferi* sensu lato species complex. BMC Genomics 17:734

Becker NS, Rollins RE, Nosenko K, Paulus A, Martin S, Krebs S, Takano A, Sato K, Kovalev SY, Kawabata H, Fingerle V, Margos G (2020) High conservation combined with high plasticity: genomics and evolution of *Borrelia bavariensis*. BMC Genomics 21:702

Berende A, ter Hofstede HJ, Vos FJ, van Middendorp H, Vogelaar ML, Tromp M, van den Hoogen FH, Donders AR, Evers AW, Kullberg BJ (2016) Randomized trial of longer-term therapy for symptoms attributed to Lyme disease. N Engl J Med 374:1209–1220

Bergey DH (1925) *Bergey's manual of determinative bacteriology*. The Williams & Wilkins Comparny, Baltimore

Bergey DH, Harrison FC, Breed RS, Hammer BW, Huntoon FM (1925) Bergey's Manual of Determinative Bacteriology, 2nd ed. The Williams & Wilkins Co, Baltimore

Berglund J, Eitrem R, Ornstein K, Lindberg A, Ringér A, Elmrud H, Carlsson M, Runehagen A, Svanborg C, Norrby R (1995) An epidemiologic study of Lyme disease in southern Sweden. N Engl J Med 333:1319–1327

Bermúdez SE, Armstrong BA, Domínguez L, Krishnavajhala A, Kneubehl AR, Gunter SM, Replogle A, Petersen JM, Lopez JE (2021) Isolation and genetic characterization of a relapsing fever spirochete isolated from *Ornithodoros puertoricensis* collected in central Panama. PLoS Negl Trop Dis 15:e0009642

Berström S, Zückert WR (2010) Structure, function and biogenesis of the *Borrelia* cell envelope. In: Samuels DS, Radolf JD (eds) Borrelia: Molecular biology, host interaction and pathogenesis. Caister Academic Press, Poole

Binetruy F, Garnier S, Boulanger N, Talagrand-Reboul É, Loire E, Faivre B, Noël V, Buysse M, Duron O (2020) A novel *Borrelia* species, intermediate between Lyme disease and relapsing fever groups, in neotropical passerine-associated ticks. Sci Rep 10:10596

Biskup UG, Strle F, Ruzic-Sabljic E (2011) Loss of plasmids of *Borrelia burgdorferi* sensu lato during prolonged in vitro cultivation. Plasmid 66:1–6

Blazejak K, Raulf MK, Janecek E, Jordan D, Fingerle V, Strube C (2018) Shifts in *Borrelia burgdorferi* (s.l.) geno-species infections in *Ixodes ricinus* over a 10-year surveillance period in the city of Hanover (Germany) and *Borrelia miyamotoi*-specific Reverse Line Blot detection. Parasit Vectors 11:304

Boden K, Lobenstein S, Hermann B, Margos G, Fingerle V (2016) *Borrelia miyamotoi*-associated neuroborreliosis in immunocompromised person. Emerg Infect Dis 22:1617–1620

Borg R, Dotevall L, Hagberg L, Maraspin V, Lotric-Furlan S, Cimperman J, Strle F (2005) Intravenous ceftriaxone compared with oral doxycycline for the treatment of Lyme neuroborreliosis. Scand J Infect Dis 37:449–454

Branda JA, Lemieux JE, Blair L, Ahmed AA, Hong DK, Bercovici S, Blauwkamp TA, Hollemon D, Ho C, Strle K, Damle NS, Lepore TJ, Pollock NR (2021) Detection of *Borrelia burgdorferi* cell-free DNA in human plasma samples for improved diagnosis of early Lyme borreliosis. Clin Infect Dis 73:e2355–e2e61

Bregnard C, Rais O, Voordouw MJ (2020) Climate and tree seed production predict the abundance of the European Lyme disease vector over a 15-year period. Parasit Vectors 13:408

Bremell D, Dotevall L (2014) Oral doxycycline for Lyme neuroborreliosis with symptoms of encephalitis, myelitis, vasculitis or intracranial hypertension. Eur J Neurol 21:1162–1167

Brown RN, Lane RS (1994) Natural and experimental *Borrelia burgdorferi* infections in woodrats and deer mice from California. J Wildl Dis 30:389–398

Brown RN, Peot MA, Lane RS (2006) Sylvatic maintenance of *Borrelia burgdorferi* (Spirochaetales) in Northern California: untangling the web of transmission. J Med Entomol 43:743–751

Brumpt E (1922) Les Spirochetoses. In: Roger GH, Widal F, Teissier PJ (eds) Nouveau Traité de Mèdecine, Masson, Paris, pp 491–531

Bryceson AD, Parry EH, Perine PL, Warrell DA, Vukotich D, Leithead CS (1970) Louse-borne relapsing fever. Q J Med 39:129–170

Buonomo A, Nucera E, Pecora V, Rizzi A, Aruanno A, Pascolini L, Ricci AG, Colagiovanni A, Schiavino D (2014) Cross-reactivity and tolerability of cephalosporins in patients with cell-mediated allergy to penicillins. J Investig Allergol Clin Immunol 24:331–337

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP (1982) Lyme disease-a tick-borne spirochetosis? Science 216:1317–1319

Butler T (2017) The Jarisch-Herxheimer reaction after antibiotic treatment of spirochetal infections: A review of recent cases and our understanding of pathogenesis. Am J Trop Med Hyg 96:46–52

Butler CM, Houwers DJ, Jongejan F, van der Kolk JH (2005) *Borrelia burgdorferi* infections with special reference to horses. A review. Vet Q 27:146–156

CAB International (2002). https://www.cabi.org/isc/datasheet/91634. *Borrelia anserina* infections: original text by M.B. Labruna

Canica MM, Nato F, du Merle L, Mazie JC, Baranton G, Postic D (1993) Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand J Infect Dis 25:441–448

Carley JG, Pope JH (1962) A new species of *Borrelia* (*B. queenslandica*) from *Rattus villosissimus* in Queensland. Aust J Exp Biol Med Sci 40:255–261. https://doi.org/10.1038/icb.1962.29. PMID: 13876596

Carlsson H, Ekerfelt C, Henningsson AJ, Brudin L, Tjernberg I (2018) Subclinical Lyme borreliosis is common in south-eastern Sweden and may be distinguished from Lyme neuroborreliosis by sex, age and specific immune marker patterns. Ticks Tick Borne Dis 9:742–748

Casjens S, Murphy M, DeLange M, Sampson L, van Vugt R, Huang WM (1997) Telomeres of the linear chromosomes of Lyme disease spirochaetes: nucleotide sequence and possible exchange with linear plasmid telomeres. Mol Microbiol 26:581–596

Casjens S, Palmer N, van Vugt R, Huang WM, Stevenson B, Rosa P, Lathigra R, Sutton G, Peterson J, Dodson RJ, Haft D, Hickey E, Gwinn M, White O, Fraser CM (2000) A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. Mol Microbiol 35:490–516

Casjens S, Eggers CH, Schwartz I (2010) *Borrelia* genomics: Chromosome, plasmids, bacteriohpages and genetic variation. In: Samuels DS, Radolf J (eds) Borrelia - Molecular biology, host interaction and pathogenesis. Caister Academic Press

Casjens SR, Mongodin EF, Qiu WG, Luft BJ, Schutzer SE, Gilcrease EB, Huang WM, Vujadinovic M, Aron JK, Vargas LC, Freeman S, Radune D, Weidman JF, Dimitrov GI, Khouri HM, Sosa JE, Halpin RA, Dunn JJ, Fraser CM (2012) Genome stability of Lyme disease spirochetes: comparative genomics of *Borrelia burgdorferi* plasmids. PLoS One 7:e33280

Casjens SR, Gilcrease EB, Vujadinovic M, Mongodin EF, Luft BJ, Schutzer SE, Fraser CM, Qiu WG (2017) Plasmid diversity and phylogenetic consistency in the Lyme disease agent *Borrelia burgdorferi*. BMC Genomics 18:165

Casjens SR, Di L, Akther S, Mongodin EF, Luft BJ, Schutzer SE, Fraser CM, Qiu WG (2018) Primordial origin and diversification of plasmids in Lyme disease agent bacteria. BMC Genomics 19:218

Cerar D, Cerar T, Ruzić-Sabljić E, Wormser GP, Strle F (2010) Subjective symptoms after treatment of early Lyme disease. Am J Med 123:79–86

Cetin E, Sotoudeh M, Auer H, Stanek G (2006) Paradigm Burgenland: risk of *Borrelia burgdorferi* sensu lato infection indicated by variable seroprevalence rates in hunters. Wien Klin Wochenschr 118:677–681

Chaconas G, Kobryn K (2010) Structure, function, and evolution of linear replicons in *Borrelia*. Annu Rev Microbiol 64:185–202

Chaconas G, Castellanos M, Verhey TB (2020) Changing of the guard: How the Lyme disease spirochete subverts the host immune response. J Biol Chem 295:301–313

Charon NW, Cockburn A, Li C, Liu J, Miller KA, Miller MR, Motaleb MA, Wolgemuth CW (2012) The unique paradigm of spirochete motility and chemotaxis. Annu Rev Microbiol 66:349–370

Coipan EC, Fonville M, Tijsse-Klasen E, van der Giessen JW, Takken W, Sprong H, Takumi K (2013) Geodemographic analysis of *Borrelia burgdorferi* sensu lato using the 5S-23S rDNA spacer region. Infect Genet Evol 17:216–222

Coipan EC, Jahfari S, Fonville M, Oei GA, Spanjaard L, Takumi K, Hovius JW, Sprong H (2016) Imbalanced presence of *Borrelia burgdorferi* s.l. multilocus sequence types in clinical manifestations of Lyme borreliosis. Infect Genet Evol 42:66–76

Collares-Pereira M, Couceiro S, Franca I, Kurtenbach K, Schafer SM, Vitorino L, Goncalves L, Baptista S, Vieira ML, Cunha C (2004) First isolation of *Borrelia lusitaniae* from a human patient. J Clin Microbiol 42:1316–1318

Comstedt P, Jakobsson T, Bergström S (2011) Global ecology and epidemiology of *Borrelia garinii* spirochetes. Infect Ecol Epidemiol 1:9545

Cooper GL, Bickford AA (1993) Spirochetosis in California game chickens. *Avian Dis* 37:1167–1171

Cuellar J, Dub T, Sane J, Hytönen J (2020) Seroprevalence of Lyme borreliosis in Finland 50 years ago. Clin Microbiol Infect 26:632–636

Cutler SJ (2010) Relapsing fever--a forgotten disease revealed. J Appl Microbiol 108:1115–1122

Cutler SJ (2015) Relapsing fever borreliae: a global review. Clin Lab Med 35:847–865

Cutler SJ, Fekade D, Hussein K, Knox KA, Melka A, Cann K, Emilianus AR, Warrell DA, Wright DJ (1994) Successful in-vitro cultivation of *Borrelia recurrentis*. Lancet 343:242

Cutler SJ, Moss J, Fukunaga M, Wright DJ, Fekade D, Warrell D (1997) *Borrelia recurrentis* characterization and comparison with relapsing-fever, Lyme-associated, and other *Borrelia* spp. Int J Syst Bacteriol 47:958–968

Dai Q, Restrepo BI, Porcella SF, Raffel SJ, Schwan TG, Barbour AG (2006) Antigenic variation by *Borrelia hermsii* occurs through recombination between extragenic repetitive elements on linear plasmids. Mol Microbiol 60:1329–1343

Davis GE (1948) The spirochetes. Annu Rev Microbiol 2(1 vol):305–334. https://doi.org/10.1146/annurev.mi.02.100148.001513. PMID: 18104349

Davis GE (1952) Observations on the biology of the argasid tick, Ornithodoros brasiliensis Aragao, 1923; with the recovery of a spirochete, *Borrelia brasiliensis*, n. sp. J Parasitol 38:473–476

Davis GE (1956) A relapsing fever spirochete, *Borrelia mazzottii* (sp. nov.) from Ornithodoros talaje from Mexico. Am J Hyg 63(1):13–17. https://doi.org/10.1093/oxfordjournals.aje.a119787. PMID: 13282883

Davis GE (1957) Order IX. *Spirochaetales* Buchanan 1918. In: Breed RS, Murray EGD, Smith NR (eds) Bergey's Manual of Determinative Bacteriology, seventh edition, The Williams & Wilkins Co, Baltimore, pp 892–907

de Carvalho IL, Fonseca JE, Marques JG, Ullmann A, Hojgaard A, Zeidner N, Nuncio MS (2008) Vasculitis-like syndrome associated with *Borrelia lusitaniae* infection. Clin Rheumatol 27: 1587–1591

De Michelis S, Sewell HS, Collares-Pereira M, Santos-Reis M, Schouls LM, Benes V, Holmes EC, Kurtenbach K (2000) Genetic diversity of *Borrelia burgdorferi* sensu lato in ticks from mainland Portugal. J Clin Microbiol 38:2128–2133

Dersch R, Hottenrott T, Senel M, Lehmensiek V, Tumani H, Rauer S, Stich O (2015) The chemokine CXCL13 is elevated in the cerebrospinal fluid of patients with neurosyphilis. Fluids Barriers CNS 12:12

Dessau RB, Fingerle V, Gray J, Hunfeld KP, Jaulhac B, Kahl O, Kristoferitsch W, Stanek G, Strle F (2014) The lymphocyte transformation test for the diagnosis of Lyme borreliosis has currently not been shown to be clinically useful. Clin Microbiol Infect 20:O786–O787

Dessau RB, van Dam AP, Fingerle V, Gray J, Hovius JW, Hunfeld KP, Jaulhac B, Kahl O, Kristoferitsch W, Lindgren PE, Markowicz M, Mavin S, Ornstein K, Rupprecht T, Stanek G, Strle F (2018) To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis. Clin Microbiol Infect 24:118–124

Dsouli N, Younsi-Kabachii H, Postic D, Nouira S, Gern L, Bouattour A (2006) Reservoir role of lizard *Psammodromus algirus* in transmission cycle of *Borrelia burgdorferi* sensu lato (Spirochaetaceae) in Tunisia. J Med Entomol 43:737–742

Due C, Fox W, Medlock JM, Pietzsch M, Logan JG (2013) Tick bite prevention and tick removal. Bmj 347:f7123

Eggers CH, Samuels DS (1999) Molecular evidence for a new bacteriophage of Borrelia burgdorferi. J Bacteriol 181:7308–7313

Eggers CH, Kimmel BJ, Bono JL, Elias AF, Rosa P, Samuels DS (2001) Transduction by phiBB-1, a bacteriophage of *Borrelia burgdorferi*. J Bacteriol 183:4771–4778

Ehounoud CB, Yao KP, Dahmani M, Achi YL, Amanzougaghene N, Kacou N'Douba A, N'Guessan JD, Raoult D, Fenollar F, Mediannikov O (2016) Multiple pathogens including potential new species in tick vectors in Côte d'Ivoire. PLoS Negl Trop Dis 10:e0004367

Eisen L (2020) Vector competence studies with hard ticks and *Borrelia burgdorferi* sensu lato spirochetes: a review. Ticks Tick Borne Dis 11:101359

Eisen L, Dolan MC (2016) Evidence for personal protective measures to reduce human contact with blacklegged ticks and for environmentally based control methods to suppress host-seeking blacklegged ticks and reduce infection with Lyme disease spirochetes in tick vectors and rodent reservoirs. J Med Entomol 53:1063–1092

Eisen L, Eisen RJ, Mun J, Salkeld DJ, Lane RS (2009) Transmission cycles of *Borrelia burgdorferi* and *B. bissettii* in relation to habitat type in northwestern California. J Vector Ecol 34:81–91

Elbir H, Raoult D, Drancourt M (2013) Relapsing fever borreliae in Africa. Am J Trop Med Hyg 89: 288–292

Elbir H, Abi-Rached L, Pontarotti P, Yoosuf N, Drancourt M (2014) African relapsing Fever borreliae genomospecies revealed by comparative genomics. Front Public Health 2:43

Elbir H, Sitlani P, Bergstrom S, Barbour AG (2017) Chromosome and megaplasmid sequences of *Borrelia anserina* (Sakharoff 1891), the agant of avian spirochetosis and type species of the genus. Genome Announc 5:e00018

Elelu N (2018) Tick-borne relapsing fever as a potential veterinary medical problem. Vet Med Sci 4: 271–279

Enkelmann J, Böhmer M, Fingerle V, Siffczyk C, Werber D, Littmann M, Merbecks SS, Helmeke C, Schroeder S, Hell S, Schlotthauer U, Burckhardt F, Stark K, Schielke A, Wilking H (2018) Incidence of notified Lyme borreliosis in Germany, 2013-2017. Sci Rep 8:14976

Estrada-Peña A, Sprong H, Cabezas-Cruz A, de la Fuente J, Ramo A, Coipan EC (2016) Nested coevolutionary networks shape the ecological relationships of ticks, hosts, and the Lyme disease bacteria of the *Borrelia burgdorferi* (s.l.) complex. Parasit Vectors 9:517

Euzeby JP (2010) Validation list no. 135. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 60:1985–1986

Evans J (2000) Lyme disease. Curr Opin Rheumatol 12:311–317

Fallon BA, Keilp JG, Corbera KM, Petkova E, Britton CB, Dwyer E, Slavov I, Cheng J, Dobkin J, Nelson DR, Sackeim HA (2008) A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology 70:992–1003

Fotso Fotso A, Angelakis E, Mouffok N, Drancourt M, Raoult D (2015) Blood-Borne *Candidatus Borrelia* algerica in a Patient with Prolonged Fever in Oran, Algeria. Am J Trop Med Hyg 93:1070–1073

Faulde MK, Rutenfranz M, Keth A, Hepke J, Rogge M, Görner A (2015) Pilot study assessing the effectiveness of factory-treated, long-lasting permethrin-impregnated clothing for the prevention of tick bites during occupational tick exposure in highly infested military training areas, Germany. Parasitol Res 114:671–678

Fedorova N, Kleinjan JE, James D, Hui LT, Peeters H, Lane RS (2014) Remarkable diversity of tick or mammalian-associated Borreliae in the metropolitan San Francisco Bay Area, California. Ticks Tick Borne Dis 5(6):951–961. https://doi.org/10.1016/j.ttbdis.2014.07.015. PMID: 25129859

Felsenfeld O (1965) Borreliae, human relapsing fever, and parasite-vector-host relationships. Bacteriol Rev 29:46–74

Figoni J, Chirouze C, Hansmann Y, Lemogne C, Hentgen V, Saunier A, Bouiller K, Gehanno JF, Rabaud C, Perrot S, Caumes E, Eldin C, de Broucker T, Jaulhac B, Roblot F, Toubiana J, Sellal F, Vuillemet F, Sordet C, Fantin B, Lina G, Gocko X, Dieudonné M, Picone O, Bodaghi B, Gangneux JP, Degeilh B, Partouche H, Lenormand C, Sotto A, Raffetin A, Monsuez JJ, Michel C, Boulanger N, Cathebras P, Tattevin P (2019) Lyme borreliosis and other tick-borne diseases. Guidelines from the French Scientific Societies (I): prevention, epidemiology, diagnosis. Med Mal Infect 49:318–334

Fingerle V, Schulte-Spechtel UC, Ruzic-Sabljic E, Leonhard S, Hofmann H, Weber K, Pfister K, Strle F, Wilske B (2008) Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. Int J Med Microbiol 298:279–290

Fingerle V, Pritsch M, Wachtler M, Margos G, Ruske S, Jung J, Loscher T, Wendtner C, Wieser A (2016) "*Candidatus* Borrelia kalaharica" detected from a febrile traveller returning to Germany from vacation in Southern Africa. PLoS Negl Trop Dis 10:e0004559

Fischer L, Korfel A, Pfeiffer S, Kiewe P, Volk HD, Cakiroglu H, Widmann T, Thiel E (2009) CXCL13 and CXCL12 in central nervous system lymphoma patients. Clin Cancer Res 15: 5968–5973

Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 390:580–586

Fryland L, Wilhelmsson P, Lindgren PE, Nyman D, Ekerfelt C, Forsberg P (2011) Low risk of developing *Borrelia burgdorferi* infection in the south-east of Sweden after being bitten by a *Borrelia burgdorferi*-infected tick. Int J Infect Dis 15:e174–e181

Fukunaga M, Takahashi Y, Tsuruta Y, Matsushita O, Ralph D, McClelland M, Nakao M (1995) Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int J Syst Bacteriol 45:804–810

Fukunaga M, Hamase A, Okada K, Nakao M (1996) *Borrelia tanukii* sp. nov. and *Borrelia turdae* sp. nov. found from ixodid ticks in Japan: rapid species identification by 16S rRNA gene-targeted PCR analysis. Microbiol Immunol 40:877–881

Fürst B, Glatz M, Kerl H, Müllegger RR (2006) The impact of immunosuppression on erythema migrans. A retrospective study of clinical presentation, response to treatment and production of *Borrelia* antibodies in 33 patients. Clin Exp Dermatol 31:509–514

Gajdacs M (2019) Taxonomy and nomenclature of bacteria with clinical and scientific importance: current concepts for pharmacists and pharmaceutical scientists. Acta Pharmaceutica Hungarica 89:99–108

Gevers D, Dawyndt P, Vandamme P, Willems A, Vancanneyt M, Swings J, De Vos P (2006) Stepping stones towards a new prokaryotic taxonomy. Philos Trans R Soc Lond B Biol Sci 361: 1911–1916

Ginsberg HS, Buckley PA, Balmforth MG, Zhioua E, Mitra S, Buckley FG (2005) Reservoir competence of native North American birds for the lyme disease spirochete, *Borrelia burgdorferi*. J Med Entomol 42:445–449

Girard YA, Fedorova N, Lane RS (2011) Genetic diversity of *Borrelia burgdorferi* and detection of *B. bissettii*-like DNA in serum of north-coastal California residents. J Clin Microbiol 49:945–954

Goddard J (2018) Tick-Borne diseases. In: Georgiev VS (ed) Infectious diseases and arthropods. Humana Press, Totowa

Goossens HA, Nohlmans MK, van den Bogaard AE (1999) Epstein-Barr virus and cytomegalovirus infections cause false-positive results in IgM two-test protocol for early Lyme borreliosis. Infection 27:231

Gray JS, Kahl O, Lane RS, Levin ML, Tsao JI (2016) Diapause in ticks of the medically important *Ixodes ricinus* species complex. Ticks Tick Borne Dis 7:992–1003

Guerrier G, Doherty T (2011) Comparison of antibiotic regimens for treating louse-borne relapsing fever: a meta-analysis. Trans R Soc Trop Med Hyg 105:483–490

Gugliotta JL, Goethert HK, Berardi VP, Telford SR 3rd. (2013) Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. N Engl J Med 368:240–245

Güner ES, Hashimoto N, Kadosaka T, Imai Y, Masuzawa T (2003) A novel, fast-growing *Borrelia* sp. isolated from the hard tick *Hyalomma aegyptium* in Turkey. Microbiology 149:2539–2544

Guner ES, Watanabe M, Hashimoto N, Kadosaka T, Kawamura Y, Ezaki T, Kawabata H, Imai Y, Kaneda K, Masuzawa T (2004) *Borrelia turcica* sp. nov., isolated from the hard tick *Hyalomma aegyptium* in Turkey. Int J Syst Evol Microbiol 54:1649–1652

Guyard C, Raffel SJ, Schrumpf ME, Dahlstrom E, Sturdevant D, Ricklefs SM, Martens C, Hayes SF, Fischer ER, Hansen BT, Porcella SF, Schwan TG (2013) Periplasmic flagellar export apparatus protein, FliH, is involved in post-transcriptional regulation of *FlaB*, motility and virulence of the relapsing fever spirochete *Borrelia hermsii*. PLoS One 8:e72550

Hahn CN, Mayhew IG, Whitwell KE, Smith KC, Carey D, Carter SD, Read RA (1996) A possible case of Lyme borreliosis in a horse in the UK. Equine Vet J 28:84–88

Han H, Liu J, Wen H, Li Z, Lei S, Qin X, . . . Yu X (2020) Pathogenic New World Relapsing Fever Borrelia in a Myotis Bat, Eastern China, 2015. Emerg Infect Dis 26(12):3083–3085. https://doi.org/10.3201/eid2612.191450

Hanincova K, Taragelova V, Koci J, Schafer SM, Hails R, Ullmann AJ, Piesman J, Labuda M, Kurtenbach K (2003) Association of *Borrelia garinii* and *B. valaisiana* with songbirds in Slovakia. Appl Environ Microbiol 69:2825–2830

Hansen K, Crone C, Kristoferitsch W (2013) Lyme neuroborreliosis. Handb Clin Neurol 115:559–575

Hartemink NA, Randolph SE, Davis SA, Heesterbeek JA (2008) The basic reproduction number for complex disease systems: defining R(0) for tick-borne infections. Am Nat 171:743–754

Henningsson AJ, Lager M, Bränström R, Tjernberg I, Skogman BH (2018) The chemokine CXCL13 in cerebrospinal fluid in children with Lyme neuroborreliosis. Eur J Clin Microbiol Infect Dis 37:1983–1991

Henningsson AJ, Asgeirsson H, Hammas B, Karlsson E, Parke Å, Hoornstra D, Wilhelmsson P, Hovius JW (2019) Two cases of *Borrelia miyamotoi* meningitis, Sweden, 2018. Emerg Infect Dis 25:1965–1968

Herzer P (1991) Joint manifestations of Lyme borreliosis in Europe. Scand J Infect Dis Suppl 77:55–63
Hillerdal H, Henningsson AJ (2021) Serodiagnosis of Lyme borreliosis-is IgM in serum more harmful than helpful? Eur J Clin Microbiol Infect Dis 40:1161–1168
Hinnebusch J, Bergstrom S, Barbour AG (1990) Cloning and sequence analysis of linear plasmid telomeres of the bacterium *Borrelia burgdorferi*. Mol Microbiol 4:811–820
Hinnebusch BJ, Barbour AG, Restrepo BI, Schwan TG (1998) Population structure of the relapsing fever spirochete *Borrelia hermsii* as indicated by polymorphism of two multigene families that encode immunogenic outer surface lipoproteins. Infect Immun 66:432–440
Hoch M, Wieser A, Loscher T, Margos G, Purner F, Zuhl J, Seilmaier M, Balzer L, Guggemos W, Rack-Hoch A, von Both U, Hauptvogel K, Schonberger K, Hautmann W, Sing A, Fingerle V (2015) Louse-borne relapsing fever (*Borrelia recurrentis*) diagnosed in 15 refugees from northeast Africa: epidemiology and preventive control measures, Bavaria, Germany, July to October 2015. Euro Surveill 20:30046
Hoen AG, Margos G, Bent SJ, Diuk-Wasser MA, Barbour A, Kurtenbach K, Fish D (2009) Phylogeography of *Borrelia burgdorferi* in the eastern United States reflects multiple independent Lyme disease emergence events. Proc Natl Acad Sci U S A 106:15013–15018
Hoffman HA, Jackson TW (1946) Spirochetosis in turkeys. J Am Vet Med Assoc 109:481–486
Hoffman HA, Jackson TW, Rucker JC (1946) Spirochetosis in turkeys (a preliminary report). J Am Vet Med Assoc 108:329–332
Hofmann H, Fingerle V, Hunfeld KP, Huppertz HI, Krause A, Rauer S, Ruf B (2017) Cutaneous Lyme borreliosis: Guideline of the German Dermatology Society. Ger Med Sci 15:Doc14
Hovind-Hougen K (1974) Electron microscopy of *Borrelia merionesi* and B*orrelia recurrentis*. Acta Pathol Microbiol Scand B Microbiol Immunol 82:799–809
Hovind-Hougen K (1995) A morphological characterization of *Borrelia anserina*. Microbiology (Reading) 141(Pt 1):79–83
Hovius JW, de Wever B, Sohne M, Brouwer MC, Coumou J, Wagemakers A, Oei A, Knol H, Narasimhan S, Hodiamont CJ, Jahfari S, Pals ST, Horlings HM, Fikrig E, Sprong H, van Oers MH (2013) A case of meningoencephalitis by the relapsing fever spirochaete *Borrelia miyamotoi* in Europe. Lancet 382:658
Huegli D, Moret J, Rais O, Moosmann Y, Erard P, Malinverni R, Gern L (2011) Prospective study on the incidence of infection by *Borrelia burgdorferi* sensu lato after a tick bite in a highly endemic area of Switzerland. Ticks Tick Borne Dis 2:129–136
Hunfeld KP, Rödel R, Wichelhaus TA (2003) In vitro activity of eight oral cephalosporins against *Borrelia burgdorferi*. Int J Antimicrob Agents 21:313–318
Hunfeld KP, Wichelhaus TA, Rödel R, Acker G, Brade V, Kraiczy P (2004) Comparison of in vitro activities of ketolides, macrolides, and an azalide against the spirochete *Borrelia burgdorferi*. Antimicrob Agents Chemother 48:344–347
Hunfeld KP, Ruzic-Sabljic E, Norris DE, Kraiczy P, Strle F (2005) In vitro susceptibility testing of *Borrelia burgdorferi* sensu lato isolates cultured from patients with erythema migrans before and after antimicrobial chemotherapy. Antimicrob Agents Chemother 49:1294–1301
Ivanova LB, Tomova A, Gonzalez-Acuna D, Murua R, Moreno CX, Hernandez C, Cabello J, Cabello C, Daniels TJ, Godfrey HP, Cabello FC (2014) *Borrelia chilensis*, a new member of the *Borrelia burgdorferi* sensu lato complex that extends the range of this genospecies in the Southern Hemisphere. Environ Microbiol 16:1069–1080
Iyer R, Caimano MJ, Luthra A, Axline D Jr, Corona A, Iacobas DA, Radolf JD, Schwartz I (2015) Stage-specific global alterations in the transcriptomes of Lyme disease spirochetes during tick feeding and following mammalian host adaptation. Mol Microbiol 95:509–538
Jakab Á, Kahlig P, Kuenzli E, Neumayr A (2022) Tick borne relapsing fever - a systematic review and analysis of the literature. PLoS Negl Trop Dis 16:e0010212
Jaulhac B, Saunier A, Caumes E, Bouiller K, Gehanno JF, Rabaud C, Perrot S, Eldin C, de Broucker T, Roblot F, Toubiana J, Sellal F, Vuillemet F, Sordet C, Fantin B, Lina G, Sobas C, Gocko X, Figoni J, Chirouze C, Hansmann Y, Hentgen V, Cathebras P, Dieudonné M, Picone O, Bodaghi B, Gangneux JP, Degeilh B, Partouche H, Lenormand C, Sotto A,

Raffetin A, Monsuez JJ, Michel C, Boulanger N, Lemogne C, Tattevin P (2019) Lyme borreliosis and other tick-borne diseases. Guidelines from the French scientific societies (II). Biological diagnosis, treatment, persistent symptoms after documented or suspected Lyme borreliosis. Med Mal Infect 49:335–346

Jiang B-G, Wu A-Q, Jiang J-F, Yuan T-T, Xu Q, Lv C-L, Chen J-J, Sun Y, Fang L-Q, Ruan X-D et al (2021) Molecular Detection of Novel Borrelia Species, Candidatus Borrelia javanense, in Amblyomma javanense Ticks from Pangolins. Pathogens 10:728. https://doi.org/10.3390/pathogens10060728

Johnson RC, Schmidt GP, Hyde FW, Steigerwalt AG, Brenner DJ (1984) *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. Int J System Bacteriol 34:496–497

Johnson RC, Burgdorfer W, Lane RS, Barbour AG, Hayes SF, Hyde FW (1987) *Borrelia coriaceae* sp. nov.: putative agent of epizootic bovine abortion. Int J Syst Bacteriol 37:72–74

Jolley KA, Maiden MC (2010) BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595

Kaenkan W, Nooma W, Chelong IA, Baimai V, Trinachartvanit W, Ahantarig A (2020) Reptile-associated *Borrelia* spp. In Amblyomma ticks, Thailand. Ticks Tick Borne Dis 11:101315

Kahl O, Gern L, Eisen L, Lane RS (2002) Ecological research on *Borrelia burgdorferi* sensu lato: terminology and some methodological pitfalls. In: Gray JS, Kahl O, Lane RS, Stanek G (eds) Lyme borreliosis: biology, epidemiology and control. CABI Publishing, Wallingford

Kahlig P, Neumayr A, Paris DH (2021) Louse-borne relapsing fever-A systematic review and analysis of the literature: Part 2-Mortality, Jarisch-Herxheimer reaction, impact on pregnancy. PLoS Negl Trop Dis 15:e0008656

Kalmar Z, Cozma V, Sprong H, Jahfari S, D'Amico G, Marcutan DI, Ionica AM, Magdas C, Modry D, Mihalca AD (2015) Transstadial transmission of *Borrelia turcica* in *Hyalomma aegyptium* ticks. PLoS One 10:e0115520

Karimi Y, Hovind-Hougen K, Birch-Andersen A, Asmar M (1979) *Borrelia persica* and *B. baltazardi* sp. nov.: experimental pathogenicity for some animals and comparison of the ultrastructure. Ann Microbiol (Paris) 130b:157–168

Kawabata H, Masuzawa T, Yanagihara Y (1993) Genomic analysis of *Borrelia japonica* sp. nov. isolated from *Ixodes ovatus* in Japan. Microbiol Immunol 37(11):843–848. https://doi.org/10.1111/j.1348-0421.1993.tb01714.x. PMID: 7905183

Kenedy MR, Scott EJ 2nd, Shrestha B, Anand A, Iqbal H, Radolf JD, Dyer DW, Akins DR (2016) Consensus computational network analysis for identifying candidate outer membrane proteins from *Borrelia* spirochetes. BMC Microbiol 16:141

King DP, Chen CI, Blanchard MT, Aldridge BM, Anderson M, Walker R, Maas J, Hanks D, Hall M, Stott JL (2005) Molecular identification of a novel deltaproteobacterium as the etiologic agent of epizootic bovine abortion (foothill abortion). J Clin Microbiol 43:604–609

Kingry LC (2021) Genomics of Relapsing Fever Spirochetes. In: Radolf JD, Samuels DS (eds) Lyme disease and relapsing fever spirochetes: genomics, molecular biology, host interactions and disease pathogenesis. Caister Academic Press, Poole

Kingry LC, Batra D, Replogle A, Rowe LA, Pritt BS, Petersen JM (2016) Whole genome sequence and comparative genomics of the novel Lyme borreliosis causing pathogen, *Borrelia mayonii*. PLoS One 11:e0168994

Kingry LC, Replogle A, Batra D, Rowe LA, Sexton C, Dolan M, Connally N, Petersen JM, Schriefer ME (2017a) Toward a complete North American *Borrelia miyamotoi* genome. Genome Announc 5:e01557

Kingry LC, Replogle A, Dolan M, Sexton C, Padgett KA, Schriefer ME (2017b) Chromosome and large linear plasmid sequences of a *Borrelia miyamotoi* strain isolated from *Ixodes pacificus* ticks from California. Genome Announc 5:e00960

Kingry LC, Anacker M, Pritt B, Bjork J, Respicio-Kingry L, Liu G, Sheldon S, Boxrud D, Strain A, Oatman S, Berry J, Sloan L, Mead P, Neitzel D, Kugeler KJ, Petersen JM (2018) Surveillance for and discovery of *Borrelia* species in US patients suspected of tickborne illness. Clin Infect Dis 66:1864–1871

Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, Trevino RP, Norton D, Levy L, Wall D, McCall J, Kosinski M, Weinstein A (2001) Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 345:85–92

Kneubehl AR, Krishnavajhala A, Leal SM, Replogle AJ, Kingry LC, Bermúdez SE, Labruna MB, Lopez JE (2022) Comparative genomics of the Western Hemisphere soft tick-borne relapsing fever borreliae highlights extensive plasmid diversity. BMC Genomics 23:410

Kobryn K, Chaconas G (2014) Hairpin telomere resolvases. Microbiol Spectr 2

Koedel U, Fingerle V, Pfister HW (2015) Lyme neuroborreliosis-epidemiology, diagnosis and management. Nat Rev Neurol 11:446–456

Krämer F, Hüsken R, Krüdewagen EM, Deuster K, Blagburn B, Straubinger RK, Butler J, Fingerle V, Charles S, Settje T, Schunack B, Stanneck D (2020) Prevention of transmission of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* by *Ixodes* spp. ticks to dogs treated with the Seresto® collar (imidacloprid 10% + flumethrin 4.5%). Parasitol Res 119:299–315

Krause PJ, Narsimhan S, Wormser GP, Rollend L, Fikrig E, Lepore T, Barbour A, Fish D (2013) Human *Borrelia miyamotoi* Infection in the United States. N Engl J Med 368:290–291

Krause PJ, Fish D, Narasimhan S, Barbour AG (2015) *Borrelia miyamotoi* infection in nature and in humans. Clin Microbiol Infect 21:631–639

Kristoferitsch W (1991) Neurological manifestations of Lyme borreliosis: clinical definition and differential diagnosis. Scand J Infect Dis Suppl 77:64–73

Kristoferitsch W, Aboulenein-Djamshidian F, Jecel J, Rauschka H, Rainer M, Stanek G, Fischer P (2018) Secondary dementia due to Lyme neuroborreliosis. Wien Klin Wochenschr 130:468–478

Krupka I, Straubinger RK (2010) Lyme borreliosis in dogs and cats: background, diagnosis, treatment and prevention of infections with *Borrelia burgdorferi* sensu stricto. Vet Clin North Am Small Anim Pract 40:1103–1119

Krupp LB, Hyman LG, Grimson R, Coyle PK, Melville P, Ahnn S, Dattwyler R, Chandler B (2003) Study and treatment of post Lyme disease (STOP-LD): a randomized double masked clinical trial. Neurology 60:1923–1930

Kugeler KJ, Eisen RJ (2020) Challenges in predicting Lyme disease risk. JAMA Netw Open 3: e200328

Kugeler KJ, Schwartz AM, Delorey MJ, Mead PS, Hinckley AF (2021) Estimating the frequency of Lyme disease diagnoses, United States, 2010-2018. Emerg Infect Dis 27:616–619

Kuleshov KV, Koetsveld J, Goptar IA, Markelov ML, Kolyasnikova NM, Sarksyan DS, Toporkova MG, Kirdyashkina NP, Shipulin GA, Hovius JW, Platonov AE (2018) Whole-genome sequencing of six *Borrelia miyamotoi* clinical strains isolated in Russia. Genome Announc 6:e01424

Kuleshov KV, Margos G, Fingerle V, Koetsveld J, Goptar IA, Markelov ML, Kolyasnikova NM, Sarksyan DS, Kirdyashkina NP, Shipulin GA, Hovius JW, Platonov AE (2020) Whole genome sequencing of *Borrelia miyamotoi* isolate Izh-4: reference for a complex bacterial genome. BMC Genomics 21:16

Kumagai Y, Sato K, Taylor KR, Zamoto-Niikura A, Imaoka K, Morikawa S, Ohnishi M, Kawabata H (2018) A relapsing fever group *Borrelia* sp. is widely distributed among wild deer in Japan. Ticks Tick Borne Dis 9:465–470

Kurtenbach K, Hanincova K, Tsao JI, Margos G, Fish D, Ogden NH (2006) Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat Rev Microbiol 4:660–669

Labandeira-Rey M, Skare JT (2001) Decreased infectivity in *Borrelia burgdorferi* strain B31 is associated with loss of linear plasmid 25 or 28-1. Infect Immunol 69:446–455

Lakos A, Solymosi N (2010) Maternal Lyme borreliosis and pregnancy outcome. Int J Infect Dis 14: e494–e498

Lantos PM, Rumbaugh J, Bockenstedt LK, Falck-Ytter YT, Aguero-Rosenfeld ME, Auwaerter PG, Baldwin K, Bannuru RR, Belani KK, Bowie WR, Branda JA, Clifford DB, DiMario FJ, Halperin JJ, Krause PJ, Lavergne V, Liang MH, Meissner HC, Nigrovic LE, Nocton JJJ, Osani MC, Pruitt AA, Rips J, Rosenfeld LE, Savoy ML, Sood SK, Steere AC, Strle F, Sundel R, Tsao J, Vaysbrot EE, Wormser GP, Zemel LS (2021) Clinical practice guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 guidelines for the prevention, diagnosis and treatment of Lyme disease. Clin Infect Dis 72:1–8

Le Fleche A, Postic D, Girardet K, Peter O, Baranton G (1997) Characterization of *Borrelia lusitaniae* sp. nov. by 16S ribosomal DNA sequence analysis. Int J Syst Bacteriol 47:921–925

Lebert H (1876) Rückfallthyphus und biliöses Typhoid. In: Vogel FCW (ed) Handbuch der akuten Infectionskrankheiten. Verlag von F. C. W. Vogel, Leipzig

Lepennetier G, Hracsko Z, Unger M, Van Griensven M, Grummel V, Krumbholz M, Berthele A, Hemmer B, Kowarik MC (2019) Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases. J Neuroinflammation 16:219

Lescot M, Audic S, Robert C, Nguyen TT, Blanc G, Cutler SJ, Wincker P, Couloux A, Claverie JM, Raoult D, Drancourt M (2008) The genome of *Borrelia recurrentis*, the agent of deadly louse-borne relapsing fever, is a degraded subset of tick-borne *Borrelia duttonii*. PLoS Genet 4:e1000185

Li Z-M, Xiao X, Zhou C-M, Liu J-X, Gu XL, Fang L-Z et al (2021) Human-pathogenic relapsing fever Borrelia found in bats from Central China phylogenetically clustered together with relapsing fever borreliae reported in the New World. PLoS Negl Trop Dis 15(3):e0009113. https://doi.org/10.1371/journal.pntd.0009113

Lin T, Gao L, Seyfang A, Oliver JH Jr (2005) '*Candidatus* Borrelia texasensis', from the American dog tick *Dermacentor variabilis*. Int J Syst Evol Microbiol 55:685–693

Lin T, Gao L, Zhang C, Odeh E, Jacobs MB, Coutte L, Chaconas G, Philipp MT, Norris SJ (2012) Analysis of an ordered, comprehensive STM mutant library in infectious *Borrelia burgdorferi*: insights into the genes required for mouse infectivity. PLoS One 7:e47532

Lin T, Troy EB, Hu LT, Gao L, Norris SJ (2014) Transposon mutagenesis as an approach to improved understanding of *Borrelia* pathogenesis and biology. Front Cell Infect Microbiol 4:63

Lindgren E, Jaenson TGT (2006) Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. http://www.euro.who.int/__data/assets/pdf_file/0006/96819/E89522.pdf

Littman MP, Goldstein RE, Labato MA, Lappin MR, Moore GE (2006) ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. J Vet Intern Med 20:422–434

Ljøstad U, Skogvoll E, Eikeland R, Midgard R, Skarpaas T, Berg A, Mygland A (2008) Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. Lancet Neurol 7:690–695

Loh SM, Gillett A, Ryan U, Irwin P, Oskam C (2017) Molecular characterization of '*Candidatus* Borrelia tachyglossi' (family Spirochaetaceae) in echidna ticks, *Bothriocroton concolor*. Int J Syst Evol Microbiol 67:1075–1080

Lopez JE, Krishnavahjala A, Garcia MN, Bermudez S (2016) Tick-borne relapsing fever spirochetes in the Americas. Vet Sci 3:16

Luft BJ, Dattwyler RJ, Johnson RC, Luger SW, Bosler EM, Rahn DW, Masters EJ, Grunwaldt E, Gadgil SD (1996) Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann Intern Med 124:785–791

Maraspin V, Lotric-Furlan S, Cimperman J, Ruzić-Sabljić E, Strle F (1999) Erythema migrans in the immunocompromised host. Wien Klin Wochenschr 111:923–932

Maraspin V, Cimperman J, Lotric-Furlan S, Logar M, Ruzić-Sabljić E, Strle F (2006) Erythema migrans in solid-organ transplant recipients. Clin Infect Dis 42:1751–1754

Maraspin V, Ružić-Sabljić E, Pleterski-Rigler D, Strle F (2011) Pregnant women with erythema migrans and isolation of borreliae from blood: course and outcome after treatment with ceftriaxone. Diagn Microbiol Infect Dis 71:446–448

Maraspin V, Bogovič P, Rojko T, Ogrinc K, Ružić-Sabljić E, Strle F (2019) Early Lyme borreliosis in patients treated with tumour necrosis factor-alfa inhibitors. J Clin Med 8:1857

Marconi RT, Liveris D, Schwartz I (1995) Identification of novel insertion elements, restriction fragment length polymorphism patterns, and discontinuous 23S rRNA in Lyme disease spirochetes: phylogenetic analyses of rRNA genes and their intergenic spacers in *Borrelia japonica* sp. nov. and genomic group 21038 (*Borrelia andersonii* sp. nov.) isolates. J Clin Microbiol 33 (9):2427–2434. https://doi.org/10.1128/jcm.33.9.2427-2434.1995. PMID: 7494041; PMCID: PMC228430

Margos G, Gatewood AG, Aanensen DM, Hanincova K, Terekhova D, Vollmer SA, Cornet M, Piesman J, Donaghy M, Bormane A, Hurn MA, Feil EJ, Fish D, Casjens S, Wormser GP,

Schwartz I, Kurtenbach K (2008) MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. Proc Natl Acad Sci U S A 105: 8730–8735

Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B, Bormane A, Vitorino L, Collares-Pereira M, Drancourt M, Kurtenbach K (2009) A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 75:5410–5416

Margos G, Hojgaard A, Lane RS, Cornet M, Fingerle V, Rudenko N, Ogden N, Aanensen DM, Fish D, Piesman J (2010) Multilocus sequence analysis of *Borrelia bissettii* strains from North America reveals a new *Borrelia* species, *Borrelia kurtenbachii*. Ticks Tick Borne Dis 1(4):151–158. https://doi.org/10.1016/j.ttbdis.2010.09.002. PMID: 21157575; PMCID: PMC3000690

Margos G, Wilske B, Sing A, Hizo-Teufel C, Cao WC, Chu C, Scholz H, Straubinger RK, Fingerle V (2013) *Borrelia bavariensis* sp. nov. is widely distributed in Europe and Asia. Int J Syst Evol Microbiol 63(Pt 11):4284–4288. https://doi.org/10.1099/ijs.0.052001-0. PMID: 23838444

Margos G, Castillo-Ramirez S, Hoen AG (2012a) Phylogeography of Lyme borreliosis-group spirochetes and methicillin-resistant *Staphylococcus aureus*. Parasitology 139:1952–1965

Margos G, Tsao JI, Castillo-Ramirez S, Girard YA, Hamer SA, Hoen AG, Lane RS, Raper SL, Ogden NH (2012b) Two boundaries separate *Borrelia burgdorferi* populations in North America. Appl Environ Microbiol 78:6059–6067

Margos G, Piesman J, Lane RS, Ogden NH, Sing A, Straubinger RK, Fingerle V (2014) *Borrelia kurtenbachii* sp. nov., a widely distributed member of the *Borrelia burgdorferi* sensu lato species complex in North America. Int J Syst Evol Microbiol 64(Pt 1):128–130. https://doi.org/10.1099/ijs.0.054593-0. PMID: 24048870

Margos G, Stockmeier S, Hizo-Teufel C, Hepner S, Fish D, Dautel H, Sing A, Dzaferovic E, Rieger M, Jungnick S, Binder K, Straubinger RK, Fingerle V (2015a) Long-term in vitro cultivation of *Borrelia miyamotoi*. Ticks Tick Borne Dis 6:181–184

Margos G, Chu CY, Takano A, Jiang BG, Liu W, Kurtenbach K, Masuzawa T, Fingerle V, Cao WC, Kawabata H (2015b) *Borrelia yangtzensis* sp. nov., a rodent-associated species in Asia, is related to *Borrelia valaisiana*. Int J Syst Evol Microbiol 65(11):3836–3840. https://doi.org/10.1099/ijsem.0.000491. PMID: 28875924

Margos G, Lane RS, Fedorova N, Koloczek J, Piesman J, Hojgaard A, Sing A, Fingerle V (2016) *Borrelia bissettiae* sp. nov. and *Borrelia californiensis* sp. nov. Prevail in Diverse Enzootic Transmission Cycles. Int J Syst Evol Microbiol 66:1447

Margos G, Hepner S, Mang C, Marosevic D, Reynolds SE, Krebs S, Sing A, Derdakova M, Reiter MA, Fingerle V (2017a) Lost in plasmids: next generation sequencing and the complex genome of the tick-borne pathogen *Borrelia burgdorferi*. BMC Genomics 18:422

Margos G, Sing A, Fingerle V (2017b) Published data do not support the notion that *Borrelia valaisiana* is human pathogenic. Infection 45:567–569

Margos G, Fedorova N, Kleinjan JE, Hartberger C, Schwan TG, Sing A, Fingerle V (2017c) *Borrelia lanei* sp. nov. extends the diversity of *Borrelia* species in California. Int J Syst Evol Microbiol 67(10):3872–3876. https://doi.org/10.1099/ijsem.0.002214. PMID: 28884668; PMCID: PMC5737112

Margos G, Gofton A, Wibberg D, Dangel A, Marosevic D, Loh SM, Oskam C, Fingerle V (2018) The genus *Borrelia* reloaded. PLoS One 13:e0208432

Margos G, Becker NS, Fingerle V, Sing A, Ramos JA, Carvalho IL, Norte AC (2019a) Core genome phylogenetic analysis of the avian associated *Borrelia turdi* indicates a close relationship to *Borrelia garinii*. Mol Phylogenet Evol 131:93–98

Margos G, Fingerle V, Reynolds S (2019b) *Borrelia bavariensis*: Vector switch, niche invasion, and geographical spread of a tick-borne bacterial parasite. Front Ecol Evol 7:401

Margos G, Fedorova N, Becker NS, Kleinjan JE, Marosevic D, Krebs S, Hui L, Fingerle V, Lane RS (2020a) *Borrelia maritima* sp. nov., a novel species of the *Borrelia burgdorferi* sensu lato complex, occupying a basal position to North American species. Int J Syst Evol Microbiol 70:849–856

Margos G, Pantchev N, Globokar M, Lopez J, Rodon J, Hernandez L, Herold H, Salas N, Civit A, Fingerle V (2020b) First cases of natural infections with *Borrelia hispanica* in two dogs and a cat from Europe. Microorganisms 8:1251

Margos G, Fingerle V, Cutler S, Gofton A, Stevenson B, Estrada-Peña A (2020c) Controversies in bacterial taxonomy: The example of the genus *Borrelia*. Ticks Tick Borne Dis 11(2):101335. https://doi.org/10.1016/j.ttbdis.2019.101335. PMID: 31836459

Markowicz M, Reiter M, Gamper J, Stanek G, Stockinger H (2021a) Persistent anti-*Borrelia* IgM antibodies without Lyme borreliosis in the clinical and immunological context. Microbiol Spectr 9:e0102021

Markowicz M, Schötta AM, Höss D, Kundi M, Schray C, Stockinger H, Stanek G (2021b) Infections with tickborne pathogens after tick bite, Austria, 2015-2018. Emerg Infect Dis 27: 1048–1056

Marosevic D, Margos G, Wallich R, Wieser A, Sing A, Fingerle V (2017) First insights in the variability of *Borrelia recurrentis* genomes. PLoS Negl Trop Dis 11:e0005865

Masuzawa T (2004) Terrestrial distribution of the Lyme borreliosis agent *Borrelia burgdorferi* sensu lato in East Asia. Jpn J Infect Dis 57:229–235

Masuzawa T, Takada N, Kudeken M, Fukui T, Yano Y, Ishiguro F, Kawamura Y, Imai Y, Ezaki T (2001) *Borrelia sinica* sp. nov., a lyme disease-related *Borrelia species* isolated in China. Int J Syst Evol Microbiol 51:1817–1824

McCall PJ, Hume JC, Motshegwa K, Pignatelli P, Talbert A, Kisinza W (2007) Does tick-borne relapsing fever have an animal reservoir in East Africa? Vector Borne Zoonotic Dis 7:659–666

McCoy BN, Maïga O, Schwan TG (2014) Detection of *Borrelia theileri* in *Rhipicephalus geigyi* from Mali. Ticks Tick Borne Dis 5:401–403

McNeil E, Hinshaw WR, Kissling RE (1949) A study of *Borrelia anserina* infection (spirochetosis) in turkeys. J Bacteriol 57:191–206

Mechai S, Margos G, Feil EJ, Lindsay LR, Ogden NH (2015) Complex population structure of *Borrelia burgdorferi* in southeastern and south central Canada as revealed by phylogeographic analysis. Appl Environ Microbiol 81:1309–1318

Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Pena A, George JC, Golovljova I, Jaenson TG, Jensen JK, Jensen PM, Kazimirova M, Oteo JA, Papa A, Pfister K, Plantard O, Randolph SE, Rizzoli A, Santos-Silva MM, Sprong H, Vial L, Hendrickx G, Zeller H, Van Bortel W (2013) Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit Vectors 6:1

Merkac MI, Tomazic J, Strle F (2015) Lyme neuroborreliosis in a patient treated with TNF-alpha inhibitor. Infection 43:759–762

Mikkilä HO, Seppälä IJ, Viljanen MK, Peltomaa MP, Karma A (2000) The expanding clinical spectrum of ocular Lyme borreliosis. Ophthalmology 107:581–587

Mitani H, Talbert A, Fukunaga M (2004) New World relapsing fever *Borrelia* found in *Ornithodoros porcinus* ticks in central Tanzania. Microbiol Immunol 48:501–505

Molloy PJ, Telford SR 3rd, Chowdri HR, Lepore TJ, Gugliotta JL, Weeks KE, Hewins ME, Goethert HK, Berardi VP (2015) *Borrelia miyamotoi* disease in the northeastern United States: A case series. Ann Intern Med 163:91–98

Mongodin EF, Casjens SR, Bruno JF, Xu Y, Drabek EF, Riley DR, Cantarel BL, Pagan PE, Hernandez YA, Vargas LC, Dunn JJ, Schutzer SE, Fraser CM, Qiu WG, Luft BJ (2013) Inter- and intra-specific pan-genomes of *Borrelia burgdorferi* sensu lato: genome stability and adaptive radiation. BMC Genomics 14:693

Moody KD, Terwilliger GA, Hansen GM, Barthold SW (1994) Experimental *Borrelia burgdorferi* infection in *Peromyscus leucopus*. J Wildl Dis 30:155–161

Morgenstern K, Baljer G, Norris DE, Kraiczy P, Hanssen-Hübner C, Hunfeld KP (2009) In vitro susceptibility of *Borrelia spielmanii* to antimicrobial agents commonly used for treatment of Lyme disease. Antimicrob Agents Chemother 53:1281–1284

Mueller I, Freitag MH, Poggensee G, Scharnetzky E, Straube E, Ch Schoerner H, Hlobil HJ, Hagedorn G, Stanek A, Schubert-Unkmeir DE, Norris JG, Hunfeld KP (2012) Evaluating frequency, diagnostic quality, and cost of Lyme borreliosis testing in Germany: a retrospective model analysis. Clin Dev Immunol 2012:595427

Mun J, Eisen RJ, Eisen L, Lane RS (2006) Detection of a *Borrelia miyamotoi* sensu lato relapsing-fever group spirochete from *Ixodes pacificus* in California. J Med Entomol 43:120–123

Muñoz-Leal S, Ramirez DG, Luz HR, Faccini JLH, Labruna MB (2020) "*Candidatus* Borrelia ibitipoquensis," a *Borrelia* valaisiana-related genospecies characterized from *Ixodes paranaensis* in Brazil. Microb Ecol 80:682–689

Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I (2010) EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol 17(8-16):e1–e4

Naddaf SR, Ghazinezhad B, Bahramali G, Cutler SJ (2012) Phylogenetic analysis of the spirochete *Borrelia microti*, a potential agent of relapsing fever in Iran. J Clin Microbiol 50(9):2873–2876. https://doi.org/10.1128/JCM.00801-12. PMID: 22718931; PMCID: PMC3421807

Nefedova VV, Korenberg EI, Gorelova NB, Kovalevskii YV (2004) Studies on the transovarial transmission of *Borrelia burgdorferi* sensu lato in the taiga tick *Ixodes persulcatus*. Folia Parasitology 51:67–71

Newman EA, Eisen L, Eisen RJ, Fedorova N, Hasty JM, Vaughn C, Lane RS (2015) *Borrelia burgdorferi* sensu lato spirochetes in wild birds in northwestern California: associations with ecological factors, bird behavior and tick infestation. PLoS One 10:e0118146

Nguala S, Baux E, Patrat-Delon S, Saunier F, Schemoul J, Tattevin P, Cazorla C, Eldin C, Bouiller K, Raffetin A (2021) Methodological quality assessment with the AGREE II scale and a comparison of European and American guidelines for the treatment of Lyme Borreliosis: a systematic review. Pathogens 10:972

Nizič T, Velikanje E, Ružić-Sabljić E, Arnež M (2012) Solitary erythema migrans in children: comparison of treatment with clarithromycin and amoxicillin. Wien Klin Wochenschr 124:427–433

Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC (1994) Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med 330:229–234

Nordstrand A, Bunikis I, Larsson C, Tsogbe K, Schwan TG, Nilsson M, Bergström S (2007) Tickborne relapsing fever diagnosis obscured by malaria, Togo. Emerg Infect Dis 13:117–123

Norris SJ (2006) Antigenic variation with a twist--the *Borrelia* story. Mol Microbiol 60:1319–1322

Norris SJ, Howell JK, Garza SA, Ferdows MS, Barbour AG (1995) High- and low-infectivity phenotypes of clonal populations of in vitro-cultured *Borrelia burgdorferi*. Infect Immunol 63: 2206–2212

Norris DE, Johnson BJ, Piesman J, Maupin GO, Clark JL, W. C. th Black. (1997) Culturing selects for specific genotypes of *Borrelia burgdorferi* in an enzootic cycle in Colorado. J Clin Microbiol 35:2359–2364

Norte AC, Margos G, Becker NS, Albino Ramos J, Núncio MS, Fingerle V, Araújo PM, Adamík P, Alivizatos H, Barba E, Barrientos R, Cauchard L, Csörgő T, Diakou A, Dingemanse NJ, Doligez B, Dubiec A, Eeva T, Flaisz B, Grim T, Hau M, Heylen D, Hornok S, Kazantzidis S, Kováts D, Krause F, Literak I, Mänd R, Mentesana L, Morinay J, Mutanen M, Neto JM, Nováková M, Sanz JJ, Pascoal da Silva L, Sprong H, Tirri IS, Török J, Trilar T, Tyller Z, Visser ME, Lopes de Carvalho I (2020) Host dispersal shapes the population structure of a tick-borne bacterial pathogen. Mol Ecol 29:485–501

Norte AC, Boyer PH, Castillo-Ramirez S, Chvostáč M, Brahami MO, Rollins RE, Woudenberg T, Didyk YM, Derdakova M, Núncio MS, Carvalho IL, Margos G, Fingerle V (2021) The population structure of *Borrelia lusitaniae* is reflected by a population division of its *Ixodes* vector. Microorganisms 9:933

Ogden NH, Lindsay LR, Hanincova K, Barker IK, Bigras-Poulin M, Charron DF, Heagy A, Francis CM, O'Callaghan CJ, Schwartz I, Thompson RA (2008) Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Appl Environ Microbiol 74:1780–1790

Ogrinc K, Hernández SA, Korva M, Bogovič P, Rojko T, Lusa L, Chiumento G, Strle F, Strle K (2022) Unique clinical, immune, and genetic signature in patients with borrelial meningoradiculoneuritis. Emerg Infect Dis 28:766–776

Ohlenbusch A, Matuschka FR, Richter D, Christen HJ, Thomssen R, Spielman A, Eiffert H (1996) Etiology of the acrodermatitis chronica atrophicans lesion in Lyme disease. J Infect Dis 174:421–423

Okeyo M, Hepner S, Rollins RE, Hartberger C, Straubinger RK, Marosevic D, Bannister SA, Bormane A, Donaghy M, Sing A, Fingerle V, Margos G (2020) Longitudinal study of prevalence and spatio-temporal distribution of *Borrelia burgdorferi* sensu lato in ticks from three defined habitats in Latvia, 1999-2010. Environ Microbiol 22:5033–5047

Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F (2006) Climate, deer, rodents, and acorns as determinants of variation in lyme-disease risk. PLoS Biol 4:e145

Pachner AR, Steere AC (1985) The triad of neurologic manifestations of Lyme disease: meningitis, cranial neuritis, and radiculoneuritis. Neurology 35:47–53

Pages F, Dautel H, Duvallet G, Kahl O, de Gentile L, Boulanger N (2014) Tick repellents for human use: prevention of tick bites and tick-borne diseases. Vector Borne Zoonotic Dis 14:85–93

Pancewicz SA, Garlicki AM, Moniuszko-Malinowska A, Zajkowska J, Kondrusik M, Grygorczuk S, Czupryna P, Dunaj J (2015) Diagnosis and treatment of tick-borne diseases recommendations of the Polish Society of Epidemiology and Infectious Diseases. Przegl Epidemiol 69(309-16):421–428

Panetta JL, Šíma R, Calvani NED, Hajdušek O, Chandra S, Panuccio J, Šlapeta J (2017) Reptile-associated *Borrelia* species in the goanna tick (*Bothriocroton undatum*) from Sydney, Australia. Parasit Vectors 10:616

Pantchev N, Pluta S, Huisinga E, Nather S, Scheufelen M, Vrhovec MG, Schweinitz A, Hampel H, Straubinger RK (2015) Tick-borne diseases (borreliosis, anaplasmosis, babesiosis) in German and Austrian dogs: Status quo and review of distribution, transmission, clinical findings, diagnostics and prophylaxis. Parasitol Res 114(Suppl 1):S19–S54

Petzke M, Schwartz I (2015) *Borrelia burgdorferi* pathogenesis and the immune response. Clin Lab Med 35:745–764

Pfister HW, Wilske B, Preac-Mursic V (1989) Clinical and serological follow-up of patients with Bannwarth´s syndrome: comparison of patients with and without penicillin treatment. Zbl. Bakt. Lyme Borreliosis *II*:276–279

Picken RN, Picken MM (2000) Molecular characterization of *Borrelia* spp. isolates from greater metropolitan Chicago reveals the presence of *Borrelia bissettii*. Preliminary report. J Mol Microbiol Biotechnol 2:505–507

Piesman J (2002) Ecology of *Borrelia burgdorferi* sensu lato in Northamerica. In: Gray JS, Kahl O, Lane RS, Stanek G (eds) Lyme borreliosis: biology of the infectious agents and epidemiology of disease. CABI Publishing, Wallingford

Piesman J, Gern L (2004) Lyme borreliosis in Europe and North America. Parasitology 129(Suppl): S191–S220

Piesman J, Schwan TG (2010) Ecology of borreliae and their arthropod vectors. In: Samuels DS, Radolf JD (eds) *Borrelia: Molecular biology, host interaction and pathogenesis*. Caister Academic Press, Poole

Pietikäinen A, Maksimow M, Kauko T, Hurme S, Salmi M, Hytönen J (2016) Cerebrospinal fluid cytokines in Lyme neuroborreliosis. J Neuroinflammation 13:273

Platonov AE, Karan LS, Kolyasnikova NM, Makhneva NA, Toporkova MG, Maleev VV, Fish D, Krause PJ (2011) Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. Emerg Infect Dis 17:1816–1823

Postic D, Ras NM, Lane RS, Hendson M, Baranton G (1998) Expanded diversity among Californian borrelia isolates and description of *Borrelia bissettii* sp. nov. (formerly *Borrelia* group DN127). J Clin Microbiol 36:3497–3504

Postic D, Garnier M, Baranton G (2007) Multilocus sequence analysis of atypical *Borrelia burgdorferi* sensu lato isolates–description of *Borrelia californiensis* sp. nov., and genomospecies 1 and 2. Int J Med Microbiol 297(4):263–271. https://doi.org/10.1016/j.ijmm.2007.01.006. PMID: 17374507

Preac Mursic V, Marget W, Busch U, Pleterski Rigler D, Hagl S (1996) Kill kinetics of *Borrelia burgdorferi* and bacterial findings in relation to the treatment of Lyme borreliosis. Infection 24:9–16

Preac-Mursic V, Wilske B, Schierz G (1986) European *Borrelia burgdorferi* isolated from humans and ticks culture conditions and antibiotic susceptibility. Zentralbl Bakteriol Mikrobiol Hyg A 263:112–118

Pritt BS, Mead PS, Johnson DK, Neitzel DF, Respicio-Kingry LB, Davis JP, Schiffman E, Sloan LM, Schriefer ME, Replogle AJ, Paskewitz SM, Ray JA, Bjork J, Steward CR, Deedon A, Lee X, Kingry LC, Miller TK, Feist MA, Theel ES, Patel R, Irish CL, Petersen JM (2016) Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. Lancet Infect Dis 16:511–512

Qiu Y, Nakao R, Hang'ombe BM, Sato K, Kajihara M, Kanchela S, Changula K, Eto Y, Ndebe J, Sasaki M, Thu MJ, Takada A, Sawa H, Sugimoto C, Kawabata H (2019) Human borreliosis caused by a new world relapsing fever *Borrelia*-like organism in the old world. Clin Infect Dis 69:107–112

Qiu Y, Squarre D, Nakamura Y, Lau ACC, Moonga LC, Kawai N, Ohnuma A, Hayashida K, Nakao R, Yamagishi J, Sawa H, Namangala B, Kawabata H (2021) Evidence of *Borrelia theileri* in wild and Ddmestic animals in the Kafue ecosystem of Zambia. Microorganisms 9:2405

Radolf JD, Samuels DS (2021) Lyme disease and relapsing fever spirochetes. Caister Academic Press, Poole

Radolf JD, Bourell KW, Akins DR, Brusca JS, Norgard MV (1994) Analysis of *Borrelia burgdorferi* membrane architecture by freeze-fracture electron microscopy. J Bacteriol 176:21–31

Radolf JD, Strle K, Lemieux JE, Strle F (2021) Lyme disease in humans. Curr Issues Mol Biol 42: 333–384

Randolph SE, Green RM, Hoodless AN, Peacey MF (2002) An empirical quantitative framework for the seasonal population dynamics of the tick *Ixodes ricinus*. Int J Parasitol 32:979–989

Rauer S, Kastenbauer S, Hofmann H, Fingerle V, Huppertz HI, Hunfeld KP, Krause A, Ruf B, Dersch R (2020) Guidelines for diagnosis and treatment in neurology - Lyme neuroborreliosis. Ger Med Sci 18:Doc03

Rauter C, Hartung T (2005) Prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes ricinus* ticks in Europe: A metaanalysis. Appl Environ Microbiol 71:7203–7216

Rebaudet S, Parola P (2006) Epidemiology of relapsing fever borreliosis in Europe. FEMS Immunol Med Microbiol 48:11–15

Replogle AJ, Sexton C, Young J, Kingry LC, Schriefer ME, Dolan M, Johnson TL, Connally NP, Padgett KA, Petersen JM (2021) Isolation of *Borrelia miyamotoi* and other Borreliae using a modified BSK medium. Sci Rep 11:1926

Richter D, Schlee DB, Matuschka FR (2003) Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent. Emerg Infect Dis 9:697–701

Richter D, Schlee DB, Allgäwer R, Matuschka FR (2004) Relationships of a novel Lyme disease spirochete, *Borrelia spielmani* sp. nov., with its hosts in Central Europe. Appl Environ Microbiol 70 (11):6414–6419. https://doi.org/10.1128/AEM.70.11.6414-6419.2004. PMID: 15528500; PMCID: PMC525186

Richter D, Postic D, Sertour N, Livey I, Matuschka FR, Baranton G (2006) Delineation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp. nov. Int J Syst Evol Microbiol 56(Pt 4):873–881. https://doi.org/10.1099/ijs.0.64050-0. PMID: 16585709

Rollend L, Fish D, Childs JE (2013) Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: a summary of the literature and recent observations. Ticks Tick Borne Dis 4:46–51

Rosa PA, Jewett MW (2021) Genetic manipulation of *Borrelia*. Curr Issues Mol Biol 42:307–332

Rosa PA, Tilly K, Stewart PE (2005) The burgeoning molecular genetics of the Lyme disease spirochaete. Nat Rev Microbiol 3:129–143

Rudenko N, Golovchenko M, Lin T, Gao L, Grubhoffer L, Oliver JH Jr (2009) Delineation of a new species of the *Borrelia burgdorferi* Sensu Lato Complex, *Borrelia americana* sp. nov. J Clin Microbiol 47(12):3875–80. https://doi.org/10.1128/JCM.01050-09. PMID: 19846628; PMCID: PMC2786643

Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH (2011) *Borrelia carolinensis* sp. nov., a novel species of the *Borrelia burgdorferi* sensu lato complex isolated from rodents and a tick from the south-eastern USA. Int J Syst Evol Microbiol 61(Pt 2):381–383. https://doi.org/10.1099/ijs.0.021436-0. PMID: 20305062; PMCID: PMC3081087

Rupprecht TA, Lechner C, Tumani H, Fingerle V (2014) CXCL13: a biomarker for acute Lyme neuroborreliosis: investigation of the predictive value in the clinical routine. Nervenarzt 85:459–464

Ruzic-Sabljic E, Lotric-Furlan S, Maraspin V, Cimperman J, Logar M, Jurca T, Strle F (2006) Comparison of isolation rate of *Borrelia burgdorferi* sensu lato in MKP and BSK-II medium. Int J Med Microbiol 296(Suppl 40):267–273

Ruzic-Sabljic E, Maraspin V, Cimperman J, Strle F, Lotric-Furlan S, Stupica D, Cerar T (2014) Comparison of isolation rate of *Borrelia burgdorferi* sensu lato in two different culture media, MKP and BSK-H. Clin Microbiol Infect 20:636–641

Schalka S, Steiner D, Ravelli FN, Steiner T, Terena AC, Marçon CR, Ayres EL, Addor FA, Miot HA, Ponzio H, Duarte I, Neffá J, Cunha JA, Boza JC, Samorano Lde P, Corrêa Mde P, Maia M, Nasser N, Leite OM, Lopes OS, Oliveira PD, Meyer RL, Cestari T, Reis VM, Rego VR (2014) Brazilian consensus on photoprotection. An Bras Dermatol 89:1–74

Schenone H (1999) Xenodiagnosis. Mem Inst Oswaldo Cruz 94(Suppl 1):289–294

Schwan TG (2021) Vector specificity of the relapsing fever spirochete *Borrelia hermsii* (Spirochaetales: Borreliaceae) for the tick *Ornithodoros hermsi* (Acari: Argasidae) involves persistent infection of the salivary glands. J Med Entomol 58:1926–1930

Schwan TG, Raffel SJ (2021) Transovarial transmission of *Borrelia hermsii* by its tick vector and reservoir host *Ornithodoros hermsi*. Microorganisms 9:1978

Schwan TG, Burgdorfer W, Garon CF (1988) Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. Infect Immunol 56:1831–1836

Schwan TG, Schrumpf ME, Hinnebusch BJ, Anderson DE Jr, Konkel ME (1996) GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. J Clin Microbiol 34:2483–2492

Schwan TG, Raffel SJ, Schrumpf ME, Porcella SF (2007) Diversity and distribution of *Borrelia hermsii*. Emerg Infect Dis 13:436–442

Schwan TG, Raffel SJ, Schrumpf ME, Gill JS, Piesman J (2009) Characterization of a novel relapsing fever spirochete in the midgut, coxal fluid, and salivary glands of the bat tick Carios kelleyi. Vector Borne Zoonotic Dis 9(6):643–647. https://doi.org/10.1089/vbz.2008.0177. PMID: 19281412; PMCID: PMC2795039

Schwartz AM, Hinckley AF, Mead PS, Hook SA, Kugeler KJ (2017) Surveillance for Lyme Disease - United States, 2008-2015. MMWR Surveill Summ 66:1–12

Schwartz I, Margos G, Casjens SR, Qiu WG, Eggers CH (2021) Multipartite genome of Lyme disease *Borrelia*: Structure, variation and prophages. Curr Issues Mol Biol 42:409–454

Scoles GA, Papero M, Beati L, Fish D (2001) A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis 1:21–34

Scott MC, Rosen ME, Hamer SA, Baker E, Edwards H, Crowder C, Tsao JI, Hickling GJ (2010) High-prevalence *Borrelia miyamotoi* infection among wild turkeys (*Meleagris gallopavo*) in Tennessee. J Med Entomol 47:1238–1242

Semenza JC, Menne B (2009) Climate change and infectious diseases in Europe. Lancet Infect Dis 9:365–375

Shang ES, Skare JT, Exner MM, Blanco DR, Kagan BL, Miller JN, Lovett MA (1998) Isolation and characterization of the outer membrane of *Borrelia hermsii*. Infect Immunol 66:1082–1091

Shaw SE, Birtles RJ, Day MJ (2001) Arthropod-transmitted infectious diseases of cats. J Feline Med Surg 3:193–209

Skerman VBD, McGowan V, Sneath PHA (1980) Approved lists of bacterial names. Int J Syst Bacteriol 30:225–420

Skerman VBD, McGowan V, Sneath PHA (1980) Approved list of bacterial names. ASM Press, Washington, DC

Skerman VBD, McGowan V, Sneath PHA (1989) Approved list of bacterial names (Amended). ASM Press, Washington, DC

Sonenshine DE, Roe M (2014) Overview: ticks, people and animals. In: Sonenshine DE, Roe M (eds) Biology of ticks. Oxford University Press, Oxford

Spielman A (1994) The emergence of Lyme disease and human babesiosis in a changing environment. Ann N Y Acad Sci 740:146–156

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33:152–155

Stackebrandt E, Frederiksen W, Garrity GM, Grimont PA, Kampfer P, Maiden MC, Nesme X, Rossello-Mora R, Swings J, Truper HG, Vauterin L, Ward AC, Whitman WB (2002) Report of the *ad hoc* committee for the re-evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 52:1043–1047

Stanek G, Strle F (2003) Lyme borreliosis. Lancet 362:1639–1647

Stanek G, Strle F (2018) Lyme borreliosis-from tick bite to diagnosis and treatment. FEMS Microbiol Rev 42:233–258

Stanek G, Strle G, Gray J, Wormser GP (2002) History and characteristics of Lyme borreliosis (Gray J, Kahl O, Lane RS, Stanek G).

Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A, Kristoferitsch W, O'Connell S, Ornstein K, Strle F, Gray J (2011) Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect 17:69–79

Stanek G, Wormser GP, Gray J, Strle F (2012) Lyme borreliosis. Lancet 379:461–473

Steere AC (1989) Lyme disease. N Engl J Med 321:586–596

Steere AC (2001) Lyme disease. N Engl J Med 345:115–125

Steere AC, Sikand VK (2003) The presenting manifestations of Lyme disease and the outcomes of treatment. N Engl J Med 348:2472–2474

Steere AC, Malawista SE, Snydman DR, Andiman WA (1976) Cluster of arthritis in children and adults in Lyme, Connecticut. Arthritis Rheum 19:824–824

Steere AC, Broderick TF, Malawista SE (1978) Erythema chronicum migrans and Lyme arthritis: epidemiologic evidence for a tick vector. Am J Epidemiol 108:312–321

Steere AC, Batsford WP, Weinberg M, Alexander J, Berger HJ, Wolfson S, Malawista SE (1980) Lyme carditis: cardiac abnormalities of Lyme disease. Ann Intern Med 93:8–16

Steere AC, Schoen RT, Taylor E (1987) The clinical evolution of Lyme arthritis. Ann Intern Med 107:725–731

Steere AC, Sikand VK, Schoen RT, Nowakowski J (2003) Asymptomatic infection with *Borrelia burgdorferi*. Clin Infect Dis 37:528–532

Steere AC, Coburn J, Glickstein L (2004) The emergence of Lyme disease. J Clin Invest 113:1093–1101

Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JW, Li X, Mead PS (2016) Lyme borreliosis. Nat Rev Dis Primers 2:16090

Steinhaus EA (1946) Insect Microbiology. Comstock Publishing Co., Ithaca, New York

Stete K, Rieg S, Margos G, Häcker G, Wagner D, Kern WV, Fingerle V (2018) Case report and genetic sequence analysis of *Candidatus* Borrelia kalaharica, Southern Africa. Emerg Infect Dis 24:1659–1664

Strle F, Stanek G (2009) Clinical manifestations and diagnosis of Lyme borreliosis. Curr Prob Dermatol 37:51–110

Strle F, Nelson JA, Ruzic-Sabljic E, Cimperman J, Maraspin V, Lotric-Furlan S, Cheng Y, Picken MM, Trenholme GM, Picken RN (1996) European Lyme borreliosis: 231 culture-confirmed cases involving patients with erythema migrans. Clin Infect Dis 23:61–65

Strle F, Videcnik J, Zorman P, Cimperman J, Lotric-Furlan S, Maraspin V (2002) Clinical and epidemiological findings for patients with erythema migrans. Comparison of cohorts from the years 1993 and 2000. Wien Klin Wochenschr 114:493–497

Strnad M, Hönig V, Růžek D, Grubhoffer L, Rego ROM (2017) Europe-wide meta-analysis of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks. Appl Environ Microbiol 83:e00609

Stupica D, Lusa L, Ruzić-Sabljić E, Cerar T, Strle F (2012) Treatment of erythema migrans with doxycycline for 10 days versus 15 days. Clin Infect Dis 55:343–350

Stupica D, Bajrović FF, Blagus R, Cerar Kišek T, Collinet-Adler S, Lah A, Levstek E, Ružić-Sabljić E (2021) Clinical manifestations and long-term outcome of early Lyme neuroborreliosis according to the European Federation of Neurological Societies diagnostic criteria (definite versus possible) in central Europe. A retrospective cohort study. Eur J Neurol 28:3155–3166

Sultan SZ, Manne A, Stewart PE, Bestor A, Rosa PA, Charon NW, Motaleb MA (2013) Motility is crucial for the infectious life cycle of *Borrelia burgdorferi*. Infect Immunol 81:2012–2021

Supriyono, Takano A, Kuwata R, Shimoda H, Hadi UK, Setiyono A, Agungpriyono S, Maeda K (2019) Detection and isolation of tick-borne bacteria (*Anaplasma* spp., *Rickettsia* spp., and *Borrelia* spp.) in *Amblyomma varanense* ticks on lizard (*Varanus salvator*). Microbiol Immunol 63:328–333. https://doi.org/10.1111/1348-0421.12721. PMID: 31209913

Swanson SJ, Neitzel D, Reed KD, Belongia EA (2006) Coinfections acquired from ixodes ticks. Clin Microbiol Rev 19:708–727

Sykes RA, Makiello P (2017) An estimate of Lyme borreliosis incidence in Western Europe. J Public Health (Oxf) 39:74–81

Takano A, Goka K, Une Y, Shimada Y, Fujita H, Shiino T, Watanabe H, Kawabata H (2010) Isolation and characterization of a novel *Borrelia* group of tick-borne borreliae from imported reptiles and their associated ticks. Environ Microbiol 12:134–146

Takano A, Fujita H, Kadosaka T, Konnai S, Tajima T, Watanabe H, Ohnishi M, Kawabata H (2011) Characterization of reptile-associated *Borrelia* sp. in the vector tick, *Amblyomma geoemydae*, and its association with Lyme disease and relapsing fever *Borrelia* spp. Environ Microbiol Rep 3:632–637

Takayama K, Rothenberg RJ, Barbour AG (1987) Absence of lipopolysaccharide in the Lyme disease spirochete, *Borrelia burgdorfer*. Infect Immunol 55:2311–2313

Talagrand-Reboul E, Boyer PH, Bergström S, Vial L, Boulanger N (2018) Relapsing fevers: Neglected tick-borne diseases. Front Cell Infect Microbiol 8:98

Teglas MB, Mapes S, Hodzic E, Nieto NC (2011) Co-infection of *Ornithodoros coriaceus* with the relapsing fever spirochete, *Borrelia coriaceae*, and the agent of epizootic bovine abortion. Med Vet Entomol 25:337–343

Telford SR 3rd, Goethert HK, Molloy PJ, Berardi VP, Chowdri HR, Gugliotta JL, Lepore TJ (2015) *Borrelia miyamotoi* disease: Neither Lyme disease nor relapsing fever. Clin Lab Med 35:867–882

Topakian R, Stieglbauer K, Nussbaumer K, Aichner FT (2008) Cerebral vasculitis and stroke in Lyme neuroborreliosis. Two case reports and review of current knowledge. Cerebrovasc Dis 26: 455–461

Trape JF, Diatta G, Arnathau C, Bitam I, Sarih M, Belghyti D, Bouattour A, Elguero E, Vial L, Mané Y, Baldé C, Prugnolle F, Chauvancy G, Mahé G, Granjon L, Duplantier JM, Durand P, Renaud F (2013) The epidemiology and geographic distribution of relapsing fever borreliosis in West and North Africa, with a review of the *Ornithodoros erraticus* complex (Acari: Ixodida). PLoS One 8:e78473

Trevisan G, Cinco M, Trevisini S, di Meo N, Ruscio M, Forgione P, Bonin S (2021) Borreliae Part 2: *Borrelia* relapsing fever group and unclassified *Borrelia*. Biology (Basel) 10:1117

Tsao JI (2009) Reviewing molecular adaptations of Lyme borreliosis spirochetes in the context of reproductive fitness in natural transmission cycles. Vet Res 40:36

Tumani H, Nölker G, Reiber H (1995) Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. Neurology 45:1663–1670

Tuuminen T, Hedman K, Söderlund-Venermo M, Seppälä I (2011) Acute parvovirus B19 infection causes nonspecificity frequently in *Borrelia* and less often in *Salmonella* and *Campylobacter* serology, posing a problem in diagnosis of infectious arthropathy. Clin Vaccine Immunol 18: 167–172

Tyler S, Tyson S, Dibernardo A, Drebot M, Feil EJ, Graham M, Knox NC, Lindsay LR, Margos G, Mechai S, Van Domselaar G, Thorpe HA, Ogden NH (2018) Whole genome sequencing and phylogenetic analysis of strains of the agent of Lyme disease *Borrelia burgdorferi* from Canadian emergence zones. Sci Rep 8:10552

van Dam AP (2011) Molecular diagnosis of *Borrelia* bacteria for the diagnosis of Lyme disease. Expert Opin Med Diagn 5:135–149

van Dam AP, Kuiper H, Vos K, Widjojokusumo A, de Jongh BM, Spanjaard L, Ramselaar AC, Kramer MD, Dankert J (1993) Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin Infect Dis 17:708–717

van Duijvendijk G, Coipan C, Wagemakers A, Fonville M, Ersöz J, Oei A, Földvári G, Hovius J, Takken W, Sprong H (2016) Larvae of *Ixodes ricinus* transmit *Borrelia afzelii* and *B. miyamotoi* to vertebrate hosts. Parasit Vectors 9:97

van Gorkom T, Sankatsing SUC, Voet W, Ismail DM, Muilwijk RH, Salomons M, Vlaminckx BJM, Bossink AWJ, Notermans DW, Bouwman JJM, Kremer K, Thijsen SFT (2018) An enzyme-linked immunosorbent spot assay measuring *Borrelia burgdorferi* B31-specific interferon Gammag-secreting T cells cannot discriminate active Lyme neuroborreliosis from past Lyme borreliosis: a prospective study in the Netherlands. J Clin Microbiol 56:e01695

Vanthomme K, Bossuyt N, Boffin N, Van Casteren V (2012) Incidence and management of presumption of Lyme borreliosis in Belgium: recent data from the sentinel network of general practitioners. Eur J Clin Microbiol Infect Dis 31:2385–2390

Vaughn MF, Funkhouser SW, Lin FC, Fine J, Juliano JJ, Apperson CS, Meshnick SR (2014) Long-lasting permethrin impregnated uniforms: a randomized-controlled trial for tick bite prevention. Am J Prev Med 46:473–480

Veinovic G, Cerar T, Strle F, Lotric-Furlan S, Maraspin V, Cimperman J, Ruzic-Sabljic E (2013) In vitro susceptibility of European human *Borrelia burgdorferi* sensu stricto strains to antimicrobial agents. Int J Antimicrob Agents 41:288–291

Veinović G, Ćakić S, Mihaljica D, Sukara R, Ružić-Sabljić E, Tomanović S (2021) In vitro efficacy of antibiotics against different *Borrelia* isolates, vol 68. Acta Microbiol Immunol Hung, pp 195–202

Vial L (2009) Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. Parasite 16:191–202

Vollmer SA, Bormane A, Dinnis RE, Seelig F, Dobson AD, Aanensen DM, James MC, Donaghy M, Randolph SE, Feil EJ, Kurtenbach K, Margos G (2011) Host migration impacts on the phylogeography of Lyme Borreliosis spirochaete species in Europe. Environ Microbiol 13:184–192

Wang G, van Dam AP, Le Fleche A, Postic D, Peter O, Baranton G, de Boer R, Spanjaard L, Dankert J (1997) Genetic and phenotypic analysis of *Borrelia valaisiana* sp. nov. (*Borrelia* genomic groups VS116 and M19). Int J Syst Bacteriol 47(4):926–932. https://doi.org/10.1099/00207713-47-4-926. PMID: 9336888

Wang H, Paesen GC, Nuttall PA, Barbour AG (1998) Male ticks help their mates to feed. Nature 391:753–754

Warrell DA (2019) Louse-borne relapsing fever (*Borrelia recurrentis* infection). Epidemiol Infect 147:e106

Weck BC, Serpa MCA, Labruna MB, Muñoz-Leal S (2022) A novel genospecies of *Borrelia burgdorferi* sensu lato associated with cricetid rodents in Brazil. Microorganisms 10:204

Wilhelmsson P, Fryland L, Lindblom P, Sjöwall J, Ahlm C, Berglund J, Haglund M, Henningsson AJ, Nolskog P, Nordberg M, Nyberg C, Ornstein K, Nyman D, Ekerfelt C, Forsberg P, Lindgren PE (2016) A prospective study on the incidence of *Borrelia burgdorferi* sensu lato infection after a tick bite in Sweden and on the Åland Islands, Finland (2008-2009). Ticks Tick Borne Dis 7:71–79

Wilking H, Stark K (2014) Trends in surveillance data of human Lyme borreliosis from six federal states in eastern Germany, 2009-2012. Ticks Tick Borne Dis 5:219–224

Wilking H, Fingerle V, Klier C, Thamm M, Stark K (2015) Antibodies against *Borrelia burgdorferi* sensu lato among adults, Germany, 2008-2011. Emerg Infect Dis 21:107–110

Wolcott KA, Margos G, Fingerle V, Becker NS (2021) Host association of *Borrelia burgdorferi* sensu lato: a review. Ticks Tick Borne Dis 12:101766

Wormser GP (2006) Hematogenous dissemination in early Lyme disease. Wien Klin Wochenschr 118:634–637

Wormser GP, Ramanathan R, Nowakowski J, McKenna D, Holmgren D, Visintainer P, Dornbush R, Singh B, Nadelman RB (2003) Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 138:697–704

Woudenberg T, Böhm S, Böhmer M, Katz K, Willrich N, Stark K, Kuhnert R, Fingerle V, Wilking H (2020) Dynamics of *Borrelia burgdorferi*-specific antibodies: seroconversion and seroreversion between two population-based, cross-sectional surveys among adults in Germany. Microorganisms 8:1859

Wright SD, Nielsen SW (1990) Experimental infection of the white-footed mouse with *Borrelia burgdorferi*. Am J Vet Res 51:1980–1987

Younsi H, Sarih M, Jouda F, Godfroid E, Gern L, Bouattour A, Baranton G, Postic D (2005) Characterization of *Borrelia lusitaniae* isolates collected in Tunisia and Morocco. J Clin Microbiol 43:1587–1593

Zhioua E, Bouattour A, Hu CM, Gharbi M, Aeschliman A, Ginsberg HS, Gern L (1999) Infection of *Ixodes ricinus* (Acari: Ixodidae) by *Borrelia burgdorferi* sensu lato in North Africa. J Med Entomol 36:216–218

Zumpt F, Organ D (1961) Strains of spirochaetes isolated from *Ornithodoros zumpti* Heisch & Guggisberg, and from wild rats in the Cape Province. A preliminary note. S Afr J Lab Clin Med 7:31–35. PMID: 13788963

Cystic and Alveolar Echinococcosis: Fraternal Twins Both in Search of Optimal Treatment

37

Dominique A. Vuitton, Laurence Millon, Tommaso Manciulli, and Enrico Brunetti

Contents

D. A. Vuitton (✉)
French National Centre for Echinococcoses, Franche-Comté University and University Hospital, Besançon, France
e-mail: dvuitton@univ-fcomte.fr

L. Millon
French National Centre for Echinococcoses, Franche-Comté University and University Hospital, Besançon, France

UMR CNRS 6249 Laboratoire Chrono-environnement, Bourgogne-Franche-Comté University, Besançon, France
e-mail: laurence.millon@univ-fcomte.fr

T. Manciulli · E. Brunetti
WHO-Collaborating Centre for Clinical Management of Cystic Echinococcosis, Division of Infectious and Tropical Diseases, University of Pavia, IRCCS San Matteo Hospital Foundation, Pavia, Italy
e-mail: tommaso.manciulli01@universitadipavia.it; enrico.brunetti@unipv.it

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_29

Abstract

The burden of the two different diseases, "cystic echinococcosis" (CE), due to several species of *Echinococcus* among the *E. granulosus sensu lato* cluster, and "alveolar echinococcosis," due to *E. multilocularis,* has long been underestimated. Other species, *E. oligarthra* and *E. vogeli,* may also cause disease in humans, similar to CE and AE, respectively. CE is usually maintained by a synanthropic domestic cycle (dog/domestic ungulates); a cycle in wild animals (fox/small mammals) allows *E. multilocularis* to subsist in nature, and environmental factors play a critical role. The larval stage of *Echinococcus* spp., the "metacestode," is characterized by a germinal layer surrounded by a laminated layer and an adventitial layer. The germinal layer forms isolated cysts (CE) or aggregated microcysts (AE), which are filled with a water-like liquid ("hydatid fluid"). The diagnosis of both diseases in humans relies on imaging: ultrasound examination (also useful for mass screening), computed tomography, and magnetic resonance imaging, which provides pathognomonic images; incidental diagnosis is frequent. WHO-US-based classification of CE cysts (CE-1 to CE-5) and staging system for AE (PNM classification) are used to choose among a variety of therapeutic options available, including surgery to remove the lesions and nonsurgical image-guided interventions to sterilize CE cysts or treat complications in CE and AE. Benzimidazoles – with a strict monitoring to ensure optimal efficacy and avoid adverse events – are still the only antiparasitic agents available. For CE as well as AE, there is a need to find new antiparasitic compounds and evaluate therapeutic strategies.

Keywords

Cestodes · *Echinococcus* spp. taxonomy · Cystic echinococcosis · Alveolar echinococcosis · Neotropical echinococcosis · Burden of diseases · Epidemiology · Parasite immunology · Ultrasound diagnosis · Medical imaging · Positron emission tomography (PET) · Serology · Molecular biology · Hepatic surgery · "PAIR" (puncture–aspiration–injection–reaspiration) · Perendoscopic procedures · Benzimidazoles · Albendazole · Mebendazole · WHO-Informal Working Group on Echinococcosis

37.1 Introduction

Echinococcosis and *Echinococcus*. Echinococcosis is the disease caused by cestode helminths of the genus *Echinococcus (E.)* (Kern et al. 2017). Actually, this generic term applies to two rather different diseases, due respectively to *E. granulosus*, "cystic echinococcosis" (CE), also previously designated as "hydatid disease," hydatidosis, or "hydatid cyst," a condition already recognized at Hippocrates' times, and to *E. multilocularis*, alveolar echinococcosis (AE), a tumor-like disease only identified 170 years ago (Virchow 1855). In addition, both species differ by their usual animal

reservoir, i.e., mostly domestic animals, for *E. granulosus*, and mostly wild animals, for *E. multilocularis*. However, for more than a century, since the characterization of the "*Taenia echinococcus*" by the German scientist Carl Theodor Ernst von Siebold and the diagnosis of the first human cases of AE in the 1850s, there was no distinction between the two species (Vuitton et al. 2011). Other species of *Echinococcus* may also cause disease in humans, albeit far more rarely and only in South America; they are *E. oligarthra* (also wrongly called *E. oligarthrus* or *oligarthus*) and *E. vogeli*, both responsible for "neotropical" echinococcosis (NE) (D'Alessandro and Rausch 2008). The clinical symptoms of "polycystic echinococcosis," due to *E. vogeli*, are very close to those of AE, and species diagnosis relies on the south-American residence of the patient and on molecular identification (Grenouillet et al. 2013). The very rare cases of *E. oligarthra* infection present as single cysts (D'Alessandro and Rausch 2008). The clinical presentation of both NE is thus similar to that of AE and CE, respectively, and these two species will not be described in detail in this chapter. Finally, a new species of *Echinococcus* was disclosed recently in wild animals and in dogs on the Tibetan plateau, China, *E. shiquicus* (Xiao et al. 2005); however, no disease in humans has been attributed to this species until now.

37.2 The *Echinococcus* Genus: a Single Genus for an Increasing Number of Species, and Two Rather Different Diseases in Humans

A New Taxonomy. A taxonomic revision of the members of the genus *Echinococcus* (Cestoda: Taeniidae) has been proposed, which follows molecular phylogeny. The full description of the genomes of *E. multilocularis* and *E. granulosus* is now available (Tsai et al. 2013) as well as the complete mitochondrial genome for all species (Nakao et al. 2013). *E. multilocularis* still appears as a single species, although variations in its genome, best detected by using the *EmsB* microsatellite marker, may identify strains and has allowed epidemiologists to track its geographical spreading in the northern hemisphere (Knapp et al. 2010). Conversely, *E. granulosus* has been split into several species, which were previously considered as strains, numbered from G1 to G10, and characterized by their most usual intermediate animal host. The phylogenetic relationships of the various "strains" of *E. granulosus* with *E. oligarthrus*, *E. vogeli*, *E. multilocularis*, and *E. shiquicus* have also been studied (Nakao et al. 2007). It is now acceptable to differentiate, within the previously named "*E. granulosus*," *E. granulosus sensu stricto* (ex-sheep strain), *E. equinus* (ex-horse strain), *E. ortleppi* (ex-cattle strain), and *E. canadensis* (ex-camel, pig, and cervid strains) (McManus 2013). *E. felidis* was also described as a distinct species, although it is phylogenetically closely related with *E. granulosus sensu stricto* (Hüttner et al. 2008). Although the definition of two species within *E. canadensis* is still debated (Nakao et al. 2015), it is now accepted to include *E. granulosus sensu stricto, E. equinus, E. ortleppi,* and *E. canadensis* within the *E. granulosus sensu lato* cluster (Wen et al. 2019). The basal positions of the

phylogenetic tree are occupied by the so-called "neotropical" endemic species, *E. oligarthra* and *E. vogeli*, whose definitive hosts are derived from carnivores that emigrated from North America after the formation of the Panamanian land bridge (Nakao et al. 2007). Nowadays, the accepted nomenclature of species should be used in any scientific publications regarding the *Echinococcus* genus. An international consensus on the terminology to be used in English in this field, in order to avoid inappropriate words and expressions used in the past, has been found after a 2-year process of "formalized consensus," and published in 2020 (Vuitton et al. 2020). Adaptation of the international terminology to the French language has been recently published (Bellanger et al. 2021a). It was also established that only three names are now formally accepted for the human diseases associated with infections by *Echinococcus* spp. metacestodes: Cystic echinococcosis (CE) due to *E. granulosus s.l.*, alveolar echinococcosis (AE) due to *E. multilocularis*, and neotropical echinococcosis (NE) due to *E. vogeli* or *E. oligartrha*.

As no restriction to a single animal host has been identified for the various "new" species described from the previously named "*E. granulosus*," and no marked differences have been identified between the clinical presentation of CE depending on the species, for the following description, "*E. granulosus*" will be used as a general name for all three new "zoonotic" species, *E. granulosus sensu stricto*, *E. ortleppi*, and *E. canadensis*. *E. equinus* has never been recognized to infect humans; it thus cannot be considered as "zoonotic"; and the pathogenicity of *E. felidis* to humans is unknown (Vuitton et al. 2020).

Cystic Versus Alveolar: Similar Structure of the Metacestode, but Different Lesions, Depending on the Immune Response of the Intermediate Host. The larval stage of *Echinococcus* spp., also called "metacestode," is characterized by the ***germinal layer***, a syncitial monolayer of parasite cells surrounded by the ***laminated layer***, an acellular layer of polysaccharides which is the interface between the parasite and the host's cellular immune response while allowing exchanges between them (Vuitton and Gottstein 2010). The germinal layer forms "buds," then "vesicles" (cysts), which are filled with a water-like liquid ("hydatid fluid" or "cyst/vesicle fluid"). These cysts may be single (typically for CE) or multiple and aggregated (typically for AE, hence the adjective "alveolar," i.e., composed of alveoli), and small (from 1 mm to 1 cm, typically for AE) or large (from 1 cm to 20 cm, typically for CE) (Eckert and Deplazes 2004). Fertility is characterized by the growth and budding of "protoscoleces" from the germinal layer, and their release into the cyst fluid. In all species of *Echinococcus*, the "protoscolex" is that multicellular organism that ensures the transformation of the larva into an adult worm in the intestine of the definitive hosts after a process of evagination when the cysts are ingested, with the offal of domestic intermediate hosts (for CE) or with the rodent intermediate hosts as prey (for AE) by the appropriate carnivores. Hundreds to thousands of worms then settle in the small intestine of the definitive host where they attach through their hooks and suckers, reach maturity, characterized by egg-containing last segments within 2–3 weeks, and release them into the environment with the feces for an average of 1 month (Eckert et al. 2001; Torgerson et al. 2011; Kern et al. 2017). Each species of *Echinococcu*s was in the past carefully described based on the shape and

length of its segments, hooklets, and suckers, since the morphology of the adult stage (worm) was thought to be species-specific: The molecular taxonomy has now shown that different species can have similar morphology, and subtle differences are not important for species differentiation, which is now based on molecular biology (Thompson and Eckert 1983; Eckert et al. 2001; Lymbery 2017). The most striking differences lie in the histological aspect of the respective metacestodes. In CE, cells of the host's immune response have nearly totally disappeared when the cyst is disclosed in an intermediate host, either animal at slaughtering or human at diagnosis, and the cyst with its germinal and laminated layer is surrounded by paucicellular fibrous area, the **adventitial layer** (Vuitton et al. 2020). The cyst may contain hundreds of protoscoleces. After damage to the germinal layer (rupture or fissure) occurs, the cyst may either degenerate or produce "daughter cysts," usually inside and more rarely outside the germinal layer, which gives a "multivesiculated" appearance to the lesions, quite different from the "multi-cystic" aspect of NE or from the "alveolar" aspect of AE (Rogan et al. 2006; da Silva 2019) (Fig. 1). Protoscoleces may also be disseminated into the peritoneal or pleural cavity when a liver or lung cyst ruptures or after surgical opening (Kern et al. 2017; Vuitton et al. 2020). In AE, the laminated layer is surrounded by a layer of epithelium-like macrophages ("epithelioid cells"), then concentric layers of cells of the immune response, including macrophages, lymphocytes, eosinophils, and giant cells, of cells involved in fibrosis development, such as fibroblasts and myofibroblasts, and of collagen bundles and various components of the extracellular matrix (Ricard-Blum et al. 1992; Ricard-Blum et al. 1996; Grenard et al. 2001). This "granulomatous" periparasitic infiltrate is usually bordered by T-lymphocytes, at the immediate proximity of the liver or lung parenchyma. In AE, the extent of the periparasitic infiltrate and the presence or absence of protoscoleces in the parasite vesicles depend on the susceptibility of the

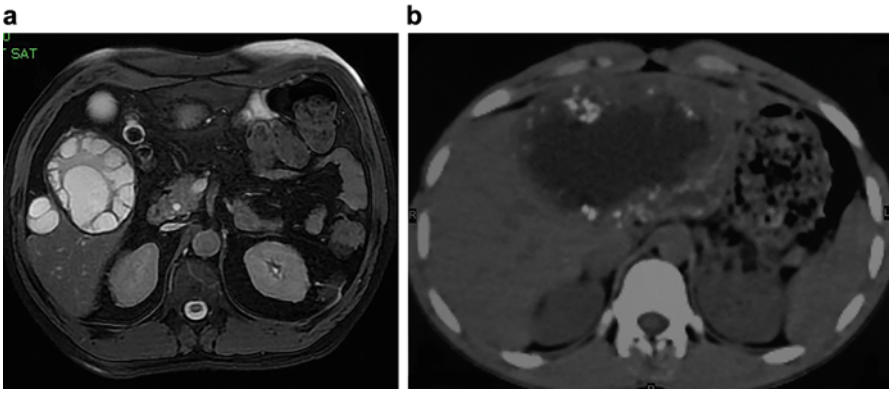

Fig. 1 Cystic (CE) and alveolar (AE) echinococcosis typical images. (**a**) CE: daughter cysts in a liver cyst, as shown at magnetic resonance imaging; (**b**) AE: necrotic cavity (pseudocyst) in the center of a huge lesion invading the right and left liver, as well as the liver hilum; at the periphery of the hypodense necrotic cavity, hyperdense calcifications are well shown in the pseudotumoral lesion by computer tomography (CT)

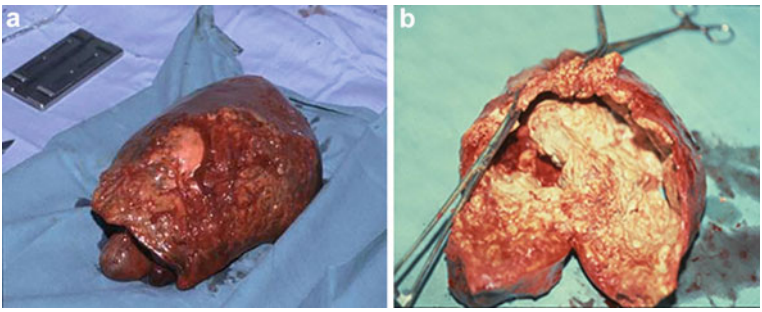

Fig. 2 Alveolar echinococcosis; macroscopical view after surgical hepatectomy. (**a**) Fibrous aspect of the lesions at the surface of the liver. (**b**) Necrotic cavity in the center of a very advanced lesion

host to the development of the metacestode, and may thus be different in the various species of intermediate hosts (Vuitton 2003; Vuitton and Gottstein 2010). Necrosis may also be observed in the periparasitic infiltrate; in degenerating lesions, especially after years of evolution in humans, massive necrosis may occur in the center of lesions, with the constitution of necrotic cavities, which give a "pseudocystic" appearance to AE lesions; however, such pseudocysts have irregular walls and are filled with solid and liquid necrotic debris, and are thus in no way similar to the real "cysts" of CE (Kern et al. 2017; Vuitton et al. 2020) (Figs. 1 and 2).

37.3 The Parasite Life Cycle and the Burden of *Echinococcus* spp. in Animals

E. granulosus sensu lato. The adult cestode develops in the small intestine of the definitive host, a carnivore (Kern et al. 2017; Romig et al. 2017; Wen et al. 2019) (Fig. 3). The last segments (proglottids) of this worm (3–5 mm long) contain oncospheres (eggs); they are released into the intestine, and the oncospheres are dispersed on the grass with the feces of the host. When the oncospheres are eaten by the intermediate host (usually a mammal), the hexacanth embryo, released into the duodenum, passes through the intestinal wall and most usually settles in the liver or in the lung as "cysts" (also called "hydatids" by parasitologists). Domestic animals such as sheep, goats, cattle, horses, camels, swines, caribous, or reindeers serve as intermediate hosts, with some but not strict specificity depending on the species within *E. granulosus sensu lato* (Vuitton et al. 2020). Commonly, dogs are infected by eating raw infective offal containing parasite cysts. Humans become accidentally infected either by touching dogs with contaminated hair, or by ingesting vegetables, water, or soil infected by dog feces (Fig. 3). Less commonly, the cycle involves a wild carnivore such as the wolf, jackal, or coyote and a wild herbivore such as wild elk, caribou, reindeer, or other cervids. As mentioned above, the animal species specificity of the various parasite species is not strict. Cattle, for instance, may be infected by *E. granulosus sensu stricto* or by *E. canadensis* as well as by *E. ortleppi*.

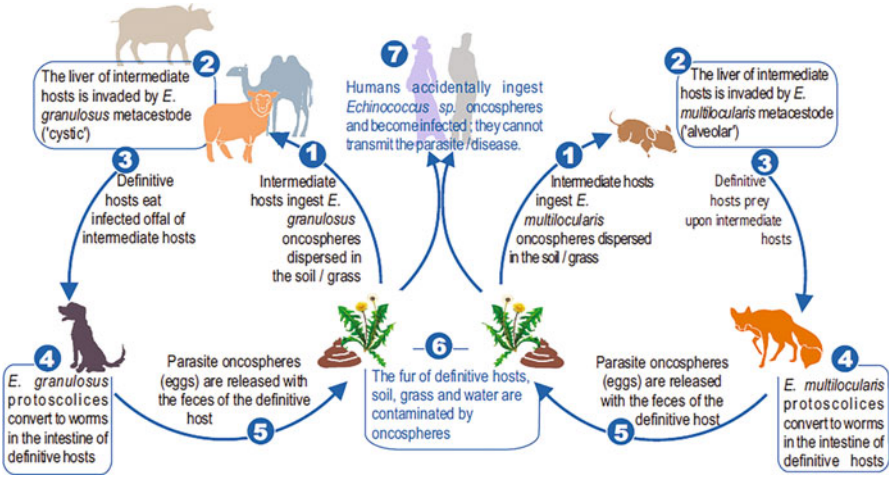

Fig. 3 Parasite cycles of *E. granulosus* (left cycle) and *E. multilocularis* (right cycle)

CE is usually maintained by the synanthropic domestic cycle (dog/domestic ungulate) and represents a persistent zoonosis in rural livestock-raising areas where humans cohabit with dogs fed on raw livestock offal. Infection of dogs by the worms of *E. granulosus* does not cause specifically identified disease or symptoms. Infection of domestic ungulates by the metacestode (larval form) of *E. granulosus s.l.* is characterized by single or multiple cysts, usually in the liver or the lung of the animals. The burden of *E. granulosus* infection in domestic animals has long been neglected and was considered to be minimal, since generally the infected animals do not present any overt disease. Moreover, in rural family-type farming, the fact that cysts might affect offal was not considered to be of great economical importance. However, more recent evaluation estimates the annual livestock production loss of at least US $141,605,195 and possibly up to US $2,190,132,464 (Budke et al. 2006).

Risk factors for domestic animal infection by *E. granulosus* metacestodes have been reviewed in an exhaustive analysis of the current literature (Otero-Abad and Torgerson 2013). Prevalence of CE differs between study locations or different livestock origin; there are seasonal variations in CE prevalence, as found by abattoir meat inspection; high altitudes and increasing annual rainfall are significant risk factors, as well as the age of the host for many farm species: higher CE prevalence is observed in old animals compared to young ones, and the number of cysts in a farm animal also increases with age. The gender of the intermediate host has also been identified as a possible determinant of CE, with females more at risk in most studies. Among host species, on a farm, small ruminants have frequently been observed with the highest rates of infection; but cattle have also been identified in many studies as bearing the highest prevalence of CE of those observed in farm species. Farm location and, especially, management factors are important determinants for infection: e.g., pigs reared in intensive conditions had significantly lower CE prevalence compared to pigs

reared in free-range conditions or on family-owned farms, while sheep and goats from mixed-farming systems showed higher rates of hydatid infection compared to small ruminants from pastoral systems (Otero-Abad and Torgerson 2013).

Livestock represents the parasite reservoir, while the infection of definitive hosts, mainly dogs, is responsible for the maintenance of the parasite cycle in animals and for the transmission to humans. The most important risk factor is the potential access of dogs to raw (uncooked) and infected offal, and thus depends on their food sources, access to the location where animals are slaughtered, access to livestock carcasses, rural location of dogs, whether dogs are free to roam (with a higher risk for stray dogs, in rural as well as in periurban areas, close to dumps or open-air abattoirs), the type of dog, its age (younger dogs being more at risk), and also the knowledge of the owners about echinococcosis and their socioeconomic background; dog's gender does not seem to be a significant risk factor (Otero-Abad and Torgerson 2013). Conversely, indoor or chained dogs are less at risk. Avoiding home slaughtering and preventing consumption of livestock offal by dogs, by proper disposal of carcasses by incineration/burial, or as more recently proposed, by boiling cyst-containing livers or lungs (Li et al. 2014), protects dogs, thus humans, from infection.

E. multilocularis. A cycle in wild animals allows *E. multilocularis* to subsist in nature (Fig. 3). The adult cestode usually develops as a worm (2–4 mm long) in the small intestine of the fox (Deplazes and Eckert 2001; Conraths and Deplazes 2015). The last segments are released into the intestine; the oncospheres (eggs) they contain are dispersed on the ground with the feces where they contaminate the grass. Oncospheres are surrounded by an envelope which allows them to resist very low temperatures (to −40 °C), but they die at +60 °C. When eggs are eaten by the intermediate host, usually a wild rodent, i.e., voles of a number of different species, depending on the endemic area, and in the lagomorph *Ochotona curzoniae* , on the Tibetan plateau of China (Vuitton et al. 2003), the hexacanth embryo is released into the duodenum, passes through the intestinal wall, and enters the liver where it usually settles. AE lesions consist of a mass of small vesicles produced by a single or several embryos, which may occupy a considerable part of the liver. The metacestode may then directly invade all organs and tissues close to the liver, or disseminate in microthrombi to any possible organs through the hepatic veins, heart, and pulmonary and systemic arteries. The life cycle is completed when the intermediate host, containing infected larvae with protoscoleces, is eaten by foxes. Dogs are the most common definitive hosts in some areas, such as China (Vuitton et al. 2003; Guo et al. 2021). Less commonly, other carnivores including raccoon dogs, coyotes, and cats can also serve as definitive hosts (Torgerson et al. 2011; Romig et al. 2017).

As for *E. granulosus* infection, gut infection by worms of *E. multilocularis* species does not result in clinical symptoms or disease, in foxes as well as in dogs or other definitive hosts. As most of the intermediate hosts are wild rodents and lagomorphs, there is no special veterinary or economic impact from the infection of the intermediate hosts by the metacestode. However, in all endemic areas, including Europe and Asia, larval infection with symptoms close to those observed in humans has more and more often been recognized in a number of domestic animals, such as horses, pigs, boars, or even dogs which appear to also serve occasionally as

intermediate hosts (Vuitton et al. 2003; Scharf et al. 2004; Ueno et al. 2012; Böttcher et al. 2013). In addition, AE cases observed in zoo animals, such as monkeys or lemurs, have received much attention in the recent years in Europe and Japan (Rehmann et al. 2005; Umhang et al. 2016), and AE was also observed in exotic pets, such as chinchillas (Staebler et al. 2007).

Environmental factors play a critical role in *E. multilocularis* infection in foxes, resulting in a heterogeneous geographical distribution of the parasite (Giraudoux et al. 2013; Romig et al. 2017). From continental to regional scales, AE forms discrete patches of endemicity within which transmission hotspots of much larger prevalence may occur. Each transmission ecosystem has its own characteristics, and lies on a subtle interplay between altitude, climate, landscape characteristics, land use, and predator/prey relationship (Giraudoux et al. 2013; Romig et al. 2017; Knapp et al. 2018; Guo et al. 2021). Regional meteorological conditions, such as low temperatures or high annual precipitations, have been reported as being associated with the infection rates. The importance of the availability and predation level on potential intermediate hosts for the successful transmission of *E. multilocularis* is well demonstrated. Changes in land use, such as promotion of permanent pastures, deforestation, or privatization of the land, are prone to influence *E. multilocularis* infection in small mammals and foxes, by favoring periods of high densities of small mammals with exclusive preying of foxes on these potentially infected mammals (Giraudoux et al. 2003; Wang et al. 2004, 2006a, 2016; Giraudoux et al. 2019). Involvement of dogs in the life cycle of *E. multilocularis*, as it occurs in rural western China, has been shown to be responsible for higher prevalence of AE in humans than fox infection alone (Wang et al. 2016; Cai et al. 2021). On the other hand, despite a higher prevalence in foxes from rural areas when compared with urban areas of Europe, there is a high infection pressure frequently reported in the periphery of the cities, and fox – as well as coyotes – urbanization has become a new threat, also by considerably increasing the human population at risk (Deplazes et al. 2004; Robardet et al. 2008; Robardet et al. 2011; Catalano et al. 2012; Luong et al. 2018).

37.4 Epidemiology of Echinococcosis in the World

CE in the World. The disease is prominent in rural areas where humans, dogs, and sheep/goat and cattle coexist closely, with poor housing conditions and low level of sanitation and is most common in the regions of the world where raising livestock is a major industry. The estimated minimum global human burden of human CE averages 285,000 disability-adjusted life years (DALYs) or an annual loss of US $194,000,000 (Budke et al. 2006). CE is found in the entire Mediterranean littoral including North Africa; former USSR; East Africa, with highest prevalence found in Turkana region, in Kenya, and in Sudan; and South America, particularly Uruguay, Argentina, and Chile, but also parts of Peru and Brazil (Deplazes et al. 2017). Highest prevalence is observed in Western China, where an average of 1–5% of the population may harbor a cyst when screened systematically using ultrasound examination of the liver (Torgerson et al. 2011). Recent data from China and

Kyrgyzstan suggest a "hot spot" distribution for CE, with some territories or communities being at higher risk than others (Paternoster et al. 2020). The disease is present in Nepal, India, Pakistan, and Bangladesh; it is still frequent in North Africa, Turkey, and all Middle-East countries, where it had been identified since Greek/Roman Antiquity. It is re-emerging in several countries where it had been partially controlled after the Second World War, and especially in all endemic ex-USSR territories, e.g., Bulgaria, Romania, Europe, and all central Asia republics (Torgerson et al. 2011; Deplazes et al. 2017; Mustapayeva et al. 2020; Paternoster et al. 2020; Tamarozzi et al. 2020b; Mustapayeva et al. 2021; Colpani et al. 2021).

Because of active control campaigns, New Zealand and Australia, which were highly endemic in the past, are now far less infected, and an increasing proportion of CE human cases are in immigrants from endemic areas (Torgerson et al. 2011; Deplazes et al. 2017). This is also true for the United States as well as for most of countries of the European Union, including Italy, Spain, and Portugal, which were still highly endemic in the past. Nevertheless, recent epidemiological studies show that CE remains endemic in these countries, especially in Italy where, out of the 436 patients with CE seen in a reference center, 248 (56.9%) were born in Italy, while 188 (43.1%) were foreign-born (Zammarchi et al. 2020; Vola et al. 2022). Strict regulations on slaughtering and control campaigns have contributed to decrease very significantly the transmission to humans despite persistent low level infection in domestic animals which can still be tracked by veterinary inspection, especially in Spain, Greece, Southern Italy, and France (especially in Sicily, Sardinia, and Corsica) (Brundu et al. 2014; Deplazes et al. 2017; Bosco et al. 2021). The diagnosis should thus be evoked for any suspect cystic lesion of the liver when it occurs in immigrants or travellers. A special situation occurs in the United Kingdom, where low level of infection by *E. granulosus sensu stricto* in sheep and in humans is still present in Wales, but most of animal infection is due to *E. equinus*, with horses as intermediate hosts and apparently no transmission to humans (Romig et al. 2006). Nonpublished results from abattoir surveillance including species genotyping seem to indicate a potential resurgence of *E. ortleppi* infection including human cases in Europe, e.g., in France, which should raise attention of public health authorities (Grenouillet et al. 2014; Basmaciyan et al. 2018).

AE in the World. Although always limited to the Northern hemisphere and restricted to specific geographical areas characterized by their ability to sustain the proper functioning of *E. multilocularis* lifecycle among its definitive and intermediate hosts, AE endemic regions have never ceased to extend, from the discovery of the first human case in 1852 in southern Germany. Initially only diagnosed in the mountainous areas of Jura and Alps in central Europe (from Eastern France to Western Austria), and in Russia, from Moscow area and Siberia to the far Eastern region of Kamchatka, AE cases were subsequently recognized during the first half of the twentieth century in Alaska, in Turkey, and in northern Japan, where the parasite was "artificially" introduced by foxes imported from the Kuril islands to Rebun island, then to Hokkaido, to fight agriculture pests [see references on the history of AE epidemiology in (Vuitton et al. 2011; Eckert and Thompson 2017)]. Since the 1940s, the epidemiological situation seemed quite stable, with AE considered to be a

rare disease, even in the endemic area. The rare occurrence and low prevalence of AE in so-called "endemic areas," at usually less than 10/100,000 in regions with the highest contamination pressure (infection of 70% of foxes) was explained both by the rare encounter of humans with infected fox feces, and by the natural resistance of humans as intermediate host for the parasite (Vuitton 2003; Vuitton and Gottstein 2010). This might also explain why, despite mild-level infection in all Central/Northern states of the United States and Southern provinces of Canada, so few AE cases had been observed in North America (Massolo et al. 2014).

However, the situation has changed radically since the 1980s. Western China is now likely to be the world region with the highest number of human AE cases (Vuitton et al. 2003; Craig and Echinococcosis Working Group in China 2006). Nine provinces and autonomous regions are concerned, including Qinghai, Xinjiang, Gansu, Sichuan, Tibet, and Ningxia in Western China where human cases and animal epidemiology are very well documented, and where both types of echinococcosis are found. In Inner Mongolia in North-Western China, as well as Heilongjiang and Jilin in North-Eastern China, *E. multilocularis* infection of animals and human AE cases have been observed, but the epidemiological situation is less known. Prevalence of the disease may reach 10% of the population in some "high risk" districts of Gansu and Ningxia, 100 times higher than in the previously known endemic areas of Europe. China is, with Turkey and central Asia, the only region in the world where CE and AE are known to coexist in some communities, and sometimes in the same patient (Wen et al. 1992; Yang et al. 2006a; Ran et al. 2019), or in the same definitive host (Vuitton et al. 2003). Estimates show that in China there are about 380,000 cases of echinococcosis (including CE and AE), and 50 million people are at risk of *Echinococcus* spp. infection (Wang et al. 2012). From the analysis combining human capital method with DALYs to analyze the indirect CE economic burden on a total of 2'018 CE patients attending the hospitals in Xinjiang, China, between 2004 and 2008, the per-person direct medical cost for the treatment of echinococcosis was estimated to be US$ 1493.12 and the per-person direct nonmedical cost to be US$ 19.67. The indirect economic cost was US$ 1435.96 per person, and the disability-adjusted life-years (DALY) lost was approximately 1.03 DALY/person (Wang et al. 2012; Kern et al. 2017).

Since the 1990s, the recognized extent of the endemic area has progressed a lot in Europe, with fox *E. multilocularis* infection disclosed in all countries, except the United Kingdom, Spain, and Portugal. It also now reaches Western France, Northern Germany, and Eastern Austria, regions that were not previously known to host *E. multilocularis*-infected foxes. Emergence (or recent recognition?) of the disease in the Baltic States is the most striking epidemiological finding of the beginning of the twenty-first century (Marcinkutė et al. 2015). Lithuania in particular now definitely appears to be a major endemic area, with 80 patients diagnosed between 1997 and 2006 for a total population of 3,535,547 inhabitants, 57% of foxes, several farmer dogs, and various intermediate hosts found infected by *E. multilocularis* (Vuitton et al. 2015; Deplazes et al. 2017). Meanwhile, in Japan and in Europe, fox *E. multilocularis* infection has reached the city, thanks to the increased number and the "urbanization" of foxes, which poses new and unresolved questions for the

prevention of AE (Deplazes et al. 2004; Robardet et al. 2008); and dogs and cats are increasingly recognized to be significant definitive hosts (Robardet et al. 2011; Knapp et al. 2018; Da Silva et al. 2020).

Finally, the second decade of the twenty-first century has seen the emergence of North America, especially Canada, as an endemic area for human AE; ecological studies point to the role of semiurban coyotes in the vicinity of cities in Alberta; and to the responsibility of European strains of *E. multilocularis* (and not the autochthonous "Alaskan" strains) in human infection, often revealed in immunodeficient patients (Catalano et al. 2012; Massolo et al. 2014, 2019; Liccioli et al. 2015; Mori et al. 2019).

In fact, the immunodeficient status of the human population in a given endemic area has become one of the parameters which must be taken into account in epidemiological studies. Changes in the sensitivity of the human hosts to *E. multilocularis* infection and larval development are also involved in the increased incidence of AE; this is particularly obvious in the emergence of cases in North America, and this contributes to the increased incidence of cases seen in European endemic areas since the beginning of the twenty-first century (Vuitton et al. 2019; Wen et al. 2019). Such AE cases found in immunosuppressed patients appear to be more and more frequent; a systematic study from the French registry of human AE cases has shown that their incidence was significantly higher than the overall increased incidence of AE cases observed in the French endemic areas in the last two decades (Chauchet et al. 2014). A recent study in a Swiss endemic area has confirmed that new diagnoses have increased fourfold in immunocompetent and ten-fold in immunocompromised patients in the past decade (Lachenmayer et al. 2019). Cases in patients with primary immune deficiency or AIDS are rare (Sailer et al. 1997; Zingg et al. 2004; Chauchet et al. 2014; Haskologlu et al. 2020); most of cases of "opportunistic AE" are observed in patients with immunosuppressive treatments (chemo- and/or immunotherapy) for malignant or chronic inflammatory diseases, or to avoid organ rejection after transplantation (Chauchet et al. 2014; Lachenmayer et al. 2019).

37.4.1 Diagnosis and Follow-up of Echinococcosis in Humans

Clinical Presentation of CE. CE presents usually as a cyst ("hydatid cyst"), in the liver or in the lung, which may remain clinically silent for a long time and is often discovered incidentally during routine abdominal ultrasound (US) examination or chest X-ray/CT scan. All other anatomical locations are possible but rarer, as shown in a large collection of cases by the Australian Hydatid Register in 1976 where the most frequent locations of 1802 cysts were liver (63%), lung (25%), muscles, (5%), bones (3%), kidney (2%), spleen, brain (1%), and heart, breast, prostate, parotid and pancreas (<1%) (Torgerson et al. 2011). Similar figures are given from more recent series of cases in China and in Europe (Wen and Yang 1997; Vola et al. 2022). For the diagnosis of liver cysts, the following signs and symptoms may be observed: right upper quadrant discomfort; urticaria; episodes of itching; right upper quadrant

palpable mass. Clinical symptoms are usually absent until the cyst has reached 10 cm in diameter; a cyst is rarely palpable until it has reached 15–20 cm. Physical examination of the liver may be normal or may disclose an enlarged and regular liver. If the cyst is located in the anterior liver, a round, painless tumor can be palpated (Kern et al. 2017).

A complication is most often at the origin of hepatic CE diagnosis (Kern et al. 2017). The observed signs and symptoms are mainly jaundice by compression of or rupture into the bile ducts, or anaphylactic shock, eosinophilia, urticaria, and/or acute abdominal pain in case of cyst rupture in the peritoneal cavity. Cyst rupture may be favored by any abdominal trauma, often related to sport practice in children of endemic areas. Compression of the common bile duct, portal or hepatic veins, or inferior vena cava is uncommon. Rupture of the cyst (usually into the bile ducts) is more common: depending on the case series, at diagnosis, 30–40% of hepatic cysts diagnosed in hospital settings have ruptured or become infected (Fica et al. 2012). Among lung cysts referred to surgeons, complicated cysts are also frequent, including lung abscess, pleural involvement, pneumonitis and fibrosis in 10.38%, 13.21%, 7.55%, and 11.32% cases, respectively (Ghoshal et al. 2012). However, when a cyst is found at mass screening in asymptomatic subjects, in more than 75% of cases its latent asymptomatic evolution without complications may last more than 10 years (Frider et al. 1999; Wang et al. 2006b). In a recent study in India, 26% of liver cysts were either ruptured or infected when referred to surgeons (Malik et al. 2010). Cysts located near the diaphragm can erode it and extend into the pleural and pericardial cavities, the lung, or the bronchi through perforation. Cysts close to the peritoneal cavity may rupture into the peritoneum or into the duodenum, stomach, colon, or right renal pelvis. These ruptures may lead to extrahepatic CE by the dissemination of *E. granulosus* germinal layer fragments and of protoscoleces, and favor the development of "daughter cysts" in or out the initial cyst (Rogan et al. 2006; Vuitton et al. 2020). More commonly, the rupture of cyst occurs into bile ducts, and is revealed by cholestatic jaundice, cholangitis, or biliary pain. Some ruptures into bile ducts may be clinically silent and are thus only disclosed during an operation.

Cough, hiccups, and chest pain are the main symptoms of lung cysts when there are not diagnosed by chance on a chest X-ray (Ghoshal et al. 2012). Cyst rupture is also a frequent opportunity for diagnosing lung cysts: rupture in the bronchi may be followed by elimination of cyst fluid and materials (membranes, protoscoleces) by cough.

In endemic areas, mass screening programs have been implemented for the past 30 years, using US and serology, and have become a common circumstance of diagnosis. Such mass screening campaigns have shown that liver cysts had a very slow and limited growth: in more than half of the cysts there were no modifications in cyst size during the 10- to 12-year period of observation, in one third growth was slight (<3 cm) and in only one case (7%) the cyst grew by 4 cm. Mean cyst growth in all cases with a prolonged follow-up was 0.7 cm (Frider et al. 1999; Wang et al. 2006b). While serology alone has been used in serosurveys to detect the presence of CE, the presence of false positive and false negative results has been demonstrated to cause an unacceptable rate of false positive and false negative results and should be avoided (see below) (Torgerson and Deplazes 2009).

Imaging Diagnosis of CE. The two key procedures for indirect diagnosis are US and serology. US can be used to recognize cysts as small as 1 cm in diameter, and may be used for diagnosis in hospital settings as well as for mass screening (Macpherson et al. 2003; Casulli et al. 2020). It usually shows one or several round masses with a well-defined contour, which may be empty or filled with echogenic structures corresponding to daughter cysts. US can be used to recognize the detachment of the endocyst that appears as a wavy line inside the cavity. When cysts become infected, they are diffusely hyperechogenic and no longer exhibit characteristic features. Several classifications of US images in CE have then been proposed with various degrees of complexity, after the first US characterization of CE cysts by Gharbi et al. (1981). The WHO-Informal Working Group on Echinococcosis (IWGE) has promoted a unified cyst classification (Fig. 4), which is currently used as a standard to compare data from mass screenings and results of therapeutic interventions (WHO Informal Working Group 2003). This classification groups the cysts according to their activity (Hosch et al. 2008); however, stage CE3a, characterized by the detachment of membranes may either progress to development of daughter cysts (CE2) or to degeneration (CE4 and 5) (Stojković et al. 2018). In addition, when the classification is used to assess any therapeutic intervention, the diameter of cysts (in cm) rather than semiquantitative values should be given (Wang et al. 2003).

On computed tomography (CT), unilocular cysts with their spherical or oval structure of near-water density are easily recognized. Conversely, multilocular cysts may have several CT patterns depending on the space occupied by daughter cysts inside the cyst. Abscesses or necrotic tumors may mimic CE cysts. In such circumstances, with negative serological results, only aspiration cytology can establish the diagnosis of hydatid cyst (Hira et al. 1988; Rinaldi et al. 2014). CE cyst aspiration with a fine needle under ultrasound guidance is currently considered as medically

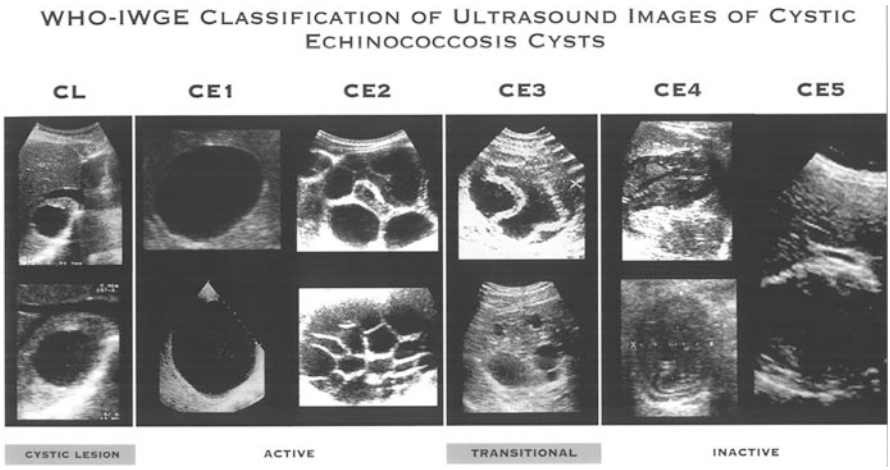

Fig. 4 International classification of Cystic echinococcosis cysts based on ultrasound images (according to WHO-Informal Working Group on Echinococcosis; in WHO-IWGE, 2003)

and ethically acceptable (Echinococcosis 2001). The appropriate procedure aimed at preventing and/or treating any anaphylactic complication and protoscolex spillage should be followed. A point-of-care examination of cystic fluid to determine the presence of PSCs or of *Echinococcus* spp. antigens can be employed to determine whether the puncture should be followed by other steps of the Puncture, Aspiration, Injection and Re-aspiration (PAIR) if the diagnosis of CE is confirmed (see below).

Magnetic resonance imaging (MRI) was shown to reproduce the ultrasound-defined features of CE better than CT and could be of use if for any reason US is not available (Stojkovic et al. 2012). FluoroDeoxyGlucose (FDG)-positron emission tomography (PET) seems to have little value for the routine diagnosis or follow-up of CE but may be of help for differential diagnosis with malignant lung tumors (Kurt et al. 2008)(56); however, peripheral FDG uptake was described in a case with liver cyst, with a typical "doughnut aspect" (Demir et al. 2008).

Clinical Presentation of AE. Before the 1980s in the European endemic area, AE was frequently recognized at an advanced stage and misdiagnosed as liver neoplasia: jaundice was the most frequent presenting symptom, in nearly 50% of the cases; it was either progressive jaundice related to hilum involvement, associated with pruritus, or intermittent jaundice with pain and fever related to superimposed bacterial biliary infection (Bresson-Hadni et al. 2000). Hepatomegaly, generally massive, was also a possible revealing symptom in about a quarter of the cases. This is still the situation in endemic areas of China (Ayifuhan et al. 2012; Aji et al. 2018) except in those regions where a systematic mass screening of the population is implemented.

During the past 30 years in Europe and Japan, changes in the revealing symptoms have occurred, because of disclosure of less severe and asymptomatic cases (Sato et al. 1997; Bresson-Hadni et al. 2000; Piarroux et al. 2011). From the French registry of human AE cases, more than 60% AE cases recorded in the last decade were asymptomatic when diagnosis was made (Bresson-Hadni et al. 2021b) (Siles-Lucas et al. 2017). Less than 25% of cases are revealed by jaundice; and hepatomegaly is observed in only 15% of the cases. Discomfort in the right upper quadrant is a revealing symptom in about 30% of cases. The contrast between a hepatomegaly mimicking a liver carcinoma or advanced cirrhosis and a good clinical status raises the suspicion of AE in endemic areas. Erratic clinical signs and symptoms generally due to metastasis or extrahepatic location of the parasite may also be observed at presentation (Kern et al. 2017). Diagnosis may be made during a surgical operation or an US, CT, or PET examination for another reason, or as the result of mass screening in an endemic area. Asymptomatic cases are more frequently disclosed in immune suppressed patients, especially when organ location/dissemination of cancer, lymphoma or leukemia is looked for during patient's follow-up, when pre-treatment evaluation is performed for systemic inflammatory/autoimmune diseases, or during the follow-up of patients after organ transplantation; evolution of AE seems faster in these patients; clinical symptoms, if any, may mimic liver abscess; and both imaging and serological diagnoses may be more difficult to interpret (Gruener et al. 2008; Chauchet et al. 2014; Vuitton et al. 2019; Lachenmayer et al. 2019).

The most frequent complications of AE are bacterial or fungal infection of the bile ducts and/or of the central necrotic area of lesions, with sepsis and septic shock (Piarroux et al. 2011; Kern et al. 2017; Ambregna et al. 2017). Locoregional extension or a hematogenous spread of parasitic tissue with distant metastases may cause a variety of symptoms ranging from dyspnea and bile-tinged sputum to seizures and stroke as well as skin nodules or bone pain or fractures. Unlike what happens with CE, anaphylactic reactions as revealing symptoms are extremely rare; the occurrence of such symptoms is always associated with systemic dissemination of parasitic fragments through the vessels. Bleeding from oesophagogastric varices related to portal hypertension, secondary to biliary cirrhosis or to chronic parasitic Budd-Chiari syndrome or portal thrombosis have become rare because of a more systematic prevention and treatment of such varices (Piarroux et al. 2011; Kern et al. 2017).

Imaging Diagnosis of AE. US and CT remain the basic imaging techniques in AE. US is the current screening method of choice for diagnosis and regular follow-up imaging in AE, as well as for mass screening (Bartholomot et al. 2002; Yang et al. 2006b; Kantarci et al. 2012; Liu et al. 2014; Kern et al. 2017). In two-third of the cases, the lesion is characterized by irregular limits and heterogeneous content with juxtaposition of hyperechogenic and hypoechogenic areas. The hyperechogenic fibrous tissue often contains scattered calcifications. Less typical US aspects include: 1) small hemangioma-like hyperechogenic nodules which correspond to a lesion at its earlier stage (currently more frequent in asymptomatic immunosuppressed patients; 2) pseudocystic lesions which correspond to huge AE lesions with massive necrosis; the surrounding hyperechogenic ring and the irregular lining should suggest the diagnosis of AE; 3) small calcified lesions which can correspond to either an abortive form of the disease or to a small-size developing AE (Bresson-Hadni et al. 2006). US combined with color Doppler can also provide useful information on biliary and vascular involvement (Liu et al. 2014; Kern et al. 2017). The limits of US examination lie in the calcification of the lesion which may prevent a proper evaluation of the lesion and its real extent. The various features of liver AE at US examination have been analyzed to propose a classification of the images (Kratzer et al. 2015); unlike the WHO-IWGE classification of CE US images, this classification, however, is not widely used although it could provide a better comparison of various series of AE patients, and help ultrasonographists to have a more systematic approach to US diagnosis. Contrast-Enhanced Ultrasonography (CEUS) is used only in specialized centers. Preliminary results showed that Levovist®-CEUS did not provide useful information (Ehrhardt et al. 2007); but using Sonovue® as a micro-bubble contrast agent could bring a very significant improvement both for the diagnosis and the follow-up of AE patients, by delineating the periparasitic micro-vascularized content of the lesions (Tao et al. 2011). Comparison of various imaging approaches to the "activity" of the lesions showed that CEUS could be a suitable substitute for FDG-PET, with the advantage of being readily portable, widely available, nonirradiating and far less expensive (Yangdan et al. 2021). Such comparisons should certainly be confirmed by further studies.

The typical CT aspect is a tumor-like lesion with irregular lining and heterogeneous content with a mosaïc of various densities: scattered, hyperdense calcifications and hypodense areas corresponding to necrosis and/or active parasitic tissue. No

significant enhancement is observed within the lesion after bolus administration of intravenous contrast medium; however, enhancement of the periphery of the lesions is sometimes observed. An intrahepatic bile duct dilation in the controlateral lobe of the liver is the hallmark of infiltration of the hilum by the parasitic process. Hypertrophy of the controlateral lobe is also usual. Following the classification of the US aspects of the liver lesions of AE (Kratzer et al. 2015), the same team has characterized CT images, with the definition of 5 types of "primary morphology," and 6 "patterns of calcification" (Graeter et al. 2016); this classification has the ambition of describing all possible morphological types of lesions at CT and may be useful to radiologists, especially in terms of comparison between lesion types observed in various settings (Graeter et al. 2020). No particular relationship has been found with the international PNM classification and staging which, on the other hand, had the ambition of proposing a prognosis, thus, a different objective. However, the description of the calcification patterns points out the morphological variety of such calcifications, which was not underlined in the past and could have pathophysiological significance: actually, the presence of microcalcifications (also described as "faethery calcifications" or "cloudy calcifications") at CT is strongly correlated to hypermetabolic activity of AE hepatic lesions at FDG-PET/CT independently of the presence of macrocalcifications (Brumpt et al. 2019).

MRI imaging may facilitate the diagnosis in uncertain cases with noncalcified lesions. It is the best technique to characterize the different components of the parasitic lesion and to study the extension to adjacent structures. It shows the typical aspect of multiple small cysts, best observed on T2-weighted images: such aggregations of microcysts are pathognomonic of AE, and may be used to assess diagnosis, especially in patients with small lesions with hemangioma-like or metastasis-like aspects at CT, and in those with a negative serology such as immunodeficient patients (Chauchet et al. 2014). Like the above mentioned "microcalcifications" inside the lesions, microcysts present on MR T2-weighted images may be regarded as a surrogate marker of the metabolic activity of the lesions (Azizi et al. 2015). Cholangio-MR imaging has now advantageously replaced the classical per-cutaneous cholangiography. It is an important part of the preoperative evaluation, as it provides information on the relationship between AE lesion and the biliary tree, and it may be completed by Endoscopic Retrograde Cholangio-Pancreatography (ERCP), which is often the first step of perendoscopic interventions, as described below (Tamarozzi et al. 2014; Ambregna et al. 2017).

PET using [18 F] FDG has been evaluated to assess the viability of the lesions, at diagnosis and in the follow-up of inoperable AE patients under long-term benzimidazoles therapy (Ehrhardt et al. 2007). Although FDG uptake does not actually reveal the metacestode, but is mostly associated with the periparasitic infiltrate by immune cells, this indirect approach may be useful and increased FDG uptake by an AE lesion in the liver is best correlated with parasitic metabolic activity when "delayed" FDG uptake images are analyzed, i.e., 3 h after FDG injection (Caoduro et al. 2013). It may be stressed that the similar increase of FDG uptake at PET-CT observed in AE and in a variety of solid primary or secondary tumors and malignant hematological disorders may be a source of difficult differential diagnosis, especially in those cases

with AE as an opportunistic infection when an abnormal lesion is found in the liver during the follow-up of patients with malignant diseases (Chauchet et al. 2014).

37.5 Immunological and Molecular Diagnosis of Echinococcosis

Serological tests may confirm the diagnosis of echinococcosis, although there is currently no standardized, highly sensitive and specific as well as inexpensive test available for antibody detection of CE or AE. Cross-reactivity is observed between both cestodes, and most tests for CE can be used for the diagnosis of AE and vice versa. In those endemic countries where both diseases may be found in the same area, a differential diagnosis could theoretically be useful; but most often, distinction is made by imaging. Cellular tests have no value for the routine diagnosis of both diseases (Bresson-Hadni et al. 1989). The intradermal Casoni test is no longer used because of its lack of sensitivity and specificity as well as because of safety issues (risk of anaphylactic shock).

Serological Tests. Detection of specific antibodies in serum uses antigens obtained from *E. granulosus* (hydatid fluid or protoscoleces) or *E. multilocularis* (protoscoleces or parasitic extracts), and/or more purified antigens or recombinant proteins from either *Echinococcus* sp. (Yang et al. 2008; Zhang et al. 2012; Craig et al. 2015; Kern et al. 2017; Gottstein et al. 2019; Vola et al. 2019; Tamarozzi et al. 2021). From the available literature and clinical experience, it may be stated that: 1) complement fixation tests are no longer used because of their poor sensitivity and specificity; 2) indirect hemagglutination and latex tests, using crude *Echinococcus* extracts, are both relatively inexpensive and sensitive, but poorly specific; 3) immunoelectrophoresis or immunosyneresis are specific, but poorly sensitive and time-consuming; they are no longer used; 4) ELISA tests, using crude antigens from *E. granulosus* or *E. multilocularis*, are sensitive, but poorly specific; 5) ELISA tests using specific antigenic fractions or recombinant antigens such as antigen 5 or antigen B of *Echinococcus granulosus*, *E. multilocularis* Em2 or Em18 fractions, and recombinant Em3/10/Em 18, are more specific; 6) Western blots use either crude extracts from *E. granulosus* or *E. multilocularis* (more sensitive) or purified fractions or recombinant antigens (more specific). Combination of antigens in a single test (as the $Em2^{plus}$, commercialized by Bordier Affinity Products, Crissier, Switzerland, or the rapid DOT-immunogold assay, commercialized by Xinjiang Key Lab, Urumqi, P.R. of China) (Feng et al. 2010), rules of interpretation for the Western blot (e.g., from *E. multilocularis* extract, as that commercialized by LDBio, Lyon, France) (Liance et al. 2000) or a combined Western Blot and Line blot assay (Deininger and Wellinghausen 2019), have attempted to combine positive and differential diagnosis. By evaluating the "best" serological tests on ultrasound and/or CT-confirmed lesions, overall sensitivity reaches 80% for liver hydatid cysts (it is lower for lung cysts, averaging 70%) and 95% for alveolar echinococcosis (it is lower in immune suppressed patients, averaging 85%); specificity may reach 90% for both (Ito and Craig 2003). However, diagnostic efficiency of serology is limited both by 1) the reduced capacity of some infected patients to develop specific antibodies (or some isotypes), for genetic or acquired reasons (such as immunosuppression) and 2) the absence of release of specific antigens by the cysts in CE, which

decreases sensitivity, and by the existence of infected nondiseased persons in endemic areas, which decreases specificity. Positive serological results in individuals at mass screening account for at least five different situations: 1) "patent," overt disease with symptoms, 2) "latent," nonapparent disease; 3) calcified dead lesions in the liver; 4) CE cyst in the lung or other organs and rare cases of isolated extrahepatic AE despite no US lesions in the liver; and, 5) no parasitic lesions at all (Bartholomot et al. 2002; Yang et al. 2007; Yang et al. 2008). Negative serology with patent CE or AE lesions have been found in all mass screening surveys and is the rule in all published hospital case series (Yang et al. 2007; Zhang et al. 2012). Serology should thus never been used as a "first intent" test, but always combined with ultrasound imaging in mass screening, and only be used as a confirmation test for a suspected diagnosis based on imaging techniques in clinical settings.

Molecular Tests. Molecular identification of both *E. granulosus* and *E. multilocularis* is mostly based on PCR using mitochondrial DNA probes, but also on nuclear sequences, including microsatellites. It may be used, if echinococcosis is suspected despite negative serology, on liver needle aspiration (for CE) and/or liver biopsy (for AE) (Dybicz et al. 2013), and it may be performed retrospectively, on formalin-fixed paraffin-embedded (FFPE)samples (Schneider et al. 2008; Simsek et al. 2011; Grenouillet et al. 2013). The use of such techniques is particularly precious in immunodeficient patients with negative serology and confusing images.

Time of storage could be critical for obtaining successful PCR. Recommendations were recently proposed (Knapp et al. 2022) for initial screening of either frozen or FFPE samples, using a short marker such as the 16S-84 bp target (Knapp et al. 2014), then different specific PCR systems in a second step for identification of *E. multilocularis* (Georges et al. 2004; Schneider et al. 2008) or *E. granulosus* (Stefanić et al. 2004; Trachsel et al. 2007). Additionally, the pan-Echinococcus 12S-268 bp target (Roelfsema et al. 2016) can be used to test for the putative occurrence of other species (e.g., *E. ortleppi* or *E. canadensis* in Europe). Molecular biology techniques also allow genotype studies both for CE (Boubaker et al. 2013; Alvarez Rojas et al. 2014) and AE (Massolo et al. 2019; Knapp et al. 2020). In addition, molecular biology techniques are useful in epidemiological studies to identify the actual species of *E. granulosus* involved in human contamination from the animal cycle (Romig et al. 2017; Massolo et al. 2019) Several techniques have been described to identify worms or eggs of *Echinococcus* spp. in the feces of definitive hosts. The most recent techniques aimed at avoiding the recurrent problem of RNAses and other inhibitors present in the fecal samples, while identifying the species of the animal host; the use of such techniques is currently revolutionizing epidemiological studies of *E. multilocularis* infection in the animal hosts (Umhang et al. 2016; Knapp et al. 2018, 2021; Da Silva et al. 2020; Herzig et al. 2021).

37.6 Antiparasitic Drugs and Echinococcosis

Two Orphan Drugs for Two Neglected Diseases. Compared to most of parasitic diseases and/or zoonoses, for which an appropriate and efficacious medical treatment is available, echinococcosis can rarely be cured by antiparasite chemotherapy

alone, safely and within an acceptable time schedule, and the available drugs are extremely limited (Pawlowski et al. 2001; Junghanss et al. 2008; Brunetti et al. 2010; Brunetti et al. 2011; Manciulli et al. 2018). In addition, there have been nearly no well-designed clinical trials for any medical treatment modality in either form of echinococcosis (Kern 2006). Only two benzimidazole compounds, used since the beginning of the 1980s, have proven effective against CE and AE: mebendazole (MBZ) (4.5 g/day) and albendazole (ABZ) (10–15 mg/kg/day) (WHO Informal Working Group on Echinococcosis 1996; Brunetti et al. 2010; Vuitton and Bresson-Hadni 2014). Both drugs have a poor bioavailability, mostly due to poor intestinal absorption; the most practical way to improve absorption is to combine ABZ with a fatty meal. ABZ (i.e., ABZ sulfoxide, the active compound originating from hepatic metabolism), however, may reach higher plasma levels than MBZ (Horton 2003; Junghanss et al. 2008; Vuitton and Bresson-Hadni 2014). For this reason, and because it is approved by the drug agencies of most countries, opposite to MBZ, ABZ is the most widely used drug for the treatment of echinococcosis. Both MBZ and ABZ must be given continuously, without interruption, for the period of treatment assigned to each case depending on the type of disease and treatment association; discontinuous administration of both drugs, as initially recommended by the pharmaceutical company and most of country drug regulation agencies, should not be used any longer (Tamarozzi et al. 2020a). Praziquantel (PZQ) is the only other drug of use in echinococcosis, since it is the only one with a demonstrated effect on *Echinococcus* sp. oncospheres, thus of possible use for deworming definitive hosts, dogs and fox (by baiting) and interrupting the parasite cycle (Gemmell et al. 1977). PZQ also exerts a toxic effect on *E. granulosus* protoscoleces (Richards et al. 1988), and may be used as adjuvant therapy together with albendazole in CE with systemic dissemination and/or specific locations, such as bone (Taylor and Morris 1989; Mohamed et al. 1998; Monge-Maillo et al. 2017; Monge-Maillo et al. 2019; Cattaneo et al. 2019); however, there are no sound clinical trials to support the use of such a combined therapy. Nitazoxanide has also been used for the treatment of CE, although its use has mostly been reported as a salvage therapy in bone CE, with contrasting results (Monge-Maillo et al. 2017; Monge-Maillo et al. 2019; Cattaneo et al. 2019).

Adverse Effects of Benzimidazoles. Adverse events of benzimidazoles occur in 6–20% of treated patients; they look more frequent when associated treatments are needed for other conditions, especially immune suppressants (Horton 2003; Chauchet et al. 2014; Tamarozzi et al. 2020a). General complaints include headache, nausea, anorexia, vomiting, abdominal pain and itching, and weight gain; in a few cases, idiosyncratic/allergic reactions have been observed (Horton 2003). A significant, but usually reversible alopecia occurs in about 5% of cases, favored by cholestasis and/or portal hypertension. In the first weeks of treatment, more severe side-effects may be observed, including leukopenia, as well as an increase in liver enzymes which may result from drug efficacy as well as be evidence of drug toxicity (Teggi et al. 1997; Horton 2003; Brunetti et al. 2010). A regular monitoring of a possible hepatotoxicity is therefore recommended: if aminotransferases rise over 3–4 times normal levels, discontinuation of therapy should be considered; ABZ sulfoxide plasma levels measured; and ABZ reintroduced at a lower dosage, with regular

monitoring of plasma levels (Vuitton et al. 2016). Albendazole sulfoxide levels should be regularly monitored when albendazole is used for a long period of time, as is the case in patients with AE or disseminated multiorgan CE; as the drug interfere with a variety of other drugs (Horton 2003) and other xenobiotics such as tobacco, cannabis or even liquorice, as recently stressed (Bresson-Hadni et al. 2021a), variations in drug systemic concentrations may explain adverse effects as well as lack of efficacy; measurement-guided modifications of ABZ dosage may widely improve both safety and efficacy of treatments (Vuitton and Bresson-Hadni 2014; Tamarozzi et al. 2020a; Bresson-Hadni et al. 2021a). ABZ has been shown to be teratogenic in rats and rabbits, and therefore, it should be avoided during pregnancy (at least during the first trimester) and lactation (Horton 2003). Although they are closely related drugs, MBZ may be better tolerated by patients with ABZ side-effects and vice versa; a switch to the other drug can thus be recommended before withdrawing chemotherapy definitively (Bresson-Hadni et al. 2011).

Candidate Drugs? All attempts at using other drugs for the treatment of echinococcosis have been unsuccessful until now, including promising drugs such as nitazoxanide, the effects of which were not convincing in humans after a preclinical trial (Stettler et al. 2004; Reuter et al. 2006; Kern et al. 2008). Several candidates have been proposed, from experimental *in vitro* and *in vivo* studies (Hemphill et al. 2007; Hemphill et al. 2014); none of them has reached phase I/II trials in humans. Several candidates from traditional medicine, including Chinese traditional medicines and others, have been studied from in vitro, experimental or clinical observations, but unfortunately have never been moved to approved treatment modalities (Siles-Lucas et al. 2018; Karpstein et al. 2021; Wang et al. 2021). Amphotericin B as salvage treatment for AE patients with intolerance or resistance to benzimidazoles effectively halted parasite progression in a small series of patients, but its IV administration precludes its use for the treatment of a chronic disease (Reuter et al. 2003).

The Advent of Biotherapies? Because of the exquisite sensitivity of *Echinococcus* larvae to the immune response of the host, efficacy of immune therapy may be anticipated; because of the efficacy of such therapies in cancer and the similarities between malignant tumors and the lesions of AE, as well as the severity of this disease, much attention has been given to a possible modulation of the immune system in order to inhibit the growth of *E. multilocularis*-induced lesions. The proof of concept was given two decades ago for interferon-alpha, with nearly complete prevention of *E. multilocularis* infection in experimental mice, and impressive regression of hepatic lesions in a patient with AE (Harraga et al. 1999; Godot et al. 2003); however, no clinical trials have been implemented to confirm these preliminary successes with interferons. More recently, experimental studies have focused on a possible intervention of immunological "check-points" in the tolerance of the host toward *Echinococcus* spp. metacestodes in order to develop biotherapeutic tools against AE and/or use biotherapies already developed for other purposes. The late stage of AE is characterized by a status of immune (T-cell) exhaustion, mostly due to the involvement of check-points similar to those observed in malignant tumors. Specific studies have addressed two inhibitory checkpoint systems, namely PD-1/PD-L1 (La et al. 2015; Wang et al. 2018; Bellanger

et al. 2020; Jebbawi et al. 2021) and TIGIT (Zhang et al. 2019, 2020, 2021). Experimental studies are very promising; however, use of check-points inhibitors (which are already used or at the preclinical stage in other indications) has not reached the preclinical stage yet for AE.

37.7 Treatment and Follow-up of CE Patients

Surgical Treatment of CE. The objective of surgery is to remove all parasitic cysts (Kern et al. 2017). For liver cysts, hepatic resection (hepatectomy) is usually only recommended for central cysts of a left lateral segment (segmentectomy). All other types of hepatic interventions are "cystectomies." Controversies still exist about the preferred operating technique of cystectomy among all proposed operations. Partial cystectomy, with an appropriate management of the residual cavity, is often preferred in endemic areas, since such a technique is easier to achieve by local surgical teams. However, without concomitant treatment by ABZ, recurrence and/or dissemination of cysts is frequent despite the measures implemented to prevent spillage of protoscoleces or of germinal layer fragments, since the cyst should be opened to perform such an operation. For any "opened cyst"-procedure, protection of the peritoneal cavity during cyst evacuation should be ensured. Partial cystectomy includes the sterilization of the cyst by injection of protoscolicidal agents, e.g., chlorhexidine, H_2O_2, 80% alcohol, or 0.5% cetrimide, then the evacuation of the cyst content. Recurrence of hepatic cysts and peritoneal dissemination of cysts are generally underestimated (2–25%), since very few series of patients with a prospective follow-up were ever reported in poor-resources endemic countries. In Kenya, among 663 patients with a surgical management of the disease, there were one intraoperative and one postoperative death, respectively, and 47 patients had repeated operations because of postoperative complications and/or recurrences (Cooney et al. 2004). To better avoid recurrence, currently various types of total cystectomy allow surgeons to completely remove the parasite without the risks of liver resection (Jerraya et al. 2015): after the liver overlying the cyst has been incised, attempts are made to excise the laminated layer intact; this may be done by following the virtual gap which exists between the inner and outer fibrous layer surrounding the cyst (Peng et al. 2006). Such a total (periadventitial) cystectomy, as proposed by Chinese surgeons, should be used whenever possible, if a surgical treatment for a noncomplicated cyst is necessary (Peng et al. 2002; Aydin et al. 2008; Mohkam et al. 2014; Chen et al. 2014; Lv et al. 2015; Zhang et al. 2020; Julien et al. 2021). The word "pericystectomy" should no longer be used to designate such an operation which removes all three layers of the cyst, including the adventitial layer, as well described by the international echinococcosis terminology which also proposes a systematic description of surgical operations for liver cyst resections according to the AORC nomenclature system (A for "Approach," O for "Opening," R for "Resection," and C for "Completeness," as shown in Fig. 5); such a standardized description should facilitate comparisons between case series, and avoid part of the biases usually unavoidable in retrospective studies (Vuitton et al. 2020;

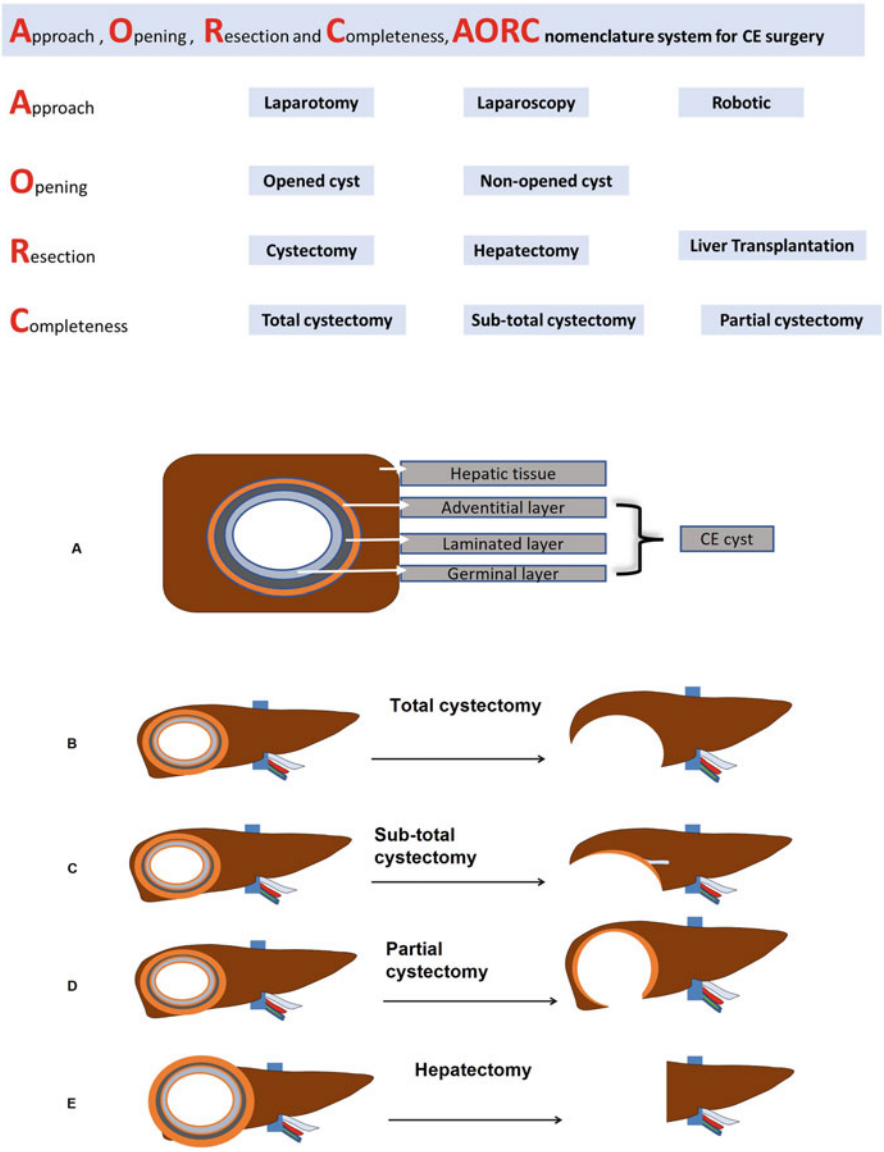

Fig. 5 The AORC (Approach/Opening/Resection/Completeness) nomenclature system for the description of surgical operations in the treatment of cystic echinococcosis (according to Vuitton et al. 2020, Parasite)

Bellanger et al. 2021a). Partial cystectomy, which removes the germinal and laminated layer of the cyst (previously named "endocystectomy"), remains widely used by surgeons; a standardized approach to this technique has been given in details; this technique includes cyst opening as part of the procedure, which needs a careful

protection of the surgical field and a long-term follow-up to check for recurrence (Al-Saeedi et al. 2019). The laparoscopic approach is now considered to be safe and is used by many surgical teams in endemic areas (Tuxun et al. 2014; Bektasoglu et al. 2019); robotic resection of hydatid cysts has also been reported (Magistri et al. 2019; Zhao et al. 2020; Yaghi et al. 2021); indocyanine green may help enhance the border between the peripheral adventitial layer and the liver parenchyma and assist surgeons to complete radical resection and reduce complications (Li et al. 2020). A meta-analysis based on 6 studies totally consisting of 1028 patients could not demonstrate a superiority of either of the surgical approaches (Sokouti et al. 2017). Another recent meta-analysis showed a higher rate of postoperative complications in the laparoscopic group, as well as lower rates of recurrence (Al-Saeedi et al. 2021). Depending on the series, recurrence rates especially were higher, similar, or lower in the laparoscopic group when surgical series were compared retrospectively (Bektasoglu et al. 2019; Bayrak and Altıntas 2019; Maitiseyiti et al. 2020). However, different types of procedures were used in the so-called "comparative" retrospective series; most studies lack important data such as cyst stage, dimension and location; and we miss prospective studies, randomized control trials, and long-term follow-up to define a strategy for the selection of cysts/patients to either of the approaches.

In any other locations (in the lung, brain, or any organ), cysts should be removed totally whenever possible (Duishanbai et al. 2011; Ghoshal et al. 2012; Ahmadinejad et al. 2020). For complicated liver cysts, a variety of techniques may be used, including surgical or perendoscopic biliary drainage (Dziri et al. 2009; Tamarozzi et al. 2014; Rinaldi et al. 2014); protoscolicidal agents should never be injected in the cyst when biliary communication is suspected; formalin and hypertonic saline must no longer be used in any cases because of the risk of caustic sclerosing cholangitis. Peritoneal dissemination and/or recurrence are difficult to treat. Liver transplantation (LT) has been exceptionally performed in patients with severe complications (Kern et al. 2017).

Nonsurgical Interventional Treatment of CE: Puncture–Aspiration–Injection–Reaspiration (PAIR). Since 1986, PAIR has been an alternative to surgery (Ben Amor et al. 1986; Gargouri et al. 1990). After percutaneous puncture under ultrasonographic guidance, a complete aspiration of the cyst is performed; the residual cavity is then filled with a scolicidal agent, usually ethanol, which is reaspired 10 min later. At the beginning of the 2000s, a review of cases treated with this technique by the WHO-IWGE and meta-analyses have concluded to the efficiency, safety and usefulness of the procedure (Echinococcosis 2001; Smego et al. 2003; Velasco-Tirado et al. 2018). A more recent meta-analysis of the comparison between PAIR and laparoscopic surgery, based on 57 studies including a total of 2832 patients has concluded to a superiority of PAIR regarding peri- and postintervention mortality and morbidity and a superiority of laparoscopic surgery regarding recurrence (Sokouti et al. 2019). However, such results are hampered by the different indications of the two techniques if the international rules for treatment decision are followed, by the specific techniques used for percutaneous puncture-associated treatment and for laparoscopic surgery, and by the different levels of expertise of the various teams involved in the collected studies. According to the

current consensus PAIR is indicated in medium-sized CE1 and CE3a cysts, and contraindicated for very large-sized cysts, and if there is communication of the cyst with the biliary tree, assessed using cysto-cholangiography, cholangio-MRI or by checking for bilirubin in the cyst content (Junghanss et al. 2008; Brunetti et al. 2010; Rinaldi et al. 2014; Kern et al. 2017). In particular, a retrospective study suggested that cysts larger than 7.5 cm have a high probability of having a biliary fistula (Kilic et al. 2008). A very limited number of anaphylactic shocks and less secondary dissemination than after surgery have been reported (Neumayr et al. 2011). Drainage may be associated with PAIR for large cysts and cysts associated with cysto-biliary communication; such a technique ("Standard Catheterization technique," S-CAT in the international echinococcosis terminology (Vuitton et al. 2020)) should be distinguished from the conventional PAIR when meta-analyses are performed; a prospective study comparing conventional PAIR and S-CAT showed that S-CAT had higher rates of major complications and length of hospital stay (Akhan et al. 2020). Other percutaneous technique have been described, which use larger tubes and vacuum aspiration, to treat cysts with daughter cysts ("Modified Catheterization techniques," Mo-CAT in the international terminology); however these procedures often have very long catheter times and selection of cases should be strict to compare favorably with surgery (Schipper et al. 2002; Kern et al. 2017; Akhan et al. 2017). Laparoscopic aspiration and sterilization of the cysts are also feasible but may be associated with spillage and recurrence, if the patients are not treated with ABZ before/after the procedure. As mentioned above, per-laparoscopic cystectomy is an option which is more and more adopted by properly trained surgical teams. Per-thoracoscopic interventions may also be performed to remove pulmonary or mediastinal cysts (Aydin et al. 2012; Alpay et al. 2012). Perendoscopic interventional procedures, including ERCP, dilation and/or stenting, have become the treatment of choice to treat postoperative biliary fistulae after cystectomy (Dziri et al. 2009; Tamarozzi et al. 2014; Jerraya et al. 2015). While PAIR has shown to be a curative procedure in cysts of appropriate stages, its diffusion has been hampered by the fear of complications, namely anaphylactic shock (Neumayr et al. 2011). While as discussed above the rate of anaphylactic complications is very low during PAIR, this remains a possibility. As such, all procedures should be performed in a hospital setting with the assistance of an intensive care specialist (Brunetti et al. 2010). Some authors have proposed the elimination of the injection step and the association of varying courses of albendazole to the treatment course (Firpo et al. 2017). However, no prospective study has been made on this variation to date.

Antiparasite Treatment and Follow-up of CE Patients. Benzimidazoles, which are parasiticidal on *E. granulosus in vitro*, are indicated for patients with multiple cysts in two or more organs and for patients with peritoneal cysts. ABZ may also be used to treat small-size cysts, since it was shown to be more effective on such cysts and when, given the natural history of CE, surgery might be disproportionate, especially in children. ABZ alone has a better effect on CE cysts than placebo or MBZ (Franchi et al. 1999). One prospective controlled trial of ABZ and PZQ (25 mg/kg/day) versus ABZ alone concluded that the combined treatment was more effective than ABZ alone (Mohamed et al. 1998). However, in randomized controlled trials, complete

disappearance of all cysts was never reached. Treatment schedules usually include "treatment cycles" of 3 month-continuous administration of ABZ, with imaging evaluation for signs of cyst degeneration after each cycle and decision to stop or prolong the treatment (Brunetti et al. 2010; Kern et al. 2017). Preintervention and postintervention antiparasitic treatment with BZM may reduce the risk of recurrence; there is no optimal scheme, but the current option is to give ABZ during 1 month before and 1–2 months after surgery (Kern et al. 2017). The most recent review of studies which compared surgery alone and surgery associated with BZM therapy was based on 22 studies with levels of evidence 2–4 which were qualitatively analyzed, and 11 randomized controlled trials which were quantitatively analyzed by meta-analysis (Velasco-Tirado et al. 2018). It confirmed that treatment outcomes were better when surgery is combined with BZM drugs given preoperation and/or postoperation. Association of ABZ with PAIR increases clinical and parasitologic efficacy (Smego et al. 2003; Velasco-Tirado et al. 2018). As a proof-of-concept, a retrospective study of PAIR without injection of protoscolecidal agent but followed by at least 1 month of ABZ administration has been performed: it showed results similar to those obtained with the classical PAIR in terms of response rate, relapse rate, and morbidity (Firpo et al. 2017); such a simplified procedure could extend the use of PAIR for the treatment of CE. A prospective comparative study should, however, be necessary to provide an optimal schedule of ABZ treatment, whatever the procedure, with and without injection. ABZ should not be administered when PAIR is performed during pregnancy (Horton 2003; Brunetti et al. 2010).

The main objective of administration of ABZ perioperatively is to prevent the development of protoscoleces after per-operative spillage. Based on experimental data (Ceballos et al. 2010, 2011, 2013), 1 month of treatment is a minimum to reach full protoscolecidal efficacy for ABZ; and adding PZQ could be a way to reduce the length of administration; however, perioperative use of PZQ to prevent protoscolex seeding, is based on a limited number of studies (Velasco-Tirado et al. 2018). A clinical study confirmed that after 28 days of administration of ABZ preoperatively, protoscoleces could still be found in some of the hydatid cysts at surgery or PAIR, and pointed out the high variability of individual albendazole sulfoxide concentrations, both in the plasma and in the cyst (Skuhala et al. 2014).

It may be anticipated that the multidisciplinary approach, which is now commonly used for AE patients, will soon also become a routine for the management of CE patients. Patients treated for CE, either by combined surgery, PAIR, S-CAT or Mo-CAT and ABZ, or by ABZ alone, should have a follow-up every 6 months during the first year after the initiation of treatment, including at least US examination. Blood cell count and amino-transferase levels should be measured every week during the first month of benzimidazole treatment, then twice a year. Recurrences (especially in the liver, the peritoneum and/or the pelvic cavity, for liver cysts) may occur several years after treatment: a retrospective study of long-term follow-up of CE patients with a care management in a single center of Spain showed 11.5% of recurrence with a median duration of recurrence's diagnosis of 12.35 years; the recurrence rate was even higher for non-liver or -lung locations (Velasco-Tirado et al. 2017). The long-term follow-up, including at least US once a year, for at least 3 years

after interruption of the antiparasite drug and/or after cystectomy, then every 2 years for 10 years, is thus needed to detect any cyst recurrence. It is clear from the experience of the National Reference Centers of many countries that such a long-term follow-up of the patients (especially children and teen agers) is not yet the rule among the surgeons of the endemic areas.

In the case of inactive cysts, the WHO-IWGE had suggested avoiding treatment ("Watch and wait" approach) on the basis of a low tendency to reactivation (Brunetti et al. 2010), and this has recently been confirmed in studies carried out at referral centers, showing that only around 1% of cysts that are spontaneously inactive eventually become active again (Stojkovic et al. 2016; Lissandrin et al. 2018).

37.8 New Biomarkers for the Follow-up of CE Cysts

We previously discussed the many limitations of serology for the diagnosis of CE cysts. It should be noted that all types of serology using the current antigens have had disappointing performances in the follow-up of CE patients (Tamarozzi et al. 2013; Stojkovic et al. 2017). Antibodies against *E. granulosus* can persist for years after cyst inactivation, whether this is spontaneous or induced by treatment. Moreover, several factors have been proven to influence serological results (Lissandrin et al. 2016; Tamarozzi et al. 2021). In the case of surgically treated patients, many studies evaluating serology as a follow-up tool report patients as being treated surgically but do not clarify the original cyst stage, or whether surgery has achieved complete removal of the cyst.

Since cyst viability determines whether the patient should undergo treatment and given the scarce availability of MRI-spectroscopy outside of select centers, biological markers of viability are being studied. Recently, some promise has been shown by cytokine assays, specifically targeting IL-4, that were able to distinguish active from inactive cysts with sufficient precision, showing a 76–95% specificity in the detection of reactivated cysts (Petrone et al. 2015, 2021). Another recently proposed marker consists of parasite-derived micro-RNAs (miRNAs). Two research groups have shown that panels of miRNAs were able to distinguish active from inactive cysts at diagnosis, pointing toward a potential role in the follow-up of patients as well as in the diagnosis in centers where US expertise is lacking (Mariconti et al. 2019). New biomarkers were recently developed for the early prediction of pediatric CE postsurgical outcomes, but defining their place in the follow-up strategy still requires additional evaluations (Ben Salah et al. 2022).

Finally, long-term follow-up of patients with a hydatid cyst disclosed at mass screening has shown that a significant percentage, especially among children, had a degenerating evolution or did not change with time (Frider et al. 1999; Solomon et al. 2017; Larrieu et al. 2019); and a "watch and wait" attitude is likely indicated in such cases with small cysts and most of uncomplicated subjects with CE4 and CE5 cysts (Junghanss et al. 2008; Brunetti et al. 2010). Cysts which have reached CE4 and even CE5 stages through ABZ treatment have been shown to move back to CE3 stage in a significant number of cases, more frequently than CE4 and CE5 cysts found at

screening; this is a reason to recommend a strict follow-up of these patients (Stojkovic et al. 2016; Stojković et al. 2018). Conversely, spontaneously inactive cysts (i.e., CE4–5 cysts that are inactive at diagnosis, in the absence of a treatment history) may be considered for a "watch and wait" approach, on the basis of a low tendency to reactivation. This has recently been confirmed in studies carried out at referral centers, showing that only around 1% of cysts that are spontaneously inactive eventually become active again (Stojkovic et al. 2016; Lissandrin et al. 2018).

37.9 Treatment and Follow-up of AE Patients

A Multidisciplinary Approach for the Treatment of Patients with AE. The therapeutic management of AE patients clearly requires a multidisciplinary approach, in which benzimidazole therapy is a common denominator (see below). A complete evaluation of the disease extension (including thoracic and brain CT) is necessary before any therapeutic decision (Vuitton 2009; Vuitton et al. 2016; Kern et al. 2017). Depending on the size of the lesion(s), its location in the liver and vascular and biliary involvement, invasion or not of adjacent organs, presence or absence of distant metastases, the options may be a curative resection or a prolonged ABZ treatment (associated with an interventional radiological or per-endoscopic procedure, if necessary, because of complications). Currently, "partial debulking" liver resections followed by continuous administration of a benzimidazole must be avoided (Kadry et al. 2005; Buttenschoen et al. 2009a, b). The PNM system of classification of AE cases, designed on the model of the TNM classification of cancers, helps clinicians to choose the appropriate treatment and the clinical teams to evaluate their results comparatively (Table 1) (Kern et al. 2006). A comprehensive algorithm, based on the possibility or not of complete resection of the lesion (assessed from imaging data and presence/absence of comorbidities) has been proposed for the care management of AE cases (Wen et al. 2019) (Fig. 6).

Surgical Treatment of AE. The only efficient treatment is partial hepatectomy when the lesions are located in liver segments accessible to resection; because the intrahepatic common bile duct is usually involved, it is often necessary to remove the bifurcation and to reconstruct the biliary tract using a Roux-en-Y loop (Kadry et al. 2005; Buttenschoen et al. 2009a; Ayifuhan et al. 2012; Kern et al. 2017; Wen et al. 2019; Yang et al. 2019). Currently, in endemic areas of Europe, 1/3 of patients with AE may benefit from a curative resection of their "parasitic tumor." In very severe cases, with life-threatening complications and no other options, liver transplantation may be proposed. Allogeneic liver transplantation is associated with a risk of recurrence, or of progression of extrahepatic locations, because of immunosuppression (Koch et al. 2003). Such a risk may be alleviated by early ABZ treatment after transplantation; and unexpected long-term survival of more than 20 years have been published in patients with residual lesions after liver transplantation (Bresson-Hadni et al. 2011). Continuous administration of ABZ is essential to prevent recurrences in the liver or in other organs (Zavoikin et al. 2020). *Ex vivo* liver resection followed by

Table 1 PNM classification and staging of alveolar echinococcosis (according to WHO-Informal Working Group on Echinococcosis; in Kern et al. 2006)

A. PNM classification of AE cases (at presentation)
P Hepatic localization of the parasite
P X Primary tumor cannot be assessed
P 0 No detectable tumor in the liver
P 1 Peripheral lesions without proximal vascular and/or biliary involvement
P 2 Central lesions with proximal vascular and/or biliary involvement of one lobe
P 3 Central lesions with hilum vascular or biliary involvement of both lobes and/or with involvement of two hepatic veins
P 4 Any liver lesion with extension along the vessels and the biliary tree
N Extra hepatic involvement of neighboring organs [diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall (muscles, skin, bone), pancreas, regional lymph nodes, liver ligaments, kidney]
N X Not evaluable
N 0 No regional involvement
N 1 Regional involvement of contiguous organs or tissues
M The absence or presence of distant metastasis [lung, distant lymph nodes, spleen, CNS, orbital, bone, skin, muscle, kidney, distant peritoneum and retroperitoneum]
M X Not completely evaluated
M 0 No metastasis
M 1 Metastasis
(a) For classification, the plane projecting between the bed of the gall bladder and the inferior vena cava divides the liver in two lobes; (b) Vessels mean inferior vena cava, portal vein, and arteries; (c) Chest X-ray and cerebral CT negative.
B. PNM stage grouping of alveolar echinococcosis
Stage I P1 N0 M0
Stage II P2 N0 M0
Stage IIIa P3 N0 M0
Stage IIIb P1–3 N1 M0
P4 N0 M0
Stage IV P4 N1 M0
Any P any N and/or M1

auto-transplantation (ELRA) is an alternative which has been developed to allow easier resection of large-sized lesions with vascular involvement (Wen et al. 2011; Aji et al. 2018; Wen et al. 2019). Until now, the experience with this type of technique comes only from Chinese hepatic surgery teams, which confronted cases in relatively young patients with large sized-lesions, biliary and vascular complications, and difficulties to ensure life-long ABZ treatment, a situation currently rarely encountered in European endemic areas (Beldi et al. 2019). Long-term evaluation has been now been published by several Chinese teams. Performed by highly experienced teams specialized in hepatic surgery, with sound imaging and technological support (He et al. 2015; Qin et al. 2016, 2021; Chen et al. 2021), this type of operation, evaluated on more than 200 cases in several distinct centers within

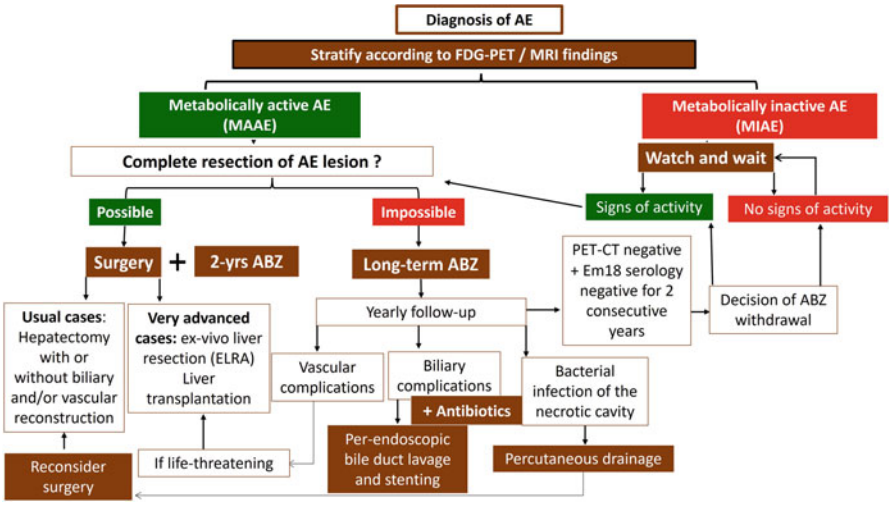

Fig. 6 Therapeutic algorithm for the care management of patients with alveolar echinococcosis (according to Wen et al., Clin Microbiol Rev., 2019)

the 10 years after its introduction in the surgical arsenal to treat severe AE cases, has results similar to or better than allotransplantation (Aji et al. 2018; Hwang et al. 2018; Shen et al. 2019; Yang et al. 2020). If the selection of patients has been careful to avoid primary liver dysfunction in the perioperative period, the obvious advantages are to avoid the resort to a liver donor and to life-long immunosuppressive antirejection treatment, and to only require the usual 2-year ABZ treatment when the resection of the lesions has been complete and thus curative (Wen et al. 2019).

Nonsurgical Interventional Treatment of AE. When curative resection is not possible, palliative surgery should be replaced by percutaneous or perendoscopic procedures. They consist of percutaneous radiological drainages of huge centroparasitic abscesses or of dilated intrahepatic bile duct above a hilum stenosis (Bresson-Hadni et al. 2006; Vuitton et al. 2016); this may be a first step before surgery (Yang et al. 2019). The best option is to push the drain beyond the stenosis to obtain an external/internal biliary drainage. Such drains may be maintained for years; combined with chemotherapy, they have allowed prolonged survival in initially very severe AE cases; shrinking of the initial necrotic "pseudocystic" cavity after percutaneous puncture and drainage may also make reconsider hepatic resection after a few months of treatment with ABZ (Vuitton et al. 2016; Kern et al. 2017; Wen et al. 2019). Biliary endo-prosthesis/stent insertion is an alternative which is currently more and more frequently used for the drainage of the bile ducts, often associated with temporary antibiotics administration to treat bacterial superinfection (Tamarozzi et al. 2014). Evaluated by a European survey, perendoscopic procedures were shown to be an efficient and safe alternative to surgery to treat AE biliary complications; insertion of multiple plastic stents delays stent occlusion and leads to effective and prolonged bile duct patency (Ambregna et al. 2017). Preoperative use of

perendoscopic procedures to treat bile duct obstruction by the AE lesions has become an important step in the care management of AE patients with jaundice and/or cholangitis by improving the nutritional status and reducing perioperative morbidity (Tamarozzi et al. 2014; Kern et al. 2017; Ambregna et al. 2017; Wen et al. 2019).

Antiparasite Treatment and Follow-up of AE Patients. MBZ and ABZ have only a parasitostatic effect *in vitro* and cannot kill *E. multilocularis* in most AE cases; however, their benefit for patients' survival and quality of life has been well assessed in the 1990s (Ammann 1991; Ammann et al. 1994; Ishizu et al. 1997). There are no comparative studies, but ABZ is currently preferred because it reduces the cost by >40%, is easier for patients to take and is now licensed for AE in many countries (Reuter et al. 2000; Piarroux et al. 2011). In case of curative surgery, ABZ should be initiated before the operation and maintained for at least 2 years to avoid recurrence (Brunetti et al. 2010; Kern et al. 2017). In inoperable cases, long-term chemotherapy (i.e., for life) may significantly prolong survival (10-year survival of approximately 80%, compared with less than 25% in historical controls) (Brunetti et al. 2010). Even in very severe cases, on the waiting list for liver transplantation, ABZ may have a dramatic efficacy in at least 15–20% of patients; antiparasitic treatment is thus always indicated (Ocak et al. 2021). A more personalized medical treatment of inoperable AE patients seems now to be possible, thanks to the combination of sequential FDG-PET evaluation with delayed acquisition of images, 3 h after FDG injection (Caoduro et al. 2013), the presence/absence of microcysts at MRI (Azizi et al. 2015), and of specific serological markers such as antibodies against *E. multilocularis* Em18 antigen, the decrease of which is best associated with absence or lack of viability of the metacestode (Tappe et al. 2009; Crouzet et al. 2010; Tappe et al. 2010; Bardonnet et al. 2013). New markers are currently under active research to better assess the "activity/viability" of the parasitic lesions (Gottstein et al. 2014; Valot et al. 2017; Bellanger et al. 2021b).

Taking antiparasitic drugs for years, and often for life, iterative stays at the hospital for complications and/or follow-up, major surgical operations, deeply impact on the patients' quality of life. A recent study has tried to evaluate the patients' quality of life after liver resection versus long-term ABZ treatment: it showed no statistically significant differences in patients with AE dependent on the applied treatment strategy (Schmidberger et al. 2019); however, there was a slight advantage in the physical and mental scores of the patients treated with surgery; furthermore, for 13 of the 25 surgically treated patients, some aspects of the health-related quality of life improved significantly after surgery. Discontinuation of BZM after many years of treatment could be tried in selected cases when all "activity markers" are negative (Ammann et al. 1998; Reuter et al. 2004; Bresson-Hadni et al. 2011; Bardonnet et al. 2013; Gottstein et al. 2014). In liver-transplanted patients, ABZ must be initiated before the operation, reintroduced as soon as possible after transplantation, and maintained for at least 2 years if all AE lesions were removed with the liver, and life-long in case of metacestode remnants or new AE foci discovered during follow-up (Bresson-Hadni et al. 2011).

Whatever the type of treatment, including or not surgery, all patients with AE should have a regular follow-up (every 3 months, then 6 months, then year, depending on the clinical status and the occurrence or not of complications). The follow-up should include US and serology, blood cell count and aminotransferase levels, and ideally FDG-PET, during the period of benzimidazole treatment; a yearly follow-up should be maintained for at least 5 years after benzimidazole withdrawal (Vuitton and Bresson-Hadni 2014; Kern et al. 2017). Monitoring of ABZ sulfoxide is also essential, both to evaluate patient's adherence to treatment, to suspect interference with associated drugs or other xenobiotics, and to properly adjust ABZ dosage in case of apparent resistance to treatment or of adverse effects (Vuitton 2009; Bresson-Hadni et al. 2021a).

37.10 Prevention and Control

As regard to the single individual, prevention of CE relies only on hygienic measures, such as washing hands before eating, avoiding contact between mouth and nonwashed hands, thorough washing of raw vegetables, and use of a safe water source; chlorination does not inactivate contaminating eggs (Craig et al. 2017; Lightowlers et al. 2021).

Control programs for CE are complex, with multiple targets, and require considerable investment of time (minimum 10 years of "attack phase" followed by a "consolidation" and "maintenance" phase) and resources, together with a consensual coordination of various actors (professionals and decision-makers in human and animal health, police, legislation, education, etc.) (Craig et al. 2017). The principal points of intervention have been individuated in: i) veterinary public health actions such as control of livestock movements and slaughter, including inspection of organs and proper disposal of infected viscera and dead animals; ii) registration of owned dog and control of stray dog population; however, dog culling practices should be carefully evaluated (Johansen and Penrith 2009); iii) accurate estimation of baseline epidemiological data in the animal and human population; iv) regular treatment of dogs with praziquantel, at least every 6 weeks; v) education of the owners about safe feeding of dogs and animal husbandry, and of the whole community about the purpose and importance of the program; vi) making CE a notifiable disease and introduce an appropriate supporting legislation (Barnes et al. 2012; Craig et al. 2017). So far, only four CE control programs have been successful, all on islands (Iceland, New Zealand, Tasmania, and Falkland Islands); and after an apparent success, one failed in Cyprus. Although purely education has not been found efficacious in reducing infection prevalence with the noticeable exception of the Iceland program, this is a pivotal measure in all control programs, as CE is generally not perceived as a serious condition for both animals and humans by communities and policy makers, and populations do not adhere to control measures aimed at animals to prevent a disease in humans (Huang et al. 2011). The introduction of livestock vaccination using the highly effective EG95 vaccine could be a very useful tool to shorten control program length (Lightowlers et al. 2021).

As regards the single individual, prevention of AE relies on similar measures as for CE: *E. multilocularis* eggs are extremely resistant to any chemical and to low temperatures (e.g., those reached by family-use freezers); they are only sensitive to heating, hence the advice to cook any fruit/vegetable collected in pastures/meadows or in family gardens exposed to fox and/or dog feces. Regular praziquantel treatment of dogs follows the same rule as for CE control, at family level, and was used in Alaska on highly endemic islands such as St Lawrence (Rausch et al. 1990). For decades, it was, however, considered that *E. multilocularis*, being a parasite which circulated in wild life, was globally beyond control (Roberts and Aubert 1995). A few control programs have targeted endemic rural areas, with variable results depending on the endemic country (e.g., Alaska vs. Europe vs. Japan); the most ambitious program of control has been established in PR China, focusing both on CE and AE which often coexist in the same areas of Western China (Craig et al. 2017); however, despite a major governmental involvement, especially because of the nomadic style of life of most of the populations at risk, results are not always at the level of the investment (van Kesteren et al. 2015). Most of the recent control programs have concerned urban foxes, using fox baiting with PZQ, with results that apparently varied according to contamination pressure in the rural areas surrounding the targeted city (Deplazes et al. 2004; Comte et al. 2013); a meta-analysis of studies in highly endemic areas of Europe or Japan showed that monthly fox baiting with PZQ resulted in a sharp and statistically significant decrease in parasite prevalence; nevertheless, when foxes were not fully dewormed, the parasite showed a strong capacity to rapidly recover its initial prevalence (Umhang et al. 2019). Conversely, consequent effort of fox culling in a city located in a French endemic area for *E. multilocularis* not only failed to decrease the fox population but resulted in an increase in *E. multilocularis* prevalence from 40% to 55% while remaining stable in an adjacent control area; this paradoxical finding was likely due to increased immigration of infected foxes from the rural surroundings (Comte et al. 2017).

37.11 Conclusion

Echinococcoses are highly neglected diseases although they affect populations nearly everywhere in the world, including developed and technically advanced countries with high-level public health systems. An efficient antiparasitic treatment of short duration with few adverse events still looks far from reach. However, even based on non-satisfactory levels of evidence, the therapeutic strategy and patients' life expectancy have considerably improved in the last 30 years. Among the major advances of the last decade, we may list (1) a genome-based definition of *Echinococcus* species involved in zoonotic and human diseases, with a totally revised taxonomy and the development of molecular biology-based epidemiology and transmission studies; (2) a consensus between the major actors in the field (researchers and clinicians) on a revised terminology which will enhance mutual understanding and international cooperation; (3) progress in deciphering immunological mechanisms involved in alveolar echinococcosis, leading to possible

immune interventions to complement antiparasitic drug approach; (4) better immunological and imaging tools for the follow-up of patients, both for CE and AE; (5) ambitious surgical techniques with proven efficacy, such as ex vivo liver resection and auto-transplantation, for advanced cases of AE, and better definition of the advantages, risks, and indications of the various interventional techniques for CE.

Prevention remains largely limited to individual measures, and even in those countries, such as China, which have implemented nation-wide programs, control of echinococcoses faces numerous obstacles because of the multifactorial aspects of *Echinococcus* spp. transmission in animals and humans. Echinococcoses were thought to be diseases of the rural environment and doomed to disappear because of societal changes, and improved hygiene and standard of living. However, cystic echinococcosis is still present in developed as well as developing countries; and since the beginning of the twenty-first century, *E. multilocularis* infection of definitive hosts has considerably increased in cities and extended its endemic areas, including in North America which was little concerned before. In addition, alveolar echinococcosis may now be listed among the opportunistic infections, and more and more individuals with therapeutic immune suppression are and will become vulnerable to *E. multilocularis* infection. Physicians should be better informed of this eventuality, and more studies are needed to assess the best strategy to tackle this emerging public health problem with a "One Health" perspective.

References

Ahmadinejad M, Hashemi M, Azizallahi N (2020) Evaluation of prognostic factors associated with postoperative complications following pulmonary hydatid cyst surgery. Open Respir Med J 14: 16–21. https://doi.org/10.2174/1874306402014010016

Aji T, Dong J-H, Shao Y-M, Zhao J-M, Li T, Tuxun T, Shalayiadang P, Ran B, Jiang T-M, Zhang R-Q, He Y-B, Huang J-F, Wen H (2018) Ex vivo liver resection and autotransplantation as alternative to allotransplantation for end-stage hepatic alveolar echinococcosis. J Hepatol 69: 1037–1046. https://doi.org/10.1016/j.jhep.2018.07.006

Akhan O, Salik AE, Ciftci T, Akinci D, Islim F, Akpinar B (2017) Comparison of long-term results of percutaneous treatment techniques for hepatic cystic echinococcosis types 2 and 3b. AJR Am J Roentgenol 208:878–884. https://doi.org/10.2214/AJR.16.16131

Akhan O, Erdoğan E, Ciftci TT, Unal E, Karaağaoğlu E, Akinci D (2020) Comparison of the long-term results of puncture, aspiration, injection and re-aspiration (PAIR) and catheterization techniques for the percutaneous treatment of CE1 and CE3a liver hydatid cysts: a prospective randomized trial. Cardiovasc Intervent Radiol 43:1034–1040. https://doi.org/10.1007/s00270-020-02477-7

Alpay L, Lacin T, Atinkaya C, Kıral H, Demir M, Baysungur V, Okur E, Yalcınkaya I (2012) Video-assisted thoracoscopic removal of pulmonary hydatid cysts. Eur J Cardiothorac Surg 42: 971–975. https://doi.org/10.1093/ejcts/ezs338

Al-Saeedi M, Khajeh E, Hoffmann K, Ghamarnejad O, Stojkovic M, Weber TF, Golriz M, Strobel O, Junghanss T, Büchler MW, Mehrabi A (2019) Standardized endocystectomy technique for surgical treatment of uncomplicated hepatic cystic echinococcosis. PLoS Negl Trop Dis 13:e0007516. https://doi.org/10.1371/journal.pntd.0007516

Al-Saeedi M, Ramouz A, Khajeh E, El Rafidi A, Ghamarnejad O, Shafiei S, Ali-Hasan-Al-Saegh S, Probst P, Stojkovic M, Weber TF, Hoffmann K, Mehrabi A (2021) Endocystectomy as a conservative surgical treatment for hepatic cystic echinococcosis: A systematic review with

single-arm meta-analysis. PLoS Negl Trop Dis 12;15(5):e0009365. https://doi.org/10.1371/journal.pntd.0009365. PMID: 33979343; PMCID: PMC8143402

Alvarez Rojas CA, Romig T, Lightowlers MW (2014) Echinococcus granulosus sensu lato genotypes infecting humans – review of current knowledge. Int J Parasitol 44:9–18. https://doi.org/10.1016/j.ijpara.2013.08.008

Ambregna S, Koch S, Sulz MC, Grüner B, Öztürk S, Chevaux J-B, Sulima M, de Gottardi A, Napoléon B, Abergel A, Bichard P, Boytchev I, Deprez P, Dumortier J, Frossard J-L, Kull E, Meny B, Moradpour D, Prat F, Vanbiervliet G, Thevenot T, Vuitton DA, Bresson-Hadni S, Vuitton L (2017) A European survey of perendoscopic treatment of biliary complications in patients with alveolar echinococcosis. Expert Rev Anti-Infect Ther 15:79–88. https://doi.org/10.1080/14787210.2017.1252260

Ammann RW (1991) Improvement of liver resectional therapy by adjuvant chemotherapy in alveolar hydatid disease. Swiss Echinococcosis Study Group (SESG). Parasitol Res 77:290–293

Ammann RW, Ilitsch N, Marincek B, Freiburghaus AU (1994) Effect of chemotherapy on the larval mass and the long-term course of alveolar echinococcosis. Swiss Echinococcosis Study Group. Hepatology 19:735–742

Ammann RW, Fleiner-Hoffmann A, Grimm F, Eckert J (1998) Long-term mebendazole therapy may be parasitocidal in alveolar echinococcosis. J Hepatol 29:994–998

Aydin U, Yazici P, Onen Z, Ozsoy M, Zeytunlu M, Kiliç M, Coker A (2008) The optimal treatment of hydatid cyst of the liver: radical surgery with a significant reduced risk of recurrence. Turk J Gastroenterol 19:33–39

Aydin Y, Ogul H, Turkyilmaz A, Eroglu A (2012) Surgical treatment of mediastinal cysts: report on 29 cases. Acta Chir Belg 112:281–286

Ayifuhan A, Tuerganaili A, Jun C, Ying-Mei S, Xiang-Wei L, Hao W (2012) Surgical treatment for hepatic alveolar echinococcosis: report of 50 cases. Hepato-Gastroenterology 59:790–793. https://doi.org/10.5754/hge10545

Azizi A, Blagosklonov O, Lounis A, Berthet L, Vuitton D-A, Bresson-Hadni S, Delabrousse E (2015) Alveolar echinococcosis: correlation between hepatic MRI findings and FDG-PET/CT metabolic activity. Abdom Imaging 40:56–63. https://doi.org/10.1007/s00261-014-0183-0

Bardonnet K, Vuitton DA, Grenouillet F, Mantion GA, Delabrousse E, Blagosklonov O, Miguet J-P, Bresson-Hadni S (2013) 30-yr course and favorable outcome of alveolar echinococcosis despite multiple metastatic organ involvement in a non-immune suppressed patient. Ann Clin Microbiol Antimicrob 12:1. https://doi.org/10.1186/1476-0711-12-1

Barnes TS, Deplazes P, Gottstein B, Jenkins DJ, Mathis A, Siles-Lucas M, Torgerson PR, Ziadinov I, Heath DD (2012) Challenges for diagnosis and control of cystic hydatid disease. Acta Trop 123:1–7. https://doi.org/10.1016/j.actatropica.2012.02.066

Bartholomot G, Vuitton DA, Harraga S, Shi DZ, Giraudoux P, Barnish G, Wang YH, MacPherson CNL, Craig PS (2002) Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in Central China. Am J Trop Med Hyg 66:23–29

Basmaciyan L, Burlet B, Ramla S, Blot M, Mahy S, Aubriot-Lorton M-H, Valot S, Grelat M, Sautour M, Grenouillet F, Knapp J, Millon L, Piroth L, Martin L, Dalle F (2018) First case of human primary vertebral cystic echinococcosis due to *Echinococcus ortleppi*. J Clin Med 7:E443. https://doi.org/10.3390/jcm7110443

Bayrak M, Altıntas Y (2019) Current approaches in the surgical treatment of liver hydatid disease: single center experience. BMC Surg 19:95. https://doi.org/10.1186/s12893-019-0553-1

Bektasoglu HK, Hasbahceci M, Tasci Y, Aydogdu I, Malya FU, Kunduz E, Dolay K (2019) Comparison of laparoscopic and conventional Cystotomy/partial cystectomy in treatment of liver Hydatidosis. Biomed Res Int 2019:1212404. https://doi.org/10.1155/2019/1212404

Beldi G, Vuitton D, Lachenmayer A, Heyd B, Dufour J-F, Richou C, Candinas D, Bresson-Hadni S (2019) Is ex vivo liver resection and autotransplantation a valid alternative treatment for end-stage hepatic alveolar echinococcosis in Europe? J Hepatol 70:1030–1031. https://doi.org/10.1016/j.jhep.2018.12.011

Bellanger A-P, Courquet S, Pallandre J-R, Godet Y, Millon L (2020) Echinococcus multilocularis vesicular fluid induces the expression of immune checkpoint proteins in vitro. Parasite Immunol 42:e12711. https://doi.org/10.1111/pim.12711

Bellanger AP, Achour K, Barkati S, Bastid C, Bresson-Hadni S, Delaere B, Dziri C, Gottstein B, Kachani M, Mantion G, Umhang G, Wallon M, Vuitton DA (2021a) Terminologie à utiliser pour l'étude et la prise en charge des échinococcoses : adaptation du consensus international à la langue française. Bull Acad Natl Med 205:1028–1059. https://doi.org/10.1016/j.banm.2021.06.016

Bellanger A-P, Wang J, Gbaguidi-Haore H, Barrera C, Bresson-Hadni S, Zlobec I, Lachenmayer A, Richou C, Turco C, Gottstein B, Millon L, Beldi G (2021b) Investigating new serological and tissue markers for the follow-up of patients operated for alveolar echinococcosis. Parasite Immunol 43:e12827. https://doi.org/10.1111/pim.12827

Ben Amor N, Gargouri M, Gharbi HA, Golvan YJ, Ayachi K, Kchouck H (1986) Trial therapy of inoperable abdominal hydatid cysts by puncture. Ann Parasitol Hum Comp 61:689–692

Ben Salah E, Barrera C, Sakly W, Mosbahi S, Balliau T, Franche N, Gottstein B, Ben Youssef S, Mekki M, Babba H, Millon L (2022) Novel biomarkers for the early prediction of pediatric cystic echinococcosis post-surgical outcomes. J Infect 84:87–93. https://doi.org/10.1016/j.jinf.2021.09.023

Bosco A, Alves LC, Cociancic P, Amadesi A, Pepe P, Morgoglione ME, Maurelli MP, Ferrer-Miranda E, Santoro KR, Nascimento Ramos RA, Rinaldi L, Cringoli G (2021) Epidemiology and spatial distribution of Echinococcus granulosus in sheep and goats slaughtered in a hyperendemic European Mediterranean area. Parasit Vectors 14:421. https://doi.org/10.1186/s13071-021-04934-9

Böttcher D, Bangoura B, Schmäschke R, Müller K, Fischer S, Vobis V, Meiler H, Wolf G, Koller A, Kramer S, Overhoff M, Gawlowska S, Schoon H-A (2013) Diagnostics and epidemiology of alveolar echinococcosis in slaughtered pigs from large-scale husbandries in Germany. Parasitol Res 112:629–636. https://doi.org/10.1007/s00436-012-3177-2

Boubaker G, Macchiaroli N, Prada L, Cucher MA, Rosenzvit MC, Ziadinov I, Deplazes P, Saarma U, Babba H, Gottstein B, Spiliotis M (2013) A multiplex PCR for the simultaneous detection and genotyping of the *Echinococcus granulosus* complex. PLoS Negl Trop Dis 7: e2017. https://doi.org/10.1371/journal.pntd.0002017

Bresson-Hadni S, Vuitton DA, Lenys D, Liance M, Racadot E, Miguet JP (1989) Cellular immune response in Echinococcus multilocularis infection in humans. I. Lymphocyte reactivity to Echinococcus antigens in patients with alveolar echinococcosis. Clin Exp Immunol 78:61–66

Bresson-Hadni S, Vuitton DA, Bartholomot B, Heyd B, Godart D, Meyer JP, Hrusovsky S, Becker MC, Mantion G, Lenys D, Miguet JP (2000) A twenty-year history of alveolar echinococcosis: analysis of a series of 117 patients from eastern France. Eur J Gastroenterol Hepatol 12:327–336

Bresson-Hadni S, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet J-P, Mantion GA, Vuitton DA (2006) Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. Parasitol Int 55(Suppl):S267–S272. https://doi.org/10.1016/j.parint.2005.11.053

Bresson-Hadni S, Blagosklonov O, Knapp J, Grenouillet F, Sako Y, Delabrousse E, Brientini M-P, Richou C, Minello A, Antonino A-T, Gillet M, Ito A, Mantion GA, Vuitton DA (2011) Should possible recurrence of disease contraindicate liver transplantation in patients with end-stage alveolar echinococcosis? A 20-year follow-up study. Liver Transpl 17:855–865. https://doi.org/10.1002/lt.22299

Bresson-Hadni S, Montange D, Richou C, Brumpt E, Fillion A, Bartholomot B, Blagosklonov O, Delabrousse E, Grenouillet F, Vuitton D-A, Millon L (2021a) Tobacco, cannabis, and liquorice: hidden players altering albendazole metabolism in patients with hepatic alveolar echinococcosis. J Hepatol 74:471–473. https://doi.org/10.1016/j.jhep.2020.10.032

Bresson-Hadni S, Spahr L, Chappuis F (2021b) Hepatic alveolar echinococcosis. Semin Liver Dis 41:393–408. https://doi.org/10.1055/s-0041-1730925

Brumpt E, Blagosklonov O, Calame P, Bresson-Hadni S, Vuitton DA, Delabrousse E (2019) AE hepatic lesions: correlation between calcifications at CT and FDG-PET/CT metabolic activity. Infection 47:955–960. https://doi.org/10.1007/s15010-019-01328-z

Brundu D, Piseddu T, Stegel G, Masu G, Ledda S, Masala G (2014) Retrospective study of human cystic echinococcosis in Italy based on the analysis of hospital discharge records between 2001 and 2012. Acta Trop 140:91–96. https://doi.org/10.1016/j.actatropica.2014.08.011

Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 114:1–16. https://doi.org/10.1016/j.actatropica.2009.11.001

Brunetti E, Garcia HH, Junghanss T, International CE Workshop in Lima, Peru, 2009 (2011) Cystic echinococcosis: chronic, complex, and still neglected. PLoS Negl Trop Dis 5:e1146. https://doi.org/10.1371/journal.pntd.0001146

Budke CM, Deplazes P, Torgerson PR (2006) Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis 12:296–303. https://doi.org/10.3201/eid1202.050499

Buttenschoen K, Carli Buttenschoen D, Gruener B, Kern P, Beger HG, Henne-Bruns D, Reuter S (2009a) Long-term experience on surgical treatment of alveolar echinococcosis. Langenbeck's Arch Surg / Deutsche Gesellschaft Für Chirurgie 394:689–698. https://doi.org/10.1007/s00423-008-0392-5

Buttenschoen K, Gruener B, Carli Buttenschoen D, Reuter S, Henne-Bruns D, Kern P (2009b) Palliative operation for the treatment of alveolar echinococcosis. Langenbeck's Arch Surg 394: 199–204. https://doi.org/10.1007/s00423-008-0367-6

Cai H, Zhang J, Zhang X, Guan Y, Ma X, Cao J, Ma J, Liu N, Wu H, Liu Y, Liu J, Wang W, Lei W, Shi K, Zhang Q, Zhang X, Zhan P, Shen Y (2021) Prevalence of *Echinococcus* species in wild foxes and stray dogs in Qinghai Province, China. Am J Trop Med Hyg:tpmd210622. https://doi.org/10.4269/ajtmh.21-0622

Caoduro C, Porot C, Vuitton DA, Bresson-Hadni S, Grenouillet F, Richou C, Boulahdour H, Blagosklonov O (2013) The role of delayed 18F-FDG PET imaging in the follow-up of patients with alveolar echinococcosis. J Nucl Med 54:358–363. https://doi.org/10.2967/jnumed.112.109942

Casulli A, Siles-Lucas M, Cretu CM, Vutova K, Akhan O, Vural G, Cortés Ruiz A, Brunetti E, Tamarozzi F (2020) Achievements of the HERACLES project on cystic echinococcosis. Trends Parasitol 36:1–4. https://doi.org/10.1016/j.pt.2019.10.009

Catalano S, Lejeune M, Liccioli S, Verocai GG, Gesy KM, Jenkins EJ, Kutz SJ, Fuentealba C, Duignan PJ, Massolo A (2012) *Echinococcus multilocularis* in urban coyotes, Alberta, Canada. Emerg Infect Dis 18:1625–1628. https://doi.org/10.3201/eid.1810.120119

Cattaneo L, Manciulli T, Cretu C-M, Giordani MT, Angheben A, Bartoloni A, Zammarchi L, Bartalesi F, Richter J, Chiodini P, Godbole G, Junghanss T, Stojkovic M, Sammarchi L, Dore R, Vercelli A, Benazzo F, Cuzzocrea F, Tamarozzi F, Brunetti E (2019) Cystic echinococcosis of the bone: a European Multicenter study. Am J Trop Med Hyg 100:617–621. https://doi.org/10.4269/ajtmh.18-0758

Ceballos L, Elissondo C, Sánchez Bruni S, Confalonieri A, Denegri G, Alvarez L, Lanusse C (2010) Chemoprophylactic activity of flubendazole in cystic echinococcosis. Chemotherapy 56: 386–392. https://doi.org/10.1159/000316827

Ceballos L, Elissondo C, Sánchez Bruni S, Denegri G, Lanusse C, Alvarez L (2011) Comparative performances of flubendazole and albendazole in cystic echinococcosis: ex vivo activity, plasma/cyst disposition, and efficacy in infected mice. Antimicrob Agents Chemother 55: 5861–5867. https://doi.org/10.1128/AAC.05105-11

Ceballos L, Virkel G, Elissondo C, Canton C, Canevari J, Murno G, Denegri G, Lanusse C, Alvarez L (2013) A pharmacology-based comparison of the activity of albendazole and flubendazole against *Echinococcus granulosus* metacestode in sheep. Acta Trop 127:216–225. https://doi.org/10.1016/j.actatropica.2013.05.004

Chauchet A, Grenouillet F, Knapp J, Richou C, Delabrousse E, Dentan C, Millon L, Di Martino V, Contreras R, Deconinck E, Blagosklonov O, Vuitton DA, Bresson-Hadni S, FrancEchino Network (2014) Increased incidence and characteristics of alveolar echinococcosis in patients with immunosuppression-associated conditions. Clin Infect Dis 59:1095–1104. https://doi.org/10.1093/cid/ciu520

Chen X, Chen X, Shao Y, Zhao J, Li H, Wen H (2014) Clinical outcome and immune follow-up of different surgical approaches for human cyst hydatid disease in liver. Am J Trop Med Hyg 91: 801–805. https://doi.org/10.4269/ajtmh.14-0177

Chen J, Wei L, Chen T-W, Li R, Zhang X-M, Deng C-M, Zhang Y, Xiong J, Li X-Z, Li Z-H (2021) Triphase contrast-enhanced CT to evaluate indications for autologous liver transplantation in

patients with end-stage hepatic alveolar echinococcosis. Sci Rep 11:22096. https://doi.org/10.1038/s41598-021-01586-8

Colpani A, Achilova O, D'Alessandro GL, Budke CM, Mariconti M, Muratov T, Vola A, Mamedov A, Giordani MT, Urukov X, De Silvestri A, Suvonkulov U, Brunetti E, Manciulli T (2021) Trends in the surgical incidence of cystic echinococcosis in Uzbekistan from 2011 to 2018. Am J Trop Med Hyg:tpmd210261. https://doi.org/10.4269/ajtmh.21-0261

Comte S, Raton V, Raoul F, Hegglin D, Giraudoux P, Deplazes P, Favier S, Gottschek D, Umhang G, Boué F, Combes B (2013) Fox baiting against *Echinococcus multilocularis*: contrasted achievements among two medium size cities. Prev Vet Med 111:147–155. https://doi.org/10.1016/j.prevetmed.2013.03.016

Comte S, Umhang G, Raton V, Raoul F, Giraudoux P, Combes B, Boué F (2017) Echinococcus multilocularis management by fox culling: an inappropriate paradigm. Prev Vet Med 147:178–185. https://doi.org/10.1016/j.prevetmed.2017.09.010

Conraths FJ, Deplazes P (2015) Echinococcus multilocularis: epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet Parasitol 213:149–161. https://doi.org/10.1016/j.vetpar.2015.07.027

Cooney RM, Flanagan KP, Zehyle E (2004) Review of surgical management of cystic hydatid disease in a resource limited setting: Turkana, Kenya. Eur J Gastroenterol Hepatol 16:1233–1236. https://doi.org/10.1097/00042737-200411000-00024

Craig PS, Echinococcosis Working Group in China (2006) Epidemiology of human alveolar echinococcosis in China. Parasitol Int 55(Suppl):S221–S225. https://doi.org/10.1016/j.parint.2005.11.034

Craig P, Mastin A, van Kesteren F, Boufana B (2015) *Echinococcus granulosus*: epidemiology and state-of-the-art of diagnostics in animals. Vet Parasitol 213:132–148. https://doi.org/10.1016/j.vetpar.2015.07.028

Craig PS, Hegglin D, Lightowlers MW, Torgerson PR, Wang Q (2017) Echinococcosis: control and prevention. Adv Parasitol 96:55–158. https://doi.org/10.1016/bs.apar.2016.09.002

Crouzet J, Grenouillet F, Delabrousse E, Blagosklonov O, Thevenot T, Di Martino V, Piarroux R, Mantion GA, Bresson-Hadni S (2010) Personalized management of patients with inoperable alveolar echinococcosis undergoing treatment with albendazole: usefulness of positron-emission-tomography combined with serological and computed tomography follow-up. Clin Microbiol Infect 16:788–791. https://doi.org/10.1111/j.1469-0691.2009.02924.x

D'Alessandro A, Rausch RL (2008) New aspects of neotropical polycystic (*Echinococcus vogeli*) and unicystic (*Echinococcus oligarthrus*) echinococcosis. Clin Microbiol Rev 21:380–401. https://doi.org/10.1128/CMR.00050-07

da Silva AM (2019) Hydatid cyst/cystic echinococcosis: anatomical and surgical nomenclature and method to quantify the cyst content solidification. - PubMed - NCBI. https://www-ncbi-nlm-nih-gov.gate2.inist.fr/pubmed/22040484. Accessed 9 Aug 2019

Da Silva AM, Courquet S, Raoul F, Rieffel D, Giraudoux P, Millon L, Knapp J (2020) Assessment of the exposure to *Echinococcus multilocularis* associated with carnivore faeces using real-time quantitative PCR and flotation technique assays. Int J Parasitol 50:1195–1204. https://doi.org/10.1016/j.ijpara.2020.07.008

Deininger S, Wellinghausen N (2019) Evaluation of a new combined Western and line blot assay (EUROLINE-WB) for diagnosis and species identification of *Echinococcus* infection in humans. GMS Infect Dis 7:Doc01. https://doi.org/10.3205/id000041

Demir H, Halac M, Yilmaz S, Isgoren S, Sonmezoglu K, Uslu I (2008) A doughnut sign of a hydatid cyst of the liver on F-18 FDG PET/CT. Clin Nucl Med 33:876–877. https://doi.org/10.1097/RLU.0b013e31818bf2df

Deplazes P, Eckert J (2001) Veterinary aspects of alveolar echinococcosis – a zoonosis of public health significance. In: Veterinary parasitology, pp 65–87

Deplazes P, Hegglin D, Gloor S, Romig T (2004) Wilderness in the city: the urbanization of *Echinococcus multilocularis*. Trends Parasitol 20:77–84

Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, Antolova D, Schurer JM, Lahmar S, Cringoli G, Magambo J, Thompson RCA, Jenkins EJ (2017) Global distribution of alveolar and cystic echinococcosis. Adv Parasitol 95:315–493. https://doi.org/10.1016/bs.apar.2016.11.001

Duishanbai S, Geng D, Liu C, Guo H-R, Hao Y-J, Liu B, Wang Y-X, Luo K, Zhou K, Wen H, Research Group of Hydatid Diseases (2011) Treatment of intracranial hydatid cysts. Chin Med J 124:2954–2958

Dybicz M, Gierczak A, Dąbrowska J, Rdzanek Ł, Michałowicz B (2013) Molecular diagnosis of cystic echinococcosis in humans from Central Poland. Parasitol Int 62:364–367. https://doi.org/10.1016/j.parint.2013.03.005

Dziri C, Haouet K, Fingerhut A, Zaouche A (2009) Management of cystic echinococcosis complications and dissemination: where is the evidence? World J Surg 33:1266–1273. https://doi.org/10.1007/s00268-009-9982-9

Echinococcosis W-IWG on (2001) PAIR: puncture, aspiration, injection, reaspiration: an option for the treatment of cystic echinococcosis. WHO, Geneva

Eckert J, Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 17:107–135

Eckert J, Thompson RCA (2017) Historical aspects of echinococcosis. Adv Parasitol 95:1–64. https://doi.org/10.1016/bs.apar.2016.07.003

Eckert J, Deplazes P, Craig PS, Gemmell M, Gottstein B, Heath D, Jenkins D, Kamiya M, Lightowlers M (2001) Echinococcosis in animals: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (eds) WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organization for Animal Health (OIE), Paris, pp 72–99

Ehrhardt AR, Reuter S, Buck AK, Haenle MM, Mason RA, Gabelmann A, Kern P, Kratzer W (2007) Assessment of disease activity in alveolar echinococcosis: a comparison of contrast enhanced ultrasound, three-phase helical CT and [(18)F] fluorodeoxyglucose positron emission tomography. Abdom Imaging 32:730–736. https://doi.org/10.1007/s00261-006-9173-1

Feng X, Wen H, Zhang Z, Chen X, Ma X, Zhang J, Qi X, Bradshaw H, Vuitton D, Craig PS (2010) Dot immunogold filtration assay (DIGFA) with multiple native antigens for rapid serodiagnosis of human cystic and alveolar echinococcosis. Acta Trop 113:114–120. https://doi.org/10.1016/j.actatropica.2009.10.003

Fica A, Soto A, Slater J, Peralta M, Humeres R, Castro M, González J, Weitzel T (2012) Clinical presentation of hydatid disease during 15 years: a case series from Santiago, Chile. Rev Chil Infectol 29:183–191. https://doi.org/10.4067/S0716-10182012000200011

Firpo G, Vola A, Lissandrin R, Tamarozzi F, Brunetti E (2017) Preliminary evaluation of percutaneous treatment of Echinococcal cysts without injection of Scolicidal agent. Am J Trop Med Hyg 97:1818–1826. https://doi.org/10.4269/ajtmh.17-0468

Franchi C, Di Vico B, Teggi A (1999) Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. Clin Infect Dis 29:304–309. https://doi.org/10.1086/520205

Frider B, Larrieu E, Odriozola M (1999) Long-term outcome of asymptomatic liver hydatidosis. J Hepatol 30:228–231

Gargouri M, Ben Amor N, Ben Chehida F, Hammou A, Gharbi HA, Ben Cheikh M, Kchouk H, Ayachi K, Golvan JY (1990) Percutaneous treatment of hydatid cysts (*Echinococcus granulosus*). Cardiovasc Intervent Radiol 13:169–173

Gemmell MA, Johnstone PD, Oudemans G (1977) The effect of praziquantel on *Echinococcus granulosus*, *Taenia hydatigena* and *Taenia ovis* infections in dogs. Res Vet Sci 23:121–123

Georges S, Villard O, Filisetti D, Mathis A, Marcellin L, Hansmann Y, Candolfi E (2004) Usefulness of PCR analysis for diagnosis of alveolar echinococcosis with unusual localizations: two case studies. J Clin Microbiol 42:5954–5956. https://doi.org/10.1128/JCM.42.12.5954-5956.2004

Gharbi HA, Hassine W, Brauner MW, Dupuch K (1981) Ultrasound examination of the hydatic liver. Radiology 139:459–463. https://doi.org/10.1148/radiology.139.2.7220891

Ghoshal AG, Sarkar S, Saha K, Sarkar U, Kundu S, Chatterjee S, Kundu S (2012) Hydatid lung disease: an analysis of five years cumulative data from Kolkata. J Assoc Physicians India 60:12–16

Giraudoux P, Craig PS, Delattre P, Bao G, Bartholomot B, Harraga S, Quéré JP, Raoul F, Wang Y, Shi D, Vuitton DA (2003) Interactions between landscape changes and host communities can regulate Echinococcus multilocularis transmission. Parasitology 127(Suppl):S121–S131

Giraudoux P, Raoul F, Afonso E, Ziadinov I, Yang Y, Li L, Li T, Quéré J-P, Feng X, Wang Q, Wen H, Ito A, Craig PS (2013) Transmission ecosystems of *Echinococcus multilocularis* in China and Central Asia. Parasitology 140:1655–1666. https://doi.org/10.1017/S0031182013000644

Giraudoux P, Zhao Y, Afonso E, Yan H, Knapp J, Rogan MT, Shi D, Jia W, Craig PS (2019) Long-term retrospective assessment of a transmission hotspot for human alveolar echinococcosis in mid-West China. PLoS Negl Trop Dis 13:e0007701. https://doi.org/10.1371/journal.pntd.0007701

Godot V, Harraga S, Podoprigora G, Liance M, Bardonnet K, Vuitton DA (2003) IFN alpha-2a protects mice against a helminth infection of the liver and modulates immune responses. Gastroenterology 124:1441–1450

Gottstein B, Wang J, Blagosklonov O, Grenouillet F, Millon L, Vuitton DA, Müller N (2014) *Echinococcus* metacestode: in search of viability markers. Parasite 21:63. https://doi.org/10.1051/parasite/2014063

Gottstein B, Lachenmayer A, Beldi G, Wang J, Merkle B, Vu XL, Kurath U, Müller N (2019) Diagnostic and follow-up performance of serological tests for different forms/courses of alveolar echinococcosis. Food Waterborne Parasitol 16:e00055. https://doi.org/10.1016/j.fawpar.2019.e00055

Graeter T, Kratzer W, Oeztuerk S, Haenle MM, Mason RA, Hillenbrand A, Kull T, Barth TF, Kern P, Gruener B (2016) Proposal of a computed tomography classification for hepatic alveolar echinococcosis. World J Gastroenterol 22:3621–3631. https://doi.org/10.3748/wjg.v22.i13.3621

Graeter T, Bao H, Delabrousse E, Brumpt E, Shi R, Li W, Jiang Y, Schmidberger J, Kratzer W, Liu W, XUUB consortium (2020) Hepatic alveolar echinococcosis: comparative computed tomography study between two Chinese and two European centres. Food Waterborne Parasitol 19:e00082. https://doi.org/10.1016/j.fawpar.2020.e00082

Grenard P, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S (2001) Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. J Hepatol 35:367–375. https://doi.org/10.1016/s0168-8278(01)00135-0

Grenouillet F, Frider B, Alvarez Rodriguez J, Amante M, Pestalardo ML, Cazorla A, Bresson-Hadni S, Millon L (2013) Molecular diagnosis of polycystic echinococcosis due to *Echinococcus vogeli* in a Paraguayan immigrant in Argentina. J Clin Microbiol 51:3151–3153. https://doi.org/10.1128/JCM.00871-13

Grenouillet F, Umhang G, Arbez-Gindre F, Mantion G, Delabrousse E, Millon L, Boué F (2014) *Echinococcus ortleppi* infections in humans and cattle, France. Emerg Infect Dis 20:2100–2102. https://doi.org/10.3201/eid2012.140641

Gruener B, Cretu C, Brunetti E, Menezes C, Haerter G, Grobusch M, Kern P (2008) Accelerated larval growth of *Echinococcus* spp in the immunodeficient host? Am J Trop Med Hyg 79:118

Guo B, Zhang Z, Guo Y, Guo G, Wang H, Ma J, Chen R, Zheng X, Bao J, He L, Wang T, Qi W, Tian M, Wang J, Zhou C, Giraudoux P, Marston CG, McManus DP, Zhang W, Li J (2021) High endemicity of alveolar echinococcosis in Yili prefecture, Xinjiang autonomous region, the People's Republic of China: infection status in different ethnic communities and in small mammals. PLoS Negl Trop Dis 15:e0008891. https://doi.org/10.1371/journal.pntd.0008891

Harraga S, Godot V, Bresson-Hadni S, Pater C, Beurton I, Bartholomot B, Vuitton DA (1999) Clinical efficacy of and switch from T helper 2 to T helper 1 cytokine profile after interferon alpha2a monotherapy for human echinococcosis. Clin Infect Dis 29:205–206. https://doi.org/10.1086/520157

Haskologlu S, Dogu F, Gollu Bahadır G, Akyuzluer S, Ciftci E, Altun D, Keles S, Kologlu M, Ikinciogullari A (2020) An unexpected infection in loss-of-function mutations in STAT3: malignant alveolar echinococcosis in liver. Iran J Allergy Asthma Immunol 19:667–675. https://doi.org/10.18502/ijaai.v19i6.4936

He Y-B, Bai L, Jiang Y, Ji X-W, Tai Q-W, Zhao J-M, Zhang J-H, Liu W-Y, Wen H (2015) Application of a three-dimensional reconstruction technique in liver autotransplantation for end-stage hepatic alveolar echinococcosis. J Gastrointest Surg 19:1457–1465. https://doi.org/10.1007/s11605-015-2842-z

Hemphill A, Spicher M, Stadelmann B, Mueller J, Naguleswaran A, Gottstein B, Walker M (2007) Innovative chemotherapeutical treatment options for alveolar and cystic echinococcosis. Parasitology 134:1657–1670. https://doi.org/10.1017/S0031182007003198

Hemphill A, Stadelmann B, Rufener R, Spiliotis M, Boubaker G, Müller J, Müller N, Gorgas D, Gottstein B (2014) Treatment of echinococcosis: albendazole and mebendazole – what else? Parasite 21:70. https://doi.org/10.1051/parasite/2014073

Herzig M, Maksimov P, Staubach C, Romig T, Knapp J, Gottstein B, Conraths FJ (2021) Red foxes harbor two genetically distinct, spatially separated *Echinococcus multilocularis* clusters in Brandenburg, Germany. Parasit Vectors 14:535. https://doi.org/10.1186/s13071-021-05038-0

Hira PR, Shweiki H, Lindberg LG, Shaheen Y, Francis I, Leven H, Behbehani K (1988) Diagnosis of cystic hydatid disease: role of aspiration cytology. Lancet 2:655–657. https://doi.org/10.1016/s0140-6736(88)90470-9

Horton J (2003) Albendazole for the treatment of echinococcosis. Fundam Clin Pharmacol 17:205–212

Hosch W, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE (2008) Metabolic viability assessment of cystic echinococcosis using high-field 1H MRS of cyst contents. NMR Biomed 21:734–754. https://doi.org/10.1002/nbm.1252

Huang L, Huang Y, Wang Q, Xiao N, Yi D, Yu W, Qiu D (2011) An agent-based model for control strategies of *Echinococcus granulosus*. Vet Parasitol 179:84–91. https://doi.org/10.1016/j.vetpar.2011.01.047

Hüttner M, Nakao M, Wassermann T, Siefert L, Boomker JDF, Dinkel A, Sako Y, Mackenstedt U, Romig T, Ito A (2008) Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. Int J Parasitol 38:861–868. https://doi.org/10.1016/j.ijpara.2007.10.013

Hwang R, Liou P, Kato T (2018) Ex vivo liver resection and autotransplantation: an emerging option in selected indications. J Hepatol 69:1002–1003. https://doi.org/10.1016/j.jhep.2018.09.005

Ishizu H, Uchino J, Sato N, Aoki S, Suzuki K, Kuribayashi H (1997) Effect of albendazole on recurrent and residual alveolar echinococcosis of the liver after surgery. Hepatology 25:528–531. https://doi.org/10.1002/hep.510250305

Ito A, Craig PS (2003) Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. Trends Parasitol 19:377–381. https://doi.org/10.1016/s1471-4922(03)00200-9

Jebbawi F, Bellanger A-P, Lunström-Stadelmann B, Rufener R, Dosch M, Goepfert C, Gottstein B, Millon L, Grandgirard D, Leib SL, Beldi G, Wang J (2021) Innate and adaptive immune responses following PD-L1 blockade in treating chronic murine alveolar echinococcosis. Parasite Immunol 43:e12834. https://doi.org/10.1111/pim.12834

Jerraya H, Khalfallah M, Osman SB, Nouira R, Dziri C (2015) Predictive factors of recurrence after surgical treatment for liver hydatid cyst. Surg Endosc 29:86–93. https://doi.org/10.1007/s00464-014-3637-0

Johansen MV, Penrith M-L (2009) Has culling been properly assessed as a valid and justified control intervention measure for zoonotic diseases? PLoS Negl Trop Dis 3:e541. https://doi.org/10.1371/journal.pntd.0000541

Julien C, Le Treut YP, Bourgouin S, Palen A, Hardwigsen J (2021) Closed cyst resection for liver hydatid disease: a new standard. J Gastrointest Surg 25:436–446. https://doi.org/10.1007/s11605-019-04509-1

Junghanss T, da Silva AM, Horton J, Chiodini PL, Brunetti E (2008) Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. Am J Trop Med Hyg 79:301–311

Kadry Z, Renner EC, Bachmann LM, Attigah N, Renner EL, Ammann RW, Clavien P-A (2005) Evaluation of treatment and long-term follow-up in patients with hepatic alveolar echinococcosis. Br J Surg 92:1110–1116. https://doi.org/10.1002/bjs.4998

Kantarci M, Bayraktutan U, Karabulut N, Aydinli B, Ogul H, Yuce I, Calik M, Eren S, Atamanalp SS, Oto A (2012) Alveolar echinococcosis: spectrum of findings at cross-sectional imaging. Radiographics 32:2053–2070. https://doi.org/10.1148/rg.327125708

Karpstein T, Chaudhry S, Bresson-Hadni S, Hayoz M, Boubaker G, Hemphill A, Rufener R, Kaethner M, Schindler I, Aebi Y, Cunha AS, Largiadèr CR, Lundström-Stadelmann B (2021) Maca against echinococcosis?-a reverse approach from patient to in vitro testing. Pathogens 10: 1335. https://doi.org/10.3390/pathogens10101335

Kern P (2006) Medical treatment of echinococcosis under the guidance of good clinical practice (GCP/ICH). Parasitol Int 55(Suppl):S273–S282. https://doi.org/10.1016/j.parint.2005.11.040

Kern P, Wen H, Sato N, Vuitton DA, Gruener B, Shao Y, Delabrousse E, Kratzer W, Bresson-Hadni S (2006) WHO classification of alveolar echinococcosis: principles and application. Parasitol Int 55(Suppl):S283–S287. https://doi.org/10.1016/j.parint.2005.11.041

Kern PA, Abboud P, Kern W, Stich A, Bresson-Hadni S, Guérin B (2008) Critical appraisal of nitazoxanide for the treatment of alveolar echinococcosis. Am J Trop Med Hyg 79(6):125

Kern P, Menezes da Silva A, Akhan O, Müllhaupt B, Vizcaychipi KA, Budke C, Vuitton DA (2017) The Echinococcoses: diagnosis, clinical management and burden of disease. Adv Parasitol 96: 259–369. https://doi.org/10.1016/bs.apar.2016.09.006

Kilic M, Yoldas O, Koc M, Keskek M, Karakose N, Ertan T, Gocmen E, Tez M (2008) Can biliary-cyst communication be predicted before surgery for hepatic hydatid disease: does size matter? Am J Surg 196:732–735. https://doi.org/10.1016/j.amjsurg.2007.07.034

Knapp J, Bart JM, Maillard S, Gottstein B, Piarroux R (2010) The genomic *Echinococcus* microsatellite EmsB sequences: from a molecular marker to the epidemiological tool. Parasitology 137:439–449. https://doi.org/10.1017/S0031182009991612

Knapp J, Millon L, Mouzon L, Umhang G, Raoul F, Ali ZS, Combes B, Comte S, Gbaguidi-Haore H, Grenouillet F, Giraudoux P (2014) Real time PCR to detect the environmental faecal contamination by *Echinococcus multilocularis* from red fox stools. Vet Parasitol 201:40–47. https://doi.org/10.1016/j.vetpar.2013.12.023

Knapp J, Giraudoux P, Combes B, Umhang G, Boué F, Said-Ali Z, Aknouche S, Garcia C, Vacheyrou M, Laboissière A, Raton V, Comte S, Favier S, Demerson J-M, Caillot C, Millon L, Raoul F (2018) Rural and urban distribution of wild and domestic carnivore stools in the context of *Echinococcus multilocularis* environmental exposure. Int J Parasitol 48: 937–946. https://doi.org/10.1016/j.ijpara.2018.05.007

Knapp J, Gottstein B, Bretagne S, Bart J-M, Umhang G, Richou C, Bresson-Hadni S, Millon L (2020) Genotyping *Echinococcus multilocularis* in human alveolar echinococcosis patients: an EmsB microsatellite analysis. Pathogens 9:E282. https://doi.org/10.3390/pathogens9040282

Knapp J, Da Silva AM, Courquet S, Millon L (2021) Assessment of the genetic diversity of *Echinococcus multilocularis* from Copro-isolated eggs. Pathogens 10:1296. https://doi.org/10.3390/pathogens10101296

Knapp J, Lallemand S, Monnien F, Felix S, Valmary-Degano S, Courquet S, Demonmerot F, Heyd B, Turco C, Doussot A, Bourgeois L, Bresson-Hadni S, Richou C, Millon L (2022) Molecular diagnosis of alveolar echinococcosis in patients based on frozen and formalin-fixed paraffin-embedded tissue samples. Parasite 29:4. https://doi.org/10.1051/parasite/2022004. Epub 2022 Feb 2. PMID: 35113014; PMCID: PMC8812296

Koch S, Bresson-Hadni S, Miguet J-P, Crumbach J-P, Gillet M, Mantion G-A, Heyd B, Vuitton D-A, Minello A, Kurtz S, European Collaborating Clinicians (2003) Experience of liver transplantation for incurable alveolar echinococcosis: a 45-case European collaborative report. Transplantation 75:856–863. https://doi.org/10.1097/01.TP.0000054230.63568.79

Kratzer W, Gruener B, Kaltenbach TEM, Ansari-Bitzenberger S, Kern P, Fuchs M, Mason RA, Barth TFE, Haenle MM, Hillenbrand A, Oeztuerk S, Graeter T (2015) Proposal of an ultrasonographic classification for hepatic alveolar echinococcosis: echinococcosis multilocularis Ulm classification-ultrasound. World J Gastroenterol 21:12392–12402. https://doi.org/10.3748/wjg.v21.i43.12392

Kurt Y, Sücüllü I, Filiz AI, Urhan M, Akin ML (2008) Pulmonary echinococcosis mimicking multiple lung metastasis of breast cancer: the role of fluoro-deoxy-glucose positron emission tomography. World J Surg Oncol 6:7. https://doi.org/10.1186/1477-7819-6-7

La X, Zhang F, Li Y, Li J, Guo Y, Zhao H, Pang N, Ma X, Wen H, Fan H, Ding J (2015) Upregulation of PD-1 on $CD4^+CD25^+$ T cells is associated with immunosuppression in liver of mice infected with Echinococcus multilocularis. Int Immunopharmacol 26:357–366. https://doi.org/10.1016/j.intimp.2015.04.013

Lachenmayer A, Gebbers D, Gottstein B, Candinas D, Beldi G (2019) Elevated incidence of alveolar echinococcosis in immunocompromised patients. Food Waterborne Parasitol 16: e00060. https://doi.org/10.1016/j.fawpar.2019.e00060

Larrieu E, Uchiumi L, Salvitti JC, Sobrino M, Panomarenko O, Tissot H, Mercapide CH, Sustercic J, Arezo M, Mujica G, Herrero E, Labanchi JL, Grizmado C, Araya D, Talmon G, Galvan JM, Sepulveda L, Seleiman M, Cornejo T, Echenique H, Del Carpio M (2019) Epidemiology, diagnosis, treatment and follow-up of cystic echinococcosis in asymptomatic carriers. Trans R Soc Trop Med Hyg 113:74–80. https://doi.org/10.1093/trstmh/try112

Li J, Wu C, Wang H, Liu H, Vuitton DA, Wen H, Zhang W (2014) Boiling sheep liver or lung for 30 minutes is necessary and sufficient to kill *Echinococcus granulosus* protoscoleces in hydatid cysts. Parasite 21:64. https://doi.org/10.1051/parasite/2014064. Epub 2014 Dec 2. PMID: 25456565; PMCID: PMC4251422

Li Y-P, Ma Z-G, Tuxun T, Li Z-D, Meng Y, Chen X (2020) The application of laparoscopy combined with indocyanine green fluorescence imaging technique for hepatic cystic echinococcosis. BMC Surg 20:249. https://doi.org/10.1186/s12893-020-00911-8

Liance M, Janin V, Bresson-Hadni S, Vuitton DA, Houin R, Piarroux R (2000) Immunodiagnosis of *Echinococcus* infections: confirmatory testing and species differentiation by a new commercial Western Blot. J Clin Microbiol 38:3718–3721. https://doi.org/10.1128/JCM.38.10.3718-3721.2000

Liccioli S, Giraudoux P, Deplazes P, Massolo A (2015) Wilderness in the "city" revisited: different urbes shape transmission of Echinococcus multilocularis by altering predator and prey communities. Trends Parasitol 31:297–305. https://doi.org/10.1016/j.pt.2015.04.007

Lightowlers MW, Gasser RB, Hemphill A, Romig T, Tamarozzi F, Deplazes P, Torgerson PR, Garcia HH, Kern P (2021) Advances in the treatment, diagnosis, control and scientific understanding of taeniid cestode parasite infections over the past 50 years. Int J Parasitol 51: 1167–1192. https://doi.org/10.1016/j.ijpara.2021.10.003

Lissandrin R, Tamarozzi F, Piccoli L, Tinelli C, De Silvestri A, Mariconti M, Meroni V, Genco F, Brunetti E (2016) Factors influencing the serological response in hepatic *Echinococcus granulosus* infection. Am J Trop Med Hyg 94:166–171. https://doi.org/10.4269/ajtmh.15-0219

Lissandrin R, Tamarozzi F, Mariconti M, Manciulli T, Brunetti E, Vola A (2018) Watch and wait approach for inactive Echinococcal cyst of the liver: an update. Am J Trop Med Hyg 99: 375–379. https://doi.org/10.4269/ajtmh.18-0164

Liu W, Delabrousse É, Blagosklonov O, Wang J, Zeng H, Jiang Y, Wang J, Qin Y, Vuitton DA, Wen H (2014) Innovation in hepatic alveolar echinococcosis imaging: best use of old tools, and necessary evaluation of new ones. Parasite 21:74. https://doi.org/10.1051/parasite/2014072

Luong LT, Chambers JL, Moizis A, Stock TM, St Clair CC (2018) Helminth parasites and zoonotic risk associated with urban coyotes (*Canis latrans*) in Alberta. Canada J Helminthol 94:e25. https://doi.org/10.1017/S0022149X1800113X

Lv H, Jiang Y, Peng X, Zhang S, Wu X, Yang H, Zhang H (2015) Subadventitial cystectomy in the management of biliary fistula with liver hydatid disease. Acta Trop 141:223–228. https://doi.org/10.1016/j.actatropica.2014.06.006

Lymbery AJ (2017) Phylogenetic pattern, evolutionary processes and species delimitation in the genus Echinococcus. Adv Parasitol 95:111–145. https://doi.org/10.1016/bs.apar.2016.07.002

Macpherson CNL, Bartholomot B, Frider B (2003) Application of ultrasound in diagnosis, treatment, epidemiology, public health and control of *Echinococcus granulosus* and *E. multilocularis*. Parasitology 127(Suppl):S21–S35

Magistri P, Pecchi A, Franceschini E, Pesi B, Guadagni S, Catellani B, Assirati G, Guidetti C, Guerrini GP, Tarantino G, Ballarin R, Codeluppi M, Morelli L, Coratti A, Di Benedetto F (2019) Not just minor resections: robotic approach for cystic echinococcosis of the liver. Infection 47: 973–979. https://doi.org/10.1007/s15010-019-01333-2

Maitiseyiti A, Ma Z, Meng Y, Tian G, Kalifu B, Lu S, Chen X (2020) Mid-term outcomes of laparoscopic Total cystectomy versus open surgery for complicated liver hydatid cysts. Surg Laparosc Endosc Percutan Tech 31:44–50. https://doi.org/10.1097/SLE.0000000000000822

Malik AA, Bari SU, Amin R, Jan M (2010) Surgical management of complicated hydatid cysts of the liver. World J Gastrointest Surg 2:78–84. https://doi.org/10.4240/wjgs.v2.i3.78

Manciulli T, Vola A, Mariconti M, Lissandrin R, Maestri M, Budke CM, Tamarozzi F, Brunetti E (2018) Shortage of Albendazole and its consequences for patients with cystic echinococcosis treated at a referral Center in Italy. Am J Trop Med Hyg 99:1006–1010. https://doi.org/10.4269/ajtmh.18-0245

Marcinkutė A, Šarkūnas M, Moks E, Saarma U, Jokelainen P, Bagrade G, Laivacuma S, Strupas K, Sokolovas V, Deplazes P (2015) *Echinococcus* infections in the Baltic region. Vet Parasitol 213:121–131. https://doi.org/10.1016/j.vetpar.2015.07.032

Mariconti M, Vola A, Manciulli T, Genco F, Lissandrin R, Meroni V, Rosenzvit M, Tamarozzi F, Brunetti E (2019) Role of microRNAs in host defense against *Echinococcus granulosus* infection: a preliminary assessment. Immunol Res 67:93–97. https://doi.org/10.1007/s12026-018-9041-4

Massolo A, Liccioli S, Budke C, Klein C (2014) *Echinococcus multilocularis* in North America: the great unknown. Parasite 21:73. https://doi.org/10.1051/parasite/2014069

Massolo A, Klein C, Kowalewska-Grochowska K, Belga S, MacDonald C, Vaughan S, Girgis S, Giunchi D, Bramer SA, Santa MA, Grant DM, Mori K, Duignan P, Slater O, Gottstein B, Müller N, Houston S (2019) European *Echinococcus multilocularis* identified in patients in Canada. N Engl J Med 381:384–385. https://doi.org/10.1056/NEJMc1814975

McManus DP (2013) Current status of the genetics and molecular taxonomy of *Echinococcus* species. Parasitology 140:1617–1623. https://doi.org/10.1017/S0031182013000802

Mohamed AE, Yasawy MI, Al Karawi MA (1998) Combined albendazole and praziquantel versus albendazole alone in the treatment of hydatid disease. Hepato-Gastroenterology 45:1690–1694

Mohkam K, Belkhir L, Wallon M, Darnis B, Peyron F, Ducerf C, Gigot J-F, Mabrut J-Y (2014) Surgical management of liver hydatid disease: subadventitial cystectomy versus resection of the protruding dome. World J Surg 38:2113–2121. https://doi.org/10.1007/s00268-014-2509-z

Monge-Maillo B, Chamorro Tojeiro S, López-Vélez R (2017) Management of osseous cystic echinococcosis. Expert Rev Anti-Infect Ther 15:1075–1082. https://doi.org/10.1080/14787210.2017.1401466

Monge-Maillo B, Olmedo Samperio M, Pérez-Molina JA, Norman F, Mejía CR, Tojeiro SC, López-Vélez R (2019) Osseous cystic echinococcosis: a case series study at a referral unit in Spain. PLoS Negl Trop Dis 13:e0007006. https://doi.org/10.1371/journal.pntd.0007006

Mori K, Liccioli S, Marceau D, Massolo A (2019) A community analysis approach to parasite transmission in multi-host systems: assemblages of small mammal prey and *Echinococcus multilocularis* in an urban area in North America. Int J Parasitol Parasites Wildl 9:49–55. https://doi.org/10.1016/j.ijppaw.2019.03.012

Mustapayeva A, Manciulli T, Zholdybay Z, Juskiewicz K, Zhakenova Z, Shapiyeva Z, Medetov Z, Vola A, Mariconti M, Brunetti E, Budke CM, Khalykova M, Duisenova A (2020) Incidence rates of surgically managed cystic echinococcosis in Kazakhstan, 2007–2016. Am J Trop Med Hyg 102:90–95. https://doi.org/10.4269/ajtmh.19-0572

Mustapayeva A, Luca D'Alessandro G, Doszhanova G, Colpani A, Sadybekov N, Baimakhanov Z, Assanov E, Salybekov S, Kaniyev S, Serikuly E, Tagabayeva L, Budke CM, Vola A, Mariconti M, De Silvestri A, Yalisheva S, Sadykova A, Zholdybay Z, Katarbayev A, Zhakenova Z, Brunetti E, Juszkiewicz K, Duisenova A, Manciulli T (2021) Ultrasound-based evaluation of the prevalence of abdominal cystic echinococcosis in the Turkestan region of Kazakhstan. Trans R Soc Trop Med Hyg:trab105. https://doi.org/10.1093/trstmh/trab105

Nakao M, McManus DP, Schantz PM, Craig PS, Ito A (2007) A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. Parasitology 134:713–722. https://doi.org/10.1017/S0031182006001934

Nakao M, Lavikainen A, Yanagida T, Ito A (2013) Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). Int J Parasitol 43:1017–1029. https://doi.org/10.1016/j.ijpara.2013.06.002

Nakao M, Lavikainen A, Hoberg E (2015) Is *Echinococcus intermedius* a valid species? Trends Parasitol 31:342–343. https://doi.org/10.1016/j.pt.2015.04.012

Neumayr A, Troia G, de Bernardis C, Tamarozzi F, Goblirsch S, Piccoli L, Hatz C, Filice C, Brunetti E (2011) Justified concern or exaggerated fear: the risk of anaphylaxis in percutaneous treatment of cystic echinococcosis-a systematic literature review. PLoS Negl Trop Dis 5:e1154. https://doi.org/10.1371/journal.pntd.0001154

Ocak S, Poyanlı A, Güllüoğu M, İbiş C, Tekant Y, Özden İ (2021) Dramatic response to albendazole in transplantation candidates with unresectable hepatic alveolar hydatid disease. Clin Case Rep 9:e04666. https://doi.org/10.1002/ccr3.4666

Otero-Abad B, Torgerson PR (2013) A systematic review of the epidemiology of echinococcosis in domestic and wild animals. PLoS Negl Trop Dis 7:e2249. https://doi.org/10.1371/journal.pntd.0002249

Paternoster G, Boo G, Wang C, Minbaeva G, Usubalieva J, Raimkulov KM, Zhoroev A, Abdykerimov KK, Kronenberg PA, M onenber B, Furrer R, Deplazes P, Torgerson PR (2020) Epidemic cystic and alveolar echinococcosis in Kyrgyzstan: an analysis of national surveillance data. Lancet Glob Health 8:e603–e611. https://doi.org/10.1016/S2214-109X(20)30038-3

Pawlowski Z, Eckert J, Vuitton D, Ammann R, Kern P, Craig P, Dar K, De Rosa F, Filice C, Gottstein B, Grimm F, MacPherson C, Sato N, Todorov T, Uchino J, von Sinner W, Wen H (2001) Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (eds) WHO/OIE manual on Echinococcosis in humans and animals: a public health problem of global concern. World Organization for Animal Health (OIE), Paris, pp 20–72

Peng X, Zhang S, Niu J (2002) Total subadventitial cystectomy for the treatment of 30 patients with hepatic hydatid cysts. Chin J General Surg (Chin) 17:529–530

Peng X, Li J, Wu X, Zhang S, Niu J, Chen X, Yao J, Sun H (2006) Detection of Osteopontin in the pericyst of human hepatic Echinococcus granulosus. Acta Trop 100:163–171. https://doi.org/10.1016/j.actatropica.2006.08.013

Petrone L, Vanini V, Petruccioli E, Ettorre GM, Busi Rizzi E, Schininà V, Girardi E, Ludovisi A, Gómez-Morales MÁ, Pozio E, Teggi A, Goletti D (2015) IL-4 specific-response in whole blood associates with human cystic echinococcosis and cyst activity. J Infect 70:299–306. https://doi.org/10.1016/j.jinf.2014.10.009

Petrone L, Tamarozzi F, Vola A, Gomez Morales MA, Ludovisi A, Najafi Fard S, Mariconti M, Brunetti E, Goletti D (2021) Accuracy of an experimental whole-blood test for detecting reactivation of echinococcal cysts. PLoS Negl Trop Dis 15:e0009648. https://doi.org/10.1371/journal.pntd.0009648

Piarroux M, Piarroux R, Giorgi R, Knapp J, Bardonnet K, Sudre B, Watelet J, Dumortier J, Gérard A, Beytout J, Abergel A, Mantion G, Vuitton DA, Bresson-Hadni S (2011) Clinical features and evolution of alveolar echinococcosis in France from 1982 to 2007: results of a survey in 387 patients. J Hepatol 55:1025–1033. https://doi.org/10.1016/j.jhep.2011.02.018

Qin Y, Li X, Zhang Q, Xie B, Ji X, Li Y, Yiblayan A, Wen H (2016) Analysis of the clinical value of (18)F-FDG PET/CT in hepatic alveolar echinococcosis before and after autologous liver transplantation. Exp Ther Med 11:43–48. https://doi.org/10.3892/etm.2015.2857

Qiu Y, Yang X, Wang T, Shen S, Yang Y, Huang B, Wang W (2021) Learning curve of ex vivo liver resection and autotransplantation in treating end-stage hepatic alveolar echinococcosis: a RA-CUSUM analysis. Front Surg 8:753968. https://doi.org/10.3389/fsurg.2021.753968

Ran B, Wang M, Jian W, Jiang T, Zhang R, Guo Q, Zhang W, Wen H, Shao Y, Aji T (2019) Simultaneous occurrence of hepatic alveolar and cystic echinococcosis. J Helminthol 94:e80. https://doi.org/10.1017/S0022149X19000385

Rausch RL, Wilson JF, Schantz PM (1990) A programme to reduce the risk of infection by Echinococcus multilocularis: the use of praziquantel to control the cestode in a village in the hyperendemic region of Alaska. Ann Trop Med Parasitol 84:239–250. https://doi.org/10.1080/00034983.1990.11812463

Rehmann P, Gröne A, Gottstein B, Völlm J, Sager H, Janovsky M, Bacciarini LN (2005) Detection of *Echinococcus multilocularis* infection in a colony of cynomolgus monkeys (*Macaca fascicularis*) using serology and ultrasonography. J Vet Diagn Investig 17:183–186

Reuter S, Jensen B, Buttenschoen K, Kratzer W, Kern P (2000) Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. J Antimicrob Chemother 46:451–456

Reuter S, Buck A, Grebe O, Nüssle-Kügele K, Kern P, Manfras BJ (2003) Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. Antimicrob Agents Chemother 47:3586–3591. https://doi.org/10.1128/AAC.47.11.3586-3591.2003

Reuter S, Buck A, Manfras B, Kratzer W, Seitz HM, Darge K, Reske SN, Kern P (2004) Structured treatment interruption in patients with alveolar echinococcosis. Hepatology 39:509–517. https://doi.org/10.1002/hep.20078

Reuter S, Manfras B, Merkle M, Härter G, Kern P (2006) In vitro activities of itraconazole, methiazole, and nitazoxanide versus *Echinococcus multilocularis* larvae. Antimicrob Agents Chemother 50:2966–2970. https://doi.org/10.1128/AAC.00476-06

Ricard-Blum S, Bresson-Hadni S, Vuitton DA, Ville G, Grimaud JA (1992) Hydroxypyridinium collagen cross-links in human liver fibrosis: study of alveolar echinococcosis. Hepatology 15: 599–602. https://doi.org/10.1002/hep.1840150408

Ricard-Blum S, Bresson-Hadni S, Guerret S, Grenard P, Volle PJ, Risteli L, Grimaud JA, Vuitton DA (1996) Mechanism of collagen network stabilization in human irreversible granulomatous liver fibrosis. Gastroenterology 111:172–182. https://doi.org/10.1053/gast.1996.v111.pm8698196

Richards KS, Riley EM, Taylor DH, Morris DL (1988) Studies on the effect of short term, high dose praziquantel treatment against protoscoleces of ovine and equine *Echinococcus granulosus* within the cyst, and in vitro. Trop Med Parasitol 39:269–272

Rinaldi F, Brunetti E, Neumayr A, Maestri M, Goblirsch S, Tamarozzi F (2014) Cystic echinococcosis of the liver: a primer for hepatologists. World J Hepatol 6:293–305. https://doi.org/10.4254/wjh.v6.i5.293

Robardet E, Giraudoux P, Caillot C, Boue F, Cliquet F, Augot D, Barrat J (2008) Infection of foxes by *Echinococcocus multilocularis* in urban and suburban areas of Nancy, France: influence of feeding habits and environment. Parasite 15:77–85. https://doi.org/10.1051/parasite/2008151077

Robardet E, Giraudoux P, Caillot C, Augot D, Boue F, Barrat J (2011) Fox defecation behaviour in relation to spatial distribution of voles in an urbanised area: an increasing risk of transmission of *Echinococcus multilocularis*? Int J Parasitol 41:145–154. https://doi.org/10.1016/j.ijpara.2010.08.007

Roberts MG, Aubert MF (1995) A model for the control of *Echinococcus multilocularis* in France. Vet Parasitol 56:67–74. https://doi.org/10.1016/0304-4017(94)00655-v

Roelfsema JH, Nozari N, Pinelli E, Kortbeek LM (2016) Novel PCRs for differential diagnosis of cestodes. Exp Parasitol 161:20–26. https://doi.org/10.1016/j.exppara.2015.12.010

Rogan MT, Hai WY, Richardson R, Zeyhle E, Craig PS (2006) Hydatid cysts: does every picture tell a story? Trends Parasitol 22:431–438. https://doi.org/10.1016/j.pt.2006.07.003

Romig T, Dinkel A, Mackenstedt U (2006) The present situation of echinococcosis in Europe. Parasitol Int 55(Suppl):S187–S191. https://doi.org/10.1016/j.parint.2005.11.028

Romig T, Deplazes P, Jenkins D, Giraudoux P, Massolo A, Craig PS, Wassermann M, Takahashi K, de la Rue M (2017) Ecology and life cycle patterns of *Echinococcus* species. Adv Parasitol 95: 213–314. https://doi.org/10.1016/bs.apar.2016.11.002

Sailer M, Soelder B, Allerberger F, Zaknun D, Feichtinger H, Gottstein B (1997) Alveolar echinococcosis of the liver in a six-year-old girl with acquired immunodeficiency syndrome. J Pediatr 130:320–323

Sato N, Namieno T, Furuya K, Takahashi H, Yamashita K, Uchino J, Suzuki K (1997) Contribution of mass screening system to resectability of hepatic lesions involving *Echinococcus multilocularis*. J Gastroenterol 32:351–354

Scharf G, Deplazes P, Kaser-Hotz B, Borer L, Hasler A, Haller M, Flückiger M (2004) Radiographic, ultrasonographic, and computed tomographic appearance of alveolar echinococcosis in dogs. Vet Radiol Ultrasound 45:411–418

Schipper HG, Laméris JS, van Delden OM, Rauws EA, Kager PA (2002) Percutaneous evacuation (PEVAC) of multivesicular echinococcal cysts with or without cystobiliary fistulas which

contain non-drainable material: first results of a modified PAIR method. Gut 50:718–723. https://doi.org/10.1136/gut.50.5.718

Schmidberger J, Steinbach J, Schlingeloff P, Kratzer W, Grüner B (2019) Surgery versus conservative drug therapy in alveolar echinococcosis patients in Germany – a health-related quality of life comparison. Food Waterborne Parasitol 16:e00057. https://doi.org/10.1016/j.fawpar.2019.e00057

Schneider R, Gollackner B, Edel B, Schmid K, Wrba F, Tucek G, Walochnik J, Auer H (2008) Development of a new PCR protocol for the detection of species and genotypes (strains) of *Echinococcus* in formalin-fixed, paraffin-embedded tissues. Int J Parasitol 38:1065–1071. https://doi.org/10.1016/j.ijpara.2007.11.008

Shen S, Kong J, Qiu Y, Zhang S, Qin Y, Wang W (2019) Ex vivo liver resection and auto-transplantation versus allotransplantation for end-stage hepatic alveolar echinococcosis. Int J Infect Dis 79:87–93. https://doi.org/10.1016/j.ijid.2018.11.016

Siles-Lucas M, Casulli A, Conraths FJ, Müller N (2017) Laboratory diagnosis of *Echinococcus* spp. in human patients and infected animals. Adv Parasitol 96:159–257. https://doi.org/10.1016/bs.apar.2016.09.003

Siles-Lucas M, Casulli A, Cirilli R, Carmena D (2018) Progress in the pharmacological treatment of human cystic and alveolar echinococcosis: compounds and therapeutic targets. PLoS Negl Trop Dis 12:e0006422. https://doi.org/10.1371/journal.pntd.0006422

Simsek S, Kaplan M, Ozercan IH (2011) A comprehensive molecular survey of *Echinococcus granulosus* in formalin-fixed paraffin-embedded tissues in human isolates in Turkey. Parasitol Res 109:411–416. https://doi.org/10.1007/s00436-011-2269-8

Skuhala T, Trkulja V, Runje M, Vukelic D, Desnica B (2014) Albendazolesulphoxide concentrations in plasma and hydatid cyst and prediction of parasitological and clinical outcomes in patients with liver hydatidosis caused by *Echinococcus granulosus*. Croat Med J 55:146–155. https://doi.org/10.3325/cmj.2014.55.146

Smego RA, Bhatti S, Khaliq AA, Beg MA (2003) Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. Clin Infect Dis 37:1073–1083. https://doi.org/10.1086/378275

Sokouti M, Sadeghi R, Pashazadeh S, Abadi SEH, Sokouti M, Rezaei-Hachesu P, Ghojazadeh M, Sokouti B (2017) A systematic review and meta-analysis on the treatment of liver hydatid cyst: comparing laparoscopic and open surgeries. Arab J Gastroenterol 18:127–135. https://doi.org/10.1016/j.ajg.2017.09.010

Sokouti M, Sadeghi R, Pashazadeh S, Abadi SEH, Sokouti M, Ghojazadeh M, Sokouti B (2019) A systematic review and meta-analysis on the treatment of liver hydatid cyst using meta-MUMS tool: comparing PAIR and laparoscopic procedures. Arch Med Sci 15:284–308. https://doi.org/10.5114/aoms.2018.73344

Solomon N, Kachani M, Zeyhle E, Macpherson CNL (2017) The natural history of cystic echinococcosis in untreated and albendazole-treated patients. Acta Trop 171:52–57. https://doi.org/10.1016/j.actatropica.2017.03.018

Staebler S, Steinmetz H, Keller S, Deplazes P (2007) First description of natural *Echinococcus multilocularis* infections in chinchilla (*Chinchilla laniger*) and Prevost's squirrel (*Callosciurus prevostii borneoensis*). Parasitol Res 101:1725–1727. https://doi.org/10.1007/s00436-007-0717-2

Stefanić S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR, Mathis A (2004) Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. Parasitol Res 92:347–351. https://doi.org/10.1007/s00436-003-1043-y

Stettler M, Rossignol JF, Fink R, Walker M, Gottstein B, Merli M, Theurillat R, Thormann W, Dricot E, Segers R, Hemphill A (2004) Secondary and primary murine alveolar echinococcosis: combined albendazole/nitazoxanide chemotherapy exhibits profound anti-parasitic activity. Int J Parasitol 34:615–624. https://doi.org/10.1016/j.ijpara.2004.01.006

Stojkovic M, Rosenberger K, Kauczor H-U, Junghanss T, Hosch W (2012) Diagnosing and staging of cystic echinococcosis: how do CT and MRI perform in comparison to ultrasound? PLoS Negl Trop Dis 6:e1880. https://doi.org/10.1371/journal.pntd.0001880

Stojkovic M, Rosenberger KD, Steudle F, Junghanss T (2016) Watch and wait Management of Inactive Cystic Echinococcosis - does the path to inactivity matter – analysis of a prospective patient cohort. PLoS Negl Trop Dis 10:e0005243. https://doi.org/10.1371/journal.pntd.0005243

Stojkovic M, Adt H-M, Rosenberger K, Boubaker G, Hernandez-Gonzalez A, Junghanss T, Zwahlen M, Siles-Lucas M (2017) Follow-up of surgically treated patients with cystic echinococcosis: can novel recombinant antigens compete with imaging? Analysis of a patient cohort. Tropical Med Int Health 22:614–621. https://doi.org/10.1111/tmi.12859

Stojković M, Weber TF, Junghanss T (2018) Clinical management of cystic echinococcosis: state of the art and perspectives. Curr Opin Infect Dis 31:383–392. https://doi.org/10.1097/QCO.0000000000000485

Tamarozzi F, Sako Y, Ito A, Piccoli L, Grisolìa A, Itoh S, Gatti S, Meroni V, Genco F, Brunetti E (2013) Recombinant AgB8/1 ELISA test vs. commercially available IgG ELISA test in the diagnosis of cystic echinococcosis. Parasite Immunol 35:433–440. https://doi.org/10.1111/pim.12050

Tamarozzi F, Vuitton L, Brunetti E, Vuitton DA, Koch S (2014) Non-surgical and non-chemical attempts to treat echinococcosis: do they work? Parasite 21:75. https://doi.org/10.1051/parasite/2014071

Tamarozzi F, Horton J, Muhtarov M, Ramharter M, Siles-Lucas M, Gruener B, Vuitton DA, Bresson-Hadni S, Manciulli T, Brunetti E (2020a) A case for adoption of continuous albendazole treatment regimen for human echinococcal infections. PLoS Negl Trop Dis 14:e0008566. https://doi.org/10.1371/journal.pntd.0008566

Tamarozzi F, Legnardi M, Fittipaldo A, Drigo M, Cassini R (2020b) Epidemiological distribution of *Echinococcus granulosus* s.l. infection in human and domestic animal hosts in European Mediterranean and Balkan countries: a systematic review. PLoS Negl Trop Dis 14:e0008519. https://doi.org/10.1371/journal.pntd.0008519

Tamarozzi F, Silva R, Fittipaldo VA, Buonfrate D, Gottstein B, Siles-Lucas M (2021) Serology for the diagnosis of human hepatic cystic echinococcosis and its relation with cyst staging: a systematic review of the literature with meta-analysis. PLoS Negl Trop Dis 15:e0009370. https://doi.org/10.1371/journal.pntd.0009370

Tao S, Qin Z, Hao W, Yongquan L, Lanhui Y, Lei Y (2011) Usefulness of gray-scale contrast-enhanced ultrasonography (SonoVue®) in diagnosing hepatic alveolar echinococcosis. Ultrasound Med Biol 37:1024–1028. https://doi.org/10.1016/j.ultrasmedbio.2011.04.014

Tappe D, Frosch M, Sako Y, Itoh S, Grüner B, Reuter S, Nakao M, Ito A, Kern P (2009) Close relationship between clinical regression and specific serology in the follow-up of patients with alveolar echinococcosis in different clinical stages. Am J Trop Med Hyg 80:792–797

Tappe D, Sako Y, Itoh S, Frosch M, Grüner B, Kern P, Ito A (2010) Immunoglobulin G subclass responses to recombinant Em18 in the follow-up of patients with alveolar echinococcosis in different clinical stages. Clin Vaccine Immunol 17:944–948. https://doi.org/10.1128/CVI.00026-10

Taylor DH, Morris DL (1989) Combination chemotherapy is more effective in postspillage prophylaxis for hydatid disease than either albendazole or praziquantel alone. Br J Surg 76:954. https://doi.org/10.1002/bjs.1800760927

Teggi A, Giattino M, Franchi C, Lastilla M (1997) A hypothesis on the significance of an increase in serum transaminases in patients with hydatidosis treated with benzimidazole carbamates. Recenti Prog Med 88:452–458

Thompson RC, Eckert J (1983) Observations on *Echinococcus multilocularis* in the definitive host. Z Parasitenkd 69:335–345

Torgerson PR, Deplazes P (2009) Echinococcosis: diagnosis and diagnostic interpretation in population studies. Trends Parasitol 25:164–170. https://doi.org/10.1016/j.pt.2008.12.008

Torgerson P, Macpherson C, Vuitton D (2011) Cystic echinococcosis. In: Palmer SR, Soulsby L, Torgerson P, Brown DWG (eds) Oxford textbook on Zoonoses. Biology, clinical practice, and public control, 2nd edn. Oxford University Press, Oxford, pp 1–62

Trachsel D, Deplazes P, Mathis A (2007) Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134:911–920. https://doi.org/10.1017/S0031182007002235

Tsai IJ, Zarowiecki M, Holroyd N, Garciarrubio A, Sánchez-Flores A, Brooks KL, Tracey A, Bobes RJ, Fragoso G, Sciutto E, Aslett M, Beasley H, Bennett HM, Cai X, Camicia F, Clark R, Cucher M, De Silva N, Day TA, Deplazes P, Estrada K, Fernández C, Holland PWH, Hou J, Hu S, Huckvale T, Hung SS, Kamenetzky L, Keane JA, Kiss F, Koziol U, Lambert O, Liu K, Luo X, Luo Y, Macchiaroli N, Nichol S, Paps J, Parkinson J, Pouchkina-Stantcheva N, Riddiford N, Rosenzvit M, Salinas G, Wasmuth JD, Zamanian M, Zheng Y, Taenia solium Genome Consortium, Cai J, Soberón X, Olson PD, Laclette JP, Brehm K, Berriman M (2013) The genomes of four tapeworm species reveal adaptations to parasitism. Nature 496:57–63. https://doi.org/10.1038/nature12031

Tuxun T, Zhang J-H, Zhao J-M, Tai Q-W, Abudurexti M, Ma H-Z, Wen H (2014) World review of laparoscopic treatment of liver cystic echinococcosis–914 patients. Int J Infect Dis 24:43–50. https://doi.org/10.1016/j.ijid.2014.01.012

Ueno M, Kuroda N, Yahagi K, Ohtaki T, Kawanaka M (2012) Analysis of antibody responses by commercial western blot assay in horses with alveolar echinococcosis. J Vet Med Sci 74:813–815

Umhang G, Lahoreau J, Hormaz V, Boucher J-M, Guenon A, Montange D, Grenouillet F, Boue F (2016) Surveillance and management of *Echinococcus multilocularis* in a wildlife park. Parasitol Int 65:245–250. https://doi.org/10.1016/j.parint.2016.01.008

Umhang G, Possenti A, Colamesta V, d'Aguanno S, La Torre G, Boué F, Casulli A (2019) A systematic review and meta-analysis on anthelmintic control programs for *Echinococcus multilocularis* in wild and domestic carnivores. Food Waterborne Parasitol 15:e00042. https://doi.org/10.1016/j.fawpar.2019.e00042

Valot B, Rognon B, Prenel A, Baraquin A, Knapp J, Anelli M, Richou C, Bresson-Hadni S, Grenouillet F, Wang J, Vuitton DA, Gottstein B, Millon L (2017) Screening of antigenic vesicular fluid proteins of *Echinococcus multilocularis* as potential viability biomarkers to monitor drug response in alveolar echinococcosis patients. Proteomics Clin Appl 11. https://doi.org/10.1002/prca.201700010

van Kesteren F, Qi X, Tao J, Feng X, Mastin A, Craig PS, Vuitton DA, Duan X, Chu X, Zhu J, Wen H (2015) Independent evaluation of a canine echinococcosis control programme in Hobukesar County, Xinjiang, China. Acta Trop 145:1–7. https://doi.org/10.1016/j.actatropica.2015.01.009

Velasco-Tirado V, Romero-Alegría Á, Belhassen-García M, Alonso-Sardón M, Esteban-Velasco C, López-Bernús A, Carpio-Perez A, Jimenez López MF, Muñoz Bellido JL, Muro A, Cordero-Sanchez M, Pardo-Lledias J, Muñoz-Bellvis L (2017) Recurrence of cystic echinococcosis in an endemic area: a retrospective study. BMC Infect Dis 17:455. https://doi.org/10.1186/s12879-017-2556-9

Velasco-Tirado V, Alonso-Sardón M, Lopez-Bernus A, Romero-Alegría Á, Burguillo FJ, Muro A, Carpio-Pérez A, Muñoz Bellido JL, Pardo-Lledias J, Cordero M, Belhassen-García M (2018) Medical treatment of cystic echinococcosis: systematic review and meta-analysis. BMC Infect Dis 18:306. https://doi.org/10.1186/s12879-018-3201-y

Virchow R (1855) Die multilokuläre, ulzerierende Echinokokkengeschwulst der Leber. Verhandlungen der Physikalisch-Medizinischen Gesellschaft, pp 84–95

Vola A, Manciulli T, De Silvestri A, Lissandrin R, Mariconti M, Siles-Lucas M, Brunetti E, Tamarozzi F (2019) Diagnostic performances of commercial ELISA, indirect Hemagglutination, and Western Blot in differentiation of hepatic Echinococcal and non-Echinococcal lesions: a retrospective analysis of data from a single referral Centre. Am J Trop Med Hyg 101:1345–1349. https://doi.org/10.4269/ajtmh.19-0556

Vola A, Mariconti M, Lissandrin R, Cuzzocrea F, Bortolotto C, Sammarchi L, Vercelli A, Rinaldi P, Maestri M, Manciulli T, Brunetti E (2022) Prospective collection of clinical data on cystic echinococcosis: experience with the European registry of cystic echinococcosis in Pavia, Italy. Trans R Soc Trop Med Hyg 116:157–162. https://doi.org/10.1093/trstmh/trab097

Vuitton DA (2003) The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? Acta Trop 85:119–132

Vuitton DA (2009) Benzimidazoles for the treatment of cystic and alveolar echinococcosis: what is the consensus? Expert Rev Anti-Infect Ther 7:145–149. https://doi.org/10.1586/14787210.7.2.145

Vuitton D, Bresson-Hadni S (2014) Alveolar echinococcosis: evaluation of therapeutic strategies. Expert Opin Orphan Drugs 2:67–86

Vuitton DA, Gottstein B (2010) Echinococcus multilocularis and its intermediate host: a model of parasite-host interplay. J Biomed Biotechnol 2010:923193. https://doi.org/10.1155/2010/923193

Vuitton DA, Zhou H, Bresson-Hadni S, Wang Q, Piarroux M, Raoul F, Giraudoux P (2003) Epidemiology of alveolar echinococcosis with particular reference to China and Europe. Parasitology 127(Suppl):S87–S107

Vuitton DA, Wang Q, Zhou H-X, Raoul F, Knapp J, Bresson-Hadni S, Wen H, Giraudoux P (2011) A historical view of alveolar echinococcosis, 160 years after the discovery of the first case in humans: part 1. What have we learnt on the distribution of the disease and on its parasitic agent? Chin Med J 124:2943–2953

Vuitton DA, Demonmerot F, Knapp J, Richou C, Grenouillet F, Chauchet A, Vuitton L, Bresson-Hadni S, Millon L (2015) Clinical epidemiology of human AE in Europe. Vet Parasitol 213: 110–120. https://doi.org/10.1016/j.vetpar.2015.07.036

Vuitton DA, Azizi A, Richou C, Vuitton L, Blagosklonov O, Delabrousse E, Mantion GA, Bresson-Hadni S (2016) Current interventional strategy for the treatment of hepatic alveolar echinococcosis. Expert Rev Anti-Infect Ther 14:1179–1194. https://doi.org/10.1080/14787210.2016.1240030

Vuitton DA, Bresson-Hadni S, Chauchet A, Richou C, Grenouillet F, Delabrousse E, Millon L, Mantion G (2019) L'échinococcose alvéolaire au XXIe siècle: une maladie infectieuse opportuniste? Bulletin De l'Academie Nationale De Medecine 203:94–101

Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, Tuxun T, Wen H, Menezes da Silva A, World Association of Echinococcosis (2020) International consensus on terminology to be used in the field of echinococcoses. Parasite 27:41. https://doi.org/10.1051/parasite/2020024

Wang Y, Zhang X, Bartholomot B, Liu B, Luo J, Li T, Wen X, Zheng H, Zhou H, Wen H, Davaadorj N, Gambolt L, Mukhar T, al-Qaoud K, Abdel-Hafez S, Giraudoux P, Vuitton DA, Fraser A, Rogan MT, Craig PS (2003) Classification, follow-up and recurrence of hepatic cystic echinococcosis using ultrasound images. Trans R Soc Trop Med Hyg 97:203–211. https://doi.org/10.1016/s0035-9203(03)90121-0

Wang Q, Vuitton DA, Qiu J, Giraudoux P, Xiao Y, Schantz PM, Raoul F, Li T, Yang W, Craig PS (2004) Fenced pasture: a possible risk factor for human alveolar echinococcosis in Tibetan pastoralist communities of Sichuan, China. Acta Trop 90:285–293. https://doi.org/10.1016/j.actatropica.2004.02.004

Wang Q, Vuitton DA, Xiao Y, Budke CM, Campos-Ponce M, Schantz PM, Raoul F, Yang W, Craig PS, Giraudoux P (2006a) Pasture types and *Echinococcus multilocularis*, Tibetan communities. Emerg Infect Dis 12:1008–1010

Wang Y, He T, Wen X, Li T, Waili A, Zhang W, Xu X, Vuitton DA, Rogan MT, Wen H, Craig PS (2006b) Post-survey follow-up for human cystic echinococcosis in Northwest China. Acta Trop 98:43–51

Wang L, Wen H, Feng X, Jiang X, Duan X (2012) Analysis of economic burden for patients with cystic echinococcosis in five hospitals in Northwest China. Trans R Soc Trop Med Hyg 106: 743–748. https://doi.org/10.1016/j.trstmh.2012.09.003

Wang Q, Yu W-J, Zhong B, Shang J-Y, Huang L, Mastin A, Renqingpengcuo null, Huang Y, Zhang G-J, He W, Giraudoux P, Wu W-P, Craig PS (2016) Seasonal pattern of *Echinococcus* re-infection in owned dogs in Tibetan communities of Sichuan, China and its implications for control. Infect Dis Poverty 5:60. https://doi.org/10.1186/s40249-016-0155-4

Wang J, Jebbawi F, Bellanger A-P, Beldi G, Millon L, Gottstein B (2018) Immunotherapy of alveolar echinococcosis via PD-1/PD-L1 immune checkpoint blockade in mice. Parasite Immunol 40:e12596. https://doi.org/10.1111/pim.12596

Wang S, Ma Y, Wang W, Dai Y, Sun H, Li J, Wang S, Li F (2021) Status and prospect of novel treatment options toward alveolar and cystic echinococcosis. Acta Trop 226:106252. https://doi.org/10.1016/j.actatropica.2021.106252

Wen H, Yang WG (1997) Public health importance of cystic echinococcosis in China. Acta Trop 67: 133–145. https://doi.org/10.1016/s0001-706x(97)00051-x

Wen H, Tian WL, Zou PF, Xiang MX (1992) A rare case of mixed cystic and alveolar hydatidosis. Trans R Soc Trop Med Hyg 86:290–291

Wen H, Dong J-H, Zhang J-H, Zhao J-M, Shao Y-M, Duan W-D, Liang Y-R, Ji X-W, Tai Q-W, Aji T, Li T (2011) Ex vivo liver resection followed by autotransplantation for end-stage hepatic alveolar echinococcosis. Chin Med J 124:2813–2817

Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W, McManus DP (2019) Echinococcosis: advances in the 21st century. Clin Microbiol Rev 32. https://doi.org/10.1128/CMR.00075-18

WHO Informal Working Group (2003) International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. Acta Trop 85: 253–261

WHO Informal Working Group on Echinococcosis (WHO-IWGE) (1996) Guidelines for treatment of cystic and alveolar echinococcosis in humans. Bull World Health Organ 74:231–242

Xiao N, Qiu J, Nakao M, Li T, Yang W, Chen X, Schantz PM, Craig PS, Ito A (2005) *Echinococcus shiquicus* n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. Int J Parasitol 35: 693–701. https://doi.org/10.1016/j.ijpara.2005.01.003

Yaghi M, Zorkot M, Kanso M, Faraj W (2021) Robotic resection for hydatid disease of the liver. BMJ Case Rep 14:e241681. https://doi.org/10.1136/bcr-2021-241681

Yang YR, Liu XZ, Vuitton DA, Bartholomot B, Wang YH, Ito A, Craig PS, McManus DP (2006a) Simultaneous alveolar and cystic echinococcosis of the liver. Trans R Soc Trop Med Hyg 100: 597–600. https://doi.org/10.1016/j.trstmh.2005.09.016

Yang YR, Sun T, Li Z, Zhang J, Teng J, Liu X, Liu R, Zhao R, Jones MK, Wang Y, Wen H, Feng X, Zhao Q, Zhao Y, Shi D, Bartholomot B, Vuitton DA, Pleydell D, Giraudoux P, Ito A, Danson MF, Boufana B, Craig PS, Williams GM, McManus DP (2006b) Community surveys and risk factor analysis of human alveolar and cystic echinococcosis in Ningxia Hui autonomous region, China. Bull World Health Organ 84:714–721

Yang YR, Craig PS, Ito A, Vuitton DA, Giraudoux P, Sun T, Williams GM, Huang Z, Li Z, Wang Y, Teng J, Li Y, Huang L, Wen H, Jones MK, McManus DP (2007) A correlative study of ultrasound with serology in an area in China co-endemic for human alveolar and cystic echinococcosis. Tropical Med Int Health 12:637–646. https://doi.org/10.1111/j.1365-3156.2007.01834.x

Yang YR, Craig PS, Vuitton DA, Williams GM, Sun T, Liu TX, Boufana B, Giraudoux P, Teng J, Li Y, Huang L, Zhang W, Jones MK, McManus DP (2008) Serological prevalence of echinococcosis and risk factors for infection among children in rural communities of southern Ningxia, China. Tropical Med Int Health 13:1086–1094. https://doi.org/10.1111/j.1365-3156.2008.02101.x

Yang C, He J, Yang X, Wang W (2019) Surgical approaches for definitive treatment of hepatic alveolar echinococcosis: results of a survey in 178 patients. Parasitology 146:1414–1420. https://doi.org/10.1017/S0031182019000891

Yang C, Yang H-J, Deng S-P, Zhang Y (2020) Current status of ex-vivo liver resection and autologous liver transplantation for end-stage hepatic alveolar echinococcosis. Ann. Palliat Med 9(2271–2278):10.21037/apm-20-184

Yangdan C-R, Wang C, Zhang L-Q, Ren B, Fan H-N, Lu M-D (2021) Recent advances in ultrasound in the diagnosis and evaluation of the activity of hepatic alveolar echinococcosis. Parasitol Res 120:3077–3082. https://doi.org/10.1007/s00436-021-07262-0

Zammarchi L, Gobbi F, Angheben A, Spinicci M, Buonfrate D, Calleri G, De Paola M, Bevilacqua N, Carrara S, Attard L, Vanino E, Gulletta M, Festa E, Iacovazzi T, Grimaldi A, Sepe A, Salomone Megna A, Gaiera G, Castagna A, Parodi P, Albonico M, Bisoffi Z, Castelli F, Olliaro P, Bartoloni A (2020) Schistosomiasis, strongyloidiasis and Chagas disease: the leading imported neglected tropical diseases in Italy. J Travel Med 27:taz100. https://doi.org/10.1093/jtm/taz100

Zavoikin VD, Zelya OP, Tumolskaya NI (2020) The importance of uninterrupted albendazole treatment in patients with unresectable alveolar echinococcosis undergoing liver transplantation. Transpl Infect Dis 22:e13291. https://doi.org/10.1111/tid.13291

Zhang W, Wen H, Li J, Lin R, McManus DP (2012) Immunology and immunodiagnosis of cystic echinococcosis: an update. Clin Dev Immunol 2012:101895. https://doi.org/10.1155/2012/101895

Zhang C, Lin R, Li Z, Yang S, Bi X, Wang H, Aini A, Zhang N, Abulizi A, Sun C, Li L, Zhao Z, Qin R, Li X, Li L, Aji T, Shao Y, Vuitton DA, Tian Z, Wen H (2019) Immune exhaustion of T cells in alveolar echinococcosis patients and its reversal by blocking checkpoint receptor TIGIT in a murine model. Hepatology. https://doi.org/10.1002/hep.30896

Zhang C, Lin R, Li Z, Yang S, Bi X, Wang H, Aini A, Zhang N, Abulizi A, Sun C, Li L, Zhao Z, Qin R, Li X, Li L, Aji T, Shao Y, Vuitton DA, Tian Z, Wen H (2020) Immune exhaustion of T cells in alveolar echinococcosis patients and its reversal by blocking checkpoint receptor TIGIT in a murine model. Hepatology 71:1297–1315. https://doi.org/10.1002/hep.30896

Zhang C, Wang H, Li J, Hou X, Li L, Wang W, Shi Y, Li D, Li L, Zhao Z, Li L, Aji T, Lin R, Shao Y, Vuitton DA, Tian Z, Sun H, Wen H (2021) Involvement of TIGIT in natural killer cell exhaustion and immune escape in patients and mouse model with liver Echinococcus multilocularis infection. Hepatology 74:3376–3393. https://doi.org/10.1002/hep.32035

Zhao Z-M, Yin Z-Z, Meng Y, Jiang N, Ma Z-G, Pan L-C, Tan X-L, Chen X, Liu R (2020) Successful robotic radical resection of hepatic echinococcosis located in posterosuperior liver segments. World J Gastroenterol 26:2831–2838. https://doi.org/10.3748/wjg.v26.i21.2831

Zingg W, Renner-Schneiter EC, Pauli-Magnus C, Renner EL, van Overbeck J, Schläpfer E, Weber M, Weber R, Opravil M, Gottstein B, Speck RF, Swiss HIV Cohort Study (2004) Alveolar echinococcosis of the liver in an adult with human immunodeficiency virus type-1 infection. Infection 32:299–302

Hantaviruses in a Global Perspective

38

Ellen Krautkrämer, Lukas Peintner, and Sandra Essbauer

Contents

EK, LP, and SE conceived the layout of the project. SE, LP, and EK wrote the manuscript. SE, EK, and LP created the figures and tables. SE and EK supervised the project.

E. Krautkrämer
Nephrology Center, University of Heidelberg, Heidelberg, Germany

L. Peintner · S. Essbauer (✉)
Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, German Centre for Infection Research, Munich, Germany
e-mail: sandraessbauer@bundeswehr.org

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_30

Abstract

In 2016, a new classification for hantaviruses has been established by the International Committee on Taxonomy of Viruses (ICTV). Hantaviruses are ranked as family *Hantaviridae* comprising seven genera. So far, pathogenic hantaviruses are exclusively detected in the genus *Orthohantavirus*. Orthohantaviruses are pathogens of emerging importance, and members are meanwhile described all over the globe. The knowledge on respective small mammal hosts and their associated viruses has been rapidly increasing in the last years and now includes beside rodents also insectivores, bats, and with these several associated new viruses. Usually, animals are asymptomatic reservoir carriers despite a few studies showing effects on rodent population levels. In humans, clinical symptoms of orthohantavirus infections are depending on the virus type. Orthohantaviruses in the Americas cause hantavirus cardiopulmonary syndrome (HCPS), whereas members in Eurasia cause hemorrhagic fever with renal syndrome (HFRS) of different severity. However, as the clinical outcome is often inapparent, recorded case numbers are also underestimated. The epidemiology of orthohantaviruses is multifaceted as multiple biotic and abiotic factors seem to bias the causal link to hantavirus oscillations. In conclusion, hantavirus research on orthohantavirus pathogenesis and epidemiology is complex, because the genus is characterized by a broad range of virulence and host species association.

Keywords

Orthohantavirus · Zoonosis · Infection · Host species · HFRS · HCPS

38.1 Introduction

The genus *Orthohantavirus* within the family of *Hantaviridae* of the order *Bunyavirales* is a heterogeneous genus and difficult to characterize as a whole. The virulence of orthohantaviruses differs enormously, from apathogenic to highly virulent members with high case fatality rates. In addition, the pathogenic potential of most members is unknown, because they were identified from animal host species. Two different diseases—hemorrhagic fever with renal syndrome (HFRS) and hantaviral cardiopulmonary syndrome (HCPS)—are associated with human infection (Vaheri et al. 2013). However, the infection may also represent as a mixture of both diseases (Chand et al. 2020; Hjelle et al. 1996; Gizzi et al. 2013; Schütt et al. 2004; Rasmuson et al. 2011; Linderholm et al. 1997; Vollmar et al. 2016; Sironen et al. 2017; López et al. 1996; Du et al. 2014). Furthermore, factors responsible for orthohantaviral virulence are not known, and genetically close related members of an apathogenic orthohantavirus were identified as highly pathogenic agents as observed within the species of Dobrava-Belgrade orthohantaviruses (DOBV) (Klempa et al. 2008).

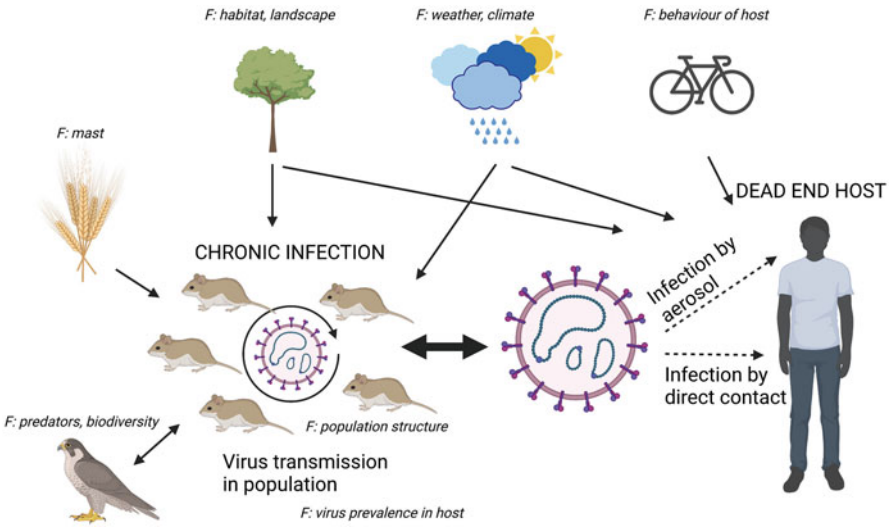

Fig. 1 Transmission cycle of orthohantaviruses and biotic and abiotic factors (F, *in italics*) that may drive viruses, rodents, and infections of humans

Therefore, characteristics associated with a certain orthohantavirus member are not necessarily valid for members of the same species or apply to the whole genus.

The natural hosts for orthohantaviruses are small mammals. For long time, rodents were thought to be the only hosts of these viruses. Orthohantaviruses are spread through the saliva, urine, and feces of animals and are typically transmitted to humans by inhalation of virus-contaminated aerosols (Fig. 1). In rare cases, direct contact of humans and rodents leads to human diseases (Heyman et al. 2012). For one orthohantavirus, the Andes virus human-to-human transmission was described, either via bodily fluids or long-term contact (see reviewed in (Toledo et al. 2021)).

In the last years, identification of further orthohantaviruses in novel host species, detection of viral genomes in patients, and the use of novel molecular and cell culture methods revealed interesting findings concerning phylogeny, epidemiology, virulence, pathogenesis, and replication of orthohantaviruses.

38.2 An Overview on Hantaviruses

38.2.1 Taxonomy

After years with a fast-growing list of hantavirus species, subspecies, genotypes, or strains, a novel taxonomy was introduced in 2016 (Laenen et al. 2019). Hantaviruses formerly graded as genus within the family of *Bunyaviridae* are now classified as family of *Hantaviridae* within the order *Bunyavirales.* The family *Hantaviridae* comprises four subfamilies and seven genera with 53 species. Table 1 shows an overview on the actual classification of the family *Hantaviridae* published in 2019 (Abudurexiti et al. 2019).

Table 1 Members of the genus Orthohantavirus (Order Bunyavirales, Family Hantaviridae, Subfamily Mammantavirinae, Genus Orthohantavirus)

Species	Virus	Disease	Host species	Geographical distribution
Andes orthohantavirus	Andes virus (ANDV)	HCPS	*Oligoryzomys longicaudatus*	Argentina, Chile, Brazil, Uruguay
	Castelo dos Sonhos virus (CASV)	HCPS	*Oligoryzomys moojeni Oligoryzomys utiaritensis*	Brazil
	Lechiguanas virus (LECV=LECHV)	HCPS	*Oligoryzomys flavescens*	Argentina
	Oran virus (ORNV)	HCPS	*Oligoryzomys longicaudatus*	Argentina
Asama orthohantavirus	Asama virus (ASAV)		*Urotrichus tapoides*	Japan
Asikkala orthohantavirus	Asikkala virus (ASIV)		*Sorex minutes*	Europe
Bayou orthohantavirus	Bayou virus (BAYV)	HCPS	*Oryzomys palustris*	USA (East Coast)
	Catacamas virus (CATV)		*Oryzomys couesi*	Honduras
Black Creek Canal orthohantavirus	Black Creek Canal virus (BCCV)	HCPS	*Sigmodon hispidus*	USA (South-East, Florida)
Bowe orthohantavirus	Bowe virus (BOWV)		*Crocidura douceti*	Guinea
Bruges orthohantavirus	Bruges virus (BRGV)		*Talpa europaea*	Belgium
Cano Delgadito orthohantavirus	Caño Delgadito virus (CADV)		*Sigmodon alstoni*	Venezuela
Cao Bang orthohantavirus	Cao Bang virus (CBNV)		*Anourosorex squamipes*	Vietnam
	Lianghe virus (LHEV)		*Anourosorex squamipes*	China
Choclo orthohantavirus	Choclo virus (CHOV)	HCPS	*Oligoryzomys fulvescens*	Panama
Dabieshan orthohantavirus	Dabieshan virus (DBSV)		*Niviventer confucianus*	China
Dobrava-Belgrade orthohantavirus	Dobrava virus (DOBV)	HFRS	*Apodemus flavicollis*	Slovenia, Croatia, Greece, Czech Republic, Slovakia, Hungary, Turkey
	Kurkino virus (KURV)	HFRS	*Apodemus agrarius*	Germany, Slovakia, Russia, Hungary, Slovenia, Croatia, Estonia
	Saaremaa virus (SAAV)		*Apodemus agrarius*	Estonia

(continued)

Table 1 (continued)

Species	Virus	Disease	Host species	Geographical distribution
	Sochi virus (SOCV)	HFRS	*Apodemus ponticus*	Russia
El Moro Canyon orthohantavirus	Carrizal virus (CARV)		*Reithrodontomys sumichrasti*	Mexico
	El Moro Canyon virus (ELMCV)		*Reithrodontomys megalotis*	Colorado
	Huitzilac virus (HUIV)		*Peromyscus beatae*	Mexico
Fugong orthohantavirus	Fugong virus (FUGV)		*Eothenomys eleusis*	China
Fusong orthohantavirus	Fusong virus (FUSV)		*Microtus fortis*	China
Hantaan orthohantavirus	Amur virus (AMRV)	HFRS	*Apodemus peninsulae*	Far eastern Russia, China
	Hantaan virus (HTNV)	HFRS	*Apodemus agrarius*	
	Soochong virus (SOOV)	HFRS	*Apodemus peninsulae*	Korea
Jeju orthohantavirus	Jeju virus (JJUV)		*Crocidura shantungensis*	South Korea
Kenkeme orthohantavirus	Kenkeme virus (KKMV)		*Sorex roboratus*	Far eastern Russia
Khabarovsk orthohantavirus	Khabarovsk virus (KHAV)		*Microtus fortis*	Far eastern Russia
	Topografov virus (TOPV)		*Lemmus sibiricus*	Sibiria
Laguna Negra orthohantavirus	Laguna Negra virus (LANV)	HCPS	*Calomys laucha*	Paraguay, Argentina, Bolivia
	Maripa virus (MARV)	HCPS	*Oligoryzomys microtis*	French Guiana
	Río Mamoré virus (RIOMV)	HCPS	*Oligoryzomys microtis*	Bolivia, Peru; Brazil
Luxi orthohantavirus	Luxi virus (LUXV)		*Eothenomys miletus*	China
Maporal orthohantavirus	Maporal virus (MAPV)		*Oligoryzomys delicatus*	Western Venezuela
Montano orthohantavirus	Montaño virus (MTNV)		*Peromyscus beatae*	Mexico
Necocli orthohantavirus	Necocli virus (NECV)		*Zygodontomys cherriei*	Colombia
Oxbow orthohantavirus	Oxbow virus (OXBV)		*Neurotrichus gibbsii*	USA
Prospect Hill orthohantavirus	Prospect Hill virus (PHV)		*Microtus pennsylvanicus*	USA

(continued)

Table 1 (continued)

Species	Virus	Disease	Host species	Geographical distribution
Puumala orthohantavirus	Hokkaido virus (HOKV)		*Myodes rufocanus*	Japan
	Muju virus (MUJV)		*Myodes regulus*	Korea
	Puumala virus (PUUV)	HFRS	*Myodes glareolus*	Europe
Robina orthohantavirus	Robina virus (ROBV)		*Pteropus alecto*	Australia
Rockport orthohantavirus	Rockport virus (RKPV)		*Scalopus aquaticus*	Rockport, Texas, USA
Sangassou orthohantavirus	Sangassou virus (SANGV)	?[a)]	*Hylomyscus simus*	Guinea
Seewis orthohantavirus	Seewis virus (SWSV)		*Sorex araneus*	Switzerland, Czech Republic, Germany, Slovakia, Finland, Hungary, Siberia
Seoul orthohantavirus	Gou virus (GOUV)	HFRS	*Rattus rattus*	China
	Seoul virus (SEOV)	HFRS	*Rattus norvegicusRattus rattus*	Asia, Africa, Europe, Americas
Sin Nombre orthohantavirus	New York virus (NYV)	HCPS	*Peromyscus leucopus*	Canada, USA (East Coast)
	Sin Nombre virus (SNV)	HCPS	*Peromyscus maniculatus Peromyscus leucopus*	USA (except East Coast)
Tatenale orthohantavirus	Tatenale virus (TATV)		*Microtus agrestis*	UK
Thailand orthohantavirus	Anjozorobe virus (ANJZV)		*Rattus rattus Eliurus majori*	Madagascar
	Serang virus (SERV)		*Rattus tanezumi*	Indonesia
	Thailand virus (THAIV)	?[a)]	*Bandicota indica*	Thailand
Tigray orthohantavirus	Tigray virus (TIGV)		*Stenocephalemys albipes*	Ethiopia
Tula orthohantavirus	Adler virus (ADLV)		*Microtus majori*	Russia
	Tula virus (TULV)	HFRS[b)]	*Microtus agrestis Microtus arvalis Microtus gregalis Microtus rossiaemeridionalis Microtus subterraneus*	Europe

(continued)

Table 1 (continued)

Species	Virus	Disease	Host species	Geographical distribution
			Lagurus lagurus *Arvicola amphibious*	
Yakeshi orthohantavirus	Yakeshi virus (YKSV)		*Sorex isodon*	China

[a]antibody detection in patients with fever of unknown origin (FUO) and HFRS-like symptoms, no genome detection (Klempa et al. 2010; Gamage et al. 2017; Pattamadilok et al. 2006)
[b]single cases of apparent infections described (Reynes et al. 2017; Hofmann et al. 2021; Zelena et al. 2019)
Adapted from Abudurexiti et al. (2019)

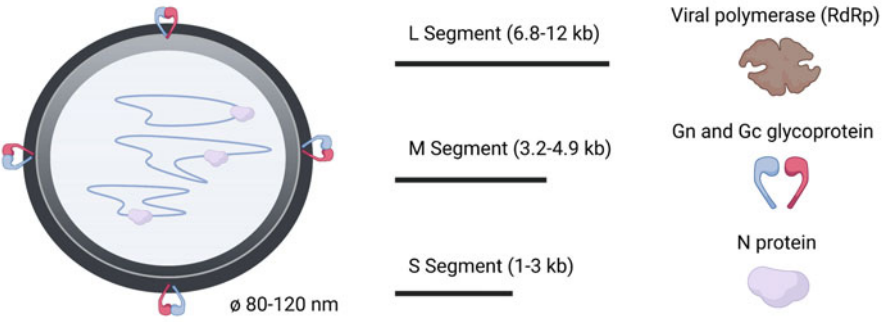

Fig. 2 Graphical structure and genome of a typical orthohantavirus. Virions are shaped spherical or oval with the glycoproteins Gn and Gc on the surface. The virions contain a tripartite negative sense RNA, wrapped by the nucleocapsid (N) protein. The large L segment encodes for the RNA-dependent RNA polymerase (RdRp, L protein), the medium (M) segment encodes for the two glycoproteins Gn and Gc, and the small (S) segment encodes for the N protein. (Image adapted from (Mittler et al. 2019, Schlegel et al. 2014))

Historically, orthohantavirus infections have been described more than 70 years ago in the 1950s as over 3000 UN soldiers suffered from the so-called Korean hemorrhagic fever (Lee 1982; Gajdusek 1956). These infections were caused by Hantaan virus (HTNV), the prototype of the genus orthohantavirus. This was the first orthohantavirus isolated and investigated in detail (Lee 1982).

Viruses of the family *Hantaviridae* have spherical or oval virions with a diameter of 80 to 120 nm (Fig. 2). The genome is a tripartite negative sense RNA. The large (L) segment encodes the viral RNA-dependent RNA polymerase (RdRp, L-protein), the medium (M) segment encodes a viral glycoprotein precursor (GPC), which is cleaved into two glycoproteins Gn and Gc. The small (S) segment encodes for the viral nucleocapsid protein (N). In some orthohantaviruses, e.g., Tula and Puumala virus, the S segment further has an open reading frame (ORF) for a nonstructural protein (Ns) (Plyusnin 2002; Binder et al. 2021). The three viral RNA segments are complexed with N proteins and form nucleocapsids. These are packed together with

an RdRp in the virus particle. The virions have a lipid envelope in which the two glycoproteins Gn and Gc are embedded. Criteria for the taxonomy of *Orthohantavirus* genus and its species are based for instance on the differences of amino acid sequences of the S and M segments (Maes et al. 2009; Laenen et al. 2019). As virus isolation is not trivial, for many described orthohantaviruses, only limited data gained directly from the reservoirs/hosts are available.

All known human pathogenic orthohantaviruses are members of the genus *Orthohantavirus* and carried by small mammals. The genus *Orthohantavirus* is built up currently of 38 species and 58 assigned viruses (Table1). However, it is not excluded that members hosted by shrews, bats, or moles of other genera of the family of *Hantaviridae* also possess pathogenic potential to humans or animals.

38.2.2 Molecular Typing and Host Virus Coevolution

Due to the late onset of specific symptoms, orthohantaviral antigens and viral RNA can only very rarely be found in human patients. Therefore, often the small mammal carriers are used to study the molecular epidemiology of these viruses. Despite the long knowledge of human cases, first viral genome data were only available in the early 1990s (Avsic-Zupanc et al. 1992; Xiao et al. 1993a, b; Pilaski et al. 1994). Reports of successful isolation of orthohantaviruses in Europe for a more proper molecular virus characterization or even pathogenicity studies are sparse. Only recently the first isolation of a Central European PUUV strain was reported (Binder et al. 2020) and may allow now the isolation and afterward in-depth characterization of, e.g., reservoir-adapted PUUV strains.

For PUUV, there exist different geographic clusters. Several northern European (e.g., north-/south-/Scandinavian, Finish, Danish) PUUV strains are separated from those found in central Europe (e.g., from Belgium, France, Germany, and Slovakia) or the Alpe-Adria area (e.g., from Austria, Slovenia, Croatia) (Avsic-Zupanc et al. 2007; Plyusnina et al. 2007, 2009; Nemirov et al. 2010; Ettinger et al. 2012). Isolation-by-distance analysis of PUUV S segment sequences (N and NSs) confirmed the strict spatial clustering in Europe (Binder et al. 2020).

Natural reassortments of segments were shown for example between members of the species Dobrava-Belgrade orthohantavirus (Kirsanovs et al. 2010) and for PUUV and DOBV (Klempa 2018). In Germany, the picture of genetic diversity of PUUV is quite more complex as at least eight geographically and phylogenetically distinct PUUV subclusters can be found here (Mertens et al. 2011c; Essbauer et al. 2007; Ulrich et al. 2008; Hofmann et al. 2008; Ettinger et al. 2012; Jeske et al. 2021).

A strict coevolution of rodent hosts and virus species was previously postulated for orthohantaviruses as these viruses group into three main clades that correspond with the rodent subfamilies (Morzunov et al. 1998; Plyusnin et al. 1996; Hughes and Friedman 2000; Plyusnin and Morzunov 2001). Meanwhile, there are several findings that do not completely support this hypothesis. One example is the detection of orthohantaviruses in nonrodent hosts that puts this theory in question (Ramsden et al.

2009). Phylogenetic analysis of TULV showed that the evolution seems to be not host-related, although different geographic genetic subclusters were shown in Europe (Schmidt-Chanasit et al. 2010; Plyusnina et al. 2007; Schlegel et al. 2012; Saxenhofer et al. 2019; Schmidt et al. 2021).

In South America, a comprehensive analysis of orthohantaviruses revealed that there exist three phylogenetic clades, the Andes/-like viruses, the Laguna Negra/-like viruses, and the Rio Mamore/-like viruses. A long coevolution of host and virus seems also to be present, and viruses of one clade are found in several host species (Firth et al. 2012). A detailed phylogenetic analysis of entire coding regions of orthohantavirus genomes showed that there exist four phylogroups in mammalian hosts, and ancient reassortment between these was postulated (Guo et al. 2013). Divergence of viruses and hosts differs for some orthohantavirus indicating a cross-species transmission during orthohantavirus evolution.

38.3 Epidemiology of Human Diseases

Today, the presence of orthohantavirus species was demonstrated in Asia, Europe, the Americas, Africa, and Australia. Pathogenic orthohantaviruses were found in Asia, Europe, and the Americas, so far.

The annual global number of orthohantavirus infections is difficult to calculate as, e.g., surveillance systems and case definitions differ between countries (Wang et al. 2021; Khismatullina et al. 2016; Knust et al. 2012). A precise estimation is also hampered by annual fluctuations in case numbers but also due to dramatic changes of case numbers by rodent control measures in China in the last decades (Sun and Zou 2018). According to actual reported cases, the most affected countries are China and Russia with averaged 14,000 and 7000 HFRS cases per year, respectively.

About 300 HCPS cases were reported in the Americas per year (Montoya-Ruiz et al. 2014; PAHO 2022; Kruger et al. 2015). However, due to the high case fatality rate, these HCPS cases are of high importance, and outbreaks of ANDV infections require special awareness due to possible person-to-person transmission (Martínez-Valdebenito et al. 2019; Martínez et al. 2020; Toledo et al. 2021).

38.3.1 Europe

In Europe, a bundle of orthohantavirus species associated with rodents and causing diseases in humans are found. First, Puumala virus (PUUV) carried by the bank vole (*Myodes glareolus*) is a virus with a broader distribution in this area. A mild form of HFRS is caused by PUUV, and lethality is below 1%. From 2014 to 2018, Finland, Sweden, and Germany reported 81% of all cases in Europe (Fig. 3). Latest numbers summarized case numbers from 29 countries are from 2018, with 1826 annual cases (Heyman et al. 2008, 2009b, 2011). Interestingly, in years with many PUUV cases and a high abundance of the virus in the hosts, a spillover of PUUV to

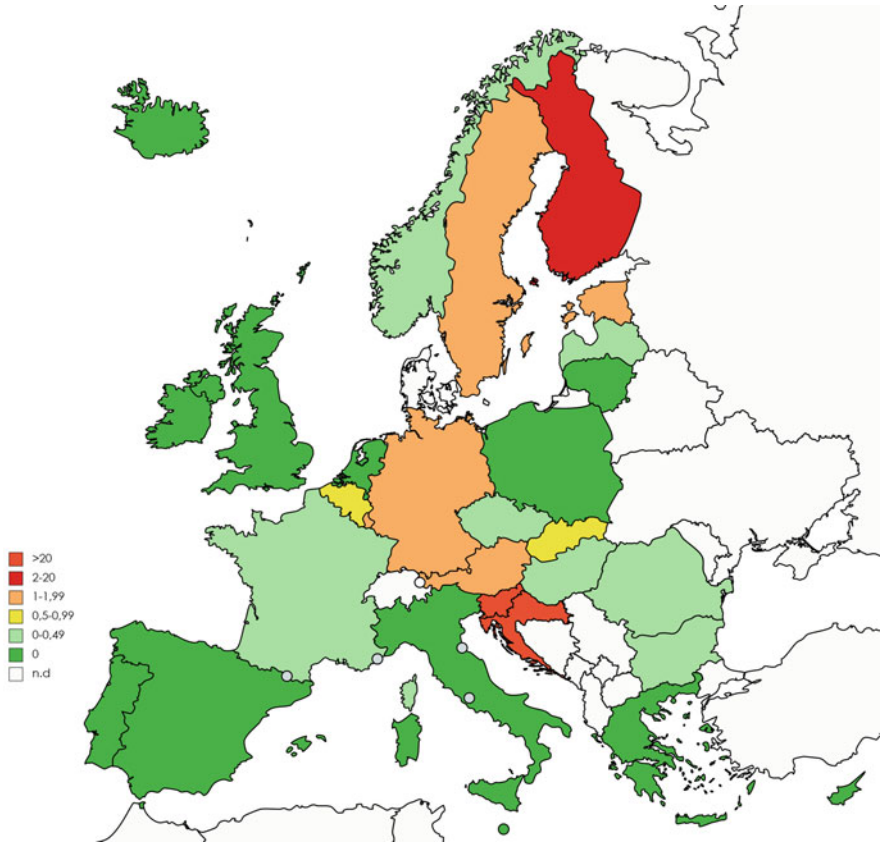

Fig. 3 Average incidence values of reported orthohantavirus infections in Europe from 2015 to 2019. (Adapted from the ECDC 5-year report 2015–2019. (ECDC 2022))

other habitat-sharing species such as *Apodemus sylvaticus* or *A. flavicollis* can be observed. The impact of the transmission of the virus from spill over from infected rodents to humans is unknown (Heyman et al. 2009b; Klingström et al. 2002a; Essbauer et al. 2006; Schlegel et al. 2009; Binder et al. 2020).

Second, species Dobrava-Belgrade orthohantavirus with its four subtypes Dobrava, Kurkino, Saaremaa, and Sochi virus is transmitted by several *Apodemus ssp.* (e.g., *A. flavicollis and A. agrarius*) (Klempa et al. 2013b). Members of the species *Dobrava-Belgrade orthohantavirus* cause HRFS with various severity from subclinical forms to life-threatening diseases (Avsic-Zupanc et al. 1999; Markotić et al. 2002; Mertens et al. 2011c) and are endemic in some central- and several southeast European countries.

Third, Tula virus (TULV) was detected in several vole species (Subfamily *Arvicolinae*: *Microtus agrestis* and *M. arvalis*) (Plyusnin et al. 1994; Vapalahti et al. 1996; Schmidt-Chanasit et al. 2010; Schmidt et al. 2021). Only limited reports

on the relevance of TULV for mild human diseases are present (Schultze et al. 2002; Klempa et al. 2003; Hofmann et al. 2021). Humans might have contact to TULV and develop specific antibodies as, e.g., found in a seroepidemiological study in Germany in forest workers (Mertens et al. 2011b).

Fourth, Seoul virus (SEOV) was giving attention in Europe. For a long time, it was unclear whether SEOV could cause disease in humans. Single reports of SEOV infections were described from 2004 up to 2021 in Europe (Heyman et al. 2004; Heyman et al. 2009a; Yasuda et al. 2021). In 2012, SEOV was detected in wild *R. norvegicus* in the United Kingdom and France. These virus sequences resembled SEOV originating from rare mild HFRS cases acquired by handling laboratory rats in the Netherlands, United Kingdom, and Belgium (Jameson et al. 2013a; Jameson et al. 2013b). In the following years, small outbreaks of SEOV were detected in *Rattus* populations, mainly pet rats, and in several European countries (Shepherd et al. 2021).

38.3.2 Asia

The continent that is mostly affected by orthohantavirus infections is Asia. High numbers of cases were reported from China, Far East Russia, and Korea (34-37). In contrast to Europe, where Nephropathia epidemica (NE) caused by PUUV—the milder form of HFRS—predominates, HFRS represents a severe public health problem in Asia. For instance, cases of HFRS were reported in nearly all Chinese provinces, with Heilongjiang in the northeast of China being the most severe endemic area with the highest annual incidence for HFRS (Guo et al. 2013). Causing agents of HFRS in Asia are HTNV and SEOV.

SEOV was first isolated from city rats (*Rattus norvegicus*) in Korea in 1981 and from laboratory rats (*Rattus norvegicus*) in Japan in 1982 (Lee et al. 1981; Lee 1982). Currently, SEOV repeatedly causes disease outbreaks in various provinces in China. Approximately, a third of the yearly cases of febrile illnesses with bleeding and kidney involvement (hemorrhagic fever with renal syndrome) are expected to be caused by SEOV annually. However, it was shown that infections with SEOV seem to demonstrate milder courses than HFRS caused by HTNV (Zhang et al. 2011).

In Russia, about 90% of infections were reported in the Volga Federal District and are described as NE caused by PUUV (Garanina et al. 2009). Several outbreaks in the Central Federal District were attributed to members of the species *Dobrava-Belgrade virus* (Klempa et al. 2008). In contrast, in the Far East region, HFRS cases caused by HTNV, SEOV, and Amur virus were observed (Yashina et al. 2000; Miyamoto et al. 2003; Peintner et al. 2021). The seasonality of HFRS cases in Russia also reveals geographic differences and seems to depend on the host reservoir species (Yashina et al. 2000). The seasonal pattern of reported hantavirus disease in humans in China and Korea shows a pronounced peak of case numbers in winter and a second minor peak in summer (Liu et al. 2012; Song et al. 2006; Huang et al. 2012).

Only a few HFRS cases were reported in other Asian countries, e.g., Indonesia, Sri Lanka, India, Thailand, and Vietnam (Jonsson et al. 2010; Huong et al. 2010). No human cases of HFRS were reported in Japan over the last 30 years, although cases were reported since 1960 and seropositive rodents were still present as demonstrated by several epizootiological surveys (Kariwa et al. 2007).

38.3.3 Americas

Hantaviruses became prominent in the United States as an outbreak of severe disease with lung manifestation (hantavirus pulmonary syndrome, HPS; hantavirus cardiopulmonary syndrome, HCPS) and death occurred in the Four-Corners Region in 1993 (Nichol et al. 1993). Sin Nombre virus (SNV) was the causative agent of this outbreak (Nichol et al. 1993; Childs et al. 1994; Duchin et al. 1994; Ksiazek et al. 1995). After the detection of SNV, many orthohantaviruses causing HCPS were detected in the North, Central, and South Americas. Most prominent, two years later in 1995, Andes virus (ANDV) was described during a severe HCPS outbreak in south-western Argentina (López et al. 1996). Remarkably, ANDV is the only orthohantavirus for which a human-to-human transmission has been demonstrated several times (Wells et al. 1997; Padula et al. 1998; Toro et al. 1998; Martinez et al. 2005; Lázaro et al. 2007; Ferres et al. 2007). A study published in 2020 describes an outbreak of HCPS by person-to-person transmission with 34 confirmed cases including 11 fatal outcomes in Argentina (Martínez et al. 2020). The outbreak was caused by one person infected via rodent-transmission and driven by three symptomatic spreaders during a social event. Spreading was probably more likely by patients with high viral loads. Control measures such as self-isolation and quarantine were imposed by local public health officials and seemed to be effective as further viral spread was prevented.

So far, the two species SNV and ANDV are the most common causes of HCPS in North, Central, and South America. Meanwhile, HCPS cases and several other hantaviruses were reported from the United States, Canada, and at least ten South and Central American States such as Argentina, Bolivia, Brazil, Chile, Ecuador, French Guiana, Panama, Paraguay, Uruguay, and Venezuela (CDC 2022; Koma et al. 2012). In general, case numbers of HCPS in Argentina, Brazil, and Chile are highest (Martinez et al. 2010; Macneil et al. 2011). In North America, HCPS in humans is also induced by, i.e., Bayou virus (Morzunov et al. 1995; Khan et al. 1995; Hjelle et al. 1996; Ksiazek et al. 1997; Torrez-Martinez et al. 1998), Black Creek Canal virus (Rollin et al. 1995; Ravkov et al. 1995; Khan et al. 1996), New York virus (Hjelle et al. 1995; Morzunov et al. 1998), and Monongahela virus (Song et al. 1996; Morzunov et al. 1998; Rhodes et al. 2000). In summary, in Northern, Central, and South America, at least 30 orthohantavirus types or genotypes have been recognized in HCPS patients or carried by *Sigmodontine* reservoir rodents. The pattern of strains as well as the genetic diversity and the distribution is multifaceted, and for many of the strains, the impact on humans to cause disease is unresolved (Macneil et al. 2011; Firth et al. 2012). Phylogeographic analyses of South American hantaviruses suggested a spread from the south-central part such as Paraguay, Brazil, Bolivia to the northern, southern, and eastern parts of this continent (Firth et al. 2012).

38.3.4 Africa and Australia

Intense studies in small mammals were initiated to find viruses and hosts in Africa. Sangassou virus (SANGV) was the first reported African orthohantavirus detected in *Muridae* collected in Guinea (Klempa et al. 2006; Klempa et al. 2012). It is the best characterized African orthohantavirus and was also isolated to grow *in vitro* in laboratory cell culture (Klempa et al. 2012). A second African murid-associated orthohantavirus is Tigray virus found in white-footed rat from Ethiopia (Meheretu et al. 2012). In addition, several further hantaviruses were described in shrews from Guinea (Klempa et al. 2007) and Côte d'Ivoire (Kang et al. 2011) and in bats from Sierra Leone (Weiss et al. 2012) and Côte d'Ivoire (Sumibcay et al. 2012; Meheretu et al. 2019), but are not yet classified by ICTV. In the last years, several studies investigated the impact of African orthohantaviruses on human health (Meheretu et al. 2021; Diagne et al. 2020; Raharinosy et al. 2018). For SANGV, a role in fever of unknown origin (FUO) and HFRS has been postulated in Guinea (Klempa et al. 2010). In a few seroepidemiological studies, a low human seroprevalence (1%–2%) was shown for African orthohantaviruses (Gonzalez et al. 1984; Klempa et al. 2010; Klempa et al. 2013b). A broad serological study on nonrodent-associated orthohantaviruses in Africa also revealed that shrew-transmitted orthohantaviruses indeed are also able to infect humans, at least in Gabon and Côte d'Ivoire (Heinemann et al. 2016). Nevertheless, further studies are needed in order to understand the impact and relevance of African orthohantaviruses for humans.

For Australia, the knowledge is even more meager than for Africa. The presence of orthohantaviruses in Australia was speculated for a long time (Bi et al. 2008), but no human cases have been reported so far. In June 2021, a complete genomic sequence of an orthohantavirus isolated from brain tissue of a black flying fox (*Pteropus alecto)* collected in Australia was published in GenBank and called Robina virus (TaxonomyBrowser 2022). An article with details on this important first detection of an Australian orthohantavirus is not yet published, but will be of interest for the whole field of orthohantavirus research.

38.4 Hantaviruses in Nonrodent Hosts, in Livestock, and in Pet Animals

38.4.1 Nonrodent Orthohantaviruses: Insectivores and Megabats as Reservoir Species

For many years, voles and mice were believed to be the only reservoir hosts for orthohantaviruses. Worldwide, intense search for *Hantaviridae* family members in bats as well as insectivores such as shrews and moles are performed, and several viruses have been discovered in the last decades. Table 2 summarizes orthohantaviruses that have been described in insectivores and megabats and that are already classified by ICTV. However, until now for nonrodent-associated orthohantaviruses, the impact on human health is not known (Carey et al. 1971; Zeller et al. 1989; Kang et al. 2009; Schlegel et al. 2012; Kim et al. 1994; Jung and Kim 1995).

Table 2 Overview on orthohantaviruses detected in different families of insectivores (Eulipotyphla) and in the family of megabats (Pteropodidae)

Order *Eulipotyphla, Soricomorpha*, Family *Soricidae* (shrews)			
Asikkala orthohantavirus	Asikkala virus (ASIV)	*Sorex minutus*	Europe
Bowe orthohantavirus	Bowe virus (BOWV)	*Crociduradouceti*	Guinea
Cao Bang orthohantavirus	Cao Bang virus (CBNV)	*Anourosorex squamipes*	Vietnam
	Lianghe virus (LHEV)	*Anourosorex squamipes*	China
Jeju orthohantavirus	Jeju virus (JJUV)	*Crocidura shantungensis*	South Korea
Kenkeme orthohantavirus	Kenkeme virus (KKMV)	Sorex roboratus	Far eastern Russia
Seewis orthohantavirus	Seewis virus (SWSV)	*Sorex araneus*	Switzerland, Czech Republic, Germany, Slovakia, Finland, Hungary, Siberia
Yakeshi orthohantavirus	Yakeshi virus (YKSV)	*Sorex isodon*	China
Order *Eulipotyphla*, *Soricomorpha*, Family *Talpidae* (moles)			
Asama orthohantavirus	Asama virus (ASAV)	*Urotrichus tapoides*	Japan
Bruges orthohantavirus	Bruges virus (BRGV)	*Talpa europaea*	Belgium
Oxbow orthohantavirus	Oxbow virus (OXBV)	*Neurotrichus gibbsii*	USA
Rockport orthohantavirus	Rockport virus (RKPV)	*Scalopus aquaticus*	Rockport, Texas, USA
Order *Chiroptera* Family *Pteropodidae* (megabats)			
Robina orthohantavirus	Robina virus (ROBV)	*Pteropus alector*	Australia

38.4.2 Evidence of Orthohantaviruses in Pet, Livestock, and Laboratory Animals

To the present knowledge, rodents are the main carriers of orthohantaviruses that have an impact on human health. Rodents may serve as prey for different birds, cats, or dogs. Further, rodents are around or in housings and also often found in buildings for livestock as buildings give some protection, hay is a good material for nesting, and food sources from livestock animals also serve rodents (Fig. 1). Therefore, it is important to know if orthohantaviruses can also be transmitted to nonrodent animals in laboratories, pets, or farm animals. Pet rats have been reported as a possible source of SEOV infections in the United Kingdom (Jameson et al. 2013a; Jameson et al. 2013b; Featherstone et al. 2013). Besides that, there exist only limited data on serological studies of different animals as for livestock such as cattle or pigs or birds of prey (Table 3). A combined study of animals' holders/handlers and animals was performed in an Austrian zoo (Juncker-Voss et al. 2004) and in the United

Table 3 Overview on investigations of orthohantavirus in pets, farm, and livestock animals (except rodents, shrews, bats)

Order	Animal species	Virus	Country	Detection	Ref.
Artiodactyla	*Bos taurus*	PUUV, HTNV	Czech Republic	Serological	(Danes et al. 1992)
Artiodactyla	*Capreolus capreolus*	PUUV	Czech Republic	Serological	(Danes et al. 1992)
Artiodactyla	*Alces alces*	PUUV	Sweden	Serological	(Ahlm et al. 2000)
Artiodactyla	*Sus scrofa**	Hantavirus	China	Serological	(Zhang et al. 2010) (Yang et al. 2004)
Carnivora	*Canis lupus**	SNV	USA	Serological	(Malecki et al. 1998)
Carnivora	*Canis lupus**	PUUV	Belgium	Serological	(Dobly et al. 2012)
Carnivora	*Vulpes vulpes*	PUUV	Belgium	Serological	(Escutenaire et al. 2000)
Carnivora	*Felis catus*	SNV	Canada, USA	Serological	(Leighton et al. 2001, Malecki et al. 1998)
Carnivora	*Felis catus*	PUUV	UK, Austria, Belgium	Serological	(Bennett et al. 1990) (Nowotny 1994, Nowotny 1996) (Dobly et al. 2012)
Lagomorpha	*Lepus europaeus*	PUUV	Czech Republic	Serological	(Danes et al. 1992)
Primates	*Macaca mulatta*	PUUV/ TULV	Germany	Serological	(Mertens et al. 2011a)
Primates	*Macaca fascicularis*	PUUV/ TULV	Germany	Serological	(Mertens et al. 2011a)
Primates	*Papio anubis*	PUUV/ TULV	Germany	Serological	(Mertens et al. 2011a)
Primates	*Macaca fascicularis*	PHV	-	e.i.: acute nephropathy, mild, transient proteinuria, azotemia	(Yanagihara et al. 1988)
Primates	*Pan troglodytes*	PHV	-	e.i.: acute nephropathy, mild, transient proteinuria, azotemia	(Yanagihara et al. 1988)
Primates	*Macaca fascicularis*	Cell-attenuated PUUV	-	e.i.: lethargy, mild proteinuria, microhematuria	(Groen et al. 1995)

(continued)

Table 3 (continued)

Order	Animal species	Virus	Country	Detection	Ref.
Primates	*Macaca fascicularis*	Bank vole-adapted PUUV	-	e.i.: loss of appetite, apathetic behavior, fever, proteinuria, biochemical markers, and immunological characteristics of HFRS	(Klingström et al. 2002b, Sironen et al. 2008)

* forma domestica; e.i., experimental infection
Adapted from Zeier et al. (2005)

Kingdom (Taori et al. 2013). Furthermore, orthohantavirus antibodies were detected in cattle (Danes et al. 1992). Serological studies further gave evidence that cats in comparison to dogs have a significantly higher orthohantavirus antibody-reactivity (Dobly et al. 2012). Table 3 summarizes the present knowledge of orthohantaviruses in pet, livestock, and laboratory animals. However, detection of orthohantavirus genome or isolation of replication-competent viruses from pets, farm, and livestock animals is missing so far and needs further investigation.

38.4.3 Do Orthohantavirus Infections Have an Impact on Animals?

As shown above, several serological studies show that animals developed antibodies against an orthohantavirus infection. Only a few studies have been performed—and mostly on rodents—in order to investigate if orthohantaviruses have an influence on the hosts. There is also a high variation of orthohantavirus prevalence in the respective rodent hosts. For example, the PUUV prevalence in rodents seems to be quite different depending on time, region, and the local orthohantavirus outbreak situation (Essbauer et al. 2006, 2013; Mertens et al. 2011c; Augot et al. 2008). Orthohantavirus infections in rodents are chronic and may have an impact on the population levels and the physiological status (Pearce-Duvet et al. 2006; Tersago et al. 2008, 2012; Luis et al. 2012). However, in summary, as described for the factors influencing the orthohantavirus epidemiology, there is also lack of data on the impact of the agents on their natural hosts.

Infection trials with some primates have shown different results, depending on the attenuation of the virus strains (Table 3). Surprisingly, infection experiments also showed that Prospect Hill virus (PHV), a North-American vole-associated virus that is nonpathogenic for humans, induced disease in *Cynomolgus macaques* (Yanagihara et al. 1988). Several nonhuman primate species seem also to be susceptible to a PUUV/TULV infection, but virus could not be isolated so far (Mertens et al. 2011a) (Table 3).

38.5 Seasonal Outbreaks, Exposition Risk Factors

The annual patterns of reported human PUUV cases in North and Central Europe are different. An investigation from 2001 to 2017 indicates a highly variable space-time disease incidence pattern. These infection rates are oscillating and show large outbreaks every 2 to 3 years with peaks in early summer (Faber et al. 2019). In Scandinavia, 3- to 4-year cycles seem to occur (Olsson et al. 2003; Vapalahti et al. 2003; Pettersson et al. 2008). Autumn and winter peaks of human cases are recorded in Fennoscandia (Rose et al. 2003; Evander and Ahlm 2009). Cycles in Central Europe do not follow a regular pattern. Varying local orthohantavirus outbreaks are reported in different years for Germany, France, and Belgium as reviewed in Essbauer et al. (2013) and Schmidt et al. (2021). Even as these countries are neighbors, outbreaks seem not to be synchronous (Essbauer et al. 2006, 2007; Faber et al. 2010; Mailles et al. 2005; Heyman et al. 2007; Ulrich et al. 2008; Koch et al. 2007; Hofmann et al. 2008; Ettinger et al. 2012). In France, most human infections are reported during late spring and summer (Vapalahti et al. 2003; Sauvage et al. 2002). In comparison, in Belgium and Germany, seasonal peaks are quite variable, and some years have exclusively summer peaks but in others also winter peaks occur (Heyman et al. 2012; Essbauer et al. 2013; Faber et al. 2010; Piechotowski et al. 2008). So far, the seasonal pattern of orthohantavirus cases in Germany and also neighboring countries seems quite unpredictable (Binder et al. 2019, 2020). In Germany, for DOBV, there might also exist two peaks, one in summer and one in winter (Hautala et al. 2002). The latter might be explained by the behavior of host, as late in season *Apodemus sp.* search for shelter in houses or garages, and therefore, humans might have enhanced contact to hosts and excreta.

In general, in Scandinavian countries (Finland, Sweden, and Norway) and Russia, PUUV incidences are much higher as in Central Europe (Fig. 3). High-endemic PUUV regions exist in most countries as, e.g., the Northern counties in Sweden (Olsson et al. 2003), the Jura for France (Augot et al. 2008), parts of South Belgium (Mailles et al. 2005) in Germany, i.e., the Swabian Alb, Main-Spessart region, Lower Bavaria, and the administrative districts of Osnabrück (Ulrich et al. 2008; Hofmann et al. 2008; Ettinger et al. 2012). Additionally, to the high oscillations, it should be mentioned that may be new regions are populated by host species. For example, several unusual urban outbreaks in the cities of Cologne, Aachen, and Osnabrück were recognized (Essbauer et al. 2007; Mailles et al. 2005; Abu Sin et al. 2007). Another example presents cities at the Swabian Alb, e.g., in 2010 and 2012, many cases were registered in the urban district of Stuttgart (Boone et al. 2012; Hautala et al. 2002). Changes in leisure behavior of city dwellers exerting more outdoor activities may also be responsible for the rise in urban cases.

The actual knowledge on factors driving orthohantaviruses in Europe was reviewed (Reusken and Heyman 2013); however, there remain many open questions on the tuning of outbreaks. Hence, several longitudinal studies were initiated in the last decade to better understand and predict outbreaks (Heyman et al. 2012; Essbauer et al. 2013; Reusken and Heyman 2013; Ulrich et al. 2008).

38.6 Hantavirus Disease

Diseases caused by pathogenic orthohantaviruses are called hemorrhagic fever with renal syndrome (HFRS) and hantaviral cardiopulmonary syndrome (HCPS) (Vaheri et al. 2013). HFRS is caused by Eurasian members, whereas HCPS is caused by orthohantaviruses of the American continents. The clinical picture of diseases is characterized by predominant involvement of kidney in HFRS and lung in HCPS. However, cases with pulmonary symptoms in infections with Eurasian orthohantaviruses and renal impairment by HCPS-causing American orthohantaviruses were also observed (Chand et al. 2020; Hjelle et al. 1996; Gizzi et al. 2013; Schütt et al. 2004; Rasmuson et al. 2011; Du et al. 2014; Linderholm et al. 1997; Vollmar et al. 2016; Sironen et al. 2017). Despite high genetic homology of orthohantaviruses, virulence of pathogenic members differs enormously, and severity of disease demonstrates an enormous variance between individuals. HCPS is associated with case fatality rates (CFR) of 21% to 39% (Alonso et al. 2019; Fonseca et al. 2018), whereas HFRS by Eurasian orthohantaviruses exhibits CFRs between 0.08% and 12% depending on the causative species (Vaheri et al. 2013; Klempa et al. 2013a). Highest CFRs between 6% and 15% are observed for SOCV, DOBV, and HTNV, whereas infections with PUUV or KURV are associated with milder courses and lower CFRs $< 1\%$ (Klempa et al. 2003). In addition, symptomatic infections with SEOV are observed in Europe, Asia, and the Americas (Zhang et al. 2011; Hofmann et al. 2008; Shepherd et al. 2021). Several case reports also describe mild signs of HFRS symptoms caused by TULV, which was formerly assigned to be nonpathogenic (Hofmann et al. 2021).

Symptomatic infections start with sudden onset of high fever and flu-like symptoms such as headache, myalgia, and gastrointestinal symptoms after several days to a few weeks after virus exposition. After this initial phase of infection, the organ-specific manifestation of the infection starts.

HFRS is associated with acute kidney injury with often massive proteinuria. Laboratory parameters that are changed involve the elevation of numbers of leukocytes, increased levels of serum creatinine, C-reactive protein, and lactate dehydrogenase activity. In contrast, numbers of platelets and serum albumin are decreased. Urinalysis reveals hematuria and proteinuria. The proteinuria is nonselective indicating a tubular and glomerular damage. In light-microscopic analysis, hantavirus disease presents as tubular-interstitial damage without obvious glomerular changes (Ferluga and Vizjak 2008). However, analysis of glomerular integrity by electron microscopy revealed structural changes of the glomerular filtration barrier (Nusshag et al. 2020; Collan et al. 1978; Boehlke et al. 2014). In addition, analysis of urine samples revealed the elevation of marker proteins for tubular and glomerular injury (Nusshag et al. 2020; Outinen et al. 2022). Besides the virus-specific virulence, differences in the clinical course between individuals are observed. Behavioral factors such as smoking or pre-existing conditions (Bergstedt Oscarsson et al. 2016; Gherasim et al. 2015; Latronico et al. 2018; Kitterer et al. 2016; Tervo et al. 2015) and genetic conditions such as HLA-haplotype influence the risk for severe courses of orthohantavirus infection (Ma et al. 2020; Mäkelä et al. 2001; Mustonen

et al. 1998). Gender-specific differences were also described. Middle-aged persons are mostly infected, and men are overrepresented (Krautkrämer et al. 2013; Faber et al. 2019; Klein et al. 2011; Hjertqvist et al. 2010; Klingström et al. 2008). Infections of children are often reported in the Americas with HCPS (Ferrés and Vial 2004), but recently also an immunocompromised boy died in Asia of a PUUV infection (Enyi et al. 2022). In Central Europe, a study revealed that infections with the Sochi virus gained an unexpectedly high fatality rate among children under 15 years of age (Dzagurova et al. 2018).

Long-term effects of an orthohantavirus infection are mostly determined by the severity of the organ involvement during the peak of the infection, but for the majority of patients, most symptoms are gone latest three months after disease onset. Due to the involvement of the kidney, many patients develop a long-term hematuria, hypertension, or proteinuria (Latus et al. 2015). Orthohantavirus-specific IgG will remain in the patients up to years after the infection. However, there is a correlation of PUUV survivors and an increased probability to develop lymphatic malignancies (Klingström et al. 2014), but no further studies were performed so far to reveal if this is PUUV specific or valid for all orthohantavirus infections.

In summary, orthohantavirus-induced diseases exhibit a broad range of symptoms and virus-specific determinants as well as individual patient-specific factors control the severity of the clinical course. It is central to gain more insights in the underlying cellular mechanism of pathogenesis for the development of antiviral strategies.

38.6.1 Pathogenesis

While most governmental research on orthohantavirus focuses on the distribution and virulence of the disease, it is also of utter importance to understand more about the biological abilities of the virus family and the mode of action of the pathophysiology. Some labs specialized on the investigation on orthohantavirus biology and were able to draw a comprehensive picture in the last decade. Understanding the molecular biological features of an infection ultimately paves the way to an effective prevention of an infection such as a vaccination or a targeted therapy dampening the symptoms of an infection.

38.6.1.1 Cell Biology

Like other viruses, orthohantaviruses are dependent on a host cell to replicate. Orthohantaviruses, entering the body either via the digestive or pulmonary system, are absorbed by cells expressing among others the integrin β3 surface receptor (CD61) such as endothelial and epithelial cells, T cells, DC cells, macrophages, and follicular dendritic cells (Noack et al. 2020).

All receptors for orthohantavirus entry were identified in *in vitro* cell culture systems. First, integrin β3 was described to mediate the entry of pathogenic orthohantaviruses (Gavrilovskaya et al. 1999; Gavrilovskaya et al. 1998). In the last years, more and more receptors and cofactors have been identified using different cell

culture models such as CD55, gC1qR, protocadherin-1, or TIM-1 (T-cell immunoglobulin and mucin domain 1) (Krautkrämer and Zeier 2008; Popugaeva et al. 2012; Buranda et al. 2010; Choi et al. 2008; Jangra et al. 2018; Mayor et al. 2020). In addition, studies demonstrated the permissibility of human and rodent cell types without integrin β3 expression (Higa et al. 2012; Raftery et al. 2014; Müller et al. 2019). In conclusion, receptor usage and entry mechanism of orthohantaviruses remain elusive. Entry may differ between cell types, host reservoir, and human cells, and between virus species. Therefore, further investigations are necessary.

Upon attachment and receptor engagement, virions ultimately fuse with the cell membrane in clathrin-coated pits by macropinocytosis of the host by changing the structure of the Gc surface protein (Torriani et al. 2019; Bauherr et al. 2020). In its open form, it is able to dimerize and expose its aromatic and polar residues who enable the fusion with the cell membrane (Bignon et al. 2019; Cifuentes-Muñoz et al. 2011). In the early endosome, the virions get uncoated, and viral ribonuclear particles (RNP) get into the cytoplasm where they are further stripped off protecting particles (Fig. 3). Orthohantaviruses provide their own RNA-dependent RNA polymerase (RdRp) that starts the initial transcription of viral RNA. However, to do so, the RdRp is depended on a yet to be defined host cell factor. Together, these two proteins perform so-called cap-snatching on 5′fragments of cellular mRNA to generate primers that are used for the initiation of the transcription of the viral genome (Jeeva et al. 2018). Subsequently, translation of the M-fragment starts at the rER, while S- and L-segments predominantly get translated at free ribosomes. An early and large quantity translation of the glycoprotein GPC is crucial to enable a continuous translation, trafficking, and assembly of other viral proteins and RNPs. Protein maturation, such as the glycosylation of the Gn/Gc glycoproteins, takes place at the Golgi apparatus. For this, the C-terminal peptides are crucial to enable efficient protein trafficking to the Golgi (Sperber et al. 2019). Gn proteins form spontaneously tri- and oligomers, while Gc proteins mostly assemble in mono- or dimers. Subsequently, Gn/Gc protein complexes self-assemble in virus like particles at a yet to be identified subcellular localizations (Acuña et al. 2014). Viral budding occurs after assembly of viral particles in the cytosol. Here, the Gn/Gc glycoproteins interact with proteins of the ESCRT machinery that releases the enveloped viruses into the extracellular fluid (Rheinemann and Sundquist 2021).

During all these steps of orthohantaviral replication cycle, the nucleocapsid (N) protein plays an outstanding role. This protein is a viral multifunctional protein that does not only facilitate stabilization of viral RNA, but also plays leading roles in genome packaging, intracellular protein transport, RNA chaperoning, DNA degradation, and intervention with the host immune response (Reuter and Krüger 2018). For instance, by remodeling Golgi structures, it establishes RNA synthesis factories (Davies et al. 2019). In addition, N protein impairs adhesion and migration capacity of infected renal cells and may contribute to renal pathogenesis (Hägele et al. 2019). All these features are mediated by its ability to interact with RNA and other viral and cellular proteins and thus qualify as a promising target for antiviral drug development (Arragain et al. 2019; Reuter and Krüger 2018).

38.6.1.2 Immune System and Cell Death Evasion

Frontline in the fight against a viral infection is the interferon (IFN) 1 system. Cells in direct contact with the virus excrete interferons as signal molecules to alert the immune system. Indeed, experiments in IFN1-deficient mice showed a high burden of orthohantavirus in all organs briefly after infection (Dowall et al. 2020). Mammalian cells identify an orthohantavirus infection by the RIG-1 receptor, a cytoplasmic receptor for dsRNA, that in turn activates the IFN1 cascade (Kell et al. 2020). Orthohantavirus, however, evolved different strategies to escape mammalian immunity. For Andes virus, viral NSs protein that antagonizes a cellular type 1 IFN response by inhibiting MAVS signaling is a key player in this evasion (Vera-Otarola et al. 2020). The effectiveness of an immune evasion of various old world or new world orthohantavirus species rests in the protein structure of their Ns or NSs proteins, respectively. Especially, NSs position 386 proved crucial in its effectiveness and may further explain the only human-to-human transmissibility of the Andes virus (Simons et al. 2019).

Mammalian cells developed a secondary line of defense against viral infection. For instance, exosomal miR145-5p, produced by orthohantavirus-infected endothelial cells, actively blocks viral replication (Wang et al. 2020). Furthermore, activated neutrophils secrete myeloperoxidase, neutrophil elastase, and IL-8, that may also drive the renal syndromes observed among patients (Strandin et al. 2018). In addition, genetic predisposition may alter the effectiveness of an immune response against orthohanta infection. It was shown that high expression of the immune modulators complement factor CFHR1 negatively and SIRBP1 positively affects the outcome of an orthohantavirus infection (Ribeiro et al. 2019).

The goal of a functioning immune system is to remove infected cells from the tissue. In case the immune system fails to extrinsically execute cell death in infected cells, concerned cells can also initiate cell death on their own, called intrinsic apoptosis (Peintner and Borner 2017). Interestingly, orthohantavirus also developed mechanisms to block the correct execution of extrinsic and intrinsic cell death (Solà-Riera et al. 2019). Central in the execution of cell death is the proteolytic protease caspase 3. It was experimentally proven, that the orthohantaviral N protein heterodimerizes with activated caspase 3 and so prevents cell death (Davies et al. 2019).

Extrinsic apoptosis induced by granzyme B or TRAIL is perturbed in orthohantavirus infected hosts by a downregulation of its surface receptor DR5 (Solà-Riera et al. 2020; Solà-Riera et al. 2019). This publication also observed an upregulation of the antiapoptotic protein Bcl2 after the infection with ANDV. This upregulation blocked the permeabilization of the outer membrane of mitochondria, a central event in intrinsic apoptosis. Hence, no activation of caspases leading to cell death was observed (Solà-Riera et al. 2020).

38.6.1.3 Recent Scientific Breakthroughs

The heterogeneity concerning the clinical picture (HFRS and HCPS) and the broad range of disease severity among orthohantaviruses even between members of the same species is one of the most interesting questions. Identifying determinants of

organ-specificity and pathogenicity is a prerequisite for the development of therapeutic strategies. Research on orthohantaviruses is hampered by the lack of a suitable small animal model and *in vitro* cell culture research was performed in non-target cells for a long time. Fortunately, the number of studies using relevant cell culture systems is increasing in the last years. Establishment of cell lines from host animal species and use of primary human target cells facilitate the characterization of steps of the orthohantaviral replication cycle. Infection of host species cell lines allows to determine the host range of rodent species for orthohantavirus infection (Essbauer et al. 2011; Binder et al. 2019). Studies in human primary cells revealed virus- and cell type specific differences in the susceptibility of target cells and functional effects that may contribute to hantavirus pathogenicity (Bourquain et al. 2019; Raftery et al. 2014; Hägele et al. 2019).

Another major hindrance of research on orthohantaviruses is the work under BSL2 and 3 conditions, which demands well-equipped laboratories to perform experiments. Also, long-term culture of virus in cell culture may change the characteristics of viruses, as frequently observed in other viruses (Strandin et al. 2020). This problem may partly be circumvented by using VSV- (vesicular stomatitis virus) or SIV- (simian immunodeficiency virus) pseudotyped viruses (Old World: (Slough et al. 2019; Higa et al. 2012), New World: (Cifuentes-Muñoz et al. 2010)). Using this recombinant virus, it was able to highlight the importance of the Gn/Gc glycoproteins in regulating viral infectivity. These tools may also be used to perform safe neutralization tests to screen for new monoclonal antibodies against the virus (Ogino et al. 2003). However, characterization of postentry steps or testing antiviral substances will still require wild-type viruses.

38.6.2 Therapy and Vaccination

Rationale of all research on human pathogens is the development of a reliable vaccine to prevent, or an effective drug treatment to fight, an infection. Hence, many laboratories aim to develop convincing treatment options against orthohantavirus infections.

Several potential drugs and drug targets have been identified over the last years. Early studies with ribavirin, for instance, proved to improve the prognosis of cardio pulmonary syndrome patients (Safronetz et al. 2011). Recently, chemical compounds of phenyl-benzotriazole derivatives or baloxavir acid were proved to massively downregulate viral burden *in vitro* (Sanna et al. 2020; Ye et al. 2019). To design more targeted drugs against surface properties of the capsid, labs use fluorescence activated cell sorting (FACS) to identify molecules from a compound library that block the entry of the virus in the target cell (Buranda et al. 2018). By analyzing the interaction of the N protein with viral RNA using advanced EM imaging, new structures on the N protein were identified that could serve as future drug binding areas (Arragain et al. 2019).

Beside all these efforts to develop potent antivirals, the gold standard of the fight against virus infections is a reliable vaccination. It is known that the intramuscular or

subcutaneous injection of cell culture produced recombinant viral Gn/Gc or N proteins induce a specific immune response in laboratory mice (Li and Klein 2012; Yu et al. 2013). Soon, it became evident that the most promising targets of antibodies are the Gc and Gn glycoproteins on the surface of orthohantaviruses, since an early binding of antibodies at these glycoproteins prevents their structural shift, which enables the binding to the host cell (Rissanen et al. 2020). Further research on the protein structure of this glycoproteins enabled the design of tailor-suited monoclonal antibodies (Levanov et al. 2019; Abdulla et al. 2021). At present, only few vaccinations made it to the market. An oral-administered vaccination targeted against SNV is used to vaccinate deer mice (*Peromyscus maniculatuts*) in North America to lower the viral burden of rodents in endemic areas (Warner et al. 2020). Vaccinations in humans are mostly driven in Asian countries or the United States and are excellently summarized elsewhere (Liu et al. 2020).

The efficacy of mononuclear antibodies against orthohantavirus is further boosted by special adjuvants. Especially, low endotoxoic lipopolysaccharide contributed to the enhancement of the immune response and—as a welcomed side effect—prolonged shelf life of the vaccine (Kurashova et al. 2020).

Summarized, there are many potential vaccinations in the pipeline targeting the different orthohantavirus species around the globe. Some of them are already being tested in preclinical studies (Dzagurova et al. 2020; Ma et al. 2020); however, there are no postexposure prophylactic vaccines in development yet (Logue et al. 2020). Furthermore, orthohantavirus is a very diverse family of virus in the order of *Bunyavirales,* and it will be very sumptuous to create a specific vaccination for each single family member. Therefore, some labs try to find a universal vaccine that targets all members of *Bunyavirales* (Ter Horst et al. 2019).

38.7 Perspective

The knowledge of the distribution and the complexity of the family of *Hantaviridae* is expanding in an unprecedented speed. Now species are found in hosts on all continents and many countries operate a tight monitoring network to identify local hotspots. Although hantaviruses are not officially endemic everywhere, practitioners should be aware, that their patients might get infected on trips abroad or within the country. Furthermore, institutes for public health need to continue their screen of hantavirus in wild animals. By monitoring the host species composition and the viral load of hosts, an impact for the human local community can be derived.

However, for many of the new described orthohantavirus species, the consequence and pathogenicity on humans are unknown. It is the effort of many researchers to generate virus isolates and grow them in cell culture systems so understand more about the biology and transmission of the virus.

Currently, there are big efforts to design an efficient pan-orthohanta vaccine. The recent successful development of SARS-CoV2 vaccines based on mRNA-technology may probably facilitate the progress for a hantaviral vaccine. However, till such a licensed vaccine is approved for the market, prevention of infection will

remain on rodent control, avoidance of contact with rodent excreta, and properly cleaning and disinfecting areas contaminated by rodent excreta.

In conclusion, despite 30 years of intense studies and growing awareness on hantaviruses, there are still a lot of open questions that will attract the curiosity of scientists for the coming years.

38.8 Cross-References

- ▶ Bat-Related Zoonoses
- ▶ Dangerous Viral Pathogens of Animal Origin: Risk and Biosecurity
- ▶ Public Health and Rodents: A Game of Cat and Mouse
- ▶ Vector-Borne Zoonoses
- ▶ Wild Birds and Zoonotic Pathogens

Conflict of Interest The authors declare no conflict of interest.

The authors declare that there is no financial or personal relationship with other people or organizations that could inappropriately influence the work. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by Bundeswehr Joint Medical Service or any other governmental institutions.

References

Abdulla F, Nain Z, Hossain MM, Syed SB, Ahmed Khan MS, Adhikari UK (2021) A comprehensive screening of the whole proteome of hantavirus and designing a multi-epitope subunit vaccine for cross-protection against hantavirus: structural vaccinology and immunoinformatics study. Microb Pathog 150:104705

Abu Sin M, Stark K, Van Treeck U, Dieckmann H, Uphoff H, Hautmann W, Bornhofen B, Jensen E, Pfaff G, Koch J (2007) Risk factors for hantavirus infection in Germany, 2005. Emerg Infect Dis 13:1364–1366

Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, De La Torre JC, De Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gão GF, Gonzalez J-PJ, Groschup MH, Günther S, Haenni A-L, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, De Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach H-P, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song J-W, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang Y-Z, Zhāng Y et al (2019) Taxonomy of the order Bunyavirales: update 2019. Arch Virol 164:1949–1965

Acuña R, Cifuentes-Muñoz N, Márquez CL, Bulling M, Klingström J, Mancini R, Lozach P-Y, Tischler ND (2014) Hantavirus Gn and Gc glycoproteins self-assemble into virus-like particles. J Virol 88

Ahlm C, Wallin K, Lundkvist A, Elgh F, Juto P, Merza M, Tärnvik A (2000) Serologic evidence of Puumala virus infection in wild moose in Northern Sweden. Am J Trop Med Hyg 62:106–111

Alonso DO, Iglesias A, Coelho R, Periolo N, Bruno A, Córdoba MT, Filomarino N, Quipildor M, Biondo E, Fortunato E, Bellomo C, Martínez VP (2019) Epidemiological description, case-fatality rate, and trends of hantavirus pulmonary syndrome: 9 years of surveillance in Argentina. J Med Virol 91:1173–1181

Arragain B, Reguera J, Desfosses A, Gutsche I, Schoehn G, Malet H (2019) High resolution cryo-em structure of the Helical Rna-Bound Hantaan virus nucleocapsid reveals its assembly mechanisms. Elife 8:E43075

Augot D, Sauvage F, Boue F, Bouloy M, Artois M, Demerson JM, Combes B, Coudrier D, Zeller H, Cliquet F, Pontier D (2008) Spatial and temporal patterning of Bank vole demography and the epidemiology of the Puumala hantavirus in Northeastern France. Epidemiol Infect 136: 1638–1643

Avsic-Zupanc T, Xiao SY, Stojanovic R, Gligic A, Van Der Groen G, Leduc JW (1992) Characterization of Dobrava virus: A hantavirus from Slovenia, Yugoslavia. J Med Virol 38:132–137

Avsic-Zupanc T, Petrovec M, Furlan P, Kaps R, Elgh F, Lundkvist A (1999) Hemorrhagic fever with renal syndrome in the Dolenjska Region Of Slovenia–A 10-year survey. Clin Infect Dis 28: 860–865

Avsic-Zupanc T, Petrovec M, Duh D, Plyusnina A, Lundkvist A, Plyusnin A (2007) Puumala hantavirus in Slovenia: analyses of S and M segment sequences recovered from patients and rodents. Virus Res 123:204–210

Bauherr S, Larsberg F, Petrich A, Sperber HS, Klose-Grzelka V, Luckner M, Azab W, Schade M, Höfer CT, Lehmann MJ, Witkowski PT, Krüger DH, Herrmann A, Schwarzer R (2020) Macropinocytosis and Clathrin-dependent endocytosis play pivotal roles for the infectious entry of Puumala virus. J Virol 94

Bennett M, Lloyd G, Jones N, Brown A, Trees AJ, Mccracken C, Smyth NR, Gaskell CJ, Gaskell RM (1990) Prevalence of antibody to hantavirus in some cat populations in Britain. Vet Rec 127:548–549

Bergstedt Oscarsson K, Brorstad A, Baudin M, Lindberg A, Forssén A, Evander M, Eriksson M, Ahlm C (2016) Human Puumala hantavirus infection in northern Sweden; increased Seroprevalence and association to risk and health factors. BMC Infect Dis 16:566

Bi Z, Formenty PBH, Roth CE (2008) Hantavirus infection: a review and global update. J Infect Dev Countries 2:3–23

Bignon EA, Albornoz A, Guardado-Calvo P, Rey FA, Tischler ND (2019) Molecular organization and dynamics of the fusion protein Gc at the hantavirus surface. Elife 8

Binder F, Lenk M, Weber S, Stoek F, Dill V, Reiche S, Riebe R, Wernike K, Hoffmann D, Ziegler U, Adler H, Essbauer S, Ulrich RG (2019) Common vole (*Microtus arvalis*) and Bank vole (*Myodes glareolus*) derived permanent cell lines differ in their susceptibility and replication kinetics of animal and zoonotic viruses. J Virol Methods 274:113729

Binder F, Drewes S, Imholt C, Saathoff M, Below DA, Bendl E, Conraths FJ, Tenhaken P, Mylius M, Brockmann S, Oehme R, Freise J, Jacob J, Ulrich RG (2020) Heterogeneous Puumala Orthohantavirus situation in endemic regions in Germany in summer 2019. Transbound Emerg Dis 67:502–509

Binder F, Gallo G, Bendl E, Eckerle I, Ermonval M, Luttermann C, Ulrich RG (2021) Inhibition of interferon I induction by non-structural protein Nss of Puumala virus and other vole-associated Orthohantaviruses: phenotypic plasticity of the protein and potential functional domains. Arch Virol 166:2999–3012

Boehlke C, Hartleben B, Huber TB, Hopfer H, Walz G, Neumann-Haefelin E (2014) Hantavirus infection with severe proteinuria and Podocyte foot-process effacement. Am J Kidney Dis 64: 452–456

Boone I, Wagner-Wiening C, Reil D, Jacob J, Rosenfeld UM, Ulrich RG, Lohr D, Pfaff G (2012) Rise in the number of notified human hantavirus infections since October 2011 in Baden-Wurttemberg, Germany. Euro Surveillance 17:20180

Bourquain D, Bodenstein C, Schürer S, Schaade L (2019) Puumala and Tula virus differ in replication kinetics and innate immune stimulation in human endothelial cells and macrophages. Viruses 11:855

Buranda T, Wu Y, Perez D, Jett SD, Bonduhawkins V, Ye C, Edwards B, Hall P, Larson RS, Lopez GP, Sklar LA, Hjelle B (2010) Recognition of decay accelerating factor and αvβ3 by inactivated

hantaviruses: toward the development of high-throughput screening flow cytometry assays. Anal Biochem 402:151–160
Buranda T, Gineste C, Wu Y, Bondu V, Perez D, Lake KR, Edwards BS, Sklar LA (2018) A high-throughput flow cytometry screen identifies molecules that inhibit hantavirus cell entry. Slas Discov 23:634–645
Carey DE, Reuben R, Panicker KN, Shope RE, Myers RM (1971) Thottapalayam virus: a presumptive arbovirus isolated from A shrew in India. Indian J Med Res 59:1758–1760
Cdc (2022) Hantavirus Surveillance [Online]. Available: https://Www.Cdc.Gov/Hantavirus/Surveillance/International.Html. Accessed 01 Feb 2022
Chand S, Thapa S, Kon S, Johnson SC, Poeschla EM, Franco-Paredes C, Rodríguez-Morales AJ, Mattar S, Henao-Martínez AF (2020) Hantavirus infection with renal failure and proteinuria, Colorado, USA, 2019. Emerg Infect Dis 26:383–385
Childs JE, Ksiazek TG, Spiropoulou CF, Krebs JW, Morzunov S, Maupin GO, Gage KL, Rollin PE, Sarisky J, Enscore RE (1994) Serologic and genetic identification of Peromyscus Maniculatus as the primary rodent reservoir for A new hantavirus in the Southwestern United States. J Infect Dis 169:1271–1280
Choi Y, Kwon Y-C, Kim S-I, Park J-M, Lee K-H, Ahn B-Y (2008) A hantavirus causing Hemorrhagic fever with renal syndrome requires Gc1qr/P32 for efficient cell binding and infection. Virology 381:178–183
Cifuentes-Muñoz N, Darlix J-L, Tischler ND (2010) Development of A Lentiviral vector system to study the role of the Andes virus glycoproteins. Virus Res 153:29–35
Cifuentes-Muñoz N, Barriga GP, Valenzuela PDT, Tischler NDYR (2011) Aromatic and polar residues spanning the candidate fusion peptide of the Andes virus Gc protein are essential for membrane fusion and infection. J Gen Virol 92:552–563
Collan Y, Lähdevirta J, Jokinen EJ (1978) Electron microscopy of Nephropathia Epidemica. Virchows Archiv A 377:129–144
Danes L, Pejcoch M, Bukovjan K, Veleba J, Halacková M (1992) Antibodies against hantaviruses in game and domestic oxen in the Czech Republic. Ceskoslovenska Epidemiol Mikrobiol Imunol 41:15–18
Davies K, Afrough B, Mankouri J, Hewson R, Edwards TA, Barr JNYR (2019) Tula Orthohantavirus Nucleocapsid protein is cleaved in infected cells and may sequester activated Caspase-3 during persistent infection to suppress apoptosis. J Gen Virol 100:1208–1221
Diagne MM, Dieng I, Granjon L, Lucaccioni H, Sow A, Ndiaye O, Faye M, Bâ K, Bâ Y, Diallo M, Faye O, Duplantier J-M, Diallo M, Handschumacher P, Faye O, Sall AA (2020) Seoul Orthohantavirus in wild black rats, Senegal, 2012–2013. Emerg Infect Dis 26:2460–2464
Dobly A, Cochez C, Goossens E, De Bosschere H, Hansen P, Roels S, Heyman P (2012) Sero-epidemiological study of the presence of hantaviruses in domestic dogs and cats from Belgium. Res Vet Sci 92:221–224
Dowall SD, Graham VA, Aram M, Findlay-Wilson S, Salguero FJ, Emery K, Hewson R (2020) Hantavirus infection in type I interferon receptor-deficient (A129) mice. J Gen Virol 101: 1047–1055
Du H, Li J, Jiang W, Yu H, Zhang Y, Wang J, Wang P, Bai X (2014) Clinical study of critical patients with Hemorrhagic fever with renal syndrome complicated by acute respiratory distress syndrome. PLoS One 9:E89740
Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, Ksiazek TG, Rollin PE, Nichol S, Umland ET (1994) Hantavirus pulmonary syndrome: A clinical description of 17 patients with A newly recognized disease. The hantavirus study group. N Engl J Med 330: 949–955
Dzagurova TK, Tkachenko EA, Ishmukhametov AA, Balovneva MV, Klempa B, Kruger DH (2018) Severe hantavirus disease in children. J Clin Virol 101:66–68
Dzagurova TK, Siniugina AA, Ishmukhametov AA, Egorova MS, Kurashova SS, Balovneva MV, Deviatkin AA, Tkachenko PE, Leonovich OA, Tkachenko EA (2020) Pre-clinical studies of inactivated polyvalent Hfrs vaccine. Front Cell Infect Microbiol 10

Ecdc (2022) Introduction to the annual epidemiological report [Online]. Available: https://Www.Ecdc.Europa.Eu/En/Surveillance-And-Disease-Data/Annual-Epidemiological-Reports/Introduction-Annual. Accessed 01 Feb 2022

Enyi L, Shuhe L, Panpan Y, Li F, Xiaolu D, Han X, Yajing X (2022) Central nervous system infection with Seoul Orthohantavirus in A child after hematopoietic stem cell transplantation: a case report. Virol J:19

Escutenaire S, Pastoret PP, Sjölander KB, Heyman P, Brochier B, Lundkvist A (2000) Evidence of Puumala hantavirus infection in red foxes (Vulpes Vulpes) in Belgium. Vet Rec 147:365–366

Essbauer S, Schmidt J, Conraths FJ, Friedrich R, Koch J, Hautmann W, Pfeffer M, Wölfel R, Finke J, Dobler G, Ulrich R (2006) A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia Epidemica in south-East Germany in 2004. Epidemiol Infect 134:1333–1344

Essbauer SS, Schmidt-Chanasit J, Madeja EL, Wegener W, Friedrich R, Petraityte R, Sasnauskas K, Jacob J, Koch J, Dobler G, Conraths FJ, Pfeffer M, Pitra C, Ulrich RG (2007) Nephropathia Epidemica in metropolitan area, Germany. Emerg Infect Dis 13:1271–1273

Essbauer SS, Krautkrämer E, Herzog S, Pfeffer M (2011) A new permanent cell line derived from the Bank vole (Myodes Glareolus) as cell culture model for zoonotic viruses. Virol J 8:339

Essbauer S, Cochez C, Marie J, Thoma B, Heyman P (2013) Epidemiology and driving factors of hantavirus infections in Western Europe. Irafms 85:25–25

Ettinger J, Hofmann J, Enders M, Tewald F, Oehme RM, Rosenfeld UM, Ali HS, Schlegel M, Essbauer S, Osterberg A, Jacob J, Reil D, Klempa B, Ulrich RG, Kruger DH (2012) Multiple synchronous outbreaks of Puumala virus, Germany, 2010. Emerg Infect Dis 18:1461–1464

Evander M, Ahlm C (2009) Milder winters in northern Scandinavia may contribute to larger outbreaks of haemorrhagic fever virus. Glob Health Action 2. https://doi.org/10.3402/Gha.V2i0.2020

Faber MS, Ulrich RG, Frank C, Brockmann SO, Pfaff GM, Jacob J, Krüger DH, Stark K (2010) Steep rise in notified hantavirus infections in Germany, April 2010. Euro Surveill 19574

Faber M, Krüger DH, Auste B, Stark K, Hofmann J, Weiss S (2019) Molecular and epidemiological characteristics of human Puumala and Dobrava-Belgrade hantavirus infections, Germany, 2001 to 2017. Eur Secur 24:1800675

Featherstone CA, Wyllie S, Frost AJ, Mcelhinney L, Fooks AR (2013) Hantavirus And Pet Rodents. Vet Rec 172:370

Ferluga D, Vizjak A (2008) Hantavirus nephropathy. J Am Soc Nephrol 19:1653–1658

Ferrés M, Vial P (2004) Hantavirus infection in children. Curr Opin Pediatr 16:70–75

Ferres M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, Hjelle B, Delgado I, Lee S-J, Mertz GJ, Andes Virus Household Contacts Study, G (2007) Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. J Infect Dis 195:1563–1571

Firth C, Tokarz R, Simith DB, Nunes MRT, Bhat M, Rosa EST, Medeiros DBA, Palacios G, Vasconcelos PFC, Lipkin WI (2012) Diversity and distribution of hantaviruses in South America. J Virol 86:13756–13766

Fonseca LX, Oliveira SVD, Duarte EC (2018) Magnitude and distribution of deaths due to hantavirus in Brazil, 2007-2015. Epidemiol E Serv De Saude: Revista Do Sistema Unico De Saude Do Brasil 27:E2017221

Gajdusek D (1956) Hemorrhagic fevers in Asia: A problem in medical ecology. Geogr Rev 46:20–42

Gamage CD, Yoshimatsu K, Sarathkumara YD, Kulendiran T, Nanayakkara N, Arikawa J (2017) Serological evidence of hantavirus infection in Girandurukotte, an area endemic for chronic kidney disease of unknown aetiology (Ckdu) in Sri Lanka. Int J Infect Dis 57:77–78

Garanina SB, Platonov AE, Zhuravlev VI, Murashkina AN, Yakimenko VV, Korneev AG, Shipulin GA (2009) Genetic diversity and geographic distribution of hantaviruses in Russia. Zoonoses Public Health 56:297–309

Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER (1998) Beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A 95:7074–7079

Gavrilovskaya IN, Brown EJ, Ginsberg MH, Mackow ER (1999) Cellular entry of hantaviruses which cause Hemorrhagic fever with renal syndrome is mediated by Beta3 Integrins. J Virol 73: 3951–3959

Gherasim A, Hjertqvist M, Lundkvist Å, Kühlmann-Berenzon S, Carlson JV, Stenmark S, Widerström M, Österlund A, Boman H, Ahlm C, Wallensten A (2015) Risk factors and potential preventive measures for Nephropatia Epidemica In Sweden 2011–2012: a case–control study. Infect Ecol Epidemiol 5. https://doi.org/10.3402/Iee.V5.27698

Gizzi M, Delaere B, Weynand B, Clement J, Maes P, Vergote V, Laenen L, Hjelle B, Verroken A, Dive A, Michaux I, Evrard P, Creytens D, Bulpa P (2013) Another case of "European hantavirus pulmonary syndrome" with severe lung, prior to kidney, involvement, and diagnosed by viral inclusions in lung macrophages. Eur J Clin Microbiol Infect Dis 32:1341–1345

Gonzalez JP, Mccormick JB, Baudon D, Gautun JP, Meunier DY, Dournon E, Georges AJ (1984) Serological evidence for Hantaan-related virus in Africa. Lancet (London, England) 2: 1036–1037

Groen J, Gerding M, Koeman JP, Roholl PJ, Van Amerongen G, Jordans HG, Niesters HG, Osterhaus AD (1995) A Macaque Model for hantavirus infection. J Infect Dis 172:38–44

Guo W-P, Lin X-D, Wang W, Tian J-H, Cong M-L, Zhang H-L, Wang M-R, Zhou R-H, Wang J-B, Li M-H, Xu J, Holmes EC, Zhang Y-Z (2013) Phylogeny and origins of hantaviruses Harbored by bats, insectivores, and rodents. PLoS Pathog 9:E1003159

Hägele S, Müller A, Nusshag C, Reiser J, Zeier M, Krautkrämer E (2019) Virus- and cell type-specific effects in Orthohantavirus infection. Virus Res 260:102–113

Hautala T, Sironen T, Vapalahti O, Pääkkö E, Särkioja T, Salmela PI, Vaheri A, Plyusnin A, Kauma H (2002) Hypophyseal Hemorrhage and Panhypopituitarism during Puumala virus infection: magnetic resonance imaging and detection of viral antigen in the Hypophysis. Clin Infect Dis 35:96–101

Heinemann P, Tia M, Alabi A, Anon J-C, Auste B, Essbauer S, Gnionsahe A, Kigninlman H, Klempa B, Kraef C, Kruger N, Leendertz FH, Ndhatz-Sanogo M, Schaumburg F, Witkowski PT, Akoua-Koffi CG, Kruger DH (2016) Human infections by non-rodent-associated hantaviruses in Africa. J Infect Dis 214:1507–1511

Heyman P, Plyusnina A, Berny P, Cochez C, Artois M, Zizi M, Pirnay JP, Plyusnin A (2004) Seoul hantavirus in Europe : first demonstration of the virus genome in wild *Rattus norvegicus* captured in France. Eur J Clin Microbiol Infect Dis 23:711

Heyman P, Cochez C, Ducoffre G, Mailles A, Zeller H, Sin MA, Koch J, Doornum GV, Koopmans M, Mossong J, Schneider F (2007) Haemorrhagic fever with renal syndrome: an analysis of the outbreaks in Belgium, France, Germany, the Netherlands and Luxembourg in 2005. Eur Secur 12:15–16

Heyman P, Vaheri A, Members E (2008) Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. Euro Surveillance 13:18925

Heyman P, Baert K, Plyusnina A, Cochez C, Lundkvist Å, Esbroeck MV, Goossens E, Vandenvelde C, Plyusnin A, Stuyck J (2009a) Serological and genetic evidence for the presence of Seoul hantavirus in Rattus Norvegicus in Flanders, Belgium. Scand J Infect Dis 41:51–56

Heyman P, Vaheri A, Lundkvist A, Avsic-Zupanc T (2009b) Hantavirus infections in Europe: from virus carriers to a major public-health problem. Expert Rev Anti-Infect Ther 7:205–217

Heyman P, Ceianu CS, Christova I, Tordo N, Beersma M, João Alves M, Lundkvist A, Hukic M, Papa A, Tenorio A, Zelená H, Essbauer S, Visontai I, Golovljova I, Connell J, Nicoletti L, Van Esbroeck M, Gjeruldsen Dudman S, Aberle SW, Avšić-Županc T, Korukluoglu G, Nowakowska A, Klempa B, Ulrich RG, Bino S, Engler O, Opp M, Vaheri A (2011) A five-year perspective on the situation of haemorrhagic fever with renal syndrome and status of the hantavirus reservoirs in Europe, 2005-2010. Euro Surveillance 16:19961

Heyman P, Thoma B, Marié J-L, Cochez C, Essbauer S (2012) In search for factors that drive hantavirus epidemics. Front Physiol:3

Higa MM, Petersen J, Hooper J, Doms RW (2012) Efficient production of Hantaan and Puumala Pseudovirions for viral tropism and neutralization studies. Virology 423:134–142

Hjelle B, Lee SW, Song W, Torrez-Martinez N, Song JW, Yanagihara R, Gavrilovskaya I, Mackow ER (1995) Molecular linkage of hantavirus pulmonary syndrome to the white-footed mouse,

Peromyscus leucopus: genetic characterization of the M genome of New York virus. J Virol 69: 8137–8141

Hjelle B, Goade D, Torrez-Martinez N, Lang-Williams M, Kim J, Harris RL, Rawlings JA (1996) Hantavirus pulmonary syndrome, renal insufficiency, and myositis associated with infection by bayou hantavirus. Clin Infect Dis 23:495–500

Hjertqvist M, Klein SL, Ahlm C, Klingström J (2010) Mortality rate patterns for Hemorrhagic fever with renal syndrome caused by Puumala virus. Emerg Infect Dis 16:1584–1586

Hofmann J, Meisel H, Klempa B, Vesenbeckh SM, Beck R, Michel D, Schmidt-Chanasit J, Ulrich RG, Grund S, Enders G, Kruger DH (2008) Hantavirus Outbreak, Germany, 2007. Emerg Infect Dis 14:850–852

Hofmann J, Kramer S, Herrlinger KR, Jeske K, Kuhns M, Weiss S, Ulrich RG, Krüger DH (2021) Tula virus as causative agent of hantavirus disease in immunocompetent person, Germany. Emerg Infect Dis 27:1234–1237

Huang L-Y, Zhou H, Yin W-W, Wang Q, Sun H, Ding F, Man T-F, Li Q, Feng Z-J (2012) The current epidemic situation and surveillance regarding Hemorrhagic fever with renal syndrome in China, 2010. Zhonghua Liu Xing Bing Xue Za Zhi = Zhonghua Liuxingbingxue Zazhi 33: 685–691

Hughes AL, Friedman R (2000) Evolutionary diversification of protein-coding genes of hantaviruses. Mol Biol Evol 17:1558–1568

Huong VTQ, Yoshimatsu K, Luan VD, Van Tuan L, Nhi L, Arikawa J, Nguyen TMN (2010) Hemorrhagic fever with renal syndrome, Vietnam. Emerging Infect Dis 16:363–365

Jameson LJ, Logue CH, Atkinson B, Baker N, Galbraith SE, Carroll MW, Brooks T, Hewson R (2013a) The continued emergence of hantaviruses: isolation of A Seoul virus implicated in human disease, United Kingdom, October 2012. Euro Surveillance 18:4–7

Jameson LJ, Taori SK, Atkinson B, Levick P, Featherstone CA, Burgt GVD, Mccarthy N, Hart J, Osborne JC, Walsh AL, Brooks TJ, Hewson R (2013b) Pet rats as A source of hantavirus in England and Wales, 2013. Eur Secur 18:20415

Jangra RK, Herbert AS, Li R, Jae LT, Kleinfelter LM, Slough MM, Barker SL, Guardado-Calvo P, Román-Sosa G, Dieterle ME, Kuehne AI, Muena NA, Wirchnianski AS, Nyakatura EK, Fels JM, Ng M, Mittler E, Pan J, Bharrhan S, Wec AZ, Lai JR, Sidhu SS, Tischler ND, Rey FA, Moffat J, Brummelkamp TR, Wang Z, Dye JM, Chandran K (2018) Protocadherin-1 is essential for cell entry by New World hantaviruses. Nature 563:559–563

Jeeva S, Mir S, Velasquez A, Weathers BA, Leka A, Wu S, Sevarany AT, Mir M (2018) Hantavirus Rdrp requires A host cell factor for cap snatching. J Virol 93

Jeske K, Jacob J, Drewes S, Pfeffer M, Heckel G, Ulrich RG, Imholt C (2021) Hantavirus–-leptospira coinfections in small mammals from Central Germany. Epidemiol Infect 149:E97

Jonsson CB, Figueiredo LTM, Vapalahti O (2010) A global perspective on hantavirus ecology, epidemiology, and disease. Clin Microbiol Rev 23:412–441

Juncker-Voss M, Prosl H, Lussy H, Enzenberg U, Auer H, Lassnig H, Müller M, Nowotny N (2004) Screening for antibodies against zoonotic agents among employees of the zoological garden of Vienna, Schönbrunn, Austria. Berliner Und Munchener Tierarztliche Wochenschrift 117: 404–409

Jung YT, Kim GR (1995) Genomic characterization of M and S Rna segments of hantaviruses isolated from bats. Acta Virol 39:231–233

Kang HJ, Arai S, Hope AG, Song J-W, Cook JA, Yanagihara R (2009) Genetic diversity and Phylogeography of Seewis virus in the Eurasian common shrew in Finland and Hungary. Virol J 6:208

Kang HJ, Kadjo B, Dubey S, Jacquet F, Yanagihara R (2011) Molecular evolution of Azagny virus, A newfound hantavirus Harbored by the west African pygmy shrew (*Crocidura obscurior*) in Côte D'ivoire. Virol J 8:373

Kariwa H, Lokugamage K, Lokugamage N, Miyamoto H, Yoshii K, Nakauchi M, Yoshimatsu K, Arikawa J, Ivanov LI, Iwasaki T, Takashima I (2007) A comparative epidemiological study of hantavirus infection in Japan and Far East Russia. Japan J Vet Res 54:145–161

Kell AM, Hemann EA, Turnbull JB, Gale M (2020) Rig-I-like receptor activation drives type I Ifn and antiviral Signaling to limit Hantaan Orthohantavirus replication. PLoS Pathog 16:E1008483

Khan AS, Spiropoulou CF, Morzunov S, Zaki SR, Kohn MA, Nawas SR, Mcfarland L, Nichol ST (1995) Fatal illness associated with A new hantavirus in Louisiana. J Med Virol 46:281–286

Khan AS, Gaviria M, Rollin PE, Hlady WG, Ksiazek TG, Armstrong LR, Greenman R, Ravkov E, Kolber M, Anapol H, Sfakianaki ED, Nichol ST, Peters CJ, Khabbaz RF (1996) Hantavirus pulmonary syndrome in Florida: association with the newly identified Black Creek canal virus. Am J Med 100:46–48

Khismatullina NA, Karimov MM, Khaertynov KS, Shuralev EA, Morzunov SP, Khaertynova IM, Ivanov AA, Milova IV, Khakimzyanova MB, Sayfullina G, Gaynullin AA, Ivanov AV, Rizvanov AA, Khaiboullina SF (2016) Epidemiological dynamics of Nephropathia Epidemica in the republic of Tatarstan, Russia, during the period of 1997-2013. Epidemiol Infect 144: 618–626

Kim GR, Lee YT, Park CH (1994) A new natural reservoir of hantavirus: isolation of hantaviruses from lung tissues of bats. Arch Virol 134:85–95

Kirsanovs S, Klempa B, Franke R, Lee MH, Schönrich G, Rang A, Kruger DH (2010) Genetic Reassortment between high-virulent and low-virulent Dobrava-Belgrade virus strains. Virus Genes 41:319–328

Kitterer D, Segerer S, Dippon J, Alscher MD, Braun N, Latus J (2016) Smoking is A risk factor for severe acute kidney injury in hantavirus-induced Nephropathia Epidemica. Nephron 134:89–94

Klein SL, Marks MA, Li W, Glass GE, Fang L-Q, Ma J-Q, Cao W-C (2011) Sex differences in the incidence and case fatality rates from Hemorrhagic fever with renal syndrome in China, 2004-2008. Clin Infect Dis 52:1414–1421

Klempa B (2018) Reassortment events in the evolution of hantaviruses. Virus Genes 54:638–646

Klempa B, Meisel H, Räth S, Bartel J, Ulrich R, Krüger DH (2003) Occurrence of renal and pulmonary syndrome in A region of Northeast Germany where Tula hantavirus circulates. J Clin Microbiol 41:4894–4897

Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Denys C, Koivogui L, Ter Meulen J, Krüger DH (2006) Hantavirus in African wood mouse, Guinea. Emerg Infect Dis 12: 838–840

Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Barrière P, Koivogui L, Ter Meulen J, Krüger DH (2007) Novel hantavirus sequences in shrew, Guinea. Emerg Infect Dis 13:520–522

Klempa B, Tkachenko EA, Dzagurova TK, Yunicheva YV, Morozov VG, Okulova NM, Slyusareva GP, Smirnov A, Kruger DH (2008) Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. Emerg Infect Dis 14:617–625

Klempa B, Koivogui L, Sylla O, Koulemou K, Auste B, Krüger DH, Ter Meulen J (2010) Serological evidence of human hantavirus infections in Guinea, West Africa. J Infect Dis 201: 1031–1034

Klempa B, Witkowski PT, Popugaeva E, Auste B, Koivogui L, Fichet-Calvet E, Strecker T, Meulen JT, Krüger DH (2012) Sangassou virus, the first hantavirus isolate from Africa, displays genetic and functional properties distinct from those of other Murinae-associated hantaviruses. J Virol 86

Klempa B, Avsic-Zupanc T, Clement J, Dzagurova TK, Henttonen H, Heyman P, Jakab F, Kruger DH, Maes P, Papa A, Tkachenko EA, Ulrich RG, Vapalahti O, Vaheri A (2013a) Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: definition of genotypes and their characteristics. Arch Virol 158:521–529

Klempa B, Koulemou K, Auste B, Emmerich P, Thomé-Bolduan C, Günther S, Koivogui L, Krüger DH, Fichet-Calvet E (2013b) Seroepidemiological study reveals regional co-occurrence of Lassa- and hantavirus antibodies in upper Guinea, West Africa. Trop Med Int Health TM & IH 18

Klingström J, Heyman P, Escutenaire S, Sjölander KB, De Jaegere F, Henttonen H, Lundkvist A (2002a) Rodent host specificity of European hantaviruses: evidence of Puumala virus interspecific Spillover. J Med Virol 68:581–588

Klingström J, Plyusnin A, Vaheri A, Lundkvist A (2002b) Wild-type Puumala hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in *Cynomolgus* macaques. J Virol 76:444–449

Klingström J, Lindgren T, Ahlm C (2008) Sex-dependent differences in plasma cytokine responses to hantavirus infection. Clin Vaccine Immunol CVI 15:885–887
Klingström J, Granath F, Ekbom A, Björkström NK, Ljunggren H-G (2014) Increased risk for lymphoma following Hemorrhagic fever with renal syndrome. Clin Infect Dis 59:1130–1132
Knust B, Macneil A, Rollin PE (2012) Hantavirus pulmonary syndrome clinical findings: evaluating A surveillance case definition. Vector Borne Zoon Dis (Larchmont, NY) 12:393–399
Koch J, Brockmann SO, Winter C, Kimmig P, Stark K (2007) Significant increase of hantavirus infections in Germany since the beginning of 2007. Euro Surveillance 12:E070503.1
Koma T, Yoshimatsu K, Taruishi M, Miyashita D, Endo R, Shimizu K, Yasuda SP, Amada T, Seto T, Murata R, Yoshida H, Kariwa H, Takashima I, Arikawa J (2012) Development of A serotyping enzyme-linked immunosorbent assay system based on recombinant truncated hantavirus Nucleocapsid proteins for New World hantavirus infection. J Virol Methods 185:74–81
Krautkrämer E, Zeier M (2008) Hantavirus causing Hemorrhagic fever with renal syndrome enters from the apical surface and requires decay-accelerating factor (Daf/Cd55). J Virol 82: 4257–4264
Krautkrämer E, Zeier M, Plyusnin A (2013) Hantavirus infection: an emerging infectious disease causing acute renal failure. Kidney Int 83:23–27
Kruger DH, Figueiredo LTM, Song J-W, Klempa B (2015) Hantaviruses–globally emerging pathogens. J Clin Virol 64:128–136
Ksiazek TG, Peters CJ, Rollin PE, Zaki S, Nichol S, Spiropoulou C, Morzunov S, Feldmann H, Sanchez A, Khan AS (1995) Identification of A new north American hantavirus that causes acute pulmonary insufficiency. Am J Trop Med Hyg 52:117–123
Ksiazek TG, Nichol ST, Mills JN, Groves MG, Wozniak A, Mcadams S, Monroe MC, Johnson AM, Martin ML, Peters CJ, Rollin PE (1997) Isolation, genetic diversity, and geographic distribution of bayou virus (Bunyaviridae: hantavirus). Am J Trop Med Hyg 57:445–448
Kurashova SS, Ishmukhametov AA, Dzagurova TK, Egorova MS, Balovneva MV, Nikitin NA, Evtushenko EA, Karpova OV, Markina AA, Aparin PG, Tkachenko PE, L'Vov VL, Tkachenko EA (2020) Various adjuvants effect on immunogenicity of Puumala virus vaccine. Front Cell Infect Microbiol 10
Laenen L, Vergote V, Calisher CH, Klempa B, Klingström J, Kuhn JH, Maes P (2019) Hantaviridae: current classification and future perspectives. Viruses 11:788
Latronico F, Mäki S, Rissanen H, Ollgren J, Lyytikäinen O, Vapalahti O, Sane J (2018) Population-based Seroprevalence of Puumala hantavirus in Finland: smoking as a risk factor. Epidemiol Infect 146:367–371
Latus J, Schwab M, Tacconelli E, Pieper F-M, Wegener D, Dippon J, Müller S, Zakim D, Segerer S, Kitterer D, Priwitzer M, Mezger B, Walter-Frank B, Corea A, Wiedenmann A, Brockmann S, Pöhlmann C, Alscher MD, Braun N (2015) Clinical course and long-term outcome of hantavirus-associated Nephropathia Epidemica, Germany. Emerg Infect Dis 21:76–83
Lázaro ME, Cantoni GE, Calanni LM, Resa AJ, Herrero ER, Iacono MA, Enria DA, Cappa SMG (2007) Clusters of hantavirus infection, Southern Argentina. Emerg Infect Dis 13:104–110
Lee HW (1982) Korean Hemorrhagic fever. Prog Med Virol 28:96–113
Lee HW, French GR, Lee PW, Baek LJ, Tsuchiya K, Foulke RS (1981) Observations on natural and laboratory infection of rodents with the etiologic agent of Korean Hemorrhagic fever. Am J Trop Med Hyg 30:477–482
Leighton FA, Artsob HA, Chu MC, Olson JG (2001) A serological survey of rural dogs and cats on the Southwestern Canadian prairie for zoonotic pathogens. Can J Public Health 92:67–71
Levanov L, Iheozor-Ejiofor RP, Lundkvist Å, Vapalahti O, Plyusnin A (2019) Defining of Mabs-neutralizing sites on the surface glycoproteins Gn and Gc of A hantavirus using vesicular stomatitis virus Pseudotypes and site-directed mutagenesis. J General Virol 100:145–155
Li W, Klein SL (2012) Seoul virus-infected rat lung endothelial cells and alveolar macrophages differ in their ability to support virus replication and induce regulatory T cell phenotypes. J Virol 86:11845–11855
Linderholm M, Sandström T, Rinnström O, Groth S, Blomberg A, Tärnvik A (1997) Impaired pulmonary function in patients with Hemorrhagic fever with renal syndrome. Clin Infect Dis 25: 1084–1089

Liu X, Jiang B, Bi P, Yang W, Liu Q (2012) Prevalence of haemorrhagic fever with renal syndrome in mainland China: analysis of National Surveillance Data, 2004-2009. Epidemiol Infect 140: 851–857

Liu R, Ma H, Shu J, Zhang Q, Han M, Liu Z, Jin X, Zhang F, Wu X (2020) Vaccines and therapeutics against hantaviruses. Front Microbiol:10

Logue J, Crozier I, Jahrling PB, Kuhn JH (2020) Post-exposure prophylactic vaccine candidates for the treatment of human risk group 4 pathogen infections. Exp Rev Vaccines 19:85–103

López N, Padula P, Rossi C, Lázaro ME, Franze-Fernández MT (1996) Genetic identification of A new hantavirus causing severe pulmonary syndrome in Argentina. Virology 220:223–226

Luis AD, Douglass RJ, Hudson PJ, Mills JN, Bjørnstad ON (2012) Sin Nombre hantavirus decreases survival of male deer mice. Oecologia 169:431–439

Ma Y, Tang K, Zhang Y, Zhang C, Zhang Y, Jin B, Ma Y (2020) Design and synthesis of Hla-A*02-restricted Hantaan virus multiple-antigenic peptide for Cd8+ T cells. Virol J 17:15

Macneil A, Ksiazek TG, Rollin PE (2011) Hantavirus pulmonary syndrome, United States, 1993–2009. Emerg Infect Dis 17:1195–1201

Maes P, Klempa B, Clement J, Matthijnssens J, Gajdusek DC, Krüger DH, Van Ranst M (2009) A proposal for new criteria for the classification of hantaviruses, based on S and M segment protein sequences. Infect Genet Evol 9:813–820

Mailles A, Sin MA, Ducoffre G, Heyman P, Koch J, Zeller H (2005) Larger than usual increase in cases of hantavirus infections in Belgium, France and Germany, June 2005. Weekly Releases (1997–2007) 10:2754

Mäkelä S, Hurme M, Ala-Houhala I, Mustonen J, Koivisto A-M, Partanen J, Vapalahti O, Vaheri A, Pasternack A (2001) Polymorphism of the cytokine genes in hospitalized patients with Puumala hantavirus infection. Nephrol Dial Transplant 16:1368–1373

Malecki TM, Jillson GP, Thilsted JP, Elrod J, Torrez-Martinez N, Hjelle B (1998) Serologic survey for hantavirus infection in domestic animals and coyotes from New Mexico and Northeastern Arizona. J Am Vet Med Assoc 212:970–973

Markotić A, Nichol ST, Kuzman I, Sanchez AJ, Ksiazek TG, Gagro A, Rabatić S, Zgorelec R, Avsic-Zupanc T, Beus I, Dekaris D (2002) Characteristics of Puumala and Dobrava infections in Croatia. J Med Virol 66:542–551

Martinez VP, Bellomo C, San Juan J, Pinna D, Forlenza R, Elder M, Padula PJ (2005) Person-to-person transmission of Andes virus. Emerg Infect Dis 11:1848–1853

Martinez VP, Bellomo CM, Cacace ML, Suárez P, Bogni L, Padula PJ (2010) Hantavirus pulmonary syndrome in Argentina, 1995–2008. Emerg Infect Dis 16:1853–1860

Martínez VP, Di Paola N, Alonso DO, Pérez-Sautu U, Bellomo CM, Iglesias AA, Coelho RM, López B, Periolo N, Larson PA, Nagle ER, Chitty JA, Pratt CB, Díaz J, Cisterna D, Campos J, Sharma H, Dighero-Kemp B, Biondo E, Lewis L, Anselmo C, Olivera CP, Pontoriero F, Lavarra E, Kuhn JH, Strella T, Edelstein A, Burgos MI, Kaler M, Rubinstein A, Kugelman JR, Sanchez-Lockhart M, Perandones C, Palacios G (2020) "Super-spreaders" and person-to-person transmission of Andes virus in Argentina. N Engl J Med 383:2230–2241

Martínez-Valdebenito C, Angulo J, Le Corre N, Marco C, Vial C, Miquel JF, Cerda J, Mertz G, Vial P, Lopez-Lastra M, Ferrés M (2019) A single-nucleotide polymorphism of αvβ3 integrin is associated with the Andes virus infection susceptibility. Viruses 11:169

Mayor J, Torriani G, Rothenberger S, Engler O (2020) T-cell immunoglobulin and mucin (Tim) contributes to the infection of human airway epithelial cells by Pseudotype viruses containing Hantaan virus glycoproteins. Virology 543:54–62

Meheretu Y, ČíŽková D, Těšíková J, Welegerima K, Tomas Z, Kidane D, Girmay K, Schmidt-Chanasit J, Bryja J, Günther S, Bryjová A, Leirs H, Goüy De Bellocq J (2012) High diversity of Rna viruses in rodents, Ethiopia. Emerg Infect Dis 18:2047–2050

Meheretu Y, Stanley WT, Craig EW, Goüy De Bellocq J, Bryja J, Leirs H, Pahlmann M, Günther S (2019) Tigray Orthohantavirus infects two related rodent species adapted to different elevations in Ethiopia. Vector Borne Zoon Dis (Larchmont, N.Y.) 19:950–953

Meheretu Y, Granberg Å, Berhane G, Khalil H, Lwande OW, Mitiku M, Welegerima K, De Bellocq JG, Bryja J, Abreha H, Leirs H, Ecke F, Evander M (2021) Prevalence of Orthohantavirus-reactive antibodies in humans and Peri-domestic rodents in northern Ethiopia. Viruses 13:1054

Mertens M, Essbauer SS, Rang A, Schröder J, Splettstoesser WD, Kretzschmar C, Krüger DH, Groschup MH, Mätz-Rensing K, Ulrich RG (2011a) Non-human primates in outdoor enclosures: risk for infection with rodent-borne hantaviruses. Vet Microbiol 147:420–425

Mertens M, Hofmann J, Petraityte-Burneikiene R, Ziller M, Sasnauskas K, Friedrich R, Niederstrasser O, Krüger DH, Groschup MH, Petri E, Werdermann S, Ulrich RG (2011b) Seroprevalence study in forestry workers of a non-endemic region in eastern Germany reveals infections by Tula and Dobrava-Belgrade hantaviruses. Med Microbiol Immunol 200:263–268

Mertens M, Kindler E, Emmerich P, Esser J, Wagner-Wiening C, Wölfel R, Petraityte-Burneikiene-R, Schmidt-Chanasit J, Zvirbliene A, Groschup MH, Dobler G, Pfeffer M, Heckel G, Ulrich RG, Essbauer SS (2011c) Phylogenetic analysis of Puumala virus subtype Bavaria, characterization and diagnostic use of its recombinant Nucleocapsid protein. Virus Genes 43:177–191

Mittler E, Dieterle ME, Kleinfelter LM, Slough MM, Chandran K, Jangra RK (2019) Chapter six - hantavirus entry: perspectives and recent advances. In: Kielian M, Mettenleiter TC, Roossinck MJ (eds) Advances in virus research. Academic Press

Miyamoto H, Kariwa H, Araki K, Lokugamage K, Hayasaka D, Cui BZ, Lokugamage N, Ivanov LI, Mizutani T, Iwasa MA, Yoshimatsu K, Arikawa J, Takashima I (2003) Serological analysis of Hemorrhagic fever with renal syndrome (Hfrs) patients in far eastern Russia and identification of the causative hantavirus genotype. Arch Virol 148:1543–1556

Montoya-Ruiz C, Diaz FJ, Rodas JD (2014) Recent evidence of hantavirus circulation in the American tropic. Viruses 6:1274–1293

Morzunov SP, Feldmann H, Spiropoulou CF, Semenova VA, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST (1995) A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. J Virol 69:1980–1983

Morzunov SP, Rowe JE, Ksiazek TG, Peters CJ, St. Jeor SC, Nichol ST (1998) Genetic analysis of the diversity and origin of hantaviruses in Peromyscus Leucopus mice in North America. J Virol 72:57–64

Müller A, Baumann A, Essbauer S, Radosa L, Krüger DH, Witkowski PT, Zeier M, Krautkrämer E (2019) Analysis of the integrin β3 receptor for pathogenic Orthohantaviruses in rodent host species. Virus Res 267:36–40

Mustonen P, Kanerva P, Vapalahti P, Vaheri (1998) Association of Hla B27 with benign clinical course of Nephropathia Epidemica caused by Puumala hantavirus. Scand J Immunol 47:277–279

Nemirov K, Leirs H, Lundkvist A, Olsson GE (2010) Puumala hantavirus and Myodes Glareolus in northern Europe: no evidence of co-divergence between genetic lineages of virus and host. J General Virol 91:1262–1274

Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ (1993) Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science (New York, N.Y.) 262:914–917

Noack D, Goeijenbier M, Reusken C, Koopmans MPG, Rockx BHG (2020) Orthohantavirus pathogenesis and cell tropism. Front Cell Infect Microbiol 10:399

Nowotny N (1994) The domestic cat: a possible transmitter of viruses from rodents to man. Lancet (London, England) 343:921

Nowotny N (1996) Serologic studies of domestic cats for potential human pathogenic virus infections from wild rodents. Zentralblatt Fur Hygiene Und Umweltmedizin = Int J Hyg Environ Med 198:452–461

Nusshag C, Stütz A, Hägele S, Speer C, Kälble F, Eckert C, Brenner T, Weigand MA, Morath C, Reiser J, Zeier M, Krautkrämer E (2020) Glomerular filtration barrier dysfunction in a self-limiting, RNA virus-induced Glomerulopathy resembles findings in idiopathic nephrotic syndromes. Sci Rep 10:19117

Ogino M, Ebihara H, Lee B-H, Araki K, Lundkvist Å, Kawaoka Y, Yoshimatsu K, Arikawa J (2003) Use of vesicular stomatitis virus Pseudotypes bearing Hantaan or Seoul virus envelope proteins in a rapid and safe neutralization test. Clin Vaccine Immunol 10

Olsson GE, Dalerum F, Hörnfeldt B, Elgh F, Palo TR, Juto P, Ahlm C (2003) Human hantavirus infections, Sweden. Emerg Infect Dis 9:1395–1401

Outinen TK, Mäkelä S, Pörsti I, Vaheri A, Mustonen J (2022) Severity biomarkers in Puumala hantavirus infection. Viruses 14:45

Padula PJ, Edelstein A, Miguel SD, López NM, Rossi CM, Rabinovich RD (1998) Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology 241:323–330

Paho (2022) Paho/Who | Pan American Health Organization [Online]. Available: https://Www.Paho.Org/En. Accessed 01 Feb 2022

Pattamadilok S, Lee BH, Kumperasart S, Yoshimatsu K, Okumura M, Nakamura I, Araki K, Khoprasert Y, Dangsupa P, Panlar P, Jandrig B, Krüger DH, Klempa B, Jäkel T, Schmidt J, Ulrich R, Kariwa H, Arikawa J (2006) Geographical distribution of hantaviruses in Thailand and potential human health significance of Thailand virus. Am J Trop Med Hyg 75:994–1002

Pearce-Duvet J, St Jeor S, Boone J, Dearing MD (2006) Changes in sin Nombre virus antibody prevalence in deer mice across seasons: the interaction between habitat, sex, and infection in deer mice. J Wildl Dis 42:819–824

Peintner L, Borner C (2017) Role of apoptosis in the development of autosomal dominant polycystic kidney disease (Adpkd). Cell Tissue Res 369:27–39

Peintner L, Wagner E, Shin A, Tukhanova N, Turebekov N, Abdiyeva K, Spaiser O, Serebrennikova Y, Tintrup E, Dmitrovskiy A, Zhalmagambetova A, Frey S, Essbauer SS (2021) Eight years of collaboration on biosafety and biosecurity issues between Kazakhstan and Germany as part of the German biosecurity programme and the G7 global partnership against the spread of weapons and materials of mass destruction. Front Public Health 9:649393

Pettersson L, Boman J, Juto P, Evander M, Ahlm C (2008) Outbreak of Puumala virus infection, Sweden. Emerg Infect Dis 14:808–810

Piechotowski I, Brockmann SO, Schwarz C, Winter CH, Ranft U, Pfaff G (2008) Emergence of hantavirus in South Germany: rodents, climate and human infections. Parasitol Res 103(Suppl 1):S131–S137

Pilaski J, Feldmann H, Morzunov S, Rollin PE, Ruo SL, Lauer B, Peters CJ, Nichol ST (1994) Genetic identification of a new Puumala virus strain causing severe Hemorrhagic fever with renal syndrome in Germany. J Infect Dis 170:1456–1462

Plyusnin A (2002) Genetics of hantaviruses: implications to taxonomy. Arch Virol 147:665–682

Plyusnin A, Morzunov SP (2001) Virus evolution and genetic diversity of hantaviruses and their rodent hosts. Curr Top Microbiol Immunol 256:47–75

Plyusnin A, Vapalahti O, Lankinen H, Lehväslaiho H, Apekina N, Myasnikov Y, Kallio-Kokko H, Henttonen H, Lundkvist A, Brummer-Korvenkontio M (1994) Tula virus: a newly detected hantavirus carried by European common voles. J Virol 68:7833–7839

Plyusnin A, Vapalahti O, Vaheri A (1996) Hantaviruses: genome structure, expression and evolution. J General Virol 77(Pt 11):2677–2687

Plyusnina A, Deter J, Charbonnel N, Cosson J-F, Plyusnin A (2007) Puumala and Tula hantaviruses in France. Virus Res 129:58–63

Plyusnina A, Ferenczi E, Rácz GR, Nemirov K, Lundkvist A, Vaheri A, Vapalahti O, Plyusnin A (2009) Co-circulation of three pathogenic hantaviruses: Puumala, Dobrava, and Saaremaa in Hungary. J Med Virol 81:2045–2052

Popugaeva E, Witkowski PT, Schlegel M, Ulrich RG, Auste B, Rang A, Krüger DH, Klempa B (2012) Dobrava-Belgrade hantavirus from Germany shows receptor usage and innate immunity induction consistent with the pathogenicity of the virus in humans. PLoS One 7:E35587

Raftery MJ, Lalwani P, KrautkrÄmer E, Peters T, Scharffetter-Kochanek K, Krüger R, Hofmann J, Seeger K, Krüger DH, Schönrich G (2014) β2 integrin mediates hantavirus-induced release of neutrophil extracellular traps. J Exp Med 211:1485–1497

Raharinosy V, Olive M-M, Andriamiarimanana FM, Andriamandimby SF, Ravalohery J-P, Andriamamonjy S, Filippone C, Rakoto DAD, Telfer S, Heraud J-M (2018) Geographical distribution and relative risk of Anjozorobe virus (Thailand Orthohantavirus) infection in black rats (*Rattus rattus*) in Madagascar. Virol J 15:83

Ramsden C, Holmes EC, Charleston MA (2009) Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for Codivergence. Mol Biol Evol 26:143–153

Rasmuson J, Pourazar J, Linderholm M, Sandström T, Blomberg A, Ahlm C (2011) Presence of activated airway T lymphocytes in human Puumala hantavirus disease. Chest 140:715–722

Ravkov EV, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST (1995) Genetic and serologic analysis of Black Creek canal virus and its association with human disease and Sigmodon Hispidus infection. Virology 210:482–489

Reusken C, Heyman P (2013) Factors driving hantavirus emergence in Europe. Curr Opin Virol 3:92–99

Reuter M, Krüger DH (2018) The Nucleocapsid protein of hantaviruses: much more than a genome-wrapping protein. Virus Genes 54:5–16

Reynes J-M, Carli D, Bour J-B, Boudjeltia S, Dewilde A, Gerbier G, Nussbaumer T, Jacomo V, Rapt M-P, Rollin PE, Septfons A (2017) Seoul virus infection in humans, France, 2014–2016. Emerg Infect Dis 23:973–977

Rheinemann L, Sundquist WI (2021) Virus Budding. Encyclopedia Virol:519–528

Rhodes LV 3rd, Huang C, Sanchez AJ, Nichol ST, Zaki SR, Ksiazek TG, Humphreys JG, Freeman JJ, Knecht KR (2000) Hantavirus pulmonary syndrome associated with Monongahela virus, Pennsylvania. Emerg Infect Dis 6:616–621

Ribeiro GE, Leon LE, Perez R, Cuiza A, Vial PA, Ferres M, Mertz GJ, Vial C (2019) Deletions in genes participating in innate immune response modify the clinical course of Andes Orthohantavirus infection. Viruses 11:680

Rissanen I, Stass R, Krumm SA, Seow J, Hulswit RJG, Paesen GC, Hepojoki J, Vapalahti O, Lundkvist Å, Reynard O, Volchkov V, Doores KJ, Huiskonen JT, Bowden TA (2020) Molecular rationale for antibody-mediated targeting of the hantavirus fusion glycoprotein. *Elife* 9:E58242

Rollin PE, Ksiazek TG, Elliott LH, Ravkov EV, Martin ML, Morzunov S, Livingstone W, Monroe M, Glass G, Ruo S (1995) Isolation of Black Creek canal virus, a new hantavirus from Sigmodon Hispidus in Florida. J Med Virol 46:35–39

Rose AMC, Vapalahti O, Lyytikäinen O, Nuorti P (2003) Patterns of Puumala virus infection in Finland. Euro Surveillance 8:9–13

Safronetz D, Haddock E, Feldmann F, Ebihara H, Feldmann H (2011) In vitro and in vivo activity of ribavirin against Andes virus infection. PLoS One 6:E23560

Sanna G, Piras S, Madeddu S, Busonera B, Klempa B, Corona P, Ibba R, Murineddu G, Carta A, Loddo R (2020) 5,6-Dichloro-2-phenyl-Benzotriazoles: new potent inhibitors of Orthohantavirus. Viruses 12:122

Sauvage F, Penalba C, Vuillaume P, Boue F, Coudrier D, Pontier D, Artois M (2002) Puumala hantavirus infection in humans and in the reservoir host, Ardennes region, France. Emerg Infect Dis 8:1509–1511

Saxenhofer M, Schmidt S, Ulrich RG, Heckel G (2019) Secondary contact between diverged host lineages entails ecological speciation in a European hantavirus. PLoS Biol 17:E3000142

Schlegel M, Klempa B, Auste B, Bemmann M, Schmidt-Chanasit J, Büchner T, Groschup MH, Meier M, Balkema-Buschmann A, Zoller H, Krüger DH, Ulrich RG (2009) Dobrava-Belgrade virus Spillover infections, Germany. Emerg Infect Dis 15:2017–2020

Schlegel M, Kindler E, Essbauer SS, Wolf R, Thiel J, Groschup MH, Heckel G, Oehme RM, Ulrich RG (2012) Tula virus infections in the Eurasian water vole in Central Europe. Vector Borne Zoon Dis (Larchmont, NY) 12:503–513

Schlegel M, Jacob J, Krüger DH, Rang A, Ulrich RG (2014) Chapter 10 - hantavirus emergence in rodents, insectivores and bats: what comes next? In: Johnson N (ed) The role of animals in emerging viral diseases. Academic Press, Boston

Schmidt S, Reil D, Jeske K, Drewes S, Rosenfeld UM, Fischer S, Spierling NG, Labutin A, Heckel G, Jacob J, Ulrich RG, Imholt C (2021) Spatial and temporal dynamics and molecular evolution of Tula Orthohantavirus in German vole populations. Viruses 13:1132

Schmidt-Chanasit J, Essbauer S, Petraityte R, Yoshimatsu K, Tackmann K, Conraths FJ, Sasnauskas K, Arikawa J, Thomas A, Pfeffer M, Scharninghausen JJ, Splettstoesser W, Wenk M, Heckel G, Ulrich RG (2010) Extensive host sharing of central European Tula virus. J Virol 84:459–474

Schultze D, Lundkvist A, Blauenstein U, Heyman P (2002) Tula virus infection associated with fever and exanthema after a wild rodent bite. Eur J Clin Microbiol Infect Dis 21:304–306

Schütt M, Meisel H, Krüger DH, Ulrich R, Dalhoff K, Dodt C (2004) Life-threatening Dobrava hantavirus infection with unusually extended pulmonary involvement. Clin Nephrol 62:54–57

Shepherd JG, Blunsum AE, Carmichael S, Smollett K, Maxwell-Scott H, Farmer ECW, Osborne J, Maclean A, Ashraf S, Shah R, Gunson R, Da Silva Filipe A, Aarons EJ, Thomson EC (2021) Seoul virus associated with pet rats, Scotland, Uk, 2019. Emerg Infect Dis 27:2677–2680

Simons MJ, Gorbunova EE, Mackow ER (2019) Unique interferon pathway regulation by the Andes virus Nucleocapsid protein is conferred by phosphorylation of serine 386. J Virol 93

Sironen T, Klingström J, Vaheri A, Andersson LC, Lundkvist Å, Plyusnin A (2008) Pathology of Puumala hantavirus infection in macaques. PLoS One 3:E3035

Sironen T, Sane J, Lokki M-L, Meri S, Andersson LC, Hautala T, Kauma H, Vuorinen S, Rasmuson J, Evander M, Ahlm C, Vaheri A (2017) Fatal Puumala hantavirus disease: involvement of complement activation and vascular leakage in the pathobiology. Open Forum Infect Dis 4:Ofx229

Slough MM, Chandran K, Jangra RK (2019) Two point mutations in Old World hantavirus glycoproteins afford the generation of highly infectious recombinant vesicular stomatitis virus vectors. MBio 10

Solà-Riera C, Gupta S, Ljunggren H-G, Klingström J (2019) Orthohantaviruses belonging to three Phylogroups all inhibit apoptosis in infected target cells. Sci Rep 9:834

Solà-Riera C, García M, Ljunggren H-G, Klingström J (2020) Hantavirus inhibits apoptosis by preventing mitochondrial membrane potential loss through up-regulation of the pro-survival factor Bcl-2. PLoS Pathog 16:E1008297

Song JW, Baek LJ, Nagle JW, Schlitter D, Yanagihara R (1996) Genetic and phylogenetic analyses of Hantaviral sequences amplified from archival tissues of deer mice (*Peromyscus maniculatus nubiterrae*) captured in the eastern United States. Arch Virol 141:959–967

Song JY, Chun BC, Kim SD, Baek LJ, Kim S-H, Sohn JW, Cheong HJ, Kim WJ, Park SC, Kim MJ (2006) Epidemiology of Hemorrhagic fever with renal syndrome in endemic area of the republic of Korea, 1995-1998. J Korean Med Sci 21:614–620

Sperber HS, Welke R-W, Petazzi RA, Bergmann R, Schade M, Shai Y, Chiantia S, Herrmann A, Schwarzer R (2019) Self-association and subcellular localization of Puumala hantavirus envelope proteins. Sci Rep 9:707

Strandin T, Mäkelä S, Mustonen J, Vaheri A (2018) Neutrophil activation in acute Hemorrhagic fever with renal syndrome is mediated by hantavirus-infected microvascular endothelial cells. Front Immunol 9

Strandin T, Smura T, Ahola P, Aaltonen K, Sironen T, Hepojoki J, Eckerle I, Ulrich RG, Vapalahti O, Kipar A, Forbes KM (2020) Orthohantavirus isolated in reservoir host cells displays minimal genetic changes and retains wild-type infection properties. Viruses 12:457

Sumibcay L, Kadjo B, Gu SH, Kang HJ, Lim BK, Cook JA, Song J-W, Yanagihara R (2012) Divergent lineage of a novel hantavirus in the Banana pipistrelle (*Neoromicia nanus*) in Côte D'ivoire. Virol J 9:34

Sun L, Zou L-X (2018) Spatiotemporal analysis and forecasting model of Hemorrhagic fever with renal syndrome in mainland China. Epidemiol Infect 146:1680–1688

Taori SK, Jameson LJ, Campbell A, Drew PJ, Mccarthy ND, Hart J, Osborne JC, Sudhanva M, Brooks TJG (2013) Uk Hantavirus, Renal Failure, And Pet Rats. Lancet (London, England) 381:1070

Taxonomybrowser (2022) Robina Orthohantavirus [Online]. Available: https://Www.Ncbi.Nlm.Nih.Gov/Taxonomy/Browser/Wwwtax.Cgi?Lvl=0&Id=2601243. Accessed 01 Feb 2022

Ter Horst S, Conceição-Neto N, Neyts J, Rocha-Pereira J (2019) Structural and functional similarities in Bunyaviruses: perspectives for pan-bunya antivirals. Rev Med Virol 29:E2039

Tersago K, Schreurs A, Linard C, Verhagen R, Van Dongen S, Leirs H (2008) Population, environmental, and community effects on local Bank vole (*Myodes glareolus*) Puumala virus infection in an area with low human incidence. Vector Borne Zoon Dis (Larchmont, NY) 8:235–244

Tersago K, Crespin L, Verhagen R, Leirs H (2012) Impact of Puumala virus infection on maturation and survival in Bank voles: a capture-mark-recapture analysis. J Wildl Dis 48:148–156

Tervo L, Mäkelä S, Syrjänen J, Huttunen R, Rimpelä A, Huhtala H, Vapalahti O, Vaheri A, Mustonen J (2015) Smoking is associated with aggravated kidney injury in Puumala hantavirus-induced haemorrhagic fever with renal syndrome. Nephrol Dial Transpl 30: 1693–1698

Toledo J, Haby MM, Reveiz L, Sosa Leon L, Angerami R, Aldighieri S (2021) Evidence for human-to-human transmission of hantavirus: a systematic review. J Infect Dis

Toro J, Vega JD, Khan AS, Mills JN, Padula P, Terry W, Yadón Z, Valderrama R, Ellis BA, Pavletic C, Cerda R, Zaki S, Shieh WJ, Meyer R, Tapia M, Mansilla C, Baro M, Vergara JA, Concha M, Calderon G, Enria D, Peters CJ, Ksiazek TG (1998) An outbreak of hantavirus pulmonary syndrome, Chile, 1997. Emerg Infect Dis 4:687–694

Torrez-Martinez N, Bharadwaj M, Goade D, Delury J, Moran P, Hicks B, Nix B, Davis JL, Hjelle B (1998) Bayou virus-associated hantavirus pulmonary syndrome in eastern Texas: identification of the Rice rat, Oryzomys Palustris, as reservoir host. Emerg Infect Dis 4:105–111

Torriani G, Mayor J, Zimmer G, Kunz S, Rothenberger S, Engler O (2019) Macropinocytosis contributes to hantavirus entry into human airway epithelial cells. Virology 531:57–68

Ulrich RG, Schmidt-Chanasit J, Schlegel M, Jacob J, Pelz HJ, Mertens M, Wenk M, Büchner T, Masur D, Sevke K, Groschup MH, Gerstengarbe FW, Pfeffer M, Oehme R, Wegener W, Bemmann M, Ohlmeyer L, Wolf R, Zoller H, Koch J, Brockmann S, Heckel G, Essbauer SS (2008) Network "rodent-borne pathogens" in Germany: longitudinal studies on the geographical distribution and prevalence of hantavirus infections. Parasitol Res 103(Suppl 1):S121–S129

Vaheri A, Henttonen H, Voutilainen L, Mustonen J, Sironen T, Vapalahti O (2013) Hantavirus infections in Europe and their impact on public health. Rev Med Virol 23:35–49

Vapalahti O, Lundkvist A, Kukkonen SK, Cheng Y, Gilljam M, Kanerva M, Manni T, Pejcoch M, Niemimaa J, Kaikusalo A, Henttonen H, Vaheri A, Plyusnin A (1996) Isolation and characterization of Tula virus, a distinct serotype in the genus hantavirus, Family Bunyaviridae. J Gen Virol 77(Pt 12):3063–3067

Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A (2003) Hantavirus infections in Europe. Lancet Infect Dis 3:653–661

Vera-Otarola J, Solis L, Lowy F, Olguín V, Angulo J, Pino K, Tischler ND, Otth C, Padula P, López-Lastra M, Williams BRG (2020) The Andes Orthohantavirus Nss protein antagonizes the type I interferon response by inhibiting Mavs Signaling. J Virol 94:E00454–E00420

Vollmar P, Lubnow M, Simon M, Müller T, Bergler T, Alois P, Thoma BR, Essbauer S (2016) Hantavirus cardiopulmonary syndrome due to Puumala virus in Germany. J Clin Virol 84:42–47

Wang X, Chen Q-Z, Zan Y-X, Wang M-R, Yan J, Guo W-W, Li K-J, Liu Y-Y, Luo F, Feng Y, Hou W, Xiong H-R (2020) Exosomal Mir-145-5p derived from Orthohantavirus-infected endothelial cells inhibits Htnv infection. FASEB J 34:13809–13825

Wang Q, Yue M, Yao P, Zhu C, Ai L, Hu D, Zhang B, Yang Z, Yang X, Luo F, Wang C, Hou W, Tan W (2021) Epidemic trend and molecular evolution of Hv family in the Main hantavirus epidemic areas from 2004 to 2016, in P.R. China. Front Cell Infect Microbiol 10: 584814–584814

Warner BM, Jangra RK, Griffin BD, Stein DR, Kobasa D, Chandran K, Kobinger GP, Safronetz D (2020) Oral vaccination with recombinant vesicular stomatitis virus expressing Sin Nombre virus glycoprotein prevents Sin Nombre virus transmission in deer mice. Front Cell Infect Microbiol 10

Weiss S, Witkowski PT, Auste B, Nowak K, Weber N, Fahr J, Mombouli J-V, Wolfe ND, Drexler JF, Drosten C, Klempa B, Leendertz FH, Kruger DH (2012) Hantavirus In Bat, Sierra Leone. Emerg Infect Dis 18:159–161

Wells RM, Sosa Estani S, Yadon ZE, Enria D, Padula P, Pini N, Mills JN, Peters CJ, Segura EL (1997) An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus pulmonary syndrome study group for Patagonia. Emerg Infect Dis 3:171–174

Xiao SY, Diglisic G, Avsic-Zupanc T, Leduc JW (1993a) Dobrava virus as a new hantavirus: evidenced by comparative sequence analysis. J Med Virol 39:152–155

Xiao SY, Spik KW, Li D, Schmaljohn CS (1993b) Nucleotide and deduced amino acid sequences of the M and S genome segments of two Puumala virus isolates from Russia. Virus Res 30:97–103

Yanagihara R, Amyx HL, Lee PW, Asher DM, Gibbs CJ, Gajdusek DC (1988) Experimental hantavirus infection in nonhuman primates. Arch Virol 101:125–130

Yang Z-Q, Yu S-Y, Nie J, Chen Q, Li Z-F, Liu Y-X, Zhang J-L, Xu J-J, Yu X-M, Bu X-P, Su J-J, Zhang Y, Tao K-H (2004) Prevalence of Hemorrhagic fever with renal syndrome virus in domestic pigs: an epidemiological investigation in Shandong Province. Di 1 Jun Yi Da Xue Xue Bao = Academic Journal Of The First Medical College Of Pla 24:1283–1286

Yashina LN, Patrushev NA, Ivanov LI, Slonova RA, Mishin VP, Kompanez GG, Zdanovskaya NI, Kuzina II, Safronov PF, Chizhikov VE, Schmaljohn C, Netesov SV (2000) Genetic diversity of hantaviruses associated with Hemorrhagic fever with renal syndrome in the Far East of Russia. Virus Res 70:31–44

Yasuda SP, Shimizu K, Koma T, Hoa NT, Le MQ, Wei Z, Muthusinghe DS, Lokupathirage SMW, Hasebe F, Yamashiro T, Arikawa J, Yoshimatsu K (2021) Immunological responses to Seoul Orthohantavirus in experimentally and naturally infected Brown rats (*Rattus norvegicus*). Viruses 13:665

Ye C, Wang D, Liu H, Ma H, Dong Y, Yao M, Wang Y, Zhang H, Zhang L, Cheng L, Xu Z, Lei Y, Zhang F, Ye W (2019) An improved enzyme-linked focus formation assay revealed Baloxavir acid as a potential antiviral therapeutic against hantavirus infection. Front Pharmacol 10

Yu L, Bai W, Wu X, Zhang L, Zhang L, Li P, Wang F, Liu Z, Zhang F, Xu Z (2013) A recombinant Pseudotyped lentivirus expressing the envelope glycoprotein of Hantaan virus induced protective immunity in mice. Virol J 10:301

Zeier M, Handermann M, Bahr U, Rensch B, Müller S, Kehm R, Muranyi W, Darai G (2005) New ecological aspects of hantavirus infection: a change of a paradigm and a challenge of prevention–a review. Virus Genes 30:157–180

Zelena H, Strakova P, Heroldova M, Mrazek J, Kastl T, Zakovska A, Ruzek D, Smetana J, Rudolf I (2019) Molecular epidemiology of hantaviruses in the Czech Republic. Emerg Infect Dis 25: 2133–2135

Zeller HG, Karabatsos N, Calisher CH, Digoutte JP, Cropp CB, Murphy FA, Shope RE (1989) Electron microscopic and antigenic studies of uncharacterized viruses. Ii. Evidence suggesting the placement of viruses in the family Bunyaviridae. Arch Virol 108:211–227

Zhang Y-Z, Zou Y, Fu ZF, Plyusnin A (2010) Hantavirus infections in humans and animals, China. Emerg Infect Dis 16:1195–1203

Zhang X, Chen H-Y, Zhu L-Y, Zeng L-L, Wang F, Li Q-G, Shao F-J, Jiang H-Q, Liu S-J, Ma Y-J, Zhu Y, Ma Y-J (2011) Comparison of Hantaan and Seoul viral infections among patients with Hemorrhagic fever with renal syndrome (Hfrs) in Heilongjiang, China. Scand J Infect Dis 43: 632–641

Human African Trypanosomiasis: The Smoldering Scourge of Africa

39

August Stich

Contents

Abstract

Human African trypanosomiasis (HAT) or sleeping sickness is a parasitic disease caused by trypanosomes, which are transmitted by tsetse flies. Two species are responsible for human infections: *Trypanosoma brucei* (*T.b.*) rhodesiense for the

A. Stich (✉)
Department of Tropical Medicine, Klinikum Würzburg Mitte, Würzburg, Germany
e-mail: august.stich@medmissio.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_31

East African, and *T.b. gambiense* for the West African sleeping sickness. A third species, *T.b. brucei*, causes Nagana disease in cattle. HAT is fatal if left untreated. In recent years, considerable progress has been achieved in terms of treatment (introduction of a new drug flexinidazole) and control (successful reduction of cases).

Keywords

Visceral Leishmaniasis · Human African Trypanosomiasis · *Trypanosoma brucei* · Congo Basin · Thick Blood Film

39.1 Historic Introduction

Human African trypanosomiasis (HAT) or sleeping sickness is one of Africa's classic tropical diseases. The first case reports go back to the fourteenth century. Until recently, the impact of HAT on health in Africa has been devastating. Many areas had long been rendered uninhabitable for people and domestic livestock. Millions may have died in Central Africa around Lake Victoria and in the Congo basin within the last 100 years.

In 1895, the British scientist Sir David Bruce (1855–1931) suggested an association between trypanosomes and the "cattle fly fever," a major problem for livestock in southern Africa. In 1902, Robert M. Forde and Everett Dutton from the Liverpool School of Tropical Medicine identified trypanosomes in the blood of a patient during a research expedition in The Gambia, and in 1903, the Italian Aldo Castellani isolated trypanosomes from the cerebrospinal fluid. In the same year, tsetse flies were identified as the vector.

Up to the 1960s, control programs had been quite successful. However, recent epidemics in the Democratic Republic of Congo, Northern Angola, South Sudan, Uganda, and other countries resulted in a major resurgence of HAT. At the turn of the twentieth century, the achievements in sleeping sickness control during colonial times had been nearly completely reversed. Fortunately, recent successes of programs run by national institutions and various dedicated nongovernmental organizations finally succeeded in a reduction of transmission in many accessible areas of central Africa. However, nobody knows the true prevalence of HAT in Africa, as many countries are still in a state of war and civil unrest with a desperate population that cannot be reached by health interventions.

Today, about 60 million people in 36 African countries are exposed to the potential risk of HAT in about 300 currently existing active foci. Today, only a few hundred new cases are reported yearly to WHO, but they are doomed if left untreated. For expatriates and international travelers, sleeping sickness has always been a rare disease, although occasional clusters of cases in tourists to Tanzania, Zambia, and Malawi are regularly reported.

There is hardly any other tropical disease that demonstrated more clearly the hypocrisy characterizing our time. On one side, trypanosomes are kept in culture and

studied extensively in numerous research laboratories. Their genome is sequenced, and many molecular, biochemical, and immunological phenomena have been discovered as a result of basic science research; scientific interest in the disease is usually restricted to its research aspects. On the other hand, diagnostic and, especially, therapeutic tools were increasingly unavailable, because patients in rural Africa were not commercially viable consumers. Global concern about the crisis of HAT in Africa is a question of scientific ethics and international solidarity. HAT can be seen as the classic example of a Neglected Tropical Disease (NTD).

Fortunately, there have been considerable developments in recent years. In 2012, WHO set out to eliminate HAT as a public health problem. In the first stage, a reduction of new cases below 2.000 per year was reached in 2020. Now, it is planned to interrupt transmission of West African HAT by 2030. This, however, will only be possible by integration of HAT control in the general package of basic and public health measures. The control of the East African HAT is even more complex due to its extensive animal reservoir, a classic example of the necessity of a modern "One Health" approach.

39.2 Taxonomic Classification

HAT is caused by subspecies of the protozoan hemoflagellate *Trypanosoma brucei*, which is transmitted to man and animals by tsetse flies (*Glossina* spp.). The distribution of the vector restricts sleeping sickness to the African continent between 14° North and 29° South.

Human disease occurs in two clinically and epidemiologically distinct forms: West African and East African sleeping sickness (Table 1). A third subspecies of the parasite, *T.b. brucei*, causes the Nagana disease in cattle, but is nonpathogenic in

Table 1 The principal features of West and East African sleeping sickness

Disease	West African sleeping sickness	East African sleeping sickness
Parasite	*Trypanosoma brucei gambiense*	*T.b. rhodesiense*
Vector	Transmitted by riverine tsetse flies (*palpalis* group)	Transmitted by savannah tsetse flies (*morsitans* group)
Clinical course	Insidious onset, slow progression, death in stage II after many months or years	Acute onset, chancre frequent, rapid course, death frequently in stage I (cardiac failure)
Diagnosis	Parasitemia scanty, Winterbottom's sign, serology	Parasitemia usually higher and easily detectable, serological tests unreliable
Treatment	See Table 3	
Epidemiology	Tendency for endemicity, man as main reservoir with evidence for several other mammal species, severe public health problem in many West and Central African countries	Wild (antelopes, e.g., bushbuck) and occasionally domestic animals as reservoir and source of case clusters and epidemic outbreaks

humans. In Uganda, the only country where all three forms occur, the areas of *gambiense* and *rhodesiense* sleeping sickness are currently about to overlap.

Trypanosoma brucei (phylum Sacromastigophora, order Kinetoplastida) is an extracellular protozoan parasite. Like *Leishmania*, it possesses a centrally placed nucleus and a kinetoplast, a distinct organelle containing extranuclear DNA. The kinetoplast is the insertion site of an undulating membrane, which extends over nearly the whole cell length and ends as a free flagellum.

The three subspecies of *T. brucei* are morphologically indistinguishable. However, they differ considerably in their interaction with their mammalian host and the epidemiological pattern of the diseases they cause. Formerly, *T.b. gambiense* and *T.b. rhodesiense* isolates were characterized either by isoenzyme analysis or by animal inoculation. The advent of molecular techniques created expectations of more reliable tools for their differentiation. However, genomic characterization has revealed several more subdivisions rather than the three expected. Whereas West African isolates proved relatively homogeneous, East African isolates from humans and animals did not simply conform to what is still called *T.b. rhodesiense* and *T.b. brucei*, but showed a complex relationship with evidence of so far undiscovered genetic exchange in the vector. Further molecular research may soon lead to a comprehensive phylogenetic tree and a deeper insight into trypanosomal evolution and biology.

39.3 Biology

Although congenital, blood-borne and mechanical transmission have been reported and may play an occasional role, the main mode of transmission is through the bite of infected tsetse flies (*Glossina* spp., order Diptera). These are biologically unique insects, which occur only in Africa in 31 distinct species and subspecies. Less than half are potential vectors of HAT. Their distinctive behavior, ecology, and chosen habitat explain many epidemiological features of sleeping sickness. Tsetse flies can live for many months in the wild, but give birth to only about eight larvae per lifetime. Both sexes feed on blood. They require warm temperatures, shade, and humidity for resting and larviposition, which makes their distribution highly localized. Recently, the mapping and monitoring of possible HAT transmission foci has become possible with the use of satellite imaging techniques.

During the blood meal on an infected mammalian host, the tsetse fly takes up trypanosomes ("short-stumpy form") into its mid-gut, where they develop into procyclic forms and multiply. After about 2 weeks, they migrate to the salivary glands as epimastigotes, where they finally develop into infective metacyclic forms. At the next blood meal, they are injected into a new vertebrate host where they appear as "long-slender" trypomastigotes and multiply by binary fission. In contrast to *Leishmania* and *T. cruzi*, *T. brucei* is an exclusively extracellular parasite.

The cyclic changes of the trypanosome into different developmental stages are accompanied by variations in morphology, metabolism, and antigenicity. Several

unique metabolic pathways have been described in trypanosomes, distinct from their host and thus qualifying as potential drug targets.

The blood stream forms of *T. brucei* are covered with a dense coat of identical glycoproteins, numbering up to about 500 aminoacids per molecule. Being highly immunogenic, they stimulate the production of specific antibodies, mainly of the IgM subclass. Once the surface glycoproteins have been recognized by host antibodies, the parasite will be attacked and destroyed through complement activation and cytokine release, giving rise to local and systemic inflammatory reactions.

However, about 2% of *T. brucei* in each new generation change the expression of their specific surface glycoprotein. The "coat" will then be different in the new clone (thus, "variant" surface glycoprotein: VSG). This phenotypic switch is done mainly by programed DNA-rearrangements, moving a transcriptionally silent VSG gene into an active, telomerically located expression site. Each *T. brucei* parasite already has the information for hundreds of different VSG genes, and within a whole trypanosome population, the potential repertoire for such different VSG copies seems to be virtually infinite.

Every new VSG copy is antigenically different, thus stimulating the production of a new IgM population. This antigenic variation is the major immune evasion strategy of the parasite, enabling the trypanosome to persist in its vertebrate host. It also reduces parasite load and prolongs the infection. But the inevitable outcome is immune exhaustion of the host (supported by additional immunosuppressive metabolites of the parasites), penetration of trypanosomes into immune-privileged sites such as the central nervous system, and finally death.

39.4 Pathogenicity and Pathology

HAT progresses much more rapidly (over weeks or months) in the East African or *rhodesiense* variety compared with a more insidious onset and protracted course that can last years in *gambiense* (West African) infection. Generally, there are many more circulating organisms in *rhodesiense* compared with *gambiense* infections, making direct parasitological diagnosis easier in East African sleeping sickness. The number of organisms in the blood also varies widely depending on the immunological response to infection. These numbers come and go in waves, because antibodies that develop to one antigenic type of trypanosome can lyse them, but are not effective in stopping multiplication of a smaller number of organisms that have a different antigenic type, and it takes time for immune systems to respond to this new variant. Host inflammatory responses mediated by cytokines are prominent and, together with immunological responses, may contribute to pathophysiology. There are two stages of infection, which are important to distinguish because stage determines the choice of treatment and subsequent risks.

A few days after an infective tsetse bite, a small papule may develop, more often in *rhodesiense* than in *gambiense* infections. As organisms multiply locally, they excite inflammatory responses, an erythematous tissue reaction with edema and lymphadenopathy can follow. This "trypanosomal chancre" is commonly found in

rhodesiense, but rarely in *gambiense* infections. Organisms then invade quickly the hemolymphatic system where they multiply. Symptoms of fever, rigors, headaches, and arthralgias follow. These may be less prominent in *gambiense* infections. Lymphadenopathy is detected frequently, especially in the posterior triangle of the neck. This "Winterbottom's sign" is diagnostically useful, because lymph juices from these glands can show parasites and help to establish a diagnosis.

After local multiplication at the site of inoculation, the trypanosomes invade the hemolymphatic system, where they can be detected after 7–10 days. During this period of spread, they are exposed to vigorous host defense mechanisms, which they evade by antigenic variation. This continuous battle between antigenic switches and humoral defense results in a fluctuating parasitemia with parasites frequently becoming undetectable, especially in *gambiense* HAT. The cyclic release of cytokines during periods of increased cell lysis results in intermittent, nonspecific symptoms: fever, chills, rigors, headache, and joint pains. These can easily be misdiagnosed as malaria, viral infection, typhoid fever, or many other conditions. Hepatosplenomegaly and generalized lymphadenopathy are common, indicating activation and hyperplasia of the reticuloendothelial system.

Trypanosomes cross the blood–brain barrier within weeks after infection in *rhodesiense* and months in *gambiense* infections. This indicates the beginning of the "sleeping sickness" phase, which is associated with personality changes, headaches, withdrawal from the environment, and other signs of neurological involvement. Patients find it harder to carry out any but the simplest of tasks and show a kind of "mental tunnel vision." There are changes in circadian rhythm as well, such as nocturnal insomnia and daytime somnolence. Unless treated, these symptoms progress and are associated with apathy, inanition, and eventually secondary infections such as pneumonias that precede death.

39.5 Clinical Manifestations

Sleeping sickness is a dreadful disease, causing great suffering to patients, their families, and the affected community. The infection often has an insidious onset, but *T. brucei*, whether the East or West African subspecies, will invariably kill if the patient is not treated in time. The natural course of HAT can be divided into different and distinct stages. Their recognition and differentiation are important for the clinical management of the patient.

39.5.1 The Trypanosomal Chancre

Tsetse bites can be quite painful, usually leaving a small and self-healing mark. In the case of a trypanosomal infection, the local reaction can be quite pronounced and longer lasting. A small raised papule will develop after about 5 days. It increases rapidly in size, surrounded by an intense erythematous tissue reaction with local edema and regional lymphadenopathy. Although some chancres have a very nasty

appearance, they are usually not very painful unless they become ulcerated and superinfected. They heal without treatment after 2–4 weeks, leaving a permanent, hyperpigmented spot.

Trypanosomal chancres occur in more than half the cases of *T.b. rhodesiense*. In *T.b. gambiense*, they are much less common and often go undetected in endemic populations.

39.5.2 Hemolymphatic Stage (HAT Stage I)

A reliable sign, particularly in *T.b. gambiense* infection, is the enlargement of lymph nodes in the posterior triangle of the neck (Winterbottom's sign). Other typical signs are a fugitive patchy or circinate rash, a myxedematous infiltration of connective tissue ("puffy face syndrome"), and an inconspicuous periostitis of the tibia with delayed hyperesthesia (Kérandel's sign).

In *T.b. rhodesiense* infection, this hemolymphatic stage is very pronounced with severe symptoms, clinically often resembling *falciparum* malaria or septicemia. Frequently, patients die within the first weeks after the onset of symptoms, mostly through cardiac involvement (myocarditis). In the early stage of *T.b. gambiense* infection, symptoms are usually infrequent and mild. Febrile episodes become less severe as the disease progresses.

Patients presenting with an appropriate history of travel in endemic areas, perhaps a history of being bitten by tsetse flies and systemic symptoms of fever should raise suspicion of HAT.

39.5.3 Meningoencephalitic Stage (HAT Stage II)

Within weeks in *T.b. rhodesiense* and months (sometimes years) in *T.b. gambiense* infection, cerebral involvement will invariably follow; trypanosomes cross the blood–brain barrier.

The onset of stage II is insidious. The exact time of central nervous system involvement cannot be determined clinically. On histology, perivascular infiltration of inflammatory cells ("cuffing") and glial proliferation can be detected, resembling cerebral endarteritis. As the disease progresses, patients complain of increasing headache and their families may detect a marked change in behavior and personality. Neurological symptoms, which follow gradually, can be focal or generalized, depending on the site of cellular damage in the central nervous system. Convulsions are common, usually indicating a poor prognosis. Periods of confusion and agitation slowly evolve toward a stage of distinct perplexity when patients lose interest in their surroundings and their own situation. Inflammatory reactions in the hypothalamic structures lead to a dysfunction in circadian rhythms and sleep regulatory systems. Sleep pattern become fragmented and finally result in a somnolent and comatose state. Progressive wasting and dehydration follow the inability to eat and drink.

In children, HAT progresses even more rapidly toward this meningoencephalitic stage. Parents often notice insomnia and behavioral changes long before the diagnosis is established.

There is no pathognomonic clinical sign of late HAT, a wide range of possible neurological and psychiatric differential diagnoses is opened instead. However, the appearance of the patient with apathy, the typical expressionless face and swollen lymph nodes at the posterior triangle of the neck, is very suggestive for HAT in endemic areas.

39.6 Laboratory Diagnosis

HAT can never be diagnosed with certainty purely on clinical grounds. Definitive diagnosis requires the detection of the parasite in chancre aspirate, blood, lymph juice, or cerebrospinal fluid using various parasitological techniques.

39.6.1 Lymph Node Aspirate

Lymph node aspiration is widely used, especially for the diagnosis of *gambiense* HAT. Fluid of enlarged lymph nodes, preferably of the posterior triangle of the neck (Winterbottom's sign), is aspirated and examined immediately at × 400 magnification without additional staining. Mobile trypanosomes can be detected for a few minutes between lymphocytes.

39.6.2 Wet Preparation, Thin and Thick Blood Film

During all stages of the disease, trypanosomes may appear in the blood stream where they can be detected in unstained wet or in stained preparations. The yield of detection is highest in the thick blood film, a technique widely used for the diagnosis of blood parasites such as *Plasmodia* or microfilaria. Giemsa or Field staining techniques are appropriate.

Especially in *gambiense* HAT, parasitemia is usually low and fluctuating, often even undetectable. Repeated examinations on successive days are sometimes necessary until trypanosomes can be documented.

39.6.3 Concentration Methods

To increase the sensitivity of blood examinations, various concentration assays have been developed. Trypanosomes tend to accumulate in the buffy coat layer after centrifugation of a blood sample. The best results have been obtained with the mAECT (mini anion exchange column technique), where trypanosomes are concentrated after passage through a cellulose column, the QBC method (quantitative buffy

coat), which was originally developed for the diagnosis of malaria, or the CTC method (capillary tube centrifugation), which is widely used in the field.

39.6.4 Nucleic Amplification Techniques

Several specific primers have been described to detect trypanosomal DNA using the polymerase chain reaction (PCR). They had been successfully applied to samples from blood, lymph juice, and CSF, mostly under conditions of a research laboratory. Until today PCR assays are still inferior to conventional parasitological techniques.

39.6.5 Serological Assays

Serology is a useful tool to detect antibodies against trypanosomes. Various test methods have been described; some of them are now commercially available. They are mainly based on ELISA technique or immunofluorescence, but provide reliable results only in *gambiense* HAT.

For rapid screening under field conditions, the CATT (card agglutination test for trypanosomiasis) is an excellent tool in areas of *T.b. gambiense* infestation. It is easy to perform and delivers results within 5 min. A visible agglutination in the CATT suggests the existence of antibodies, but does not necessarily imply overt disease. Still, any positive serological result always requires parasitological confirmation before the initiation of treatment.

Recently, novel diagnostic tests similar to the rapid detection tests (RDT) for the diagnosis of malaria or COVID-19 have been developed by the nonprofit organization FIND (Foundation for Innovative New Diagnostics) and are currently undergoing evaluation under field conditions.

39.6.6 Nonspecific Laboratory Findings

Anemia and thrombocytopenia are caused by systemic effects of cytokine release, especially of TNF-α. Hypergammaglobinemia can reach extreme levels as a result of polyclonal activation of plasma cells. IgM levels detected in HAT are among the highest observed in any infectious disease.

39.6.7 Diagnosis of Stage II

Stage determination is crucial for the correct management of a patient. This cannot be done on clinical grounds alone. Therefore, cerebrospinal fluid must be examined in every patient found positive for trypanosomes in blood or lymph aspirate. In addition, a lumbar puncture should also be performed in all patients in whom HAT is

suspected clinically even if peripheral examinations had proved negative. A minimum of 5 ml of cerebrospinal fluid is required to examine for:

- *Leucocytes*: Cerebral involvement in HAT stage II is accompanied by pleocytosis, mostly lymphocytes, in the cerebrospinal fluid. By convention a number of five cells or more per mm^3 cerebrospinal fluid defines central nervous system involvement even if the patient does not (yet) have neurological symptoms. Pathognomonic for HAT is the appearance of activated plasma cells with eosinophilic inclusions in the cerebrospinal fluid, the morular cells of Mott
- *Trypanosomes*: The chances of detecting trypanosomes in the cerebrospinal fluid increase with the level of pleocytosis and the technique used. The highest yield is obtained by cerebrospinal fluid double centrifugation and rapid microscopy at the bedside
- *Protein*: In patients with HAT, a level of 37 mg of protein per 100 ml cerebrospinal fluid (dye-binding protein assay) or more is highly suggestive of the advanced stage. Stage II HAT is characterized by an autochthonous production of IgM antibodies in the cerebrospinal fluid, which can be selectively detected if suitable laboratory facilities exist (e.g., latex IgM test)

39.7 Treatment

39.7.1 General Considerations

HAT is curable, especially if the diagnosis is made at an early stage of the disease. In the harsh reality of the African situation, however, there are many major obstacles to successful patient management:

1. Sleeping sickness is a disease of rural, remote places. The active foci of sleeping sickness are usually in far away and insecure places, which are difficult to reach. Many treatment centers work under emergency conditions with extremely restricted resources. Numerous patients, without proper access to health care, are left unattended.
2. The diagnosis is difficult. Initial diagnosis and exact staging of trypanosomiasis requires sophisticated methods that are potentially harmful to the patient and thus justified only in the hands of experienced personnel. Repetitive training programs, constant supervision, and continuous quality control are necessary but, in reality, rarely available.
3. The treatment of trypanosomiasis is extremely costly although the drugs themselves are now covered by a donation program. Invariably, demand exceeds the locally available resources. External funding and sustainable donor commitments for rural Africa is generally decreasing.
4. The treatment can be complicated, dangerous, prolonged, and usually requires hospitalization. Most patients with late-stage trypanosomiasis are severely ill and malnourished. Adverse drug reactions during treatment are difficult to assess due

to concomitant pathologies. Their management requires considerable medical skill and good nursing care. Hospitals in rural Africa are often inadequately equipped and staffed to accomplish good patient care.

5. Many drugs are not easily available. Trypanosomicidal agents were on the verge of disappearance despite increasing demand. The range of drugs is diminishing, and hardly any new treatments are in sight. This is especially worrying in view of the reported spread of drug resistance.
6. HAT treatment is not standardized. Trypanosomiasis treatment regimens vary considerably between countries and treatment centers. Results from different centers are comparable to only a very limited extent. Few properly conducted and sufficiently powered clinical trials are available to evaluate duration, dosage, and possible combinations of drugs. Sufficient infrastructure for carrying out clinical research exists in only a handful of places.
7. The price for cure of HAT is high: dangerous drugs with limited availability and prolonged treatment schedules administered in many places by poorly trained personnel in rudimentary medical facilities. Little progress has been achieved in the last 30 years.

The treatment of HAT depends on the trypanosome subspecies and the stage of the disease (Table 2).

39.7.2 Stage I Drugs

39.7.2.1 Pentamidine

Since its introduction in 1937, pentamidine has become the drug of choice for *gambiense* HAT stage I, achieving cure rates as high as 98%. However, there are frequent failures in *rhodesiense* HAT. Lower rates of cellular pentamidine uptake in *T.b. rhodesiense* may explain these differences. Some cures of stage II infections have also been reported, but cerebrospinal fluid drug levels are usually not sufficiently high to guarantee a reliable trypanosomicidal effect in the central nervous system.

Pentamidine is usually given by deep intramuscular injection, often on an outpatient basis. If hospital care and reasonable monitoring conditions are available, an intravenous infusion, given in normal saline over 2 h, might be used instead. The

Table 2 The choice of drugs in the treatment of sleeping sickness until 2019

	Gambiense sleeping sickness		*Rhodesiense* sleeping sickness	
HAT Stage I	Pentamidine		Suramin	
HAT Stage II	1st line	NECT (nifurtimox eflornithine combination therapy)	1st line	Melarsoprol
	2nd line	Melarsoprol	2nd line	+ Nifurtimox

Table 3 Dosage and principal adverse reactions of antitrypanosomal agents

	Dosage regimen	Adverse drug reactions
Pentamidine	4 mg/kg body weight intramuscular daily or on alternate days for 7–10 injections	Hypotensive reaction (common) with tachycardia, dizziness, even collapse and shock, especially after intravenous administration, close monitoring of pulse rate and blood pressure after injection is mandatory Inflammatory reactions at the site of injection (sterile abscesses, necrosis) Renal, hepatic, and pancreatic dysfunction Neurotoxicity: peripheral polyneuropathy Bone marrow depression
Suramin	Day 1: Test dose of 4–5 mg/kg body weight Day 3, 10, 17, 24 and 31: 20 mg/kg body weight, maximum dose per injection 1 g	Pyrexia (very common) Early hypersensitivity reactions such as nausea, circulatory collapse, urticaria Late hypersensitivity reactions: skin reactions (exfoliative dermatitis), hemolytic anemia Renal impairment: albuminuria, cylindruria, hematuria (high renal tissue concentrations); regular urine checks during treatment are mandatory Neurotoxicity: peripheral neuropathy Bone marrow toxicity: agranulocytosis, thrombocytopenia
Melarsoprol	New regimen Day 1–10: 2.2 mg/kg body weight (not evaluated for *T.b. rhodesiense*)	Treatment-induced encephalopathy Pyrexia Neurotoxicity: peripheral motor or sensory polyneuropathy Dermatological reactions: pruritus, urticaria, exfoliative dermatitis Cardiotoxicity Renal and hepatic dysfunction
Eflornithine	Most commonly used dosage regimen 100 mg/kg body weight at 6-h intervals for 14 days	Gastrointestinal symptoms such as nausea, vomiting, and diarrhea Bone marrow toxicity: anemia, leukopenia, thrombocytopenia Alopecia, usually toward the end of the treatment cycle Neurological symptoms such as convulsions
Nifurtimox	5 mg/kg body weight three times daily for 30 days	Abdominal discomfort such as nausea, pains, and vomiting in half of the treated patients, often leading to a disruption of the treatment course Neurological complications: convulsions Impairment of cerebellar function, polyneuropathy Skin reactions
NECT	Nifurimox 5 mg/kg every 8 h over 10 days + Eflornithine 200 mg i.v. every 12 h over 7 days	As above, but less as compared to monotherapy

main advantage of pentamidine over other drugs is the short treatment course and ease of administration. Adverse effects are related to the route of administration or its dose and are usually reversible.

In clinical medicine, pentamidine is also used as second-line therapy for visceral leishmaniasis and especially in the prophylaxis and treatment of opportunistic *Pneumocystis jirovecii* pneumonia in AIDS. Since the start of the HIV pandemic, the price of pentamidine was increased more than tenfold by producers, making it unaffordable by health institutions in low-income countries. After an intervention led by WHO, a limited amount of pentamidine is now made available for use in HAT.

39.7.2.2 Suramin

In the early twentieth century, the development of suramin, resulting from German research on the trypanosomicidal activity of various dyes ("Bayer 205"), was a major breakthrough in the field of tropical medicine. For the first time, African trypanosomiasis, at least in its early stages, became treatable without causing major harm.

Suramin is still used to treat stage I HAT, especially *rhodesiense*. Like pentamidine it does not reach therapeutic levels in cerebrospinal fluid. Suramin is injected intravenously after dilution in distilled water.

Adverse effects depend on nutritional status, concomitant illnesses (especially onchocerciasis), and the patient's clinical condition. Although life-threatening reactions have been described, serious adverse effects are rare.

39.7.3 Stage II Drugs

39.7.3.1 Melarsoprol

Until the systematic introduction of the arsenical compound melarsoprol in 1949, late-stage trypanosomiasis was virtually untreatable. Since then, it has remained the most widely used stage II antitrypanosomal drug both for *T.b. gambiense* and *rhodesiense* infections. It has saved many lives, but has a high rate of dangerous adverse effects. Increasing frequency of relapses and resistance has been reported in some parts of Congo, Angola, Sudan, and Uganda.

Melarsoprol clears trypanosomes rapidly from the blood, lymph, and cerebrospinal fluid. Its toxicity usually restricts its use to late-stage disease. It is given by slow intravenous injection; extravascular leakage must be avoided.

A new, simpler regimen is based on recently acquired knowledge of the drug's pharmacokinetics. The most important adverse effect is an acute encephalopathy, provoked around day 5–8 of the treatment course in 5–14% of all patients. There is severe headache, convulsions, rapid neurological deterioration, or deepening of coma. Characteristically, the comatose patient's eyes remain open. Most probably, this is an immune-mediated reaction precipitated by release of parasite antigens in the first days of treatment. The overall case fatality under treatment ranges between 2% and 12%, depending on the stage of disease and the quality of medical and nursing care. Simultaneous administration of glucocorticosteroids (prednisolone 1 mg/kg body weight; maximum 40 mg daily) might reduce mortality, especially

in cases with high cerebrospinal fluid pleocytosis. However, in areas where tuberculosis, amebiasis or strongyloidiasis are highly prevalent, corticosteroids have dangers of their own!

39.7.3.2 Eflornithine (DFMO)

Initially developed as antitumur agent, eflornithine (alpha-difluoro-methylornithine) was introduced in 1980 as an antitrypanosomal drug, in the hope that it might replace melarsoprol for treatment of stage II trypanosomiasis. However, exorbitant costs and limited availability have restricted its use mostly to melarsoprol-refractory cases of *gambiense* sleeping sickness. *T.b. rhodesiense* is much less sensitive due to a much higher turnover rate of the target enzyme ornithine-decarboxylase and, therefore, cannot be treated with Eflornithine.

It can be taken orally, but intravenous administration is preferred as it achieves a much higher bioavailability and success rate. Eflornithine should be administered slowly over a period of at least 30 min. Continuous 24-h administration is preferable if facilities allow.

The range of adverse reactions to eflornithine is wide, as with other cytotoxic drugs in cancer treatment. Their occurrence and intensity increase with the duration of treatment and the severity of the patient's general condition.

In the late 1990s, no pharmaceutical company has produced eflornithine for use against HAT, despite demands by WHO. The discovery of its therapeutic effect in cosmetic creams against facial hair helped to restimulate production and thus had a beneficial "spin-off effect" for HAT. In 2001, agreements were signed between WHO and two major drug producing companies which led to a "Public-Private-Partnership" (PPP) and helped to assure a sufficient supply of eflornithine and other drugs essential for the treatment of HAT. The agreement was prolonged several times on a 5-year basis.

39.7.3.3 Nifurtimox

Ten years after its introduction for the treatment of American trypanosomiasis in 1967, nifurtimox was found to be effective in the treatment of *gambiense* sleeping sickness. It has a place as second-line treatment in melarsoprol-refractory cases or in combination chemotherapy.

Nifurtimox is generally not well tolerated, but adverse effects are usually not severe. They are dose-related and rapidly reversible after discontinuation of the drug.

39.7.3.4 Combination Treatments in HAT

Melarsoprol, eflornithine, and nifurtimox interfere with trypanothione synthesis and activity at different stages. There is also experimental evidence that combinations of suramin and stage II drugs might also be beneficial. Therefore, by reducing the overall dosage of each individual component, drug combinations could perhaps reduce the frequency of serious side effects, and the development of resistance, which are such common problems in the treatment of sleeping sickness.

In 2009, a large multi-center clinical trial was published demonstrating the advantages of a nifurtimox eflornithine combination therapy (NECT) over

eflornithine monotherapy in *gambiense* HAT. NECT is now considered to be the treatment of choice in the late stage of *T.b. gambiense* infection.

39.7.3.5 Recent Developments in the Treatment of HAT

A major breakthrough was achieved in the last 10 years. When the international nongovernmental organization *Médecins sans Frontières* received the Nobel Peace Prize in 1999, the award money was used to form a new institution: *Drugs für Neglected Disease Initiative* (DNDi), which set out to bridge the gap between basic science and drug development. One major outcome was a new drug to treat HAT: flexinidazole, a nitroimidazole for oral use. In November 2018, the European Medicines Agency adopted a positive opinion for fexinidazole, and WHO followed by updating the guidelines for the treatment of West African HAT in 2019. Fexinidazole is now recommended for individuals of $\geq$ 6 years and $\geq$ 20 kg, in first and second stage T.b. gambiense infection with $<$100 leukocytes/μl in the cerebrospinal fluid. NECT remains recommended for those with with $\geq$ 100 leukocytes/μl. The use of flexinidazole for *T.b. rhodesiense* infection is still not recommended, but currently under investigation.

39.8 Preventive Measures

39.8.1 Individual Protection

Tsetse flies have a very patchy distribution. Infested strips of land are often well known to the local population and should be avoided as much as possible. HAT among tourists and occasional visitors to endemic areas is a rare but regularly reported event. Pentamidine or suramin chemoprophylaxis is historical, and can no longer be recommended.

Insect repellents are of limited use, as tsetse flies are visually oriented. Long-sleeved, bright clothing (avoidance of black or dark blue) can decrease attractiveness to these insects and is the best defense against attacking tsetse flies.

39.8.2 Control in Endemic Areas

In the past, tremendous efforts were undertaken to control the threat posed by trypanosomiasis to humans, livestock, and economic development in rural Africa. Control programs are based on the five complementary pillars given in Table 4.

Table 4 Control of HAT

1	Diagnosis and treatment of patients
2	Active case finding
3	Vector control
4	Implementation and continuation of a surveillance system
5	Training, health education and community participation

The most important strategy is active case finding. This requires mobile teams, which regularly visit villages in endemic areas. Mostly based on the results of CATT screening, patients, preferably in the early stage of the disease, are identified and treated. Gradually, the parasite reservoir is depleted. As *Glossina* is a relatively incompetent vector with infectivity rates usually below 0.1% and susceptible to control measures such as insecticide application or trapping, the combination of various approaches can lead to a complete break of the transmission cycle. This was achieved in the past in many places. However, the recent resurgence of sleeping sickness in areas ridden by war and civil unrest, in combination with the decreasing availability of drugs on the international market and the general loss of interest in health in Africa, gives rise to the fear that HAT will soon be again out of control.

References

DNDi (Drugs for Neglected Diseases Initiative). www.dndi.org

FIND (Foundation for Innovative New Diagnostics). www.finddx.org

Franco JR, Cecchi G, Paone M et al (2022) The elimination of human African trypanosomiasis: achievements in relation to WHO road map targets for 2020. PLoS Negl Trop Dis 16(1): e0010047. Published 2022 Jan 18

Krishna S, Lindner AK, Lejon V (2022) Human African trypanosomiasis: treatment and prevention. In: Post TW (ed) UpToDate. UpToDate, Waltham

Krishna S, Lindner AK, Lejon V Human (2022) African trypanosomiasis: human African trypanosomiasis: epidemiology, clinical manifestations, and diagnosis. In: Post TW (ed) UpToDate. UpToDate, Waltham

Simarro PP, Cecchi G, Paone M et al (2010) The atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. Int J Health Geogr 9:57

Simarro PP, Franco JR, Cecchi G et al (2012) Human African trypanosomiasis in non-endemic countries (2000–2010). J Travel Med 19:44

WHO (2012) Accelerating work to overcome neglected tropical diseases: a roadmap for implementation. World Health Organization, Geneva

WHO (2019) WHO interim guidelines for the treatment of gambiense human African trypanosomiasis. World Health Organization, Geneva

WHO (2020) Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030. World Health Organization

WHO, WHO Expert Committee on the Control and Surveillance of Human African Trypanosomiasis (2013, Geneva, Switzerland) (2013) Control and surveillance of human African trypanosomiasis: report of a WHO expert committee. World Health Organization

WHO Datenbank Global Health Observatory. www.who.int/data/gho/data/themes/topics/human-african-trypanosomiasis

Part VII

Waterborne Zoonoses

Cryptosporidium and Cryptosporidiosis: Trickle or Treat?

40

Lucy J. Robertson and Ian Woolsey

Contents

Abstract

Cryptosporidiosis usually manifests as gastrointestinal infection and is associated with considerable morbidity and, in some circumstances, mortality. Effective treatment that is suitable for all patients, including those that are particularly affected by infection, children and the immunocompromised, is lacking.

There are several species of *Cryptosporidium*, some of which are zoonotic. The most important of these zoonotic species is *Cryptosporidium parvum*, but others, including *C. cuniculus* (predominantly associated with rabbits), *C. meleagridis* (predominantly associated with poultry), and *C. ubiquitum* (predominantly associated with sheep and cervids), are also of public health importance. *C. hominis* is generally only infective to humans. Subtypes within species have also been identified, with varying host-specificities, and, in addition, some genotypes have been identified that may, potentially, be recognized as individual species as more information accumulates.

L. J. Robertson (✉) · I. Woolsey
Parasitology Laboratory, Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
e-mail: lucy.robertson@nmbu.no; ian.woolsey@nmbu.no

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_32

Cryptosporidium is particularly suited to waterborne transmission, but foodborne transmission has also occurred on multiple occasions and outbreaks have been documented. In this chapter various waterborne outbreaks are reviewed, particularly those associated with zoonotic transmission, and also Standard Methods for analyzing water and food samples for contamination with these parasites. Although most waterborne outbreaks of cryptosporidiosis are due to *C. hominis* (and are therefore not zoonotic), most foodborne outbreaks are apparently zoonotic. Zoonotic transmission also occurs when there is close contact between infected animals and humans, particularly veterinary students and young children on petting farms. Thus, cryptosporidiosis is an important zoonosis with the potential for causing community-wide outbreaks of disease due to both waterborne and foodborne transmission.

Keywords

Cryptosporidium · Cryptosporidiosis · Foodborne transmission · Waterborne transmission · Zoonoses

40.1 Introduction

Cryptosporidium spp. are protozoan parasites that have been reported from a large variety of different hosts globally, including humans. To date, at least 25 different species of *Cryptosporidium* have been identified and described, and of these around 50% have been reported as being infectious to humans. In addition, multiple subtypes have been identified, some of which have zoonotic potential (e.g., chipmunk genotype I; Bujila et al. 2021), and the zoonotic horse genotype (Widmer et al. 2020), but have currently not been assigned species status. Additionally, subtypes within species can have a significant bearing on adaption to host species. It is becoming increasingly apparent that within species currently considered zoonotic, some subtypes may have specific host-specificity and may even solely infect humans, and therefore have no zoonotic potential. For *C. parvum* these include *C. p. anthroponosum* (IIc) (Nader et al. 2019) and subtypes IIe and IIm, whereas infections in livestock (cattle, sheep, and goats – the main sources of human zoonotic infection) are dominated by subtypes IIa and IId worldwide (Widmer et al. 2020). Furthermore, while some subtypes are capable of infecting both humans and other animals, their proclivity for a particular host species can have a bearing on their zoonotic potential. For example, *C. ubiquitum* subtype XIIa is found in ruminants worldwide, but subtypes XIIb-XIIf are predominantly found in rodents. Not surprisingly, the most common subtype found in humans in most parts of the world is XIIa. In the USA, however, there is a different pattern, with XIIb-XIId most commonly found in humans, and these genotypes were also the most common ones in rodents in the USA and also found in drinking water sources. As people in the USA probably have limited direct contact with wild rodents, it would seem that here *C. ubiquitum* zoonotic transmission may well be through rodent-contaminated water supplies (Li et al. 2014).

However, most reported human infections involve *C. parvum* (an important zoonotic species, particularly associated with infections in calves) or *C. hominis*, which is found primarily in humans. Other species that are considered important as human pathogens include *C. meleagridis*, a zoonotic infection primarily associated with infections in turkeys, but also relatively commonly identified in children in South America, and *C. cuniculus*, another zoonotic species particularly associated with infections in rabbits (Table 1). The other species of *Cryptosporidium* with zoonotic potential tend to be associated only with sporadic cases of human infection (e.g., *C. ubiquitum* infections that are usually associated with infections in sheep and cervids, although in some locations rodents) or are particularly associated with infections in immunocompromised patients (e.g., *C. suis* infections, more commonly associated with infection in pigs, and *C. felis* infections, more commonly associated with infections in cats; however one case of probably zoonotic transmission of *C. felis* from a cat to its immunocompetent owner has been documented (Beser et al. 2015). Although prevalence data are patchy, *Cryptosporidium* infection has emerged as a global public health problem, and has been reported from all continents except Antarctica (Wang et al. 2018).

Waterborne transmission is important in cryptosporidiosis; numerous waterborne outbreaks of cryptosporidiosis have occurred worldwide due to oocyst contamination of drinking water sources. The largest waterborne illness outbreak of any kind in the USA occurred in 1993 when over 400,000 people acquired cryptosporidiosis in Milwaukee (Mac Kenzie et al. 1994). More recently, around 27,000 people suffered from waterborne cryptosporidiosis in Östersund, Sweden in 2010 (Widerström et al. 2014).

In this chapter, emphasis will be primarily directed toward those species of *Cryptosporidium* that are predominantly zoonotic (that is, not *C. hominis*, as this is associated almost exclusively with human infections), and that have also been associated with waterborne or foodborne infection. Thus, *C. parvum* will be the main focus of the chapter, but also *C. cuniculus*. In addition, more general information will be provided including on the life cycle, clinical symptoms, diagnosis, and treatment of *Cryptosporidium* infections, both from a medical and a veterinary perspective, transmission routes and the epidemiology of infection, waterborne and foodborne outbreaks of zoonotic cryptosporidiosis, and the detection of *Cryptosporidium* in these matrices.

40.2 General Information on *Cryptosporidium* and Cryptosporidiosis: Life Cycle, Clinical Presentation, Diagnosis, and Treatment

For all *Cryptosporidium* species, the life cycle is direct (no intermediate host). When viable oocysts are ingested by an appropriate susceptible host, they usually excyst in the small intestine (*C. baileyi* can be associated with intra-tracheal infection in chickens) where the resultant sporozoites invade epithelial cells – and locate epicellularly (within the cell, but not within the cytoplasm). Repeated cycles of

Table 1 Overview of the major relevant species of *Cryptosporidium* in domestic animals (livestock and pets), including clinical presentation and zoonotic potential[a]

Cryptosporidium species	Host animal	Clinical notes for host animal species	Zoonotic potential
C. andersoni	Cattle	Older post-weaned calves, yearlings, and adults – some failure to thrive. Infects abomasal gastric glands	Yes
C. baileyi	Poultry	Detected in many different anatomical sites including digestive tract, respiratory tract, and urinary tract. Has been associated with high morbidity and mortality	A few reports from immunocompromised patients
C. bovis	Cattle	Common in post-weaned calves – less pathogenic than *C. parvum*	No
C. canis	Dogs	Often asymptomatic: may be associated with diarrhea	Yes: sporadic cases diagnosed in both immunocompetent and immunocompromised patients
C. cuniculus	Rabbits	Symptoms mostly mild or lacking, but severe cases have been reported	Yes: first identified as pathogenic to human during a waterborne outbreak
C. felis	Cats	May be associated with persistent diarrhea	Yes: sporadic cases diagnosed in both immunocompetent and immunocompromised patients
C. erinacei	Hedgehogs and horses	No symptoms reported	Yes: Sporadic cases reported; one report in an immunocompetent male
C. galli	Poultry	Infects the proventriculus, and has been associated with acute diarrheal disease	No
C. meleagridis	Poultry	Mostly intestinal and generally associated with mild symptoms	Yes: cases in immunocompetent and immunocompromised reported. Occurs particularly in Peru
C. parvum	Cattle	Common in pre-weaned calves – acute onset diarrhea. Intestinal location	Yes: considerable
	Cervids	Information on species detected among farmed deer is lacking; diarrhea in young calves, possibly severe, but can also be asymptomatic	

(continued)

Table 1 (continued)

Cryptosporidium species	Host animal	Clinical notes for host animal species	Zoonotic potential
	Dogs	Often asymptomatic: may be associated with diarrhea	
	Horses	Foals seem more susceptible; both asymptomatic cases and clinical infections (diarrhea) reported	
	Pigs	Less common than in bovines and small ruminants; diarrhea and vomiting	
	Small ruminants	Relatively common in pre-weaned lambs, associated with diarrhea	
C. scrofarum	Pigs	Relatively common, mild symptoms	No
C. suis	Pigs	Relatively common, mild symptoms	Few reports from immunocompromised patients
C. tyzzeri	Mice	No clinical signs or macroscopic changes associated with experimental infection	One case report in a 25-year old female
C. ubiquitum	Small ruminants	Common in older lambs and sheep, often apparently asymptomatic	Yes: sporadic cases in both immunocompetent and immunocompromised
C. xiaoi	Small ruminants	Common in older lambs and sheep, often apparently asymptomatic	No

[a]Information in the table derived and adapted from Santín and Trout (2008), Robertson and Fayer (2012), Robertson et al. (2014), Rašková et al. (2013), Kváč et al. (2014), Kaupke et al. (2014), Ryan et al. (2014), Ren et al. (2012), Jiang et al. (2020)

asexual reproduction result in destruction of these cells and the production of enormous quantities of meronts. A sexual cycle, gametogony, results in oocyst production. The oocysts sporulate while within the host (unlike other genera of Apicomplexa such as *Eimeria* spp. or *Toxoplasma gondii*) and may excyst within the same host, resulting in re-invasion of the epithelial cells and continuation of the infection. Alternatively, the oocysts are excreted in the feces, and are immediately infectious for the next host. These small oocysts (for most species they are approximately spherical and around 3–5 μm in diameter, although there is interspecies variation) are also very robust and can survive for long periods under cool, moist conditions.

In the human host, cryptosporidiosis is an enteric disease, generally characterized by watery diarrhea, abdominal pain, nausea, and related symptoms. However, other

symptoms have been reported, including low-grade fever and headache. Immunocompromised individuals and children in developing countries are most affected by cryptosporidiosis, and the relationship of infection with growth faltering, malnutrition, and diarrheal mortality is in need of further exploration (Shirley et al. 2012). In some individuals, however, infection may be largely asymptomatic.

The spectrum of symptoms depends not only on the host (age, nutritional status, immunity), but also on parasite factors including the number ingested and species. While cryptosporidiosis is self-limiting in immunocompetent individuals, a high relapse rate has been reported in some studies, as well as post-infection sequelae, which can be both gastrointestinal and non-gastrointestinal; in particular, after an outbreak in Sweden in 2010–2011, a follow-up study found that 48% of cases reported symptoms up to 28 months after the initial infection compared to non-cases. In addition to abdominal pain, these included nausea, fatigue, headaches, and joint pain (Lilja et al. 2018). In a recent systematic review that included 3670 cases, the most common long-term sequelae were diarrhea (25%), abdominal pain (25%), nausea (24%), fatigue (24%), and headache (21%) (Carter et al. 2020). In immunocompromised patients, the symptoms are often more severe, and infection may become chronic, debilitating, and potentially life-threatening, with high volumes of diarrhea, spreading of infection beyond the primary site, and severe weight loss.

In animals, the symptoms of *Cryptosporidium* infection appear to depend highly on parasite adaptation to the host and host age/immunological status, although results from different studies vary, and for many species infections are largely asymptomatic (see Table 1). Nevertheless, infections of some domestic animals with some species of *Cryptosporidium* may result in severe infection, usually with acute diarrhea as the main symptom. In some animals, especially young animals and particularly in association with concomitant infections or conditions, this may even be fatal (Xiao and Cama 2018; Cruvinel et al. 2020; Garro et al. 2021).

For both human and animal infections, diagnosis usually depends on the demonstration of oocysts (or their antigens or DNA) in fecal samples. As the oocysts are very small, the use of a staining technique, particularly using antibodies labeled with a fluorochrome and screening with fluorescence microscopy (immunofluorescent antibody testing; IFAT), is recommended. IFAT is considered to be a gold standard, although other techniques such as modified Ziehl-Neelsen (mZN) or auramine phenol staining may also be used successfully. However, mZN detects between 70% and 87.5% of infections compared with immunofluorescent antibody stains (Ryan et al. 2018), although a pre-screening concentration stage (formol-ether or flotation on sucrose or salt) may enhance sensitivity. Immunochromatographic kits are another diagnostic option that are rapid and simple to use for diagnostics; however they may have limited sensitivity in light infections, tend to be expensive, have a relatively short shelf life, and, to date, have not been widely adopted. Some investigations have shown poor sensitivity particularly in infections with species other than *C. hominis* or *C. parvum* (Agnamey et al. 2011). However, some next-generation kits, including kits not requiring refrigeration seem to have better sensitivity (Johansen et al. 2021).

While molecular methods such as PCR were not the mainstay of the general parasitology diagnostic laboratory a decade ago, in wealthy countries with the necessary infrastructure this has now changed, and molecular assays for gastrointestinal parasites are now the norm and tend to be of superior sensitivity and specificity. Indeed, molecular techniques have been reported to detect more than double the number of infections that would be detected by mZN (Checkley et al. 2015; Zahedi and Ryan 2020). A nested PCR has been developed for the detection of all *Cryptosporidium* spp. that can be differentiated by sequencing the PCR amplicons, but this is only genus-specific and is unable to identify species (Robinson et al. 2020). However, a duplex real-time PCR assay has also been developed, able to differentiate between *C. parvum* and *C. hominis* (Robinson et al. 2020). In addition, several assays based on multiplex PCR assays are being approved by the US Food and Drug Administration (FDA) in recent years. The majority of these tests are panel assays that are suitable for automation and thus enable higher throughput and faster results. Additionally, they are suitable for the simultaneous detection of numerous enteric parasites, or even different enteric pathogens (with gastrointestinal bacteria and viruses included in the panel). These tests are expensive, currently between US$ 30 and 155 per sample with equipment costs running up to US$40,000, which is an obstacle to their implementation even for laboratories in developed countries. However, they are a very useful tool in epidemiological surveillance, research, and outbreak investigations (Ryan et al. 2017). Further work is required to assess how well these tests perform on different sample types (Ryan et al. 2018).

Successful treatments for cryptosporidiosis in both humans and animals remain elusive, although one treatment (nitazoxanide) has been FDA-approved for symptom alleviation in immunocompetent humans and has shown promise for treating cryptosporidiosis in animals. Current treatments for ruminant cryptosporidiosis include halofuginone lactate or paromomycin, both of which reduce oocyst shedding and decrease the severity of diarrhea (Innes et al. 2020). Failure of therapeutic agents being evaluated is often considered to reflect the specific taxonomic position of *Cryptosporidium*, which indicates that it is more closely related to organisms such as gregarines rather than to classical coccidia (Ryan et al. 2016). For immuncompromised patients with HIV-infection, the development of effective antiretroviral therapies has been of more value for decreasing mortality due to cryptosporidiosis than any parasite-targeted treatment, and currently supportive care and antiretroviral therapy form the basis of treatment for *Cryptosporidium* in patients with HIV/AIDs (Squire and Ryan 2017). In developing countries, however, antiretroviral therapy coverage is often limited and thus cryptosporidiosis in HIV patients, particularly in association with other insults to health, may prove fatal.

The identification of *Cryptosporidium* as one of the four main etiological agents associated with serious childhood diarrhea (Kotloff et al. 2013) indicates that there is still a need to develop an effective chemotherapy targeting this parasite. The burden of diarrhea caused by *Cryptosporidium* infection was assessed in The Global Burden of Diseases, Injuries, and Risk Factors study (GBD) 2016 along with more than 300 causes of death and disability. By systematically quantifying the morbidity and mortality associated with these causes, *Cryptosporidium* infection was identified as

the fifth leading diarrheal etiology in children under 5 years with acute infection causing more than 48,000 deaths and in excess of 4.2 disability-adjusted life-years (DALYs) lost globally in 2016. These estimates on the burden of *Cryptosporidium* infection were then supplemented via a meta-analysis approach to determine the effect on childhood diarrhea caused by *Cryptosporidium* infection on physical growth parameters. The significant burden of acute short-term diarrhea was found to be a substantial underestimation of the true burden of disease when height-for-age, weight-for-age, and weight-for-height were considered, with an additional 7.85 million DALYs associated with these longer-term effects on childhood development (Khalil et al. 2018).

40.3 Transmission Routes and Epidemiology of *Cryptosporidium* Infection

As *Cryptosporidium* oocysts are immediately infectious upon excretion (unlike the majority of classical coccidial oocysts), direct fecal oral transmission (animal-to-animal, animal-to-human, human-to-animal, and human-to-human), as well as re-infection of the excretor, is relatively common and has been well documented. Although it is often impossible to exclude entirely the possibility that transmission has occurred via a contaminated vehicle, person-to-person spread is particularly well described within families (often secondary cases after a primary outbreak infection), in institutions such as childcare nurseries, and in hospitals. With respect to zoonotic transmission, infection of veterinary students examining diarrheic calves has, in particular, been well-documented and also of children visiting petting farms where they have direct contact with young animals. There are some reports where pet-to-human transmission has been convincingly demonstrated (e.g., Beser et al. 2015). However, again infection from environmental contamination (that is, from oocyst contamination of a transmission vehicle such as food) or common source transmission to both hosts often cannot be entirely excluded in such settings.

As well as being immediately infectious, *Cryptosporidium* oocysts are also notoriously robust, being able to survive in damp, cool environments for weeks or months, and being resistant against a range of commonly used disinfectants, including chlorination at levels used by the water industry. This means that transmission via a vehicle, such as water or food, is also well-documented, particularly as this may lead to an outbreak situation in which hundreds or thousands of individuals may be infected. It should, however, be noted that oocysts do not survive prolonged freezing at below −15 °C, freeze-thawing, or desiccation and that they are also killed by cooking (EFSA 2018).

In general, clinical cryptosporidiosis occurs most often in the toddler age group (1- and 2-year olds), presumably due to lack of previous infection (and hence immunity) and due to the unhygienic behavior in this age group, especially mouthing of myriad objects. Although more commonly diagnosed in male children, possibly due to greater exposure to contaminated objects or perhaps an artifact associated with medical consultation, in adults it is women who tend to be more frequently

diagnosed with clinical cryptosporidiosis than men (Nichols 2008). This is probably due to greater exposure of women than men to children with cryptosporidiosis, who may be excreting infective oocysts, but might also reflect that drinking water consumption is often higher among women. A range of studies have explored risk factors for infection. Although these vary according to study conditions, particularly whether associated with an outbreak or with sporadic infections and whether the study has focused on a population in an industrialized or developing country, in general, the key risk factors identified include: ingestion of contaminated drinking or recreational water; contact with infected persons or animals, particularly calves and lambs; travel to areas where the disease is considered endemic; and contact with children under 6 years of age (particularly, but not exclusively, children with diarrhea) (Bouzid et al. 2013). Interestingly, several investigations have indicated a negative association between cryptosporidiosis and eating raw vegetables, while, in contrast, various outbreaks of cryptosporidiosis have been associated with consumption of fresh produce. It has been proposed that this apparently protective effect may be due to repeated exposure to low numbers of *Cryptosporidium* oocysts on raw vegetables thus allowing the development of protective immunity (Bouzid et al. 2013). Risk factors for infection will vary by region; in Ethiopia, for example, an in-depth exploration of risk factors associated with pediatric cryptosporidiosis identified use of public tap water and moderate acute malnutrition as relevant risk factors for cryptosporidiosis diarrhea, neither of which are likely to occur widely in most industrialized countries (Johansen et al. 2021). In a recent meta-analysis of risk factors for *Cryptosporidium* infections in low to middle income countries, significant reported risk factors were animal contact, household diarrhea, and open defecation; interestingly, however, poor quality drinking water was not found to be significant (Bouzid et al. 2018).

Associations have also been made between season and cryptosporidiosis (Lal et al. 2012), with infection peaks in spring and late summer/early autumn and least cases reported in winter, regardless of the country of study. It is suggested that the spring peak may reflect agricultural practices (calving and other young livestock), as well as greater potential for water contamination due to heavy rainfalls and spring snowmelt (Lal et al. 2012). In some instances, the late-summer peak may represent the return home of tourists who have been vacationing in more endemic areas, and perhaps associated with behaviors resulting in greater exposure risks, such as increased contact with recreational water.

The development and use of tools for molecular epidemiological studies have provided useful insights into the transmission of cryptosporidiosis. Small subunit (SSU) rRNA-based tools are now commonly used for genotyping *Cryptosporidium* identified in both human and animal infections, and also for oocysts isolated from water and other environmental samples (Xiao 2010). At the sub-genotyping level, one of the popular tools is the DNA sequence analysis of the 60 kDa glycoprotein (gp60) gene. This gene contains tandem repeats of the serine-coding trinucleotide TCA, TCG, or TCT at its 5′ end, along with extensive sequence differences in the non-repeat regions, and has been used to categorize *C. parvum* and *C. hominis* (the most common *Cryptosporidium* spp. causing infections in humans) to subtype

families (Xiao 2010). Besides the sequence heterogeneity of the gp60 gene, which makes it useful as a *Cryptosporidium* subtyping tool (it is one of the most polymorphic markers identified in the *Cryptosporidium* genome), it is also of biological relevance. The gp60 gene codes for a protein that is located on the surface of the apical region of invasive stages of the parasite, and thus provides a biological possibility of associating parasite characteristics, including clinical presentation, with subtype family.

The use of these and other molecular tools has revealed that, although cryptosporidiosis is generally considered an infection of global importance, there is a clear geographical distribution of *C. parvum* and *C. hominis* in human infections. Although both *C. parvum* and *C. hominis* are common in European countries, *C. parvum* is the dominant species in humans in the Middle East, while in the rest of the world, particularly in developing countries, *C. hominis* is usually the predominant species in humans (Xiao 2010). Furthermore, within-country geographic variations have also been noted, with *C. parvum* infections being more common than *C. hominis* in rural regions of the USA and Ireland (Xiao 2010). These data suggest that zoonotic cryptosporidiosis is more common in the Middle East and Europe than in developing countries, at least at present; this is also supported by some of the data from surveys of calves in some developing countries, where infection rates have sometimes been unexpectedly low (Chang'a et al. 2011; Robertson et al. 2020; Guo et al. 2021). Indeed, in Africa, zoonotic *Cryptosporidium* spp. transmission appears to occur a great deal less frequently than human–human transmission. In a recent review (Robertson et al. 2020), numerous possible reasons for this were suggested. These include that management practices predominant in Africa such as smaller herd sizes, outdoor housing, and the use of hay bedding (reducing close contact between animals and oocyst survival rates, respectively) are both associated with lower risk of transmission; that established cattle herds in Africa are predominantly of *B. indicus* breeds with evidence suggesting that these breeds may be more resistant to *C. parvum* infection, and year-round breeding also means that seasonal peaks in environmental contamination in spring, associated with lambing and calving do not occur. Furthermore, it was noted in Africa, and potentially low-income settings in general, that infants and children are likely to receive low level exposure via human transmission thus potentially inferring a certain degree of protection against later zoonotic infection (Robertson et al. 2020).

40.4 Waterborne Transmission of Zoonotic *Cryptosporidium* Species

Cryptosporidium lends itself to waterborne transmission due to various factors in its biology. These factors include the low infective dose (theoretically as low as a single oocyst, although human volunteer studies suggest tens of oocysts), the large numbers of oocysts excreted during infection – calves infected with *C. parvum* may produce as many as 6×10^7 oocysts per gram of feces, and a single infected calf may excrete 4×10^{10} oocysts during its second week of life and 6×10^{11} oocysts during

its first month of life (Uga et al. 2000; Nydam et al. 2001), and the robustness and longevity of the oocysts in damp, cool environments, including upon exposure to drinking water chlorination regimes. In addition, the small size of oocysts enables them to penetrate some of the physical barriers in water treatment. Together, these factors have resulted in numerous outbreaks of waterborne cryptosporidiosis, both associated with drinking water and also with recreational water. A review of 325 outbreaks of human disease attributed to the waterborne transmission of pathogenic protozoa (from the beginning of records up until around 2003) indicated that the majority of them (approximately 51%) were caused by *Cryptosporidium* infection (Karanis et al. 2007). Although the majority of these are suggested to be *C. parvum* infections, the lack of molecular characterization methods at the time of most of these outbreaks coupled with the fact that the majority of different species of oocysts are morphologically indistinguishable mean that it is likely that a large proportion of these outbreaks were actually due to *C. hominis* infections (and possibly some other species of *Cryptosporidium*). A follow-up review of more recent outbreaks (Baldursson and Karanis 2011) indicated that *Cryptosporidium* spp. continued to be the dominant etiological agent of waterborne outbreaks of protozoan disease between 2004 and 2010, with more than 60% of the 199 documented outbreaks due to *Cryptosporidium* infection. Although the review does not provide a species overview for these outbreaks (*C. hominis* or *C. parvum*), reference to the original papers cited in the review demonstrates that the majority of these outbreaks for which species information was available were due to *C. hominis*. However, even for outbreaks with *C. parvum*, this knowledge is insufficient to determine whether zoonotic transmission has occurred (from animals to humans, via water as the transmission vehicle), since contamination can also occur from human sewage even with *C. parvum* infections, and thus traditional epidemiological data analysis is needed in addition to molecular epidemiological data in order to determine the source of contamination. However, when the etiological agent is *C. hominis*, then zoonotic transmission is much less likely to have occurred (Nydam et al. 2005).

Nevertheless, some waterborne outbreaks of cryptosporidiosis have often been attributed to contamination of water catchments by animals, and although sometimes the indications implicating zoonotic transmission have been speculative (and later proven to be incorrect or unlikely), on other occasions the evidence has been substantial.

For example, grazing cattle or slaughterhouse effluent contaminating Lake Michigan were mentioned as two possible sources of *Cryptosporidium* oocysts in the large outbreak in Milwaukee, Wisconsin in 1993 (Mac Kenzie et al. 1994). But retrospective analysis of clinical isolates revealed that the outbreak was caused by the anthroponotic *C. hominis* (Sulaiman et al. 1998), and it is worth noting that among the most recent waterborne outbreaks in the UK (compiled by Chalmers (2012)), the majority do not indicate zoonotic transmission as *C. hominis* infection predominates.

However, during an outbreak in Scotland in 1988 substantial testing of water and environmental samples was conducted (Smith et al. 1989) – albeit with the less sophisticated methods available at that time – and they were less sensitive and

specific than those currently developed (sucrose flotation was used, for example, rather than immunomagnetic separation (IMS)), which indicated that irregular seepage of oocyst-containing water into a break-pressure tank was the most likely cause of the outbreak. This seepage was considered to have been exacerbated by heavy rains, and the occurrence of muck spreading and the spraying of cattle slurry prior to the outbreak in the vicinity of a fire clay pipe draining into the break-pressure tank indicated that this was the likely zoonotic source of oocysts (Smith et al. 1989). Another outbreak in the UK in 1992 was again associated with heavy rainfall and, in this instance, flooding of a field in which livestock (species not mentioned) grazed and thereafter drainage into a shaft associated with a groundwater drinking water supply (Bridgman et al. 1995) suggests again waterborne zoonotic transmission. It should be noted that although the traditional epidemiological investigations associated with both these outbreaks was extensive, they occurred before the introduction of reliable molecular tools that could be used to determine whether the etiological agent was potentially zoonotic, and, moreover, before *C. parvum* and *C. hominis* had been recognized as separate species.

Although zoonotic transmission of cryptosporidiosis from lambs has been definitively shown in various outbreaks associated with direct contact such as petting farms (Gormley et al. 2011; Robertson et al. 2014), and a range of waterborne outbreaks of cryptosporidiosis in the UK have implicated sheep as the source of the infection, a lack of studies in which oocysts from both grazing lambs or sheep and oocysts in water and in patient samples have been characterized has resulted in a lack of clarity over whether these really are the source of contamination (Robertson 2009). This is unfortunate, as sometimes the assumption that lambs or sheep are the source of contamination has resulted in measures being implemented that may have been unnecessary. These have included extensive boil water notices, relocation of sheep during the lambing season, and even contributed to the closure of some sheep farms, all measures that may result in anxiety and frustration for local communities (Robertson 2009). Thus, now that we have tools to help in deciding sources of contamination of water supplies, it is important to ensure that they are appropriately used.

While the focus of zoonotic transmission, including by the waterborne route, tends to focus on *C. parvum*, it should be noted that sheep are also frequently infected with *C. ubiquitum*, and this species has also been detected in a number of sporadic human cases globally (Cieloszyk et al. 2012; Elwin et al. 2012; Feltus et al. 2006; Leoni et al. 2006; Ong et al. 2002; Soba et al. 2006; Trotz-Williams et al. 2006). As this species has also been identified in storm water, wastewater, raw water, and drinking water (Jiang et al. 2005; Liu et al. 2011; Nichols et al. 2010; Van Dyke et al. 2012) and was the third most common species in raw water in Scotland, as well as the most common species identified in drinking water (Nichols et al. 2010), there is a clear potential for zoonotic waterborne transmission. Given that no such waterborne outbreaks have been described to date, it is possible that infectivity to humans is relatively limited. Likewise, deer also are relatively commonly infected with both *C. parvum* and *C. ubiquitum*, but there have been no documented cases of proven zoonotic transmission from deer. A study from Australia suggested that deer

were not likely to be a threat for zoonotic transmission of cryptosporidiosis in a specific protected drinking water supply watershed (Cinque et al. 2008); a study from the USA, however, reached the opposite conclusion and stated that deer in a particular watershed posed a potential threat regarding zoonotic cryptosporidiosis and therefore were appropriate targets for source water protection (Jellison et al. 2009). In addition, subtyping of *C. ubiquitum* samples from the USA has suggested that subtypes XIIb-XIId from wild rodents have the potential to infect humans via waterborne transmission (Li et al. 2014).

Another zoonotic species of *Cryptosporidium, C. cuniculus,* has, however, been associated with waterborne transmission. *C. cuniculus* is rarely, but sporadically, identified in human infections (e.g., of 3030 *Cryptosporidium*-positive fecal samples submitted for routine typing in the UK between 2007 and 2008, only 37 were diagnosed as *C. cuniculus* (1.2%); Chalmers et al. 2011) and transmission of *Cryptosporidium* to humans from farmed rabbits has not been recorded (Robertson et al. 2014). Indeed, an investigation exploring associations between farm animals and human patients with cryptosporidiosis did not indicate rabbits as a particular source of infection among farmed animals (Smith et al. 2010). However, a waterborne outbreak of cryptosporidiosis in England in 2008 affecting 29 people was identified to the species level in eight patients as *C. cuniculus*, and to the subtype level as subtype VaA18 (Chalmers et al. 2009). Furthermore, *Cryptosporidium* oocysts of the same species and sub-genotype were identified in the colon of a carcass of a rabbit (presumably wild) that was found in a tank at the implicated water treatment works. Thus, this outbreak, which was investigated using both traditional and molecular epidemiological tools, convincingly demonstrates a waterborne outbreak of zoonotic cryptosporidiosis.

Another of the most important zoonotic species of *Cryptosporidium* is *C. meleagridis*. This species is the third most common species in human cryptosporidiosis worldwide (Robertson et al. 2014). Studies on the prevalence and species distribution for cryptosporidiosis in humans in South America have identified that the prevalence of *C. meleagridis* infection is very similar to that of *C. parvum* (Cama et al. 2003, 2007, 2008). Although *C. meleagridis* infection is usually associated with infection in turkeys, the only documented case of zoonotic transmission from a bird source demonstrates that chickens rather than turkeys were the source of infection (Silverlås et al. 2012). Moreover, no waterborne transmission has been documented. However, as not all patients in epidemiological investigations that have been diagnosed with *C. meleagridis* infections have had contact with birds or even animals (Elwin et al. 2012), transmission via a contaminated vehicle (such as food or water) seems possible, or – alternatively – direct infection from another person with that infection.

As well as adequate water treatment, including sufficient barriers to ensure acceptable removal and/or inactivation of pathogens such as *Cryptosporidium*, one of the major mechanisms for ensuring safe water supply is implementing an appropriate catchment control policy. With respect to zoonotic *Cryptosporidium* infection, this may mean limiting or restricting access of domestic animals and wildlife to vulnerable sites, particularly watercourses, associated with drinking water supply

and possibly recreational use. Such restrictions might be of particular validity during periods of heavy rain, when wash-off contamination is likely to be highest. Environmental studies have demonstrated that contamination of surface waters with *Cryptosporidium* oocysts are significantly affected by land use, such as cattle husbandry and manure management practices, as well as seasonal and weather characteristics (Keeley and Faulkner 2008). However, such restrictions regarding grazing of domestic animals, for example, may have little impact if there is a large reservoir of infection in wild animals, and will also serve little purpose if the restricted animals are not infected with zoonotic pathogens. For example, a study in Canada that sought to investigate the contribution of dairy cattle to protozoan contamination of water sources (Budu-Amoako et al. 2012) concluded that the *Cryptosporidium* oocysts being shed were predominantly non-zoonotic *C. bovis* and *C. andersoni*, and therefore of little significance to public health. Given that pre-weaned calves are the most likely age group and species to shed *C. parvum* oocysts, it could be argued that measures to protect water catchments from young ruminants and their feces, including spreading of calf manure on fields where runoff cannot occur, should be directed toward this animal group (Robertson et al. 2014). However, it should be emphasized that water courses should be subjected to individual risk assessment and measures implemented accordingly; it should not be forgotten that even with adequate catchment protection and water treatment, unexpected events may occur that nevertheless may result in contamination (e.g., the entry of a rabbit with *C. cuniculus* infection into water treatment works). Therefore, it is necessary to follow outbreaks of diarrhea in the community, and – where appropriate – to analyze water samples for contamination.

40.5 Foodborne Transmission of Zoonotic *Cryptosporidium*

Although cryptosporidiosis is generally considered a waterborne, rather than foodborne, disease, the potential for foodborne transmission is widely acknowledged. Indeed, it has been estimated that 10% of all *Cryptosporidium* infections are foodborne (EFSA 2018). Globally, *Cryptosporidium* has been ranked the fifth most important pathogen out of 24 potentially foodborne parasites with only *Taenia solium, Echinococcus granulosus, Echinococcus multilocularis*, and *Toxoplasma gondii* exceeding it (FAO/WHO 2014) and caused 8.6 million cases of foodborne illness in 2010, 3759 deaths and 296,156 DALYs (Ryan et al. 2018). A European ranking of foodborne parasites (Bouwknegt et al. 2018) also placed *Cryptosporidium* as the fifth most important, although regionally second highest in both Northern and Western Europe; a similar exercise from India also placed *Cryptosporidium* in the second position (Robertson et al. 2015). Food contamination with *Cryptosporidium* oocysts may occur during production, processing, or preparation, and the longevity of the oocysts that is enhanced on moist and sheltered surfaces of fruit and vegetables (Gajadhar and Allen 2004; Ryan et al. 2018) means that they can survive various processing treatments, including chlorine baths and blast freezing (Duhain et al. 2012). This longevity can make trackback measures to an outbreak difficult as

there can be a long lag time between the contamination of a food item and its consumption, and, in addition, there may be a prolonged period of several days between infection and symptoms, and between symptoms and diagnosis. Washing of fresh produce may fail to remove contaminating oocysts, since they not only adhere to surfaces but may also infiltrate into leafy vegetables via stomatal openings (Macarisin et al. 2010a, b). However, a study looking at removal of *Cryptosporidium* oocysts (and other parasites) from artificially contaminated blueberries and raspberries found that simple rinsing under cold water for 1 min removed over 85% of contaminating *Cryptosporidium*, and washing in a vinegar solution or using a salad spinner to remove excess water increased removal efficiency to, on average, over 90% (Temesgen et al. 2021).

Fewer foodborne cryptosporidiosis outbreaks have been documented than waterborne outbreaks. Of the 40 reported cryptosporidiosis outbreaks (in which molecular characterization was performed) in the last 10 years, 27 have been waterborne while only 13 foodborne outbreaks have been reported (Zahedi and Ryan 2020). This could be because fewer foodborne outbreaks have occurred (because, for example, the oocysts survive less readily on food due to potential for desiccation, freezing, or heating, or because contamination of food is less likely to occur than contamination of water). Alternatively, this could reflect that fewer people tend to be infected in foodborne outbreaks (due to more restricted distribution of the contaminated product), and therefore the event may be less likely to be identified. Additionally, traceback is often hampered by the fact that the food item in question has been consumed and therefore not available for testing (Gajadhar and Allen 2004; Ryan et al. 2018). One interesting aspect of comparing foodborne outbreaks and waterborne outbreaks is that whereas waterborne outbreaks of cryptosporidiosis tend to be most frequently caused by the anthroponotic species *C. hominis*, foodborne outbreaks tend to be most frequently caused by the zoonotic species *C. parvum*. In a review considering geographical distribution of foodborne cryptosporidiosis (Robertson and Chalmers 2013), seven of the eight outbreaks in which molecular analyses were conducted on samples report *C. parvum* as the etiological agent, and only one *C. hominis.* A further outbreak of foodborne cryptosporidiosis from May 2012 in the UK (McKerr et al. 2015) was primarily epidemiologically associated with consumption of ready-to-eat pre-cut mixed leaves from a major supermarket chain and was also found to be due to *C. parvum*. Of the outbreaks in the last 10 years only 6 of the 27 waterborne outbreaks reported *C. parvum* as the etiological agent and only 1 case of *C. hominis* was reported in 13 foodborne outbreaks (Zahedi and Ryan 2020). Infection with *C. parvum* does not necessarily indicate zoonotic transmission from an animal source, since humans can also transmit *C. parvum*. However, epidemiological investigations into at least some of these outbreaks suggest that an animal source of *Cryptosporidium* is probable. Moreover, zoonotic transmission also seems likely for some of the outbreaks in which molecular analyses were not conducted, as evinced from traditional epidemiological investigations. For example, an outbreak in the USA in 2003 in which ozonated apple cider was the infection vehicle was demonstrated to be largely due to infection with two similar sub-types of *C. parvum* (for one patient, infection with *C. ubiquitum* was

recorded) (Blackburn et al. 2006). However, in two earlier outbreaks of cryptosporidiosis associated with consumption of apple cider, species identification had not been conducted (Millard et al. 1994; Anonymous 1997), although evidence from one of these outbreaks strongly suggested that animals were the probable source of infection, with calves with cryptosporidiosis grazing in the orchard from which the apples had been obtained. The first outbreak linked to apples reported outside the USA occurred in Norway in Autumn 2018. The species was *C. parvum,* GP60 subtype IIaA14G1R1, and was linked to one container of self-pressed apple juice suggesting an isolated contamination event suggesting contaminated ground at the orchard, contaminated fruit, or contamination during processing (Robertson et al. 2019). It was unclear whether human or animal contamination was most likely to be responsible for the contamination event.

Another foodborne outbreak of cryptosporidiosis was suspected to have been transmitted via the consumption of vegetables that had been sprayed with water possibly contaminated with cattle feces, with both human cases and implicated animals infected with the same subtype of *C. parvum* (Collier et al. 2011). Furthermore, it would seem likely that the outbreaks of cryptosporidiosis that have been postulated to have been caused by the consumption of inadequately pasteurized cow milk (Gelletlie et al. 1997; Harper et al. 2002) are due to zoonotic transmission, although this cannot be proven in the absence of insufficient information.

Why foodborne cryptosporidiosis should more likely be due to zoonotic transmission and why waterborne cryptosporidiosis should more likely be due to anthroponotic transmission is not entirely clear. It could, at least partly be the result of cryptosporidiosis being historically regarded as a waterborne parasite leading to under-assessment and underreporting of food as a transmission vehicle coupled with fewer international standards for testing food compared to drinking water (EFSA 2018). However, it would seem probable that greater effort is made to keep a barrier between human sewage and food production areas, but it is simply not possible to maintain this barrier between human sewage and water catchment areas. Furthermore, along the farm-to-fork food production chain there are various opportunities for contact between food products and animal feces from potentially infected animals, but relatively few opportunities for contact between human sewage and food products.

40.6 Detection of Zoonotic *Cryptosporidium* Oocysts in Water Supplies and on Food

Our understanding of the importance of the waterborne route of infection for *Cryptosporidium*, particularly exacerbated by the occurrence of community-wide outbreaks with hundreds or thousands of individuals infected, led to a need for analysis of water samples for these parasites. Such information provides a handle on the extent of contamination in different water sources and how this may be affected by factors such as weather patterns and season. Such data are also used as input for risk assessments, to evaluate whether water supplies are likely sources of infection in

outbreak situations, to determine the efficacy of different removal or inactivation measures, and to have a basis for recommending the type and extent of water treatments and interventions that are necessary for a particular water supply. However, analysis of water samples for *Cryptosporidium* oocysts is not easy. Unlike bacteria they cannot be readily cultivated, and there are no simple, easy-to-quantify indicators or appropriate surrogates. Thus, in order to detect and quantify *Cryptosporidium* contamination of water, the individual oocysts must be isolated and enumerated or their DNA (or some other marker) must be isolated and the quantity measured. In order to determine if the oocysts detected are zoonotic, molecular analyses must also be conducted, either downstream from detection or as a component of the detection method.

Over the past 30 years or so, different approaches and equipment have been investigated for their suitability at conducting this task. Method requirements include reproducibility, specificity, and sensitivity (given the low concentrations of these parasites usually occurring in water). Additionally, it is preferable for the method to be cheap, user-friendly, rapid, and with the potential to be implemented in a standard analytical laboratory.

The first documented Standard Methods for analysis of water for *Cryptosporidium* oocysts were probably the US EPA ICR (United States Environment Protection Agency Information Collection Rule) Method (US EPA 1996) and the "SCA Blue book method" (Anonymous 1990). These methods included collection of a water sample by filtration through a yarn-wound polypropylene filter; removal of all particulates from the filter using detergents and mechanical extraction; concentration of the particles by centrifugation (or settling); purification/isolation of putative parasites by buoyant density gradient centrifugation (usually using Percoll-sucrose, specific gravity of 1.10); detection of the parasites using immunofluorescent antibody testing (IFAT) in which the sample concentrate is incubated with fluorochrome-labeled monoclonal antibodies against the oocyst walls.

These methods have been improved over time, particularly with respect to the production of improved sampling techniques (different filter types and continuous-flow centrifugation) and the use of IMS for isolation of oocysts from the sample concentrate prior to detection (Anonymous 2006). Interlaboratory trials have been used for validation work for different techniques, and now the most commonly followed Standard Methods are probably US EPA Methods 1622 and 1623.1 (both of which are regularly updated and available for downloading from the US EPA homepage), and ISO Method 15,553 (Anonymous 2006). In addition, individual countries or regions have also produced versions of these methods or considered them.

State-of-the-art detection methods are coming online and include on-chip biosensors, electrochemical biosensors, capacitance, and nanotechnology such as the use of semiconductor quantum dot technology to fluorescently label oocysts. While there is no significant difference between this new technique and FITC, quantum dots are more photo-stable and generate 50% less interference with other fluorescence material in water samples. Oligonucleotide gold nanoparticles have been used for molecular detection without DNA amplification and these nanoparticles have

been employed in immuno-dot blot assays, increasing sensitivity of parasite detection 500-fold when compared to conventional ELISA. These results demonstrated good correlation with those of conventional PCR validation (Luka et al. 2021) making this method a promising candidate for future use in routine water screening assuming prices become economically competitive with current detection methods.

Methods for detection of *Cryptosporidium* contamination of foods has lagged behind that for water, although an ISO Standard that is directed toward analysis of leafy green vegetables and berry fruits has been published (ISO 18744; Anonymous 2016). This uses a similar sequence of steps as the water analysis method, but with elution of the parasites from the fresh produce by a washing procedure prior to concentration by centrifugation, isolation by IMS, and detection by IFAT. Immunomagnetic bead separation (IMS) methods have significantly improved the sensitivity of immunological methods. IFAT detection of *Cryptosporidium* on lettuce and raspberries after IMS has been used with increased sensitivity and specificity of up to 95.8% and 85.4%, respectively (Cook et al. 2006).

Approaches used for *Cryptosporidium* detection in different food matrices, including fresh produce, fruit juice, milk and dairy products, meat, and shellfish, have been extensively described (EFSA 2018; Chalmers et al. 2020); in general, the most sensitive methods require oocyst separation from the sample matrix followed by detection either by IFAT or PCR. As IFAT is the detection method described in the ISO Standard, this remains the method most commonly used and is considered the gold standard. However, there is a need for standardization and although ISO 18744 is considered a good starting point for sample surveys, further refinement to increase its range of applicability has been recommended (Chalmers et al. 2020). The methods are not considered suitable for routine analyses for food operators, and considering factors such as the variety of relevant food matrices, cost, and practicability, transition from IFAT to molecular detection has been recommended (Chalmers et al. 2020). The EFSA-funded IMPACT project is one progression toward this recommendation (Mayer-Scholl et al. 2021).

40.7 Conclusion

Cryptosporidiosis is an important protozoan disease that is associated with considerable morbidity and, in some circumstances, mortality on a global level. The main symptom is diarrhea. One of the most important facets of this infection from a clinical perspective is the absence of an effective treatment that is suitable for all patients, including children and the immunocompromised, two patient groups that are particularly affected by infection.

There are several species of *Cryptosporidium*, some of which have some degree of host-specificity, but many of which have the potential to infect humans and are considered zoonotic. The most important of these is *C. parvum*, but other species, including *C. cuniculus* (predominantly associated with rabbits), *C. meleagridis* (predominantly associated with poultry), and *C. ubiquitum* (predominantly associated with sheep and cervids, but also rodents) are also of relevance to public health.

In addition, *C. hominis* affects predominantly humans, but occasional infections in other animals have been reported. Various characteristics of *Cryptosporidium* mean that it lends itself to waterborne transmission, and many waterborne outbreaks have been documented, often affecting hundreds or thousands of individuals. The importance of the waterborne transmission route has instigated the development of Standard Methods for analyzing water samples for contamination with these parasites, and methods for food have also been developed. Although the majority of documented waterborne outbreaks of cryptosporidiosis are due to infection with *C. hominis* (and are therefore not zoonotic), the majority of foodborne outbreaks of cryptosporidiosis are apparently zoonotic, as evidenced by both traditional and molecular epidemiological investigations. In addition, zoonotic transmission has frequently been recorded when there is close contact between infected animals and humans, particularly veterinary students and young children on petting farms. Thus, cryptosporidiosis is an important zoonosis, with the potential for causing community-wide outbreaks of disease due to both waterborne and foodborne transmission.

40.8 Cross-References

► Giardiasis from a One Health Perspective

References

Agnamey P, Sarfati C, Pinel C et al (2011) Evaluation of four commercial rapid immunochromatographic assays for detection of *Cryptosporidium* antigens in stool samples: a blind multicenter trial. J Clin Microbiol 49(4):1605–1607

Anonymous (1990) Methods for the examination of waters and associated materials. Isolation and identification of *Giardia* Cysts, *Cryptosporidium* oocysts and free living pathogenic amoebae in water, etc. 1989. Department of Environment, Standing Committee of Analysts. H.M.S.O. Publication, London

Anonymous (1997) Outbreaks of *Escherichia coli* 0157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider, Connecticut and New York, October 1996. Morb Mortal Wkly Rep MMWR 46:4–8

Anonymous (2006) ISO 15553:2006 Water quality – isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts from water

Anonymous (2016) ISO 18744:2016 Microbiology of the food chain – detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits

Baldursson S, Karanis P (2011) Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. Water Res 45(20):6603–6614

Beser J, Toresson L, Eitrem R et al (2015) Possible zoonotic transmission of *Cryptosporidium felis* in a household. Infect Ecol Epidemiol 5(1):28463

Blackburn BG, Mazurek JM, Hlavsa M et al (2006) Cryptosporidiosis associated with ozonated apple cider. Emerg Infect Dis 12:684–686

Bouwknegt M, Devleesschauwer B, Graham H et al (2018) Prioritisation of food-borne parasites in Europe, 2016. Eur Secur 23(9):17–00161

Bouzid M, Hunter PR, Chalmers R et al (2013) *Cryptosporidium* pathogenicity and virulence. Clin Microbiol Rev 26(1):115–134

Bouzid M, Kintz E, Hunter PR (2018) Risk factors for *Cryptosporidium* infection in low and middle income countries: a systematic review and meta-analysis. PLOS Neglect Trop Dis 12(6): e0006553

Bridgman SA, Robertson RMP, Syed Q et al (1995) Outbreak of cryptosporidiosis associated with a disinfected groundwater supply. Epidemiol Infect 115(3):555–566

Budu-Amoako E, Greenwood SJ, Dixon BR et al (2012) *Giardia* and *Cryptosporidium* on dairy farms and the role these farms may play in contaminating water sources in Prince Edward Island, Canada. J Vet Intern Med 26(3):668–673

Bujila I, Troell K, Fischerström K et al (2021) *Cryptosporidium* chipmunk genotype I–an emerging cause of human cryptosporidiosis in Sweden. Infect Genet Evol 92:104895

Cama VA, Bern C, Sulaiman IM, Gilman RH et al (2003) *Cryptosporidium* species and genotypes in HIV-positive patients in Lima Peru. J Eukaryot Microbiol 50(Suppl):531–533

Cama VA, Ross JM, Crawford S et al (2007) Differences in clinical manifestations among *Cryptosporidium* species and subtypes in HIV-infected persons. J Infect Dis 196:684–691

Cama VA, Bern C, Roberts J et al (2008) *Cryptosporidium* species and subtypes and clinical manifestations in children Peru. Emerg Infect Dis 14:1567–1574

Carter BL, Chalmers RM, Davies AP (2020) Health sequelae of human cryptosporidiosis in industrialised countries: a systematic review. Parasit Vectors 13(1):1–14

Chalmers RM (2012) Waterborne outbreaks of cryptosporidiosis. Ann Ist Super Sanita 48(4):429–446

Chalmers RM, Robinson G, Elwin K et al (2009) *Cryptosporidium* sp. rabbit genotype a newly identified human pathogen. Emerg Infect Dis 15(5):829–830

Chalmers RM, Elwin K, Hadfield SJ et al (2011) Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus,* United Kingdom 2007–2008. Emerg Infect Dis 17(3):536–538

Chalmers RM, Robertson LJ, Dorny P et al (2020) Parasite detection in food: current status and future needs for validation. Trends Food Sci Technol 99:337–350. https://doi.org/10.1016/j.tifs.2020.03.011

Chang'a JS, Robertson LJ, Mtambo MMA et al (2011) Unexpected results from large-scale cryptosporidiosis screening study in calves in Tanzania. Ann Trop Med Parasitol 105(7):515–521

Checkley W, White AC Jr, Jaganath D (2015) A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect Dis 15(1):85–94

Cieloszyk J, Goni P, Garcia A et al (2012) Two cases of zoonotic cryptosporidiosis in Spain by the unusual species *Cryptosporidium ubiquitum* and *Cryptosporidium felis*. Enferm Infecc Microbiol Clin 30:549–551

Cinque K, Stevens MA, Haydon SR et al (2008) Investigating public health impacts of deer in a protected drinking water supply watershed. Water Sci Technol 58(1):127–132

Collier SA, Smith S, Lowe A et al (2011) Cryptosporidiosis outbreak at a summer camp – North Carolina. Morb Mortal Wkly Rep MMWR 60:918–922

Cook N, Paton CA, Wilkinson N et al (2006) Towards standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries. Part 2: validation. Int J Food Microbiol 109 (3):222–228

Cruvinel LB, Ayres H, Zapa DMB et al (2020) Prevalence and risk factors for agents causing diarrhea (coronavirus, rotavirus, *Cryptosporidium* spp., *Eimeria* spp., and nematodes helminths) according to age in dairy calves from Brazil. Trop Anim Health 52(2):777–791

Duhain GLMC, Minnaar A, Buys EM (2012) Effect of chlorine, blanching, freezing, and microwave heating on Cryptosporidium parvum viability inoculated on green peppers. J of Food Protect 75(5):936–941

EFSA Panel on Biological Hazards (BIOHAZ) (2018) Public health risks associated with foodborne parasites. EFSA J 16(12):e05495

Elwin K, Hadfield SJ, Robinson G et al (2012) The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales 2000–2008. Epidemiol Infect 140:673–683

FAO/WHO (2014) Multicriteria-based ranking for risk management of foodborne parasites. Report of a Joint FAO/WHO Expert Meeting, 3–7 September 2012 FAO Headquarters, Rome, Italy 2014

Feltus DC, Giddings CW, Schneck BL et al (2006) Evidence supporting zoonotic transmission of *Cryptosporidium* spp. in Wisconsin. J Clin Microbiol 44:4303–4308
Gajadhar AA, Allen JR (2004) Factors contributing to the public health and economic importance of waterborne zoonotic parasites. Vet Parasitol 126(1–2):3–14
Garro CJ, Morici GE, Tomazic M et al (2021) Occurrence of *Cryptosporidium* and other enteropathogens and their association with diarrhea in dairy calves of Buenos Aires province, Argentina. Vet Parasitol Reg Stud Reports 24:100567
Gelletlie R, Stuart J, Soltanpoor N et al (1997) Cryptosporidiosis associated with school milk. Lancet 350:1005–1006
Gormley FJ, Little CL, Chalmers RM et al (2011) Zoonotic cryptosporidiosis from petting farms, England and Wales, 1992–2009. Emerg Infect Dis 17:151–152
Guo Y, Li N, Ryan U et al (2021) Small ruminants and zoonotic cryptosporidiosis. Parasitol Res:1–10. https://doi.org/10.1007/s00436-021-07116-9
Harper CM, Cowell NA, Adams BC et al (2002) Outbreak of *Cryptosporidium* linked to drinking unpasteurised milk. Commun Dis Intell 26:449–450
Innes EA, Chalmers RM, Wells B et al (2020) A one health approach to tackle cryptosporidiosis. Trends Parasitol 36(3):290–303
Jellison KL, Lynch AE, Ziemann JM (2009) Source tracking identifies deer and geese as vectors of human-infectious *Cryptosporidium* genotypes in an urban/suburban watershed. Environ Sci Technol 43(12):4267–4272
Jiang J, Alderisio KA, Xiao L (2005) Distribution of *Cryptosporidium* genotypes in storm event water samples from three watersheds in New York. Appl Environ Microbiol 71:4446–4454
Jiang W, Roellig DM, Lebbad M et al (2020) Subtype distribution of zoonotic pathogen *Cryptosporidium felis* in humans and animals in several countries. Emerg Microb Infect 9(1):2446–2454
Johansen ØH, Abdissa A, Zangenberg M et al (2021) Performance and operational feasibility of two diagnostic tests for cryptosporidiosis in children (CRYPTO-POC): a clinical, prospective, diagnostic accuracy study. Lancet Infect Dis 21(5):722–730
Karanis P, Kourenti C, Smith H (2007) Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health 5(1):1–38
Kaupke A, Kwit E, Chalmers RM et al (2014) An outbreak of massive mortality among farm rabbits associated with *Cryptosporidium* infection. Vet Res Sci 97(1):85–87
Keeley A, Faulkner BR (2008) Influence of land use and watershed characteristics on protozoa contamination in a potential drinking water resources reservoir. Water Res 42(10–11):2803–2813
Khalil IA, Troeger C, Rao PC (2018) Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analyses study. Lancet Glob Health 6(7):e758–e768
Kotloff KL, Nataro JP, Blackwelder WC (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382(9888):209–222
Kváč M, Hofmannová L, Hlásková L et al (2014) *Cryptosporidium erinacei* n. sp. (Apicomplexa: Cryptosporidiidae) in hedgehogs. Vet Parasitol 201(1–2):9–17
Lal A, Hales S, French N et al (2012) Seasonality in human zoonotic enteric diseases: a systematic review. PLoS One 7(4):e31883
Leoni F, Amar C, Nichols G et al (2006) Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. J Med Microbiol 55:703–707
Li N, Xiao L, Alderisio K et al (2014) Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans. Emerg Infect Dis 20(2):217
Lilja M, Widerström M, Lindh J (2018) Persisting post-infection symptoms 2 years after a large waterborne outbreak of *Cryptosporidium hominis* in northern Sweden. BMC Res Notes 11(1):1–5
Liu A, Ji H, Wang E et al (2011) Molecular identification and distribution of *Cryptosporidium* and *Giardia duodenalis* in raw urban wastewater in Harbin, China. Parasitol Res 109:913–918
Luka G, Samiei E, Tasnim N et al (2021) Comprehensive review of conventional and state-of-the-art detection methods of *Cryptosporidium*. J Hazard Mater 421:126714

Mac Kenzie WR, Hoxie NJ, Proctor ME et al (1994) A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N Engl J Med 331(3): 161–167

Macarisin D, Bauchan G, Fayer R (2010a) Spinacia oleracea L. leaf stomata harboring *Cryptosporidium parvum* oocysts: a potential threat to food safety. Appl Environ Microb 76(2):555–559

Macarisin D, Santín M, Bauchan G, Fayer R (2010b) Infectivity of *Cryptosporidium parvum* oocysts after storage of experimentally contaminated apples. J Food Protect 73(10):1824–1829

Mayer-Scholl A, Šoba Šparl B, Deksne G (2021) The IMPACT project: standardising molecular detection methods to improve risk assessment capacity for foodborne protozoan parasites, using *Cryptosporidium* in ready-to-eat salad as a model. Apicomplexan Parasites and One Health. http://www.apicowplexa.net/wp-content/uploads/2021/06/Abstracts-June-24-2021.pdf

McKerr C, Adak GK, Nichols G et al (2015) An outbreak of *Cryptosporidium parvum* across England & Scotland associated with consumption of fresh pre-cut salad leaves, May 2012. PLoS One 10(5):e0125955. https://doi.org/10.1371/journal.pone.0125955

Millard PS, Gensheimer KF, Addiss DG et al (1994) An outbreak of cryptosporidiosis from fresh-pressed apple cider. JAMA 272:1592–1596

Nader JL, Mathers TC, Ward BJ et al (2019) Evolutionary genomics of anthroponosis in *Cryptosporidium*. Nat Microbiol 4(5):826–836

Nichols G (2008) Chapter 4: Epidemiology. In: Fayer R, Xiao L (eds) *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, pp 79–118

Nichols RA, Connelly L, Sullivan CB et al (2010) Identification of *Cryptosporidium* species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. Appl Environ Microbiol 76:5977–5986

Nydam DV, Wade SE, Schaaf SL et al (2001) Number of *Cryptosporidium parvum* oocysts or *giardia* spp cysts shed by dairy calves after natural infection. Am J Vet Res 62(10):1612–1615

Nydam DV, Lindergard G, Santucci F et al (2005) Risk of infection with *Cryptosporidium parvum* and *Cryptosporidium hominis* in dairy cattle in the New York City watershed. Am J Vet Res 66 (3):413–417

Ong CS, Eisler DL, Alikhani A et al (2002) Novel *Cryptosporidium* genotypes in sporadic cryptosporidiosis cases: first report of human infections with a cervine genotype. Emerg Infect Dis 8:263–268

Rašková V, Květoňová D, Sak B et al (2013) Human cryptosporidiosis caused by *Cryptosporidium tyzzeri* and *C. parvum* isolates presumably transmitted from wild mice. J Clin Microbiol 51(1): 360–362

Ren X, Zhao J, Zhang L et al (2012) *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). Exp Parasitol 130(3):274–281

Robertson LJ (2009) *Giardia* and *Cryptosporidium* infections in sheep and goats: a review of the potential for transmission to humans via environmental contamination. Epidemiol Infect 137: 913–921

Robertson LJ, Chalmers RM (2013) Foodborne cryptosporidiosis: is there really more in Nordic countries? Trends Parasitol 29(1):3–9

Robertson LJ, Fayer R (2012) Chapter 2: *Cryptosporidium*. In: Robertson LJ, Smith HV (eds) Foodborne protozoan parasites. Nova Publishers, New York, NY, USA, pp 33–64

Robertson LJ, Björkman C, Axén C, Fayer R (2014) Chapter 4: Cryptosporidiosis in farmed animals. In: Cacciò S, Widmer G (eds) *Cryptosporidium*: parasite and disease. Springer

Robertson LJ, Sehgal R, Goyal K (2015) An Indian multicriteria-based risk ranking of foodborne parasites. Food Res Int 77:315–319

Robertson LJ, Temesgen TT, Tysnes KR et al (2019) An apple a day: an outbreak of cryptosporidiosis in Norway associated with self-pressed apple juice. Epidemiol Infect 147:e139. https://doi.org/10.1017/S0950268819000232

Robertson LJ, Johansen ØH, Kifleyohannes T et al (2020) *Cryptosporidium* infections in Africa – how important is zoonotic transmission? A review of the evidence. Front Vet Sci 7:575881. https://doi.org/10.3389/fvets.2020.575881

Robinson G, Elwin K, Chalmers RM (2020) *Cryptosporidium* diagnostic assays: molecular detection. Methods Mol Biol 2052:11–22
Ryan U, Fayer R, Xiao L (2014) *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141(13):1667–1685
Ryan U, Paparini A, Monis P et al (2016) It's official–*Cryptosporidium* is a gregarine: what are the implications for the water industry? Water Res 105:305–313
Ryan U, Paparini A, Oskam C (2017) New technologies for detection of enteric parasites. Trends Parasitol 33(7):532–546
Ryan U, Hijjawi N, Xiao L (2018) Foodborne cryptosporidiosis. Int J Parasitol 48(1):1–12
Santín M, Trout JM (2008) Chapter 18: Livestock. In: Fayer R, Xiao L (eds) *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton
Shirley DA, Moonah SN, Kotloff KL (2012) Burden of disease from cryptosporidiosis. Curr Opin Infect Dis 25(5):555–563
Silverlås C, Mattsson JG, Insulander M et al (2012) Zoonotic transmission of *Cryptosporidium meleagridis* on an organic Swedish farm. Int J Parasitol 42:963–967
Smith HV, Patterson WJ, Hardie R et al (1989) An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. Epidemiol Infect 103(3):703–715
Smith RP, Chalmers RM, Mueller-Doblies D et al (2010) Investigation of farms linked to human patients with cryptosporidiosis in England and Wales. Prev Vet Med 94:9–17
Soba B, Petrovec M, Mioc V et al (2006) Molecular characterisation of *Cryptosporidium* isolates from humans in Slovenia. Clin Microbiol Infect 12:918–921
Squire SA, Ryan U (2017) *Cryptosporidium* and *Giardia* in Africa: current and future challenges. Parasit Vectors 10(1):1–32
Sulaiman IM, Xiao L, Yang C et al (1998) Differentiating human from animal isolates of *Cryptosporidium parvum*. Emerg Infect Dis 4:681–685
Temesgen TT, Robertson LJ, Stigum VM et al (2021) Removal of parasite transmission stages from berries using washing procedures suitable for consumers. Foods 10(2):481
Trotz-Williams LA, Martin DS, Gatei W et al (2006) Genotype and subtype analyses of *Cryptosporidium* isolates from dairy calves and humans in Ontario. Parasitol Res 99:346–352
Uga S, Matsuo J, Kono E et al (2000) Prevalence of *Cryptosporidium parvum* infection and pattern of oocyst shedding in calves in Japan. Vet Parasitol 94:27–32
US EPA (1996) ICR microbial laboratory manual. EPA/600/R-95/178. Available at: http://www.epa.gov/microbes/documents/icrmicro.pdf
Van Dyke MI, Ong CS, Prystajecky NA et al (2012) Identifying host sources, human health risk and indicators of *Cryptosporidium* and *Giardia* in a Canadian watershed influenced by urban and rural activities. J Water Health 10:311–323
Wang ZD, Liu Q, Liu HH et al (2018) Prevalence of *Cryptosporidium*, microsporidia and *Isospora* infection in HIV-infected people: a global systematic review and meta-analysis. Parasit Vectors 11(1):1–19
Widerström M, Schönning C, Lilja M et al (2014) Large outbreak of *Cryptosporidium hominis* infection transmitted through the public water supply, Sweden. Emerg Infect Dis 20(4):581
Widmer G, Carmena D, Kváč M et al (2020) Update on *Cryptosporidium* spp.: highlights from the seventh international *Giardia* and *Cryptosporidium* conference. Parasite 27:14. https://doi.org/10.1051/parasite/2020011
Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol 124(1):80–89
Xiao L, Cama VA (2018) Chapter 5: *Cryptosporidium* and cryptosporidiosis. In: Ortega Y, Sterling C (eds) Foodborne parasites. Springer, Cham
Zahedi A, Ryan U (2020) *Cryptosporidium*–an update with an emphasis on foodborne and waterborne transmission. Res Vet Sci 132:500–512. https://doi.org/10.1016/j.rvsc.2020.08.002

Giardiasis from a One Health Perspective

41

Marco Lalle and Simone M. Cacciò

Contents

Abstract

The flagellated protozoan *Giardia duodenalis* is the etiologic agent of giardiasis, a very common gastrointestinal infection of mammals, including humans, with a global distribution. The clinical manifestations of giardiasis are quite variable, and range from the absence of symptoms to acute diarrhea, characterized by dehydration, abdominal pain, flatulence, nausea, vomiting, fatigue, and weight loss to chronic infection. There is considerable genetic variation within *G. duodenalis*, and eight major genetic groups, or assemblages, have been identified (assemblages A to H). Of these, assemblages A and B infect both

M. Lalle · S. M. Cacciò (✉)
Unit of Foodborne and Neglected Parasites, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
e-mail: marco.lalle@iss.it; simone.caccio@iss.it

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_33

humans and animals, whereas assemblages C to H are mostly host-specific, and rarely reported in humans. Multiple transmission routes are possible, including direct (person-to person, animal-to-person, and animal-to-animal), and indirect (through consumption of contaminated water and food) pathways. The increasing application of molecular techniques, from multi-locus typing up to whole genome analysis, showed that infection is highly prevalent in many animal species, yet zoonotic transmission contributes modestly to human giardiasis. Nevertheless, a few animal species (e.g., beavers) have been implicated in the transmission of *Giardia* to humans, particularly through contamination of water; well-conducted studies in epidemic settings may further clarify the role of animals. Water plays a very important role in the epidemiology of giardiasis, with widespread contamination of different water sources leading to waterborne outbreaks. To understand the complex epidemiology of giardiasis, a One Health approach is needed.

Keywords

Giardia · *Giardia duodenalis* · Taxonomy · Genotyping · Epidemiology · Molecular epidemiology · Humans · Farm animals · Companion animals · Wild animals · Transmission pathways · Zoonosis · Risk factors · Water · Food · Outbreaks · Clinical signs · Pathogenesis · Treatment · One Health

41.1 An Introduction to the Organism

Species within the genus *Giardia* are unicellular flagellates that infect the gut of different classes of vertebrates. There are currently eight recognized species in the genus (Table 1), including *Giardia agilis* in amphibians, *G. ardeae* and *G. psittaci* in birds, *G. microti*, *G. muris*, and *G. cricetidarum* in rodents, *G. peramelis* in marsupials, and *G. duodenalis* (syn. *G. lamblia*, *G. intestinalis*) in many mammals, including humans (Adam 2021). The life cycle of *Giardia* is direct and involves only two stages, the trophozoite, the stage that replicates in the host and cause symptoms, and the cyst, which is the infective stage shed with host feces (Fig. 1). Infection is initiated by ingestion of cysts either by the fecal-oral route (direct person-to person, animal-to-person, or animal-to-animal contacts), or indirectly through consumption of contaminated food or water. Exposure to the acidic environment of the stomach provides the necessary stimuli for the excystation of trophozoites from the cyst in the duodenum (Gardner and Hill 2001). Trophozoites undergo repeated mitotic divisions and are eventually triggered to develop into environmentally resistant cysts in response to changes in the small intestine environment, such as reduced cholesterol, increased bile content, and slightly basic pH, as well as in response to density and host-induced stress signals (Barash et al. 2017). Cysts shed with feces are immediately infectious and able to survive for weeks to months in the environment, leading to contamination of water and food (Feng and Xiao 2011). In humans, the infective dose is low, and 10–100 cysts might be sufficient to cause infection (Rendtorff 1954).

Table 1 The species currently recognized within the *Giardia* genus, their host distribution, and distinctive morphological characteristics. The eight assemblages comprised of *G. duodenalis*, and their host distribution, are indicated

Species	Host	Distinctive morphologic features (from light or electron microscopy)	Length/width of the trophozoite (μm)
Giardia agilis	Amphibians	Long and narrow trophozoites with club-shaped median bodies	20–30/4–5
Giardia ardeae	Birds	Rounded trophozoites, prominent notch in ventral disc and rudimentary caudal flagellum. Median bodies round-oval to claw-shaped	~10/~6
Giardia psittaci	Birds	Pear-shaped trophozoites, with no ventro-lateral flange. Claw-shaped median bodies	~14/~6
Giardia muris	Rodents	Rounded trophozoites with small, round median bodies	9–12/5–7
Giardia microti	Rodents	Trophozoites similar to *G. duodenalis*. Cysts contain fully differentiated trophozoites	12–15/6–8
Giardia cricetidarum	Hamsters	Small, rounded trophozoites, more similar to *G. muris*	12–18/8–12
Giardia duodenalis		Pera-shaped trophozoites with claw-shaped median bodies	12–15/6–8
Assemblage A	Mammals, including humans		
Assemblage B	Mammals, including humans		
Assemblage C	Domestic and wild canines		
Assemblage D	Domestic and wild canines		
Assemblage E	Ruminants, pigs		
Assemblage F	Cats		
Assemblage G	Rats		
Assemblage H	Marine mammals (seals)		
Giardia peramelis	Quanda (marsupial)	Similar to *G. duodenalis*	

41.2 Taxonomy of *Giardia*

Based on morphological characters, organisms in the genus *Giardia* are classified in the phylum Metamonada, subphylum Trichozoa, superclass Eopharyngia, class Trepomonadea, subclass Diplozoa, and order Giardiida (Thompson and Monis 2012).

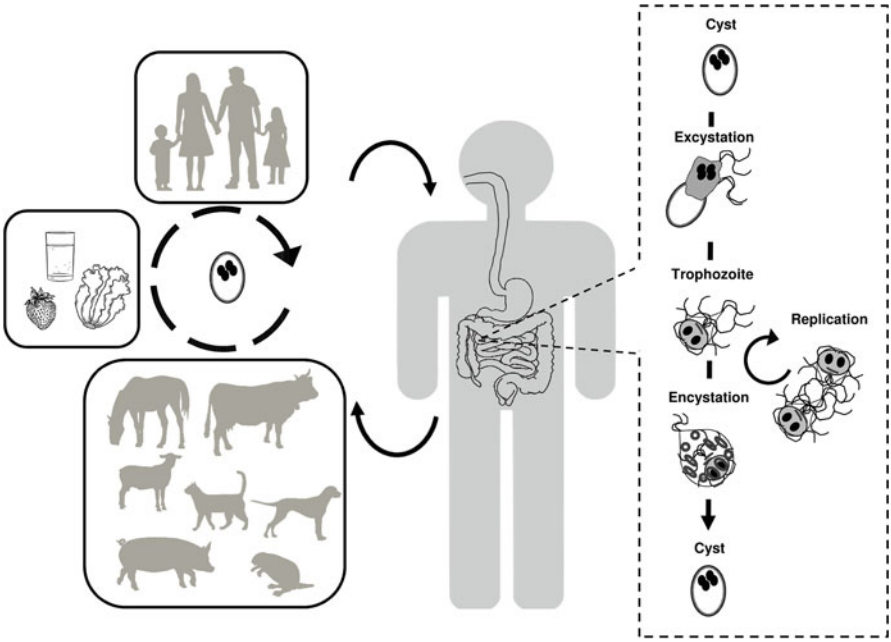

Fig. 1 Schematic representation of the transmission pathways of giardiasis and the life cycle of *Giardia*

The taxonomy of *Giardia* species has been, and still is, a subject of intense debate and controversy, and this has resulted in a confusing nomenclature with different names used for the same species (Monis et al. 2009). Early taxonomy, based on the assumption of strict host specificity (i.e., a different parasite species for each host), resulted in the description of 51 species of *Giardia*, including 30 from mammals (of which 2 from humans), 14 from birds, 4 from amphibians, 2 from reptiles, and 1 from fish (Thompson and Monis 2012). The taxonomy of *Giardia* species was reconsidered by Filice (1952), who concluded that no experimental evidence supported the validity of species named on the basis of host specificity. Filice proposed to recognize only three morphologically distinct species, namely, *Giardia muris*, *G. agilis*, and *G. duodenalis*, on the basis of the shape of internal median bodies as well as body shape and length (Filice 1952). This proposal rapidly became accepted, albeit, as acknowledged by Filice himself, many species were put under the *G. duodenalis* "umbrella," due to the lack of tools to discriminate between variants. With the development of advanced microscopic techniques, ultrastructural description of trophozoites allowed the description of two new species from birds, *Giardia ardeae* and *Giardia psittaci*, whereas *Giardia microti*, a parasite infecting various rodents, was recognized as a separate species due to the unique presence of fully differentiated trophozoites in the cysts. More recently, two additional species, *G. peramelis* in marsupials and *G. cricetidarum* in hamsters, have been described (Ryan and Zahedi 2019). Therefore, eight species are currently recognized in the genus (Table 1).

The development of techniques for the in vitro propagation of trophozoites in axenic conditions (Farthing et al. 1983) opened the possibility to characterize *Giardia* isolates from different hosts using genetic techniques. The first important series of experiments, based on polymorphisms of isoenzymes, revealed a large amount of genetic variability among *Giardia duodenalis* strains (Andrews et al. 1989). Importantly, cluster analysis of the isoenzyme data identified strongly supported groups of genetically related strains that, in most cases, were derived from specific hosts (Monis et al. 2003). DNA sequence analyses further confirmed the validity of these genetic groups, which were named assemblages to underline the fact that each group comprised of highly related but not identical strains. To date, eight assemblages are recognized, which have different host distribution: assemblages A and B are found in humans and other mammals, assemblages C and D are specific to dogs and other canids, assemblage E is found in livestock, assemblage F in cats, assemblage G in rats, and assemblage H in marine mammals (Cacciò et al. 2018; Table 1). These assemblages are separated by very large genetic distances, and are likely to represent distinct species, a conclusion reinforced by comparisons at the whole genome level of isolates from assemblages A, B, C, D, and E (Morrison et al. 2007; Franzén et al. 2009; Jerlström-Hultqvist et al. 2010; Ankarklev et al. 2015; Kooyman et al. 2019). A revision of the taxonomy and a new nomenclature at the species level has been proposed but not formally adopted (Monis et al. 2009), as controversies persisted. Therefore, the term assemblage will be used in this chapter.

As noted above, additional genetic variation exists among isolates within assemblages (i.e., intra-assemblage variability), which led to the description of sub-assemblages (Monis et al. 2003). Four sub-assemblages in assemblage A (AI, AII, AII, and AIV) and four in assemblage B (BI, BII, BIII, and BIV) were identified (Monis et al. 2003). Importantly, human isolates belonged to AI, AII, BIII, and BIV. Even the isolates belonging to sub-assemblages are not identical, and are better viewed as clusters of closely related isolates (Monis et al. 2003). However, the lack of a standardized nomenclature for the genetic variants found within sub-assemblages has created confusion, with different names used in different studies (Sulaiman et al. 2003; Lalle et al. 2005). These genetic variants are better described as genotypes, and, when multiple loci are investigated, as multi-locus genotypes (MLGs). To avoid further confusion, genotypes will be identified only at the level of the sub-assemblage to which they belong (e.g., AI, AII, BIV).

41.2.1 Molecular Typing of *Giardia duodenalis*

Like for many other pathogens, the introduction of molecular techniques has revolutionized the study of the epidemiology of giardiasis. Molecular tools are thought to provide higher sensitivity and specificity compared to microscopic or immunologic assays, and, more importantly, to allow a reliable identification of the parasite at the level of species, assemblage, sub-assemblage, and genotypes (Cacciò et al. 2008).

The first PCR assays targeted fragments of conserved eukaryotic genes, sometimes using degenerated primers (18S rRNA, glutamate dehydrogenase (*gdh*), elongation factor 1-alfa, triose phosphate isomerase (*tpi*); Monis et al. 1999), or genes

uniquely associated with the parasite (e.g., beta-giardin (*bg*); Lalle et al. 2005). These markers are still widely used for genotyping parasite isolates (Feng and Xiao 2011). In particular, a multi-locus typing scheme based on the *gdh*, *tpi*, and *bg* markers has been consistently used (Cacciò et al. 2008; Lebbad et al. 2010). Nevertheless, the limited genetic variation found in these markers is not sufficient for a full discrimination of isolates. A recent study has demonstrated the potential of a new multi-locus typing scheme, based on six highly variable genes, to distinguish isolates of sub-assemblages AI and AII, trace zoonotic infection, and identity outbreak samples (Ankarklev et al. 2018). Similarly, a new typing scheme with high resolution has been proposed for assemblage B. This scheme is based on sequence analysis of only three markers, but result in clustering the isolates with a resolution similar to that obtained by whole genome comparison (Seabolt et al. 2021). The limitations of the currently available markers and the need for new genotyping tools have been recently reviewed (Capewell et al. 2021).

41.2.2 The Complex Genetics of *Giardia duodenalis*

Giardia is a tetraploid organism (it has two diploid nuclei) and has been considered as an asexually replicating organism (Adam 2021). Replication is equational rather than reductional, which means that nuclear asymmetry is maintained throughout the replication cycle (Yu et al. 2002). Therefore, mutations are expected to be fixed independently in each nucleus and, if genetic exchanges do not occur, allelic heterozygosity should accumulate, as observed in other asexual organisms (Gladyshev and Arkhipova 2010).

Opposite to this prediction, an extremely low level (0.002%) of allelic sequence heterozygosity (ASH) was detected in the genome of an assemblage A isolate (WB isolate, sub-assemblage AI) (Morrison et al. 2007). While this was a surprising finding, whole genome sequencing (WGS) of sub-assemblage AII isolates showed a heterozygosity level of 0.25–0.35% (Ankarklev et al. 2015), and an even higher level (0.43%) was found in the genome of an assemblage B (GS isolate) (Franzén et al. 2009). Additional WGS confirmed variable levels of ASH in *G. duodenalis* assemblages, from 0.89% in assemblage C to 0.74% in assemblage D (Kooyman et al. 2019), and to 0.037% in assemblage E (Jerlström-Hultqvist et al. 2010).

ASH is often visualized as "double peaks" in chromatograms obtained from direct sequencing of PCR products. The majority of the heterozygous sites contain two different bases but some feature three or four, capturing the diversity from all of the copies of the genome. Formally, these sequencing profiles can be generated by the presence of genetically different parasites (i.e., a mixed infection) and/or by ASH in a single population of parasites. Using micromanipulation to isolate single trophozoites from in vitro culture and single cysts from human stools, Ankarklev et al. (2012) showed that ASH is present in both single trophozoites and single cysts. Additionally, different sequence patterns were observed in different cysts originated from the same human patient, thus suggesting the presence of multiple sub-assemblage infections

(Ankarklev et al. 2012). Unfortunately, this elegant approach is unsuitable for multi-locus sequence typing scheme, and difficult to apply routinely.

Certainly, mixed infections with genetically different isolates are common, as demonstrated by the use of assemblage-specific PCR assays (Almeida et al. 2010; Vanni et al. 2012). More recently, Woschke et al. (2021) analyzed longitudinal samples collected from patients in Germany, and demonstrated that reproducible pattern of ASH are discernable and of epidemiologic value.

The reliability of any typing scheme is influenced by the occurrence and extent of genetic exchanges. As mentioned above, the range of allelic heterozygosity observed in *G. duodenalis* (<0.001 to 0.89) is similar to that expected in sexually reproducing organisms (Adam 2021), and this has raised the question of whether canonical meiotic sexual reproduction, or another form of sexuality, is occurring in this parasite. Two lines of experimental evidences are pertinent to that question. First, recombination has been inferred from genotyping data of sub-assemblage AII field isolates in an endemic setting (Cooper et al. 2010), but also between sub-assemblages AI and AII in Europe (Ankarklev et al. 2018), and even between assemblages A and E (Ankarklev et al. 2018).

Second, electron microscopy studies revealed fused nuclei in forming cysts while fluorescent in situ hybridization allowed visualization of plasmid transfer between nuclei (Poxleitner et al. 2008). Based on these observations, a model in which homologous recombination occurs after nuclear envelope fusion was proposed. However, more recently it has been shown that the two nuclei of *Giardia* possess different numbers of chromosomes (aneuploidy, or near tetraploidy) with unequal distribution of individual chromosomes and genes between the two nuclei (Tůmová et al. 2019). This indicates that nuclear differentiation provides opportunities for generation of novel genetic variability.

Clearly, a better understanding of the genetics of *G. duodenalis* remains essential to improve genotyping schemes.

41.3 Epidemiology and Molecular Epidemiology of Giardiasis in Humans

Giardiasis is a very common gastrointestinal infection of humans; it has been estimated that every year about 184 million people in Asia, Africa, and Latin America have symptomatic infections (WHO 1996; Pires et al. 2015). The parasite has a global distribution, but the prevalence of infection is clearly higher in developing regions of the world, where *Giardia* is common in both children and adults (Cacciò and Sprong 2011; Pires et al. 2015).

Infection rates have been reported in both low-income countries (range 8–30%) and high-income countries (range 1–8%) (Feng and Xiao 2011). Those rates are probably higher in individuals with diarrhea, but the current epidemiological scenario is largely influenced by the fact that many countries did not report any data, by the lack of monitoring programs, and by the high rate of asymptomatic carriage of

Giardia in humans (Cacciò and Sprong 2011). This suggests that giardiasis is strongly underdiagnosed and underreported.

In humans, giardiasis is mainly a pediatric infection, with the highest prevalence observed in children aged 0–4 years. This pattern is found in both high- and low-income countries, and is thought to be due to lower hygiene and higher susceptibility of children at the first exposure to the parasite (Cacciò and Sprong 2011). A secondary peak is observed in adults aged 30–40; in this case, women represent the risk category, likely because of direct transmission of *Giardia* from children to their mothers. Other risk groups include institutionalized and adopted children, returning travelers, immigrant/refugees, and in men who have sex with men (Cacciò and Sprong 2011; Escobedo et al. 2010).

Seasonality has been observed in high-income countries. In the USA, a twofold increase in transmission of giardiasis occurs during the summer, and coincides with increased outdoor activities (e.g., swimming and camping) (Yoder et al. 2012). Similarly, a study in New Zealand (period 1997–2006) showed peaks in the late summer and early autumn (Snel et al. 2009). In Europe, higher number of cases was reported from August to October with a peak in September (European Centre for Disease Prevention and Control. Giardiasis (lambliasis). In: ECDC. Annual epidemiological report for 2017. Stockholm: ECDC 2019). Bearing in mind the delay between infection, onset of symptoms, and diagnosis (up to 5 weeks), the late summer/early autumn peak probably represents an increase in infection during late summer months, resulting from travel and outdoor recreational activities.

The routes of transmission for sporadic cases are only partially known, but studies in the USA and the UK have identified international travel, male-male sexual behavior, having contact with children in diapers, and exposure to drinking and recreational water as the most important risk factors (Reses et al. 2018; Horton et al. 2019). These studies therefore suggest that prevention measures should focus on reducing risks associated with diaper handling, sexual contact, swimming in untreated water, and drinking untreated water.

As mentioned above, humans are mostly infected by assemblages A and B, although a small number of individuals have been found infected with assemblage C, E, and F (Ryan and Zahedi 2019; Cai et al. 2021). The geographical distribution of assemblages largely overlaps, but globally assemblage B is more prevalent than assemblage A.

Genetic characterization of isolates of assemblage A is relatively straightforward, also because of the low level of ASH. Phylogenetic analysis of multiple markers (Cacciò et al. 2008; Lebbad et al. 2010; Ankarklev et al. 2018) showed three strongly supported groups, namely, sub-assemblages AI, AII, and AIII. Clearly, animal-derived isolates are grouped in sub-assemblages AI and AIII, but never in sub-assemblage AII. On the other hand, most human-derived isolates belong to sub-assemblage AII, but a few are grouped in sub-assemblage AI. The latter may represent cases of zoonotic transmission, and, when available, data on exposure substantiated contact with animals as a risk (Ankarklev et al. 2018). Of note, the high genetic variability of the markers allowed distinguishing isolates from outbreaks, which shared a specific multi-locus genotype not observed in unrelated, sporadic

cases from the same country (Ankarklev et al. 2018). The good discriminatory power of this scheme has been recently confirmed by another study (Woschke et al. 2021).

The situation is less clear for assemblage B. Indeed, while more genetic variability is present in the genotyping markers, the high level of ASH complicate the assignment of isolates to specific genotypes or multi-locus genotypes (Woschke et al. 2021).

41.4 Epidemiology and Molecular Epidemiology of Giardiasis in Animals

This section will only provide a concise account of the many studies published in recent years. Interested readers can find additional details in exhaustive review articles (Ryan and Zahedi 2019; Cai et al. 2021). As a general comment, is may be useful to recall that the current knowledge on the prevalence and distribution of genotypes of *Giardia* in animals is still incomplete, as data from many areas of the word are scanty, particularly from Central and South American countries, where giardiasis is endemic in humans (Coelho et al. 2017; Rivero et al. 2020).

41.4.1 Farm Animals

The prevalence of *Giardia* in farm animals is clearly influenced by the age of the animals tested, the study design, the sensitivity of the diagnostic methods, the geographical and climatological parameters, and management practices (Ryan and Zahedi 2019).

In cattle, the infection has a global distribution, with animal prevalence ranging from 1% to 74%, and farm prevalence from 45% to 100%. In general, the prevalence is higher in young animals compared to adults, and in dairy cattle compared to beef cattle (Ryan and Zahedi 2019).

In sheep, the animal prevalence ranges from 1.5% to 89% and the farm prevalence from 10% to 100% (Feng and Xiao 2011; Ryan and Zahedi 2019). As for cattle, the prevalence is higher in lambs than in adult sheep. In goats, the animal prevalence ranges from 4% to 43% and the farm prevalence from 66% to 95%. In pigs, the animal prevalence ranges from 0.6% to 26.9% and the farm prevalence from 10% to 84% (Feng and Xiao 2011, Ryan and Zahedi 2019). The data at the farm level, although variable, indicate that a large proportion of animals will become infected at a time (Xiao and Herd 1994).

From a public health perspective, identification of the assemblage, sub-assemblage, and genotype from animal samples is essential to infer zoonotic potential (Ryan and Cacciò 2013; Cai et al. 2021). Molecular surveys have shown that assemblage E largely predominates (prevalence >90%) in cattle, globally. In a recent review that examined studies published since 2012 (Ryan and Zahedi 2019), typing data from 2672 cattle showed assemblage E in 2426 animals, assemblage A in 207 animals, assemblage B in 29 animals, and mixed assemblages in 37 animals.

A similar distribution was found in yaks from China and in water buffaloes, with dominance of assemblage E (Ryan and Zahedi 2019; Cai et al. 2021). Importantly, the main sub-assemblage in assemblage A in cattle is AI, which is uncommon in humans, thus suggesting a minor role of cattle as zoonotic reservoir.

In sheep and goats, assemblage E clearly predominates, followed by assemblage A (Ryan and Zahedi 2019, Cai et al. 2021). Like for cattle, sub-assemblage AI is the most common variant, although sub-assemblage AII was reported in some studies (see Cai et al. 2021).

In pigs, Assemblage E is the most commonly found, and among isolates belonging to assemblage A, sub-assemblage AI was found.

In horses, the prevalence ranges from 1.5% to 17.4%, being usually higher in foals than in adults (Cai et al. 2021). Contrary to what is reported above for other farm animals, surveys conducted in Europe, South America, and Asia showed that most positive samples from horses had assemblages A and B, with identification of sub-assemblage AI.

41.4.2 Companion Animals

Giardiasis is a common infection of dogs and cats, with prevalence ranging from1.1% to 45.9% (Bouzid et al. 2015; Ryan and Zahedi 2019; Cai et al. 2021). This wide range of prevalence can be attributed to the use of diagnostic techniques with different sensitivity (microscopy, ELISA, and PCR), but also to the nature of the population studied (household, shelter, stray), the animal age, breed, spay/neuter status, and geographic location (Ryan and Zahedi 2019; Cai et al. 2021). In general, the highest prevalence is observed in younger animals and in kennel or shelter populations.

Molecular analysis of dog isolates showed that the host-specific assemblages C and D are more prevalent than the zoonotic assemblages A and B, although a remarkable number mixed infections was found (Ryan and Zahedi 2019; Cai et al. 2021).

Among cats, the host-specific assemblage F is the most prevalent, followed by assemblage A, with less frequent reports of assemblage B, C, D, and E (Ryan and Zahedi 2019).

41.4.3 Wild Animals

As mentioned above, a number of host-adapted species (*G. muris*, *G. microti*, *G. peramelis*, *G. agilis*, *G. cricetidarum*, *G. ardeae*, and *G. psittaci*; Table 1) are known to infect a wide range of wild animals. However, the zoonotic assemblages A and B are also commonly found in wild animals.

In non-human primates (NHP), data from chimpanzees, gibbons, gorillas, macaques, monkeys, and lemurs indicate a prevalence ranging from 4.8% to 57.8% (Ryan and Zahedi 2019; Cai et al. 2021). In many cases, surveys have

concerned captive animals, where transmission of *Giardia* may differ from that occurring in the natural environment. However, genotyping data have shown a similar distribution of assemblages in captive and wild NHPs, with assemblages A and B present, and the latter being more prevalent. Thus, NHP and humans share similar distribution and relative proportion of *G. duodenalis* assemblages.

In rodents, the prevalence of *G. duodenalis* infection is high, ranging from 6% to 66.3% (Cai et al. 2021). The host-specific assemblage G and the zoonotic assemblage B have been commonly found in species such as chinchillas, bamboo rats, and beavers. Beavers are of particular interest, since it was association between infection in these animals and waterborne outbreaks of human giardiasis that led the World Health Organization to classify *Giardia* as a zoonotic parasite (WHO 1979). In Canada and North America, giardiasis is also known as "beaver fever" and the role of these animals as source of human infection through contamination of drinking source water has been argued since many years, but proved difficult to confirm. Recently, a retrospective comparative genomics analysis of *Giardia* isolates following various waterborne outbreaks have shown clustering of human isolates with isolates from surface water and beavers implicated to be sources by public health (Prystajecky et al. 2015; Tsui et al. 2018). Thus, beavers can act as an amplification host or reservoir host for *G. duodenalis* in watersheds.

In wild hoofed animals, assemblage E is rare, while it is highly prevalent in livestock. Interestingly, sub-assemblage AIII appears to be largely restricted to this group of animals, albeit it has been found in a cat (Lebbad et al. 2010), but never in humans.

In wild carnivores, molecular analyses have shown that the host-adapted assemblage D is prevalent in coyotes, raccoon dogs, foxes, and wolves, but assemblages A and B have been found in most wild carnivores tested (Cai et al. 2021). The most common sub-assemblage found in these hosts is AI.

In marsupials, one of the dominant mammalian groups within watersheds in Australia, data from 31 species belonging to 9 families have indicated a prevalence ranging between 1.3% and 24.1% (Ryan and Zahedi 2019). Molecular typing has detected assemblages A and B with higher frequency compared to the host adapted species, *G. peramelis* (Ryan and Zahedi 2019). Although sporadically, assemblages C, D, and E have also been reported in marsupials, suggesting transmission between multiple hosts.

In marine mammals, including seals, dolphins, whales, and porpoise, all *G. duodenalis* assemblages, except assemblage E, have been found. These animals often harbor the host-specific assemblage H, which has never been detected in humans (Ryan and Zahedi 2019).

41.5 Remarks on Zoonotic Transmission

With the increased application of molecular typing techniques, it is becoming clear that most animal species do not act as reservoirs of *Giardia* cysts infectious to humans. This is based on the higher occurrence of host-adapted assemblages (C to H)

in animals, which are rarely described in humans (Cacciò et al. 2018; Cai et al. 2021). Moreover, the identification of sub-assemblages AI and AII in animals, and the lack of sub-assemblage AII, which seems to be largely adapted to humans, suggest that zoonotic transmission of assemblage A is limited, although possible.

The situation is less clear for assemblage B, also due to difficulties in genotyping isolates caused by high ASH, but future efforts should focus on this assemblage. High-resolution typing of isolates using whole genome data, although technically demanding, can generate relevant data, as already shown (Tsui et al. 2018). Well-designed studies that include evaluation of risk factors and consider cross-species transmission in endemic settings, such as rural areas where people, domesticated animals, and wildlife overlap (e.g., Kuthyar et al. 2021), remain important to assess zoonotic transmission.

41.6 Waterborne and Foodborne Giardiasis

Although the importance of zoonotic transmission is still to be understood, there is little doubt that a large number of different hosts can shed *Giardia* cysts in the environment, thus potentially contribute to contamination of water and food. Not surprisingly, a plethora of studies have demonstrated the ubiquitous presence of *Giardia* cysts in all water samples tested, including raw and treated wastewater, surface water, ground water, and drinking water (reviewed by Hamilton et al. 2018).

Quite logically, contamination is highest in untreated wastewater samples, and, indeed, up to 77% of the samples from published studies (1543 of 2010) were positive, with concentrations of up to 1,200,000 cysts per liter (Hamilton et al. 2018). In treated wastewater samples, the maximum concentration reported was of 51,333 cysts per liter. The number of positive samples, and the concentration in *Giardia* cysts, decreases in surface water samples, yet the reported levels range from 0.01 to 1000 cysts per liter in river water samples, from 0.004 to 16.2 cysts per liter in samples from lakes and dams, and from 0.3 to 33 cysts per liter in beach waters (Hamilton et al. 2018). Since these water sources are used for recreational activities or for the production of drinking water, contamination with *Giardia* cysts is of particular relevance for public health.

The ability of the *Giardia* cyst stage to withstand chlorine disinfection of water and to persist for long periods in the environment while maintaining viability, coupled with the low infective dose, clearly enhances its transmission (Boarato-David et al. 2017).

A recent review examined waterborne protozoan outbreaks during the period from 2017 to 2020, and found that *Giardia* has been responsible for 48 of the 251 outbreaks (Ma et al. 2022). Almost all outbreaks were reported in the USA, UK, and New Zealand, a finding most likely explained by more advanced diagnostic capabilities and existence of surveillance programs to monitor water contamination in high-income countries. Large outbreaks linked to contaminated drinking water have been reported in Portland (USA) with about 50,000 infected individuals, and in Bergen (Norway) with about 2500 infected individuals.

Food can also be a vehicle of *Giardia* transmission. The detection of parasite cysts in food is difficult, because contamination levels are expected to be low and available detection methods have sub-optimal performance (e.g., microscopy), or are costly (e.g., immunomagnetic separation). Moreover, specific characteristics of food may interfere with extraction/elution of cysts, therefore influencing recovery and leading to an underestimation of the contamination.

Nevertheless, cysts have been detected on dairy products, meat, shellfish, fruit, and vegetables (Ryan et al. 2019). Fresh produce, which are often consumed raw, is thought to represent the high-risk food.

Few outbreaks of foodborne giardiasis have been identified and investigated, all in the USA, and have been linked to various foods, with fresh produce being the most common food type. Investigations have indicated infected food handlers as the most common source of contamination (Ryan et al. 2019).

41.7 Giardiasis in Humans

Human giardiasis occurs with heterogeneous clinical manifestations and high variability in the severity of disease, ranging from the lack of apparent symptoms (subclinical or asymptomatic giardiasis) to acute or chronic illness (Farthing 1997). Symptoms usually appear within 2 weeks after ingestion of cysts and last for up to 6 weeks. Diarrhea is the commonest symptom of the acute phase accompanied by greasy stools, flatulence, abdominal discomfort, fatigue, and weight loss. Fever, nausea, vomiting, itch, and urticaria are far less common (Farthing 1997). Acute symptoms are generally self-limiting and resolve following clearance of the parasite. Although recurrent infection can be frequent in poor-hygiene settings, persistent (chronic) infection, with intermittent or no symptoms, has been observed worldwide in some patients to last far more than 2 months, up to years (Escobedo et al. 2014). Low production of specific anti-*Giardia* antibodies, malnutrition, immunosuppression, and co-infection with other pathogens have been associated with the development of chronic giardiasis (Fink and Singer 2017; Escobedo et al. 2018). Extraintestinal manifestations have been reported in up to 30% of infected patients, and include ocular pathologies, reactive and post-infectious arthritis, IgE-mediated allergies, and muscular complication due a decrease in potassium level following diarrhea (Halliez and Buret 2013). Long-term sequelae are well documented too, in both adults and children. In low-income countries and poor-hygiene settings, where giardiasis is endemic and highly prevalent in children below 2 years of age, worsening of symptoms has been reported (Platts-Mills et al. 2015). A prospective longitudinal studies on a large children cohort clearly identified giardiasis, independently of diarrheal disease (Muhsen and Levine 2012), as a risk factor to develop failure-to-thrive syndrome, malnutrition, malabsorption, retarded growth and development, and poor cognitive functions (Donowitz et al. 2016; Rogawski et al. 2017). In high-income countries, patients who experienced symptomatic giardiasis are at high risk to develop long-term post-infectious sequelae, such as post-infectious irritable bowel syndrome (PI-IBS) and chronic fatigue (CF),

with a higher provability compared to other gastrointestinal infections (Litleskare et al. 2018; Halliez and Buret 2013; Nakao et al. 2017; Dormond et al. 2016). The prevalence of PI-IBS in patients infected with *Giardia* is significantly higher compared to control groups, even 10 years after infection (Litleskare et al. 2018). Delays in diagnosis and treatment of giardiasis might cause/increase the risk of chronic infection and the development of PI-IBS and CF (Mørch et al. 2009). The complex interplay between host factors (immunological response, gut microbiota) and parasite factors (e.g., genotype/assemblage-specific pathogenic mechanisms) accounts for the extreme variability in clinical manifestations, although our understanding of this complexity is still incomplete (Fig. 2) (Allain and Buret 2020). Acute symptomatology (i.e., diarrheal disease), and also malabsorption, are the result of an alteration of the intestinal barrier function induced by the parasite, which occurs via enterocyte apoptosis, increased intestinal permeability, and disruption of microvillous brush border and villus shortening (Allain and Buret 2020). To resist peristaltic flow, *G. duodenalis* trophozoites tightly adhere to the surface of enterocytes of the small intestine epithelium, without invading the intestinal tissue. Attachment occurs mechanically via the ventral disc, a microtubule-based suckling structure, and via chemical binding mediated by specific proteins, such as giardins and lectins (Müller and von Allmen 2005). Although mechanical damages (i.e., ventral disc imprint) were observed in vivo on mouse enterocytes infected by *G. muris* (Erlandsen and

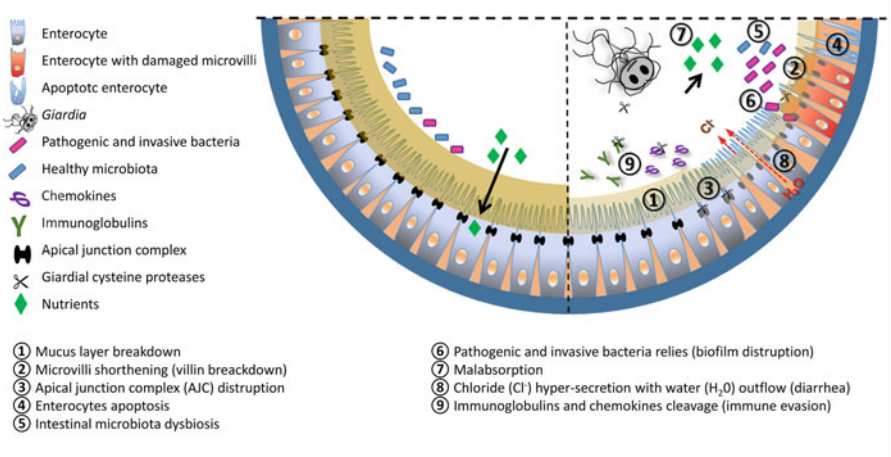

Fig. 2 *Giardia* pathogenic mechanisms. *Giardia* adheres to the mucosa of small intestine (by ventral disk and adhesive proteins) and induces disruption of the epithelial barrier via: (1) mucus layer breakdown, (2) shortening of microvilli, (3) disruption and alteration of apical junctional complexes, (4) enterocyte apoptosis. *Giardia* (5) alters the relative composition of intestinal microbiota, and (6) promotes disruption of biofilms of pathobionts. Impairment of intestinal function by Giardia leads to (7) malabsorption of water, glucose, nutrients, and electrolytes, (8) leading to chlorine hypersecretion water outflow into the intestinal lumen and diarrhea. *Giardia* survives host immune system and modulate inflammatory response by (9) cysteine protease-mediated cleavage of immunoglobulin and chemokines/cytokines

Chase 1974) or *G. duodenalis* (Khanna et al. 1990), and in vitro on MDCK cell lines incubated with different *G. duodenalis* isolates (Chávez et al. 1995), a similar damage was not observed when examining human biopsies. Nevertheless, duodenal biopsies of patients with chronic giardiasis showed villus shortening, decreased transepithelial electrical resistance (TEER) and increased paracellular permeability, altered ion and glucose transport, downregulation of tight junction proteins (i.e., claudin 1), and increased apoptosis of enterocytes (Troeger et al. 2007). Since epithelial microvilli are responsible for nutrient-coupled absorption of electrolytes (particularly Na and D-glucose), their shortening impairs the absorption of water, glucose, and electrolytes. This imbalance causes chloride hyper-secretion with water outflow into the intestinal lumen, resulting in distension, rapid peristalsis, and diarrhea (Maàyeh and Svärd 2017). *Giardia* can compromise the intestinal barrier function in several ways, particularly via the activity of excretory/secretory products (ESPs). Damaging of brush-border microvilli occurs at the expense of cytoskeleton both directly, via degradation of the key actin-binding protein villin by *Giardia* secreted cysteine proteases (CPs), and indirectly, via modulation of phosphorylation level and degradation of villin and ezrin by T-lymphocyte CD4+/CD8 + -mediated immune responses (Solaymani-Mohammadi and Singer 2013; Bhargava et al. 2015). Additionally, the agglutination properties of *Giardia* lectins on enterocyte membrane, to which the parasite adheres, might contribute to microvillus shortening (Farthing et al. 1986).

The intestinal barrier integrity can also be compromised by depletion of the mucus barrier, breakdown of apical junctional complexes (AJCs), and apoptosis of enterocytes in an ESP-mediated manner. *Giardia* can thin the mucus layer, coating the enterocytes surface, by CP-mediated cleavage of mucin-2, and can further deplete the mucin stock by triggering mucus hypersecretion from goblet cells via cleavage and activation of protease-activated receptor 2 (Amat et al. 2017; Fekete et al. 2022). The lack of the mucus barrier indeed promotes parasitic colonization and access of the trophozoites to enterocyte cell membrane. Proteins from AJCs, particularly those in tight and adherent junctions (claudins, occludins, ZO proteins, E-cadherin, β-cathenin), are targeted and degraded by *Giardia* CPs (Ma'ayeh et al. 2017; Liu et al. 2018; Ortega-Pierres and Argüello-García 2019). In a human organoid-derived in vitro model of giardiasis, disruption of tight junctions and loss of epithelial barrier function with decrease in TEER activation was observed early in response to Giardia infection, and associated to the activation of enterocytes cAMP/PKA signaling pathways (Holthaus et al. 2021). Giardia also induces enterocyte apoptosis mediated by activation of pro-apoptotic caspase-3 and -9, increased expression of pro-apoptotic Bax, decreased expression of anti-apoptotic Bcl-2, and induced proteolytic cleavage of poly(-ADP-ribose) polymerase (PARP). Impaired glucose uptake by Giardia through SGLT1 inhibition is another mechanism associated with activation of the apoptotic pathway in enterocytes (Maàyeh and Svärd 2017). However, the identity of *Giardia* molecules responsible for the activation of these different pathways is still unknown.

Pathogenesis and outcomes of giardiasis, and the composition of gut microbiota, are strictly correlated, as mainly observed in animal models. Colonization of the host

gut can be controlled by commensal microbiota that prevent establishment of the parasite (Fekete et al. 2021). In turn, during the acute phase of infection, the parasite can induce alteration in the microbiota, both at the site of infection and beyond (Fekete et al. 2021). This alteration results in an enrichment of bacterial taxa showing high metabolic flexibility and better oxygen tolerance, with lipids and arginine as energy source (Fink and Singer 2017). Moreover, the breakdown of the mucus layer induced by *Giardia* can also promote disruption of microbiota biofilms, leading to the release of pathobionts that in turn triggers an inflammatory response eventually responsible for the development of post-infection inflammatory diseases (Fekete et al. 2021). Further evidences also indicate that the microbiota may play an important role in the development of stunting associated with giardiasis (Singer et al. 2020).

Giardia infection is characterized by an absence of overt inflammatory cell infiltration, thus with a low inflammatory response of the mucosal layer (Oberhuber et al. 1997). Giardia takes advantage of multiple strategies to evade the host's immune system and modulate local inflammatory responses. The parasite possess a mechanism of antigenic variation that relies on the exposure of variant surface proteins (VSPs), which form a dense coat on the surface of the trophozoite (Gargantini et al. 2016). The VSPs repertoire is large, with up to 300 different members, and highly variable between assemblages and even among isolates (Franzén et al. 2009). Only one VSP is predominantly expressed at any time by the parasite, and its replacement during infection allows the parasite to escape the immune response (Gargantini et al. 2016). Sera from *Giardia*-infected individuals recognize VSPs, as well as other *Giardia* secreted proteins, indicating their importance in antibody-mediated immunity (Moss et al. 2014). Indeed, a significantly lower anti-VSP IgG/M response is associated with chronic infections (Hjøllo et al. 2018). Simultaneous exposure of the immune system to multiple VSPs represents a promising vaccine strategy (Serradell et al. 2016). *Giardia* is also able to neutralize antimicrobial molecules produced by the host. Secretion in the gut lumen of parasite enzymes able to metabolize arginine (i.e., arginine deiminase [ADI], ornithine carbamoyl transferase [OCT)], and of CPs result in the inhibition of nitric oxide production, by arginine consumption, and cleavage of antimicrobial defensins, respectively (Liu et al. 2019; Eckmann et al. 2000). Giardia CPs also degrade host immunoglobulins (IgG, IgA1, and IgA2) and pro-inflammatory chemokines/cytokines (CXCL1–3, CXCL8, CCL2, and CCL20) (Ortega-Pierres and Argüello-García 2019). Evidences from in vitro long-term exposure of human enterocytes to *Giardia* trophozoites and from duodenal biopsies of children (<2 years) suffering from environmental enteric dysfunction (EED), a subclinical disorder of intestinal function common in tropical countries and in settings of poverty and economic disadvantage, and giardiasis indicate that *Giardia* also induces downregulation of genes associated with host inflammatory response pathways (Roxstrom-Lindquist et al. 2005; Haberman et al. 2021). In the gerbil model of giardiasis, a switch from an initial Th1/proinflammatory response to a marked Th2 response was observed during late infection (Serradell et al. 2018).

The host immune response can control primary *G. duodenalis* infection by multiple immunological factors. Increasing evidences indicate that CD4+ T cells

are essential for parasite clearance, since T cell-deficient mice fail to control infection. In particular, a prominent role is played by the Th17 cell population, which produces IL-17 in IL-6-dependent manner. Upregulation of IL-17 during murine or bovine infections was reported, whereas IL-17 deficiency in mice results in a delay in parasite clearance (Singer et al. 2019). The complement system is also involved in activating and mediating downstream immune responses during infection. Mice deficient for the complement receptor C3aR displays a reduced T-cell response against parasite antigens following infection (Singer et al. 2019).

The role of secretory IgA in parasite clearance and in protection against reinfection is controversial. IgA are produced in response to intestinal infections by B-cells within the intestinal lamina propria, and then transported in the gut lumen by the enterocytes (Fink and Singer 2017). An increased risk of giardiasis with chronic diarrhea, malabsorption, and even lymphoid hyperplasia has been observed in patients with IgA immunodeficiency, and patients with symptomatic giardiasis had significantly lower secretory IgA levels in fecal samples (Swain et al. 2019). Evidence from the murine model of infection with *G. muris* suggested that IgA antibodies contribute to protective immunity (Fink and Singer 2017). However, while *G. muris* infection can persist in pIgR-deficient or B cell-deficient mice (with impaired IgA production), no difference in parasite clearance were observed in mice infected with *G. duodenalis*, irrespective of IgA deficiency (Fink and Singer 2017). In gerbils infected with *G. duodenalis*, the amount of fecal IgA was directly proportional to the inoculum (dose) of trophozoites, while the elimination of cysts was inversely proportional to the dose and to fecal IgA levels (Amorim et al. 2010). Mast cells (MCs) also play a role in the immune response against *Giardia*. As shown in the murine infection model, MCs are massively recruited to the small intestine during infection and are linked to IgA production, as mice deficient in the receptor protein kinase c-kit, a weak activator of MCs, failed to produce parasite-specific IgA (Fink and Singer 2017).

41.8 Giardiasis in Other Mammals

Clinical signs of giardiasis has been documented in both livestock and companion animals, although less extensively than in humans. On the other hand, and despite the availability of the murine and gerbil models, information on Giardia-induced pathogenesis in animals is fragmentary.

Infection in dogs and cats is mostly asymptomatic. When present, clinical signs in dogs range from slight abdominal discomfort to severe abdominal pain, cramps, and soft to watery diarrhea, whereas acute diarrhea and weight loss are more commonly reported in cats (Tangtrongsup and Scorza 2010; Janeczko and Griffin 2010). As observed in humans, infection is more common in puppies and in kittens than in adults, likely as the result of an immature immune system (Dixon 2021). For both animal species, shedding of cysts is similar in healthy and in symptomatic animals (Thompson et al. 2008; Tangtrongsup and Scorza 2010; Tysnes et al. 2014). As for humans, the clinical presentation of giardiasis in animals is likely influenced by strain variability, genetic predisposition, presence of other pathogens, gut microbiota

composition, nutritional status, stress, and immunosuppression (Uiterwijk et al. 2019). Noteworthy, chronic giardiasis is also observed in dogs. Study on puppies naturally infected with G. duodenalis (assemblages C and/or D) lead to the conclusion that the disease is not self-limiting and can last for over 5 months, in the absence of any treatment (Boucard et al. 2021). Moreover, a marked alteration of the gut microbiota was observed in puppies shedding high number of cysts compared to those that shed low numbers, suggesting microbiota-mediated impaired intestinal function (Boucard et al. 2021).

Infection with *Giardia* is very common in ruminants, and can be associated with diarrhea, weight loss, and malabsorption; however, asymptomatic infections are also common (Thompson et al. 2008; Geurden et al. 2010). Malabsorption has been reported in infected calves and goat kids, and related to an increased number of intraepithelial lymphocytes, suggestive of epithelial permeability, and a decreased villus to crypt ratio (Ruest et al. 1997; Koudela and Vítovec 1998). The relevance of Giardia as a causative agent of diarrhea in ruminants is still unclear, as diarrheal disease is influenced by other factors, such as co-infections with other pathogens and husbandry practices. Nevertheless, even asymptomatic infections can have a negative impact in production and growth performance in livestock. Indeed, pre-weaned dairy calves positive for Giardia showed a reduced daily gain compared to Giardia-negative calves (Santin 2020).

In sheep and goats, Giardia can cause severe diarrhea, depression, weight loss, and mortality, and infection has been associated with poor feed efficiency and decreased weight gain (Thompson et al. 2008; Geurden et al. 2010). In an outbreak caused by Giardia Assemblage B in a sheep farm, malabsorption, decreased weight gain, and impairment in feed efficiency was associated with excretion of malodorous and poorly formed feces, but not with diarrhea. A mild to severe infiltrative enteritis with eosinophils, lymphocytes, and plasma cells was detected in histological sections of the gut (Aloisio et al. 2006). Giardiasis in sheep was associated with a reduced carcass weight (Jacobson et al. 2016). In pigs, Giardia has been associated with diarrhea; however high rates of infection have also been reported in asymptomatic pigs (Armson et al. 2009; Akinkuotu et al. 2019). Characterization of the assemblage is important, since pigs infected with assemblage E showed soft/diarrheic feces, whereas those infected with assemblage A did not (Armson et al. 2009). Giardiasis has been reported also in horses but clinical signs of infection are rarely observed. When present, particularly in young and aged or immunologically suppressed horses, symptoms include mild/self-limiting diarrhea, severe in case of heavy infections, poor hair coat, and weight loss (Siwila 2017).

41.9 Treatment of Giardiasis in Human and Animals

Currently, no vaccine is available to protect humans from *Giardia* infection. Treatment options then rely on a limited pool of effective molecules, with metronidazole (MTZ) and other long acting 5-nitroimidazoles (tinidazole, secnidazole, and ornidazole) being the first-line drugs (Lalle 2010). Treatment of symptomatic cases

of giardiasis is always recommended, particularly in low-endemic areas, to alleviate disease's severity, prevent chronic infection, and reduce the risk of spreading the infection (Lalle and Hanevik 2018). The 5-nitroimidazoles are pro-drugs that can exert their toxic effects only after reduction of the nitro group into reactive nitroimidazole intermediates. As this happens in a strongly reductive environment, only anaerobic/microaerophilic organisms are targeted by the drugs (Lalle 2010; Lalle and Hanevik 2018). Standard MTZ regimen in adults consists of three doses/day of 250 mg or 500 mg of drugs for 7 or 5 days, respectively. For children, 5–7 mg of MTZ is administered three times/day for 5–7 days (Mørch and Hanevik 2020). Tinidazole, secnidazole, and ornidazole are commonly administered in a single-dose regimen of 1–2 g in adults and 20–50 mg/kg in children (Mørch and Hanevik 2020). Unpleasant side effects and an increase of cases that are refractory to MTZ treatment, particularly among returning travelers from Asia, are posing new challenges for the management of giardiasis (Lalle and Hanevik 2018). To treat cases of refractory giardiasis, different regimens of MTZ and of second-line drugs, such as albendazole and cloroquine, either individually or in combination, have been used successfully (Lalle and Hanevik 2018).

An inactivated veterinary vaccine (GiardiaVax), composed of killed trophozoites isolated from a sheep (unknown assemblage), was licensed to prevent disease and reduce cyst shedding in dogs (Siwila 2017). As the efficacy of this vaccine is contradictory in calves and cats, and even in dogs, it has been withdrawn from several markets, worldwide (Siwila 2017; Geurden et al. 2014). An oral vaccine formulation comprising VSPs immunopurified from trophozoites of the *Giardia* WB isolate expressing full VSP repertoire has proven effective to reduce clinical signs and to prevent new infections in experimentally infected gerbils, puppies, and kittens (Serradell et al. 2016). Noteworthy, immunization of dogs with this vaccine in an endemic area resulted in a decrease of *Giardia* infection among children living in the same area (Serradell et al. 2016).

Pharmacological treatment of symptomatic companion animals is widely used to reduce the potential zoonotic transmission to human. Treatment approval varies between countries. Fenbendazole is widely registered in EU countries for the treatment of dogs, and it is also recommended for cats. A combination tablet containing febantel/pyrantel/praziquantel is licensed for dogs in European countries and countries outside the EU (ESSCAP 2018). Metronidazole is also licensed in most European countries for dogs and cats, but onset of serious adverse effects of the CNS in dogs treated recurrently or with high dose might discourage its use (Robertson 2021). Currently, no drugs are approved for treatment of giardiasis in pets in USA, but fenbendazole and metronidazole are commonly used as well (Siwila 2017). No drug is licensed for treatment of giardiasis in ruminants or other livestock. Treatment with fenbendazole, albendazole, and the broad-spectrum antibiotic paromomycin is effective in improving clinical conditions (decreased diarrhea and increase weight gain) and in reducing cyst shedding. However, drug treatment of ruminants might be of limited significance, due to the high risk of reinfection associated to high environmental contamination of farm and high costs for producers (Siwila 2017; Robertson 2021).

41.10 Conclusions

The issue of zoonotic transmission has dominated the debate around *Giardia* for about 30 years, since the WHO recognized giardiasis as a zoonosis. In recent years, there have been substantial advances in our understanding of the biology, genetics, and taxonomy of *Giardia*, and a wealth of data has been generated through the molecular characterization of isolates from many hosts and from the environment. We now understand that the genetics of *Giardia* is complex, and that more complexity is found among isolates of assemblage B, possibly due to more frequent genetic exchanges. Molecular epidemiologic surveys have demonstrated that animals are more commonly infected with assemblage-specific *Giardia* parasites that are not infectious to humans. However, animals are also infected with assemblage A and B, which are zoonotic. Sub-assemblages AI and AII are found in both humans and animals, but sub-assemblage AI is preferentially found in livestock and pets whereas sub-assemblage AII is predominantly found in humans. Sub-assemblage AIII is almost exclusively found in wild hoofed animals, and is likely to be non-zoonotic. The host distribution of assemblage B is predominantly human and non-human primates, but many other mammals are also infected. Therefore, while the potential for zoonotic transmission exists, studies in well-defined epidemiologic settings are needed to confirm which animal can act as reservoir and under which conditions zoonotic transmission can occur. Further, the extent of reverse zoonotic transmission (i.e., from humans to animals) should also be evaluated in view of its importance for conservation management.

Concerning the pathology of giardiasis, current knowledge on disease outcomes in animals is limited and not linked to the different Assemblages potentially infecting both human and animal. Zoonotic transmission and differences in symptomatology are questions that certainly need to be further investigated. Some evidences suggest that vaccination of companion animals can be a valuable strategy to prevent human giardiasis, particularly in poor-hygiene settings where anthropozoonotic transmission is likely to occur and the zoonotic Assemblages A and B are mainly present. The possible role of animal reservoirs in the onset of parasite resistance to drugs (e.g., nitroimidazoles) should also receive attention.

Future studies, in particular, those aimed at further comparative genomics of assemblages, should lead to a more comprehensive understanding of the host-specificity and transmission cycles of *Giardia*.

41.11 Cross-References

- ▶ Cats – Revered and Reviled – and Associated Zoonoses
- ▶ Cryptosporidium and Cryptosporidiosis: Trickle or Treat?
- ▶ Dogs and Transmission of Infection to Man, “Respected Member of the Family?”
- ▶ Zoonoses and Poverty: The Multiple Burdens of Zoonoses in Low- and Middle-Income Countries

References

Adam RD (2021) *Giardia duodenalis*: biology and pathogenesis. Clin Microbiol Rev 34(4): e0002419. https://doi.org/10.1128/CMR.00024-19

Akinkuotu OA, Takeet MI, Otesile EB, Olufemi F, Greenwood SJ, McClure JT (2019) Prevalence and multilocus genotypes of *Giardia duodenalis* infecting pigs in Ogun state. Nigeria Infect Genet Evol 70:53–60. https://doi.org/10.1016/j.meegid.2019.02.017

Allain T, Buret AG (2020) Pathogenesis and post-infectious complications in giardiasis. Adv Parasitol 107:173–199. https://doi.org/10.1016/bs.apar.2019.12.001

Almeida A, Pozio E, Cacciò SM (2010) Genotyping of *Giardia duodenalis* cysts by new real-time PCR assays for detection of mixed infections in human samples. Appl Environ Microbiol 76(6): 1895–1901. https://doi.org/10.1128/AEM.02305-09

Aloisio F, Filippini G, Antenucci P, Lepri E, Pezzotti G, Cacciò SM, Pozio E (2006) Severe weight loss in lambs infected with *Giardia duodenalis* assemblage B. Vet Parasitol 142:154–158. https://doi.org/10.1016/j.vetpar.2006.06.023

Amat CB, Motta JP, Fekete E, Moreau F, Chadee K, Buret AG (2017) Cysteine protease-dependent mucous disruptions and differential mucin gene expression in *Giardia duodenalis* infection. Am J Pathol 187:2486–2498

Amorim RM, Silva DA, Taketomi EA, Morato MG, Mundim MJ, Ribeiro DP, Oliveira TC, Viana JC, Gomes MA, Cury MC (2010) *Giardia duodenalis*: kinetics of cyst elimination and the systemic humoral and intestinal secretory immune responses in gerbils (*Meriones unguiculatus*) experimentally infected. Exp Parasitol 125:297–303. https://doi.org/10.1016/j.exppara.2010.02.007

Andrews RH, Adams M, Boreham PF, Mayrhofer G, Meloni BP (1989) *Giardia intestinalis*: electrophoretic evidence for a species complex. Int J Parasitol 19(2):183–190. https://doi.org/10.1016/0020-7519(89)90006-4

Ankarklev J, Svärd SG, Lebbad M (2012) Allelic sequence heterozygosity in single *Giardia* parasites. BMC Microbiol 12:65. https://doi.org/10.1186/1471-2180-12-65

Ankarklev J, Franzén O, Peirasmaki D, Jerlström-Hultqvist J, Lebbad M, Andersson J, Andersson B, Svärd SG (2015) Comparative genomic analyses of freshly isolated *Giardia intestinalis* assemblage A isolates. BMC Genomics 16(1):697. https://doi.org/10.1186/s12864-015-1893-6

Ankarklev J, Lebbad M, Einarsson E, Franzén O, Ahola H, Troell K, Svärd SG (2018) A novel high-resolution multilocus sequence typing of *Giardia intestinalis* assemblage A isolates reveals zoonotic transmission, clonal outbreaks and recombination. Infect Genet Evol 60:7–16. https://doi.org/10.1016/j.meegid.2018.02.012

Armson A, Yang R, Thompson J, Johnson J, Reid S, Ryan UM (2009) *Giardia* genotypes in pigs in Western Australia: prevalence and association with diarrhea. Exp Parasitol 121(4):381–3. https://doi.org/10.1016/j.exppara.2009.01.008. PMID: 19271283

Barash NR, Nosala C, Pham JK, McInally SG, Gourguechon S, McCarthy-Sinclair B, Dawson SC (2017) *Giardia* colonizes and encysts in high-density foci in the murine small intestine. mSphere 2:e00343–e00316. https://doi.org/10.1128/mSphere.00343-16

Bhargava A, Cotton JA, Dixon BR, Gedamu L, Yates RM, Buret AG (2015) *Giardia duodenalis* surface cysteine proteases induce cleavage of the intestinal epithelial cytoskeletal protein villin via myosin light chain kinase. PLoS One 10:e0136102. https://doi.org/10.1371/journal.pone.0136102

Boarato-David E, Guimarães S, Cacciò SM (2017) Giardia duodenalis. In: Rose JB, Jiménez-Cisneros B (eds) Water and sanitation for the 21st century: health and microbiological aspects of excreta and wastewater management. Michigan State University, E. Lansing, UNESCO. https://doi.org/10.14321/waterpathogens.35

Boucard AS, Thomas M, Lebon W, Polack B, Florent I, Langella P, Bermúdez-Humarán LG (2021) Age and *Giardia intestinalis* infection impact canine gut microbiota. Microorganisms 9:1862. https://doi.org/10.3390/microorganisms9091862

Bouzid M, Halai K, Jeffreys D, Hunter PR (2015) The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. Vet Parasitol 207(3–4):181–202. https://doi.org/10.1016/j.vetpar.2014.12.011

Cacciò SM, Sprong H (2011) Epidemiology of giardiasis in humans. In: Lujan HD, Svard S (eds) *Giardia* A model organism. Springer, Wien/New York, pp 16–28

Cacciò SM, Beck R, Lalle M, Marinculic A, Pozio E (2008) Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int J Parasitol 38(13): 1523–1531. https://doi.org/10.1016/j.ijpara.2008.04.008

Cacciò SM, Lalle M, Svärd SG (2018) Host specificity in the *Giardia duodenalis* species complex. Infect Genet Evol 66:335–345. https://doi.org/10.1016/j.meegid.2017.12.001

Cai W, Ryan U, Xiao L, Feng Y (2021) Zoonotic giardiasis: an update. Parasitol Res 120(12):4199–4218. https://doi.org/10.1007/s00436-021-07325-2

Capewell P, Krumrie S, Katzer F, Alexander CL, Weir W (2021) Molecular epidemiology of *Giardia* infections in the genomic era. Trends Parasitol 37(2):142–153. https://doi.org/10.1016/j.pt.2020.09.013

Chávez B, González-Mariscal L, Cedillo-Rivera R, Martínez-Palomo A (1995) *Giardia lamblia*: in vitro cytopathic effect of human isolates. Exp Parasitol 80:133–138. https://doi.org/10.1006/expr.1995.1015

Coelho CH, Durigan M, Leal DAG, Schneider AB, Franco RMB, Singer SM (2017) Giardiasis as a neglected disease in Brazil: systematic review of 20 years of publications. PLoS Negl Trop Dis 11(10):e0006005. https://doi.org/10.1371/journal.pntd.0006005

Cooper MA, Sterling CR, Gilman RH, Cama V, Ortega Y, Adam RD (2010) Molecular analysis of household transmission of *Giardia lamblia* in a region of high endemicity in Peru. J Infect Dis 202(11):1713–1721. https://doi.org/10.1086/657142

Dixon BR (2021) *Giardia duodenalis* in humans and animals – transmission and disease. Res Vet Sci 135:283–289. https://doi.org/10.1016/j.rvsc.2020.09.034

Donowitz JR, Alam M, Kabir M, Ma JZ, Nazib F, Platts-Mills JA, Bartelt LA, Haque R, Petri WA Jr (2016) A Prospective Longitudinal Cohort to Investigate the Effects of Early Life Giardiasis on Growth and All Cause Diarrhea. Clin Infect Dis 63(6):792–7. https://doi.org/10.1093/cid/ciw391. Epub 2016 Jun 16. PMID: 27313261; PMCID: PMC4996141

Dormond M, Gutierrez RL, Porter CK (2016) *Giardia lamblia* infection increases risk of chronic gastrointestinal disorders. Trop Dis Travel Med Vaccines 2:17

Eckmann L, Laurent F, Langford TD, Hetsko ML, Smith JR, Kagnoff MF, Gillin FD (2000) Nitric oxide production by human intestinal epithelial cells and competition for arginine as potential determinants of host defense against the lumen-dwelling pathogen *Giardia lamblia*. J Immunol 164:1478–1487. https://doi.org/10.4049/jimmunol.164.3.1478

Erlandsen SL, Chase DG (1974) Morphological alterations in the microvillous border of villous epithelial cells produced by intestinal microorganisms. Am J Clin Nutr 27:1277–1286. https://doi.org/10.1093/ajcn/27.11.1277

Escobedo AA, Almirall P, Robertson LJ, Franco RM, Hanevik K, Mørch K, Cimerman S (2010) Giardiasis: the ever-present threat of a neglected disease. Infect Disord Drug Targets 10(5):329–348. https://doi.org/10.2174/187152610793180821

Escobedo AA, Hanevik K, Almirall P, Cimerman S, Alfonso M (2014) Management of chronic *Giardia* infection. Expert Rev Anti-Infect Ther 12:1143–1157

Escobedo AA, Almirall P, Hanevik K et al (2018) Giardiasis: a diagnosis that should be considered regardless of the setting. Epidemiol Infect 11:1–3

ESSCAP (2018) Guidelines GL6. Control of intestinal protozoa in dogs and cats. https://esccap.org/guidelines/GL6. Accessed 1 Feb 2022.

European Centre for Disease Prevention and Control (2019) Giardiasis (lambliasis). In: ECDC (ed) Annual epidemiological report for 2017. ECDC, Stockholm

Farthing MJ (1997) The molecular pathogenesis of giardiasis. J Pediatr Gastroenterol Nutr 24:79–88. https://doi.org/10.1097/00005176-199701000-00018

Farthing MJ, Varon SR, Keusch GT (1983) Mammalian bile promotes growth of *Giardia lamblia* in axenic culture. Trans R Soc Trop Med Hyg 77(4):467–469. https://doi.org/10.1016/0035-9203(83)90115-3

Farthing MJ, Pereira ME, Keusch GT (1986) Description and characterization of a surface lectin from *Giardia lamblia*. Infect Immun 51:661–667

Fekete E, Allain T, Siddiq A, Sosnowski O, Buret AG (2021) *Giardia* spp. and the gut microbiota: dangerous liaisons. Front Microbiol 11:618106. https://doi.org/10.3389/fmicb.2020.618106

Fekete E, Allain T, Amat CB, Mihara K, Saifeddine M, Hollenberg MD, Chadee K, Buret AG (2022) *Giardia duodenalis* cysteine proteases cleave proteinase-activated receptor-2 to regulate intestinal goblet cell mucin gene expression. Int J Parasitol. https://doi.org/10.1016/j.ijpara.2021.11.011

Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev 24(1):110–140. https://doi.org/10.1128/CMR.00033-10

Filice FP (1952) Studies on the cytology and life history of a *Giardia* from the laboratory rat. Univ Calif Publ Zool 57:53–146

Fink MY, Singer SM (2017) The intersection of immune responses, microbiota, and pathogenesis in giardiasis. Trends Parasitol 33:901–913. https://doi.org/10.1016/j.pt.2017.08.001

Franzén O, Jerlström-Hultqvist J, Castro E, Sherwood E, Ankarklev J, Reiner DS, Palm D, Andersson JO, Andersson B, Svärd SG (2009) Draft genome sequencing of *Giardia intestinalis* assemblage B isolate GS: is human giardiasis caused by two different species? PLoS Pathog 5: e1000560. https://doi.org/10.1371/journal.ppat.1000560

Gardner TB, Hill DR (2001) Treatment of giardiasis. Clin Microbiol Rev 14(1):114–128. https://doi.org/10.1128/CMR.14.1.114-128.2001

Gargantini PR, Serradell MDC, Ríos DN, Tenaglia AH, Luján HD (2016) Antigenic variation in the intestinal parasite Giardia lamblia. Curr Opin Microbiol 32:52–58. https://doi.org/10.1016/j.mib.2016.04.017. Epub 2016 May 10. PMID: 27177351

Geurden T, Vercruysse J, Claerebout E (2010) Is *Giardia* a significant pathogen in production animals? Exp Parasitol 124:98–106. https://doi.org/10.1016/j.exppara.2009.03.001

Geurden T, Olson ME, O'Handley RM, Schetters T, Bowman D, Vercruysse J (2014) World Association for the Advancement of Veterinary Parasitology (WAAVP): guideline for the evaluation of drug efficacy against non-coccidial gastrointestinal protozoa in livestock and companion animals. Vet Parasitol 204:81–86

Gladyshev EA, Arkhipova IR (2010) Genome structure of bdelloid rotifers: shaped by asexuality or desiccation? J Hered 101(Suppl 1):S85–S93. https://doi.org/10.1093/jhered/esq008

Haberman Y, Iqbal NT, Ghandikota S, Mallawaarachchi I, Braun T, Dexheimer PJ, Rahman N, Hadar R, Sadiq K, Ahmad Z, Idress R, Iqbal J, Ahmed S, Hotwani A, Umrani F, Ehsan L, Medlock G, Syed S, Moskaluk C, Ma JZ, Jegga AG, Moore SR, Ali SA, Denson LA (2021) Mucosal genomics implicate lymphocyte activation and lipid metabolism in refractory environmental enteric dysfunction. Gastroenterology 160:2055–2071.e0. https://doi.org/10.1053/j.gastro.2021.01.221

Halliez MC, Buret AG (2013) Extra-intestinal and long term consequences of *Giardia duodenalis* infections. World J Gastroenterol 19:8974–8985

Hamilton KA, Waso M, Reyneke B, Saeidi N, Levine A, Lalancette C, Besner MC, Khan W, Ahmed W (2018) *Cryptosporidium* and *Giardia* in wastewater and surface water environments. J Environ Qual 47(5):1006–1023. https://doi.org/10.2134/jeq2018.04.0132

Hjøllo T, Bratland E, Steinsland H, Radunovic M, Langeland N, Hanevik K (2018) Longitudinal cohort study of serum antibody responses towards *Giardia lamblia* variant-specific surface proteins in a non-endemic area. Exp Parasitol 191:66–72. https://doi.org/10.1016/j.exppara.2018.06.005

Holthaus D, Kraft MR, Krug SM, Wolf S, Müller A, Betancourt ED, Schorr M, Holland G, Knauf F, Schulzke JD, Aebischer T, Klotz C (2021) Dissection of barrier dysfunction in organoid-derived human intestinal epithelia induced by *Giardia duodenalis*. Gastroenterology. https://doi.org/10.1053/j.gastro.2021.11.022

Horton B, Bridle H, Alexander CL, Katzer F (2019) *Giardia duodenalis* in the UK: current knowledge of risk factors and public health implications. Parasitology 146(4):413–424. https://doi.org/10.1017/S0031182018001683

Jacobson C, Williams A, Yang R, Ryan U, Carmichael I, Campbell AJ, Gardner GE (2016) Greater intensity and frequency of *Cryptosporidium* and *Giardia* oocyst shedding beyond the neonatal

period is associated with reductions in growth, carcase weight and dressing efficiency in sheep. Vet Parasitol 228:42–51. https://doi.org/10.1016/j.vetpar.2016.08.003

Janeczko S, Griffin B (2010) *Giardia* infection in cats. Compend Contin Educ Vet 32(8):E4. PMID: 20957611

Jerlström-Hultqvist J, Franzén O, Ankarklev J, Xu F, Nohýnková E, Andersson JO, Svärd SG, Andersson B (2010) Genome analysis and comparative genomics of a *Giardia intestinalis* assemblage E isolate. BMC Genomics 11:543. https://doi.org/10.1186/1471-2164-11-543

Khanna R, Joshi K, Kum K, Malik AK, Vinayak VK (1990) An ultrastructural analysis of changes in surface architecture of intestinal mucosa following *Giardia lamblia* infection in mice. Gastroenterol Jpn 25:649–658. https://doi.org/10.1007/BF02779368

Kooyman FNJ, Wagenaar JA, Zomer A (2019) Whole-genome sequencing of dog-specific assemblages C and D of *Giardia duodenalis* from single and pooled cysts indicates host-associated genes. Microb Genom 5(12):e000302. https://doi.org/10.1099/mgen.0.000302

Koudela B, Vítovec J (1998) Experimental giardiasis in goat kids. Vet Parasitol 74(1):9–18. https://doi.org/10.1016/s0304-4017(97)00146-5

Kuthyar S, Kowalewski MM, Seabolt M, Roellig DM, Gillespie TR (2021) Molecular characterization of *Giardia duodenalis* and evidence for cross-species transmission in northern Argentina. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14220

Lalle M (2010) Giardiasis in the post genomic era: treatment, drug resistance and novel therapeutic perspectives. Infect Disord Drug Targets 10:283–294. https://doi.org/10.2174/187152610791591610

Lalle M, Hanevik K (2018) Treatment-refractory giardiasis: challenges and solutions. Infect Drug Resist 11:1921–1933. https://doi.org/10.2147/IDR.S141468

Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Cacciò SM (2005) Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol 35(2):207–213. https://doi.org/10.1016/j.ijpara.2004.10.022

Lebbad M, Mattsson JG, Christensson B, Ljungström B, Backhans A, Andersson JO, Svärd SG (2010) From mouse to moose: multilocus genotyping of *Giardia* isolates from various animal species. Vet Parasitol 168(3–4):231–239. https://doi.org/10.1016/j.vetpar.2009.11.003

Litleskare S, Rortveit G, Eide GE, Hanevik K, Langeland N, Wensaas KA (2018) Prevalence of irritable bowel syndrome and chronic fatigue 10 years after *Giardia* infection. Clin Gastroenterol Hepatol 16:1064–1072.e4

Liu J, Ma'ayeh S, Peirasmaki D, Lundström-Stadelmann B, Hellman L et al (2018) Secreted *Giardia intestinalis* cysteine proteases disrupt intestinal epithelial cell junctional complexes and degrade chemokines. Virulence 9:879–894

Liu J, Fu Z, Hellman L, Svärd SG (2019) Cleavage specificity of recombinant *Giardia intestinalis* cysteine proteases: degradation of immunoglobulins and defensins. Mol Biochem Parasitol 227:29–38. https://doi.org/10.1016/j.molbiopara.2018.10.004

Ma JY, Li MY, Qi ZZ, Fu M, Sun TF, Elsheikha HM, Cong W (2022) Waterborne protozoan outbreaks: an update on the global, regional, and national prevalence from 2017 to 2020 and sources of contamination. Sci Total Environ 806(Pt 2):150562. https://doi.org/10.1016/j.scitotenv.2021.150562

Ma'ayeh SY, Liu J, Peirasmaki D, Hörnaeus K, Bergström Lind S, Grabherr M, Bergquist J, Svärd SG (2017) Characterization of the *Giardia intestinalis* secretome during interaction with human intestinal epithelial cells: the impact on host cells. PLoS Negl Trop Dis 11:e0006120

Maàyeh S, Svärd S (2017) *Giardia* and giardiasis. In: Bruschi F (ed) Humans frontiers in parasitology: water-borne protozoa, vol 2. Bentham Books, Sharjah, pp 150–220

Monis PT, Andrews RH, Mayrhofer G, Ey PL (1999) Molecular systematics of the parasitic protozoan *Giardia intestinalis*. Mol Biol Evol 16(9):1135–1144. https://doi.org/10.1093/oxfordjournals.molbev.a026204

Monis PT, Andrews RH, Mayrhofer G, Ey PL (2003) Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. Infect Genet Evol 3(1):29–38. https://doi.org/10.1016/s1567-1348(02)00149-1

Monis PT, Caccio SM, Thompson RC (2009) Variation in *Giardia*: towards a taxonomic revision of the genus. Trends Parasitol 25(2):93–100. https://doi.org/10.1016/j.pt.2008.11.006

Mørch K, Hanevik K (2020) Giardiasis treatment: an update with a focus on refractory disease. Curr Opin Infect Dis 33:355–364. https://doi.org/10.1097/QCO.0000000000000668

Mørch K, Hanevik K, Rortveit G, Wensaas KA, Eide GE, Hausken T, Langeland N (2009) Severity of *Giardia* infection associated with post-infectious fatigue and abdominal symptoms two years after. BMC Infect Dis 9:206. https://doi.org/10.1186/1471-2334-9-206. PMID: 20003489; PMCID: PMC2808308

Morrison HG, McArthur AG, Gillin FD, Aley SB, Adam RD, Olsen GJ, Best AA, Cande WZ, Chen F, Cipriano MJ, Davids BJ, Dawson SC, Elmendorf HG, Hehl AB, Holder ME, Huse SM, Kim UU, Lasek-Nesselquist E, Manning G, Nigam A, Nixon JE, Palm D, Passamaneck NE, Prabhu A, Reich CI, Reiner DS, Samuelson J, Svard SG, Sogin ML (2007) Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. Science 317(5846):1921–1926. https://doi.org/10.1126/science.1143837

Moss DM, Priest JW, Hamlin K, Derado G, Herbein J, Petri WA Jr, Lammie PJ (2014) Longitudinal evaluation of enteric protozoa in Haitian children by stool exam and multiplex serologic assay. Am J Trop Med Hyg 90:653–660. https://doi.org/10.4269/ajtmh.13-0545

Muhsen K, Levine MM (2012) A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries. Clin Infect Dis 55 Suppl 4(Suppl 4):S271–93. https://doi.org/10.1093/cid/cis762. PMID: 23169940; PMCID: PMC3502312

Müller N, von Allmen N (2005) Recent insights into the mucosal reactions associated with *Giardia lamblia* infections. Int J Parasitol 35:1339–1347. https://doi.org/10.1016/j.ijpara.2005.07.008

Nakao JH, Collier SA, Gargano JW (2017) Giardiasis and subsequent irritable bowel syndrome: a longitudinal cohort study using health insurance data. J Infect Dis 215:798–805

Oberhuber G, Kastner N, Stolte M (1997) Giardiasis: a histologic analysis of 567 cases. Scand J Gastroenterol 32:48–51

Ortega-Pierres MG, Argüello-García R (2019) *Giardia duodenalis*: role of secreted molecules as virulent factors in the cytotoxic effect on epithelial cells. Adv Parasitol 106:129–169. https://doi.org/10.1016/bs.apar.2019.07.003

Pires SM, Fischer-Walker CL, Lanata CF, Devleesschauwer B, Hall AJ, Kirk MD, Duarte AS, Black RE, Angulo FJ (2015) Aetiology-specific estimates of the global and regional incidence and mortality of diarrhoeal diseases commonly transmitted through food. PLoS One 10(12): e0142927. https://doi.org/10.1371/journal.pone.0142927

Platts-Mills JA, Babji S, Bodhidatta L, et al. (2015) Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health 3:e564–75

Poxleitner MK, Carpenter ML, Mancuso JJ, Wang CJ, Dawson SC, Cande WZ (2008) Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. Science 319(5869):1530–1533. https://doi.org/10.1126/science.1153752

Prystajecky N, Tsui CK, Hsiao WW, Uyaguari-Diaz MI, Ho J, Tang P, Isaac-Renton J (2015) *Giardia* spp. are commonly found in mixed assemblages in surface water, as revealed by molecular and whole-genome characterization. Appl Environ Microbiol 81(14):4827–4834. https://doi.org/10.1128/AEM.00524-15

Rendtorff RC (1954) The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. Am J Hyg 59:209–220. https://doi.org/10.1093/oxfordjournals.aje.a119634

Reses HE, Gargano JW, Liang JL, Cronquist A, Smith K, Collier SA, Roy SL, Vanden Eng J, Bogard A, Lee B, Hlavsa MC, Rosenberg ES, Fullerton KE, Beach MJ, Yoder JS (2018) Risk factors for sporadic *Giardia* infection in the USA: a case-control study in Colorado and Minnesota. Epidemiol Infect 146(9):1071–1078. https://doi.org/10.1017/S0950268818001073

Rivero MR, Feliziani C, De Angelo C, Tiranti K, Salomon OD, Touz MC (2020) *Giardia* spp., the most ubiquitous protozoan parasite in Argentina: human, animal and environmental surveys reported in the last 40 years. Parasitol Res 119(10):3181–3201. https://doi.org/10.1007/s00436-020-06853-7

Robertson LJ (2021) Giardiasis in animals (Lambliasis) Merck Veterinary manual (Last full review/revision November 2021). https://www.merckvetmanual.com/digestive-system/giardiasis-giardia/giardiasis-in-animals. Accessed 1 Feb 2022

Rogawski ET, Bartelt LA, Platts-Mills JA, Seidman JC, Samie A, Havt A, Babji S, Trigoso DR, Qureshi S, Shakoor S, Haque R, Mduma E, Bajracharya S, Gaffar SMA, Lima AAM, Kang G, Kosek MN, Ahmed T, Svensen E, Mason C, Bhutta ZA, Lang DR, Gottlieb M, Guerrant RL, Houpt ER, Bessong PO (2017) MAL-ED Network Investigators. Determinants and Impact of *Giardia* Infection in the First 2 Years of Life in the MAL-ED Birth Cohort. J Pediatric Infect Dis Soc 6(2):153–160. https://doi.org/10.1093/jpids/piw082. PMID: 28204556; PMCID: PMC5907871

Roxström-Lindquist K, Ringqvist E, Palm D, Svärd S (2005) *Giardia lamblia*-induced changes in gene expression in differentiated Caco-2 human intestinal epithelial cells. Infect Immun 73 (12):8204–8. https://doi.org/10.1128/IAI.73.12.8204-8208.2005. PMID: 16299316; PMCID: PMC1307045

Ruest N, Couture Y, Faubert GM, Girard C (1997) Morphological changes in the jejunum of calves naturally infected with *Giardia* spp. and *Cryptosporidium* spp. Vet Parasitol 69:177–186. https://doi.org/10.1016/s0304-4017(96)01121-1

Ryan U, Cacciò SM (2013) Zoonotic potential of *Giardia*. Int J Parasitol 43(12–13):943–956. https://doi.org/10.1016/j.ijpara.2013.06.001

Ryan U, Zahedi A (2019) Molecular epidemiology of giardiasis from a veterinary perspective. Adv Parasitol 106:209–254. https://doi.org/10.1016/bs.apar.2019.07.002

Ryan U, Hijjawi N, Feng Y, Xiao L (2019) *Giardia*: an under-reported foodborne parasite. Int J Parasitol 49(1):1–11. https://doi.org/10.1016/j.ijpara.2018.07.003

Santin M (2020) *Cryptosporidium* and *Giardia* in Ruminants. Vet Clin North Am Food Anim Pract 36(1):223–238. https://doi.org/10.1016/j.cvfa.2019.11.005. PMID: 32029186

Seabolt MH, Konstantinidis KT, Roellig DM (2021) Hidden diversity within common protozoan parasites as revealed by a novel genomotyping scheme. Appl Environ Microbiol 87(6):e02275–e02220. https://doi.org/10.1128/AEM.02275-20. PMID: 33397705; PMCID: PMC8105021

Serradell MC, Saura A, Rupil LL, Gargantini PR, Faya MI, Furlan PJ, Lujan HD (2016) Vaccination of domestic animals with a novel oral vaccine prevents *Giardia* infections, alleviates signs of giardiasis and reduces transmission to humans. NPJ Vaccines 1:16018. https://doi.org/10.1038/npjvaccines.2016.18

Serradell MC, Gargantini PR, Saura A, Oms SR, Rupil LL, Berod L, Sparwasser T, Luján HD (2018) Cytokines, antibodies, and histopathological profiles during *Giardia* infection and variant-specific surface protein-based vaccination. Infect Immun 86:e00773–e00717. https://doi.org/10.1128/IAI.00773-17

Singer SM, Fink MY, Angelova VV (2019) Recent insights into innate and adaptive immune responses to *Giardia*. Adv Parasitol 106:171–208. https://doi.org/10.1016/bs.apar.2019.07.004

Singer SM, Angelova VV, DeLeon H, Miskovsky E (2020) What's eating you? An update on *Giardia*, the microbiome and the immune response. Curr Opin Microbiol 58:87–92. https://doi.org/10.1016/j.mib.2020.09.006

Siwila J (2017) Giardiasis: livestock and companion animals. In: Rodriguez-Morales AJ (ed) Current topics in giardiasis. IntechOpen, London. https://doi.org/10.5772/intechopen.70874. Accessed 1 Feb 2022

Snel SJ, Baker MG, Kamalesh V, French N, Learmonth J (2009) A tale of two parasites: the comparative epidemiology of cryptosporidiosis and giardiasis. Epidemiol Infect 137(11):1641–1650. https://doi.org/10.1017/S0950268809002465

Solaymani-Mohammadi S, Singer SM (2013) Regulation of intestinal epithelial cell cytoskeletal remodeling by cellular immunity following gut infection. Mucosal Immunol 6:369–378. https://doi.org/10.1038/mi.2012.80

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, Das P, Lal AA, Xiao L (2003) Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis 9(11):1444–1452. https://doi.org/10.3201/eid0911.030084

Swain S, Selmi C, Gershwin ME, Teuber SS (2019) The clinical implications of selective IgA deficiency. J Transl Autoimmun 2:100025. https://doi.org/10.1016/j.jtauto.2019.100025; Erratum in: J Transl Autoimmun. 2020;3:100041

Tangtrongsup S, Scorza V (2010) Update on the diagnosis and management of *Giardia* spp infections in dogs and cats. Top Companion Anim Med 25:155–162. https://doi.org/10.1053/j.tcam.2010.07.003

Thompson RC, Monis P (2012) *Giardia*-from genome to proteome. Adv Parasitol 78:57–95. https://doi.org/10.1016/B978-0-12-394303-3.00003-7

Thompson RC, Palmer CS, O'Handley R (2008) The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. Vet J 177:18–25. https://doi.org/10.1016/j.tvjl.2007.09.022

Troeger H, Epple HJ, Schneider T, Wahnschaffe U, Ullrich R, Burchard GD, Jelinek T, Zeitz M, Fromm M, Schulzke JD (2007) Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. Gut 56:328–335. https://doi.org/10.1136/gut.2006.100198

Tsui CK, Miller R, Uyaguari-Diaz M, Tang P, Chauve C, Hsiao W, Isaac-Renton J, Prystajecky N (2018) Beaver fever: whole-genome characterization of waterborne outbreak and sporadic isolates to study the zoonotic transmission of giardiasis. mSphere 3(2):e00090–e00018. https://doi.org/10.1128/mSphere.00090-18

Tůmová P, Dluhošová J, Weisz F, Nohýnková E (2019) Unequal distribution of genes and chromosomes refers to nuclear diversification in the binucleated *Giardia intestinalis*. Int J Parasitol 49:463–470. https://doi.org/10.1016/j.ijpara.2019.01.003

Tysnes KR, Skancke E, Robertson LJ (2014) Subclinical *Giardia* in dogs: a veterinary conundrum relevant to human infection. Trends Parasitol 30:520–527. https://doi.org/10.1016/j.pt.2014.08.007

Uiterwijk M, Nijsse R, Kooyman FNJ, Wagenaar JA, Mughini-Gras L, Ploeger HW (2019) Host factors associated with *Giardia duodenalis* infection in dogs across multiple diagnostic tests. Parasit Vectors 12:556. https://doi.org/10.1186/s13071-019-3810-3

Vanni I, Cacciò SM, van Lith L, Lebbad M, Svärd SG, Pozio E, Tosini F (2012) Detection of *Giardia duodenalis* assemblages A and B in human feces by simple, assemblage-specific PCR assays. PLoS Negl Trop Dis 6(8):e1776. https://doi.org/10.1371/journal.pntd.0001776

World Health Organisation (1979) Parasitic zoonoses. Report of a WHO expert committee with the participation of FAO. Technical Report Series No. 637. World Health Organization, Geneva

World Health Organisation (1996) The World Health Report, 1996. World Health Organization, Geneva

Woschke A, Faber M, Stark K, Holtfreter M, Mockenhaupt F, Richter J, Regnath T, Sobottka I, Reiter-Owona I, Diefenbach A, Gosten-Heinrich P, Friesen J, Ignatius R, Aebischer T, Klotz C (2021) Suitability of current typing procedures to identify epidemiologically linked human *Giardia duodenalis* isolates. PLoS Negl Trop Dis 15(3):e0009277. https://doi.org/10.1371/journal.pntd.0009277

Xiao L, Herd RP (1994) Infection pattern of *Cryptosporidium* and *Giardia* in calves. Vet Parasitol 55(3):257–62. https://doi.org/10.1016/0304-4017(93)00645-f

Yoder JS, Gargano JW, Wallace RM, Beach MJ (2012) Centers for Disease Control and Prevention (CDC). Giardiasis surveillance–United States, 2009–2010. MMWR Surveill Summ 61(5):13–23

Yu LZ, Birky CW Jr, Adam RD (2002) The two nuclei of *Giardia* each have complete copies of the genome and are partitioned equationally at cytokinesis. Eukaryot Cell 1(2):191–199. https://doi.org/10.1128/EC.1.2.191-199.2002

Leptospirosis: Messing with Our Minds- A Review of Unusual Neurological and Psychiatric Complexities

42

Scott B. Craig, Sarah J. Prior, Steven L. Weier, Glenn C. Graham, Trudi A. Collet, Frederick A. J. Moore, Glen R. Hewitson, Jamie L. McMahon, Peter R. Moore, Inga-Marie Sultana, Sonja Hall-Mendelin, and David B. McKay

Contents

Sarah J. Prior contributed equally and should be considered joint first author.

S. B. Craig (✉)
Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia

Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia
e-mail: scott.craig@health.qld.gov.au

S. J. Prior · S. L. Weier · T. A. Collet
Tasmanian School of Medicine, University of Tasmania, Bernie, TAS, Australia
e-mail: sarah.wynwood@utas.edu.au; s.weier@qut.edu.au; t.collet@qut.edu.au

G. C. Graham · D. B. McKay
Faculty of Health, University of the Sunshine Coast, Sippy Downs, QLD, Australia
e-mail: Glenn.Graham@health.qld.gov.au; dmckay@usc.edu.au

F. A. J. Moore · G. R. Hewitson · J. L. McMahon · P. R. Moore · I.-M. Sultana · S. Hall-Mendelin
Public Health Virology, Public and Environmental Health, Forensic and Scientific Services, Coopers Plains, QLD, Australia
e-mail: Frederick.Moore@health.qld.gov.au; Glen.Hewitson@health.qld.gov.au; Jamie.McMahon@health.qld.gov.au; Peter.Moore2@health.qld.gov.au; Inga.Sultana@health.qld.gov.au; Sonja.Hall-Mendelin@health.qld.gov.au

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_34

Abstract

Leptospirosis is a biphasic febrile illness common in tropical and subtropical regions. Clinically, patients may present with a mild, influenza-like illness through to syndromes revealing multiorgan failure. The aim of the current review is to focus on the rare neurological and psychiatric complexities of leptospirosis. A review of neuroleptospirosis and psychosis in leptospirosis will precede a review of the extant literature concerning acute disseminated encephalomyelitis following a leptospiral infection. Physicians working in areas where the incidence of leptospirosis is high should remain cognizant of patients with leptospirosis presenting primarily with a neurological syndrome. Such awareness will ensure a diagnosis of leptospirosis is not delayed and appropriate treatment strategies implemented.

Keywords

Infectious disease · Leptospirosis · Neuroleptospirosis · Psychosis · Acute disseminated encephalomyelitis · Neurology · Psychiatry · Psychology · Neuroscience

42.1 Introduction

Since the previous edition of this body of work, there have been advances in molecular biology that reveal that the genus *Leptospira* consists of 66 species that can be classified across four subclades. Pathogenic leptospires belong primarily in the P1 subclade of the new classification (Casanovas-Massana et al. 2020; Vincent et al. 2019). Serologically, there are more than 300 distinct leptospiral serovars arranged across 30 serogroups (World Organization for Animal Health 2022). The current chapter in this second edition of Zoonoses – Infecting Humans and Animals, is not aiming to provide an all-encompassing review of the extant leptospirosis literature. There is a plethora of excellent reviews in the medical and scientific literature reviewing leptospirosis and those looking for extensive reviews of great breadth and depth in the area are directed to the work of Ellis (1990), Levett (2001), Adler and de la Peña Moctezuma (2010), Levett and Haake (2010), Adler (2015), the first addition of this series (Craig et al. 2015), and of course the seminal review of the topic by Faine et al. (1999). The aim of the current review is to focus on the rare neurological and psychiatric complexities of leptospirosis. This will manifest itself with a review of neuroleptospirosis, which in isolation of other symptomology is a rare presentation of leptospirosis. The chapter will also offer a review of psychosis in leptospirosis and a review of the extant literature concerning acute disseminated encephalomyelitis

(ADEM) following a leptospiral infection. In discussing ADEM, Hill's (1965) perspectives of causation will be considered in assessing if ADEM following an episode of leptospirosis is a coincidence. Such a review is timely given the emergence of these neurological and psychiatric manifestations in the course of leptospirosis, and, secondly, given that altered mental status is a poor prognostic indictor.

42.2 Leptospira

In their reviews Reik (1987) and Levett (2001) describe leptospires, the causative infectious agent of leptospirosis, as 6–20 μm in length with a diameter of 0.1–0.2 μm and may consist of 30–50 closely wound spirals. Leptospires morphologically resemble a corkscrew when they are observed in scanning electron micrographs (Tulsiani et al. 2011; Willcox 1976). Leptospirosis has been reported in over 150 mammalian species (Picardeau 2013; Ko et al. 2009). The main animal reservoirs include rodents, dogs, cattle, horses, and pigs. These animals may act as maintenance hosts for adapted serovars such as serovar Canicola in dogs or serovars Ballum, Icterohaemorrhagiae, or Copenhageni in rodents. Renal colonization and shedding of leptospires in the urine of infected animals engenders the transmission of the organism to infect humans and other animals who are incidental hosts (Adler and de la Peña Moctezuma 2010; Faine et al. 1999).

42.3 Human Epidemiology

The global burden of human leptospirosis is currently unknown; however, estimates of the annual incidence range from 0.1–1 case/100,000 people in temperate areas to 100 cases/100,000 during epidemics in tropical regions (Everard and Everard 1993; Levett 2001). In addition, the incidence of leptospirosios is also higher in those environments prone to flooding (Lau et al. 2010a). An estimated 300,000–500,000 severe cases occur each year, with case fatality reports of up to 30% (WHO 2003; Hartskeerl 2006). In an attempt to develop a better understanding of the burden of leptospiral disease, the WHO (2011) estimates that the global incidence in endemic areas exceeds five severe cases per 100,000. Across Europe as a whole, the overall incidence rate in 2010 was 0.13 per 100,000 inhabitants (Dupouey et al. 2014). The Asia Pacific region has some of the highest incidence rates for leptospirosis since high population densities are potentially a risk factor for leptospirosis (Victoriano et al. 2009). This is not surprising given the frequent climatic calamities, overcrowding, poor sanitation, proximity of domestic and wild animals, and occupational risks. In Sri Lanka the annual incidence is reported as 54 per million, in Thailand the annual incidence is estimated to be 48.9 per million, and Taiwan the incidence has been reported at 4.1 per million (Pappas et al. 2008). In French Polynesia, the average incidence is 39 per 100,000 and in New Caledonia, the average incidence is 45 per 100,000 (Picardeau 2013). In Australia, the annual

incidence is 8.9 cases per million. Leptospirosis is endemic in the Caribbean islands and in many parts of Central and Southern America. Pappas et al. (2008) reported that the incidence in Trinidad and Tobago is 120.4 per million, Barbados 100.3 per million, and Jamaica 78 per million. In El Salvador, Brazil, and Argentina the incidence is 35.8, 12.8, and 9.5 per million, respectively. Given the lack of reporting in many developing areas, misdiagnosis, lack of awareness, patients failing to present for treatment, and those with subclinical infections, it is almost impossible to determine the true incidence. The source of infection in humans is usually through either direct or indirect contact with the urine of an infected animal. Further, the usual portal of entry is via compromised cutaneous or mucosal membranes (Levett 2001). Occupation is a significant risk factor as dairy and cattle farmers, veterinarians, abattoir workers, meat inspectors, rodent control workers, and other occupations where intermit contact with animals is required, all have a greater chance of direct contact with the urine of infected animals. Occupations that bring humans into indirect contact with animal urine are also at risk of infection, e.g., sewer workers, miners, soldiers, septic tank cleaners, fish farmers, gamekeepers, canal workers, rice field workers, taro farmers, banana farmers, and sugar cane workers (Faine et al. 1999; Levett 2001; Tulsiani et al. 2011). Recreational activities while traveling are also considered a risk factor for the disease (Lau et al. 2010b).

42.4 Evidence of Human-to-Human Transmission

Currently, reported evidence of human-to-human transmission is scarce. Adler and de la Peña Moctezuma (2010), p. 289) submit that "human to human transmission for practical purposes is non-existent and that leptospirosis is recognised globally as a zoonosis." However, diagnosis of such transmission has been suggested serologically. Bolin and Koellner (1988) reported the case of a 29-year-old breastfeeding mother who worked as a veterinarian and had a confirmed *L. interrogans* serovar Hardjo infection. The mother continued to breastfeed during her illness and 21 days post-onset of symptoms, the infant displayed clinical signs consistent with leptospirosis. A positive result was confirmed by the microscopic agglutination test (MAT). In another report detailing possible human-to-human transmission, Harrison and Fitzgerald (1988) discussed the possible sexual transmission of *L. interrogans* serovar Icterohaemorrhagie. The diagnosis of this condition was also confirmed serologically by MAT.

42.5 Pathogenesis

The minimum infecting dose leading to leptospirosis is elusive; however, the incubation period is assumed to be inversely correlated with the size of the inoculum. For example, a high infecting dose may engender a short incubation period when compared to a low infecting dose. Conversely, small doses may result in prolonged

incubation times which may extend into the immune phase. It is anticipated that these small infecting doses might be responsible for mild or even subclinical infection (Faine et al. 1999). Once in the blood, leptospires are capable of circulating to all tissues. Leptospires that evade phagocytic cells of the recticuloendothelial system grow in an exponential manner doubling every eight hours (Faine et al. 1999). There is evidence to suggest that phagocytosed leptospires do not survive long within the interior of the phagocyte (Tu et al. 1982; Wang et al. 1984). Virulent strains have the ability to attenuate phagocytic responses by activating apoptosis in the macrophage (Merien et al. 1998). Moreover, Adler and de la Peña Moctezuma (2010) write that the ability to resist complement and death by neutrophilic destruction may be a feature of virulent leptospires in nonimmune hosts. A number of leptospiral virulence factors such as hemolysins, fibronectin binding proteins, and numerous surface proteins such as LipL32, Lig A, Lig B, lipoprotein Loa22, and the 6 Len proteins (LenABCDEF) are postulated to play a role in pathogenesis (Adler and de la Peña Moctezuma 2010; Bulach et al. 2006; Hoke et al. 2008; Matsunaga et al. 2003; Merien et al. 2000; Picardeau et al. 2008; Ristow et al. 2007; Stevenson et al. 2007). *L. interrogans* catalase KatE and HtpG (high-temperature protein G is the bacterial homolog to the highly conserved molecular chaperone Hsp90) and CLpB, an ATP-dependent disaggregase (Lourdault et al. 2011; Kędzierska-Mieszkowska and Arent 2020), have also been shown to be virulence factors in leptospires (Eshghi et al. 2012; King et al. 2014) as have several flagellar components (Lambert et al. 2012; Wunder et al. 2016), phospholipase C (Zhao et al. 2013) and collenganse A (Kassegne et al. 2014).

42.6 Clinical Presentation

Following the initial incubation period which can be as short as 3 days or as long as one month (Haake and Levett 2015), the infection enters the acute phase of the disease which can last up to 10 days (Tulsiani et al. 2011). Clinically, during the acute phase, patients typically present with headache, fever, excruciating myalgia, and arthralgia and sometimes rigors, vomiting, photophobia, and a mucosal rash (Faine et al. 1999). Conjunctival suffusion has utility as a diagnostic indicator as it is commonly reported in leptospirosis (Haake and Levett 2015). Hemoptysis, hypotension, and bradycardia are also common presentations. These symptoms are considered nonspecific, thereby making the diagnosis of leptospirosis difficult. Hepatosplenomegaly, jaundice, liver failure, renal failure, and acute respiratory distress are common features of the more acute form of the disease (Sutliff et al. 1953; Solbrig et al. 1987; Faine et al. 1999; Levett 2001). Central to the pathology observed in leptospirosis is the damage caused to the endothelium of small blood vessels. This engenders ischemia in target organs, thus resulting in renal, hepatic, and pulmonary damage and thrombocytopenia. Levett (2001) posits that many of the clinical complications occur during the immune phase when the patient produces immunoglobulins, specific for the destruction of leptospires and the leptospires localize within tissues.

42.7 CNS Involvement

Before the central tenants of this chapter are explored it is appropriate to orientate the reader to the subject matter with a brief overview of CNS involvement in leptospirosis. In their review of 483 cases of leptospirosis, Heath et al. (1965) reported that of all the organ systems, the CNS was the most frequent system involved in leptospirosis. Heath et al. (1965) observed CNS complications such as CSF pleocytosis, meningismus, CSF protein elevation, or mental disturbance in 235 (68%) of their 483 cases. Leptospires reach the CNS rapidly and Mumford et al. (1990) posit that the neurological derangements may be due to the effects of the organism and the hosts immune reaction to the leptospires. Here Mumford et al. (1990) are noting that CNS pathology may occur in the acute and immune phases of leptospirosis. During the acute/leptospiremic phase, patients may endure an intense, analgesic-resistant headache. While the pain is mostly frontal, bitemporal or occipital pain may be reported and may persist into the immune phase (Reik 1987). Leptospires may be isolated in CSF during the acute phase, in which the CSF will usually be otherwise unremarkable and any signs of meningeal irritation are the exception rather than the norm. Reik (1987) posits that while 25% of cases may exhibit cerebral symptoms in the acute phase, these symptoms are mild and transient as are cranial nerve and peripheral nerve neuropathies experienced during this time.

The CNS derangements observed in the immune phase add to the rich tapestry in the fabric of the pathology observed in leptospirosis. Faine et al. (1999) posits that aseptic meningitis is a common complication in the immune phase. Headaches, vomiting, photophobia, neck stiffness, confusion, and delirium may emerge during this time. The CSF in the immune phase reveals a pleocytosis in 80–90% of cases with a transient granulocytosis giving way to a lymphocyte predominance. Hemorrhagic CNS complications may be diverse and multifocal with bleeding in cerebral subarachnoid, subdural, intraparenchymal, and spinal extradural regions (Reik 1987). Cranial nerve neuropathies with optic, oculomotor, glossopharyngeal, facial, or auditory nerve involvement have been reported (Gsell 1978) as have peripheral nerve abnormalities engendering brachial and lumbosacral plexitis and Guillain-Barré syndrome (Faine et al. 1999; Reik 1987). Recovery from these cranial and peripheral neuropathies may take weeks or months and in some instances recovery may be incomplete (Gsell 1978).

At autopsy there are diverse pathologic changes observed both macroscopically and microscopically in those who have succumbed to leptospirosis. While Edwards and Domm (1960) suggest that changes in the brain and meninges are minimal and nonspecific, Koppisch and Bond (1953, as cited in Gsell 1978) reported that macroscopically, narrow sulci, flattened gyri, and a yellow or greenish subarachnoid fluid and choroid plexis can be observed in some but not all instances. Arean (1962) examined the brains of 13 leptospirosis patients and posited the brain was slightly green and the convolutions were edematous and the sulci narrow. Reik (1987) posits that pathological changes in the CNS during leptospirosis include perivascular lymphocytic infiltration in the brain and spinal cord, loss of cerebellar granular cells, patchy demyelination in the cortex and pons as well as gliosis. Moreover,

Gsell (1978) posits glial cell proliferation, variable perivasal lymphocytic inflammation, and purpural hemorrhages may underpin the encephalitic process.

42.8 Neurological Presentation in Leptospirosis (Neuroleptospirosis)

The World Health Organization (2003) confirms that the clinical manifestations of leptospirosis are highly variable and the disease may present as either a mild, influenza-like illness; Weil's syndrome characterized by jaundice and renal failure; hemorrhage and myocarditis with arrhythmias; meningitis/meningoencephalitis, or pulmonary hemorrhage with respiratory failure. Panicker et al. (2001) reported on 40 patients who were admitted to hospital with an acute neurological disease, and who were subsequently found to have leptospirosis. While 13 of these 40 patients presented with headache, fever, neck stiffness, and a CSF consistent with an aseptic meningitis, 17 of the 40 patients presented with a primary paraparesis. Recent reports of patients presenting with a primary acute neurological disease with an absence of, or very few leptospiral symptoms are presented in Table 1. Inspection of Table 1 shows that the neurological presentation of the six patients is quite variable. Headache, stiff neck, and vomiting are consistent with an aseptic meningitis seen in the CSF as an elevated white blood cell count with elevated protein and glucose. In the peripheral blood a neutrophilic leucocytosis may be observed with elevated liver function tests and serum creatinine in some instances. Radiologically, X-ray and ultrasound appear to be of limited utility as they present normal findings. T2-weighted MRI showing hyperintense signals in different CNS regions may be clinically useful, however this technology may not have the specificity to differentiate CNS pathology due to a leptospiral etiology from other microbial etiologies. Physicians working in areas where the incidence of leptospirosis is high should remain cognizant of primary neuroleptospirosis as this initial presentation is uncommon. Further, in the absence of hepatic or renal involvement, a diagnosis of leptospirosis is likely to be delayed. This delay may prevent the most appropriate treatment, therefore reducing positive outcomes.

42.9 Psychosis During Leptospirosis

Watson et al. (2021, p. 1) succinctly summarize psychosis as "a highly disruptive syndrome with many aetiologies characterised primarily by delusions, hallucinations, and disorganised thought, speech and behaviour." Psychosis during an episode of leptospirosis was reported in the work of early pioneers in leptospirology (Hecker and Otto 1911, as cited in Gsell 1978; Trembur and Schallert 1916, as cited in Gsell 1978). Recent accounts of psychosis during an episode of leptospirosis are presented in Table 2. Inspection of Table 2 reveals that most case studies have reported psychosis in the immune phase of leptospirosis. The one exception was the latest case series by Semiz et al. (2005); however, while leptospires were identified in their

Table 1 Recent reports of neuroleptospirosis

Publication	Neurological symptoms	CSF	Other pathology (Blood) Radiology	Outcome
Mumford et al. (1990)	Increasing leg weakness and pain in lower limbs. Flaccid paraplegia and areflexia	Not Reported	Neutrophilic leucocytosis, thrombocytopenia, deranged LFTs Abdominal/Liver ultrasound normal Chest, abdominal, and spinal X-ray normal Nerve conduction studies normal	Succumbed
Wang et al. (2016)[a]	Neck stiffness, vomiting, and headache	WBC of 254 cells/μL 91% lymho/ mononuclear Protein = 91 mg/dl Glucose = 64 mg/dl	Hemoglobin 15.7 g/ dL Platelet count was 338,000 cells/μL WBC count was 18,670 cells/μL 81.2% segmented neutrophils creatinine 1.03 mg/ dl, AST 34 IU/L	Recovered
Bhatt et al. (2018)	Diffuse headache, decreased responsiveness to commands with decreased verbal output and inability to move limbs. Constricted pupils, nonreactive to light, neck rigidity, positive Brudzinski and Kernig's signs	WBC = 115/mm^3 Lymphocytic predominance Glucose = 60 mg/dL Protein = 53 mg/dL	MRI hyperintensity in dorsal midbrain and area surrounding the anterior commissure	Recovered
Tomacruz et al. (2019)	Altered sensorium, behavioral changes, agitated and disoriented to person, time, and place, and seizures	WBC = 5 cells/μL lymphocytic predominance Protein = 60 mg/dL Glucose = 74.55 mg/ dL	WBC = 16×10^9/L Elevated LFTs and serum creatinine Hyperintense signals located at the bilateral, frontal subcortical white matter and left external capsule	Recovered
Bandara et al. (2021)[b]	Patient 1 Headache, photophobia, and	WBC = 166 cells/μL Polymorphs 96% Protein = 52.4 mg/dL	WBC = 14×10^3/μ L (Neutrophilia) CRP = 238 mg/dL	Recovered

(continued)

Table 1 (continued)

Publication	Neurological symptoms	CSF	Other pathology (Blood) Radiology	Outcome
	vomiting and neck stiffness	Glucose = 83.6 mg/dL	Elevated LFTs and serum creatinine CT of brain – normal Ultrasound abdomen – normal	
	Patient 2 Headache, photophobia, and vomiting and neck stiffness	WBC = 64 cells/μL Polymorphs 13% Protein = 39 mg/dL Glucose = 72.9 mg/dL	WBC = 26×10^3/μL (Neutrophilia) CRP = 14 mg/dL Normal or slight elevation on LFTs Serum creatinine normal CT of brain – normal CT of chest – normal	Recovered

[a]Infecting serovar = *L. santarosai* serovar *Shermanii*
[b]Infecting serovar = *L. borgpeterenii* serovar *Tarassovi* for both patient 1 and patient 2
WBC white blood cell (count); *LFT's* liver function tests; *CRP* C-reactive protein; *MRI* magnetic resonance imaging; *CT* computed tomography

patients' blood cultures, they were also IgM reactive. Therefore, it is likely these patients were in the late acute/early immune phase. This would also be consistent with the mixed microscopic agglutination test results. Table 2 also reveals that there is no specific serovar engendering the observed psychosis, as psychosis was observed in patients infected with different serovars. Unfortunately, the majority of reported cases do not mention the infecting serovar.

While psychiatric symptoms may result from direct invasion of the central nervous system by an infectious agent, a number of lines of evidence point toward an immune-based response providing the genesis for psychosis in leptospirosis. Firstly, there is a greater risk of developing systemic autoimmune diseases following an episode of leptospirosis (Teh et al. 2020). Moreover, recently, a 13-year-old female developed neuropsychiatric and extrapyramidal features and sleep disturbances (but no definite hallucinations, illusion, or delusion) following an episode of leptospirosis. The patient was shown to produce anti-N-methyl-D-aspartate receptor (anti-NMDAR) auto-antibodies and was started on intravenous methylprednisolone and intravenous immunoglobulin. The patient showed gradual improvement over one week following immunotherapy. Furthermore, the extrapyramidal and neuropsychiatric features completely resolved within 2 weeks. Interestingly, Pollak et al. (2020) delineate numerous CNS auto-antibodies that may provide the genesis for numerous psychiatric states and report that the main psychiatric symptoms associated with anti-NMDAR antibodies include anxiety, agitation, bizarre behavior, catatonia, delusional or paranoid thoughts, visual or auditory hallucinations,

Table 2 Reports of psychosis during an episode of leptospirosis

Author	N	Phase	Serovar	Titre	Presentation/Symptoms
Bouman (1935)	2	Immune	Not reported	Patient 1. 1:3000	Agitated and walked atactically, constantly moving arms, stealing, had visual disturbances, and acts of violence. Scared and thought people were threatening him and his condition would not improve. Though he was a sexual deviant. Later believed that people had negative thoughts of him. Believed his illness was due to a political and medical conspiracy
		Immune	Not reported	Patient 2. 1: 100000	Restless, conversant but disorientated and though people were talking about him. Believed he was bad for his wife, and people wanted to kill him. He was mute, anxious, and had eating disturbances. Could be easily agitated and was at times aggressive
Edwards and Domm (1960)	1	Late acute early immune	Canicola	Patient. 1: 3200	Refused to take medication, ran down hallway screaming, escaped from the hospital, and assaulted a restaurant attendant
Avery (1976a)*	1	Late acute	Hardjo (prijitno/ bovis?)	1:12800	Paranoia, believed a bad spirit had entered him. Hebephrenic though disorder with poor association of though, Communication, and staturing. Had bodily hallucinations
Avery (1976b)* same patient	1	Immune	Not reported	Not reported	Psychotic symptoms – no further delineation of the symptoms
Marshall and Scrimgeour (1978)	1	Immune	Tarassovi	1:1500	Schizophreniform psychosis – limited delineation of symptoms auditory hallucinations, confused meaningless speech, and disorientated in time and space
Ram and Karim (1981)	2	Indeterminable	Pomona	Patient 1 not reported	Patient 1: Numerous visual hallucinations and delusions of people wanting to harm him
		Indeterminable	Not reported	Patient 2 not reported but reactive	Patient 2: Numerous visual hallucinations and delusions of people wanting to harm him, labile mood, apprehensive, and disorientated in time and space
Semiz et al. (2005)	4	Late acute/ (blood culture +)	Not reported	Patient 1 not reported	Patient 1: Increased psychomotor activity, concentration difficulties, insomnia, grandiosity, and flight of ideas.

(continued)

Table 2 (continued)

Author	N	Phase	Serovar	Titre	Presentation/Symptoms
		Early immune (IgM reactive)			EEG. Abnormalities from right temporal lobe. MRI revealed gliosis in bilateral thalamus and brachium pontis. Brief Psychiatric Rating Scale (BPRS) score of 20 and Young Mania Rating Scale (YMRS) score of 35
		Late acute/ (blood culture +) Early immune (IgM reactive)	Not reported	Patient 2 not reported	Patient 2: Aggressive behavior, nihilistic delusions, lack of insight, slowing of though and psychomotor abilities Scale for the Assessment of Negative Symptoms (SANS) = 59 Scale for the Assessment of Positive Symptoms (SAPS) = 41 BPRS = 32
		Late acute/ (blood culture +) Early immune (IgM reactive)	Not reported	Patient 3 not reported	Patient 3: Insomnia, agitated, and aggressive behavior Persecutory and grandiose delusions, flight of ideas, euphoria Distractibility, and increased psychomotor activity SANS = 22, SAPS = 43, BPRS = 35, and YMRS = 40
		Late acute/ (blood culture +) Early immune (IgM reactive)	Not reported	Patient 4 not reported	Patient 4: referential, persecutory, and influence delusions affective blunting, decreased psychomotor activity, flow of thought, and self-care. SANS = 107, SAPS = 15, and BPRS = 44

movement disorder, and seizures. Moreover, recent animal studies using a mouse model have reported that mice infused with CSF from patients with anti-NMDAR encephalitis displayed reversible psychotic-like features and dopamine receptor changes in cell surface dopamine receptors (Carceles-Cordon et al. 2020). In total the evidence presented here suggests the generation of anti-NMDAR auto-antibodies may be germane to psychiatric disturbance in leptospirosis.

Pollak et al. (2020) delineate further immune mechanisms that may be associated with psychosis. For example, Pollak et al. (2020) postulate that raised CRP, IL-6, TNF-α, and several other cytokines are increased in patients with psychosis compared with healthy controls and may qualify as biomarkers for psychosis. This is of interest to the association of leptospirosis and psychosis as IL-6 and TNF-α may be elevated in leptospirosis (Levett 2001; Haake and Levett 2015). Pollak et al. (2020) provide further avenues of immunological underpinnings associated with psychosis when they posit that there is evidence of increased microglial density and activation

as well as lymphocyte infiltration occurring in approximately 20% of brains of individuals who had schizophrenia. This latter point is of interest given psychosis is common in schizophrenia and the observed CNS pathology outlined in the previous section reporting glial cell proliferation (Gsell 1978) and perivascular lymphocytic infiltration in the brain and spinal cord are pathological features of leptospirosis.

42.10 Acute Disseminated Encephalomyelitis (ADEM) Following a Leptospiral Infection

Tenembaum (2013) posits that acute disseminated encephalomyelitis (ADEM) is an immune-mediated inflammatory demyelinating disease effecting the brain and spinal cord that can follow vaccination or systematic infection. Fever, malaise, headache, nausea, and vomiting may precede the altered consciousness and neurological symptoms. ADEM is rapidly progressive and while a full recovery is observed in most of patients, a minority of patients show residual neurological deficits even after 6 months. Tenembaum (2013) also highlights how life-threating ADEM can be with reports of respiratory failure secondary to brainstem involvement or severe impaired consciousness occurring in 11–16% of cases. Moreover, a case control study revealed neurocognitive deficits in attention, executive function, IQ, and educational achievement in children following an ADEM diagnosis before five years of age (Jacobs et al. 2004). ADEM may also be a risk for a future diagnosis of multiple sclerosis in a minority of patients (Hintzen et al. 2016). A complete understanding of the pathogenesis underpinning ADEM eludes the literature; however, an autoimmune etiology predominates with molecular mimicry, inflammatory cascades, and associations with some major histocompatibility complex (MHC) class II alleles all being postulated in the etiology (Tenembaum 2013). Treatment regimens consists of corticosteroids, intravenous immunoglobulin, and plasmapheresis (Pohl and Tenembaum 2012). Diagnosis may require MRI imaging. MRI T2-weighted images typically reveal multiple hyperintense bilateral, asymmetric patchy and poorly marginated lesions. ADEM lesions are typically observed in the subcortical and central white matter, cortical gray-white matter junctions, thalamus, basal ganglia, cerebellum, and brainstem. Spinal cord involvement may also be observed in patients with large spinal cord lesions extending over multiple segments (Pohl et al. 2016). The cerebrospinal fluid (CSF) findings of patients with ADEM are unremarkable. If a pleocytosis is observed it is usually a mild lymphomonocytic pleocytosis, with increased protein and normal glucose levels. Oligoclonal IgG bands are considered transient and a rare phenomenon (Tenembaum 2013).

The clinical presentation, CSF results, and MRI T2 observations of the extant case reports of ADEM following a leptospiral infection are presented in Table 3. Strikingly, there is consistent clinical presentation with all five patients with ADEM following leptospirosis presenting with some form of altered consciousness. The CSF results were consistent across the five case reports. CSF white cell counts were similar in four of the five patients with lymphocytes, the predominate cells, observed

Table 3 Acute disseminated encephalomyelitis (ADEM) following a leptospiral infection

Publication	Neurological symptoms	CSF	MRI	Follow-up
Alonso-Valle et al. (2001)	Confusion and left hemiparesis with facial central palsy	11 cells/mm^3 (90% Lymphocytes) Protein 168 mg/dL Glucose Normal	T2 hyperintense bilateral sub cortical matter lesions and T2 hyperintense unilateral lesion in the left cerebellum	MRI at 1 month resolution
Chandra et al. (2004)	Stuporous, responding only to painful stimuli	10 cells/mm^3 (100% lymphocytes)	T2 hyperintense lesions in the subcortical white matter, corpus callosum, thalamus, basal ganglia, and upper brainstem	MRI at 6 months resolution
Lelis et al. (2009)	Fever, fatigue, seizures, and reduced consciousness	Normal	T2 hyperintense lesions near lateral ventricles, cerebellar peduncle, and left cerebellar lobe	Sequalae at 1 month
Singh et al. (2011)	Altered sensorium, drowsy	10 cells/mm^3 (70% lymphocytes) Protein 81 mg/dL Glucose normal	T2 hyperintense lesions involving subcortical white matter, bilateral internal and external capsules, basal ganglia, corpus callosum, cerebellar hemispheres, pons, and bilateral middle cerebellar peduncles	MRI at 1.5 months resolution
Özbay et al. (2021)	Severe confusion	WBC 20 $\times$ 10^6/L Protein 1620 mg/L Glucose normal	T2 hyperintense lesions involving the thalamus, periventricular white matter, and the corpus callosum and regions distal to the internal capsule	Nil. Patient succumbed

in most instances. Similarly, while CSF protein was elevated, CSF glucose was normal. T2 MRI results also revealed all patients demonstrating hyperintense lesions throughout the CNS. Combined these results are congruent with ADEM (Tenembaum 2013). Complete resolution however was not observed in all the patients. One patient succumbed to the disease and one patient had sequalae after 1-month follow-up. It is uncertain if this patient achieved full resolution.

While randomized controlled studies may not be possible to prove pathogenic leptospires causing ADEM, it is germane to consider if leptospires are causing the immune-mediated manifestations of ADEM or is the development of ADEM following an episode of leptospirosis simply coincidental. This is a reasonable consideration and one analyzed by Ellul et al. (2020) who used Hill's (1965) nine pillars of

causation in their consideration of SARS-Cov2 and the development of neurological disorders. In applying Hill's (1965) criteria to determine causative links between leptospirosis and ADEM it can be argued that six of the nine pillars of causation are supported. Firstly, although case reports are low there is *consistency* with the link in regard to presentation and clinical accounts from different locations around the world (Asia and Europe). The relationship appears *specific* in regard to the pathology observed and neurological manifestations. *Temporality* is observed with the ADEM postinfectious syndrome, due to an adaptive immune response, proceeding not preceding leptospirosis. *Biological plausibility* is supported by the recent observation of CNS-targeted auto-antibodies such as anti-NMDAR antibodies in leptospirosis in addition to the reports of Guillain-Barre syndrome and an increased risk of systemic autoimmune rheumatic diseases following leptospirosis (Levett 2001; Panda et al. 2021; Teh et al. 2020). *Coherence* is perhaps supported by the vast CNS, PNS, cranial nerve and peripheral nerve, and CNS hemorrhagic complications observed in leptospirosis. The development of ADEM does not, in the words of Hill (1965, p. 289), "seriously conflict with the generally known facts of the natural history and biology of the disease" (leptospirosis). Further, *analogy* is supported by the finding that ADEM has been reported following infection with other bacteria such as *Mycoplasma pneumoniae*, *Borrelia burgdorferi*, and beta-hemolytic *Streptococcus* and vaccination against pertussis (Tenembaum,2013). Unequivocally, the limited number of reports (N = 5) of ADEM relative to incidence of leptospirosis in the tropics, estimated to be 10–100 per 100,000 (WHO 2003), undermines Hill's (1965) *experimental*, *strength, and biological gradient* aspects of causation. It should be noted that Hill (1965, p. 299) states that, "none of my nine viewpoints can bring indisputable evidence for or against the cause and-effect hypothesis and none can be required as a *sine qua non*." Evidence in support of *experimental*, *strength, and biological gradient* aspects of causation may change, however, as more cases of ADEM following leptospirosis come to light and/or *in vitro* or animal models are developed.

42.11 Conclusion

Understanding the pathology underpinning the neurological and psychiatric symptoms observed during an episode of leptospirosis is pivotal given that altered mental status is a poor prognostic indictor. The pathology observed in the CNS and PNS are extensive and much of the pathology may be driven by immune-mediated factors. Further case reports and investigations focusing on immune-mediated factors such as anti-NMDAR auto-antibodies are necessary to advance our understanding of the underlying pathological process in leptospirosis.

Acknowledgements We extend our thanks to the dedicated efforts of Sonia Johnson, Carol Church, and Trish Murphy and the rest of the Public and Environmental Health library staff, who were able to source the many obscure, dated journal articles reviewed in this work. We cannot express how thankful we are to you for the highest level of librarianship you provided.

References

Adler B (2015) Leptospira and leptospirosis. Springer, Dordrecht

Adler B, de la Peña Moctezuma A (2010) *Leptospira* and leptospirosis. Vet Microbiol 140(3–4):287–296

Alonso-Valle H, Muñoz R, Hernández JL, Matorras P (2001) Acute disseminated encephalomyelitis following *Leptospira* infection. Eur Neurol 46(2):104–105. https://doi.org/10.1159/000050775

Arean VM (1962) The pathologic anatomy and pathogenesis of fatal human leptospirosis (Weil's disease). Am J Pathol 40(4):393–423

Avery TL (1976a) Letter: Leptospirosis hardjo. N Z Med J 83(560):208

Avery TL (1976b) Leptospirosis hardjo. N Z Med J 83(563):336

Bandara AGNMK, Kalaivarny G, Perera N, Indrakumar J (2021) Aseptic meningitis as the initial presentation of *Leptospira borgpetersenii* serovar Tarassovi: two case reports and a literature review. BMC Infect Dis 21(1):488. https://doi.org/10.1186/s12879-021-06200-w

Bhatt M, Rastogi N, Soneja M, Biswas A (2018) Uncommon manifestation of leptospirosis: a diagnostic challenge. BMJ Case Rep 2018:bcr2018225281. https://doi.org/10.1136/bcr-2018-225281

Bolin CA, Koellner P (1988) Human-to-human transmission of Leptospira interrogans by milk. J Infect Dis 158(1):246–247. https://doi.org/10.1093/infdis/158.1.246

Bouman L (1935) Psychische stoornissan bij de ziekte van Weil. Nederlandsche Tijdschrift voor Geneeskunde 97:1078–1084

Bulach DM, Zuerner RL, Wilson P, Seemann T, McGrath A, Cullen PA, Davis J, Johnson M, Kuczek E, Alt DP, Peterson-Burch B, Coppel RL, Rood JI, Davies JK, Adler B (2006) Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. Proc Natl Acad Sci U S A 103(39):14560–14565. https://doi.org/10.1073/pnas.0603979103

Carceles-Cordon M, Mannara F, Aguilar E, Castellanos A, Planagumà J, Dalmau J (2020) NMDAR antibodies alter dopamine receptors and cause psychotic behavior in mice. Ann Neurol 88(3):603–613. https://doi.org/10.1002/ana.25829

Casanovas-Massana A, Hamond C, Santos LA, de Oliveira D, Hacker KP, Balassiano I, Costa F, Medeiros MA, Reis MG, Ko AI, Wunder EA (2020) *Leptospira yasudae* sp. nov. and *Leptospira stimsonii* sp. nov., two new species of the pathogenic group isolated from environmental sources. Int J Syst Evol Microbiol 70(3):1450–1456. https://doi.org/10.1099/ijsem.0.003480

Chandra SR, Kalpana D, Anilkumar TV, Kabeer KA, Chithra P, Bhaskaran R (2004) Acute disseminated encephalomyelitis following leptospirosis. J Assoc Physicians India 52:327–329

Craig SB, Wynwood SJ, Collet TA, Weier SL, McKay DB (2015) Leptospirosis and Leptospires – the silent Assassins. In: Zoonoses-infections affecting humans and animals. Springer, Dordrecht, pp 849–858

Dupouey J, Faucher B, Edouard S, Richet H, Kodjo A, Drancourt M, Davoust B (2014) Human leptospirosis: an emerging risk in Europe? Comp Immunol Microbiol Infect Dis 37(2):77–83. https://doi.org/10.1016/j.cimid.2013.12.002

Edwards GA, Domm BM (1960) Human leptospirosis. Medicine 39:117–156. https://doi.org/10.1097/00005792-196002000-00003

Ellis WA (1990) Leptospirosis-A review of veterinary aspects. Irish Vet News 12:6–12

Ellul M, Varatharaj A, Nicholson TR, Pollak TA, Thomas N, Easton A, Zandi MS, Manji H, Solomon T, Carson A, Turner MR, Kneen R, Galea I, Pett S, Thomas RH, Michael BD, CoroNerve Steering Committee (2020) Defining causality in COVID-19 and neurological disorders. J Neurol Neurosurg Psychiatry 91(8):811–812. https://doi.org/10.1136/jnnp-2020-323667

Eshghi A, Lourdault K, Murray GL, Bartpho T, Sermswan RW, Picardeau M, Adler B, Snarr B, Zuerner RL, Cameron CE (2012) *Leptospira interrogans* catalase is required for resistance to H_2O_2 and for virulence. Infect Immun 80(11):3892–3899. https://doi.org/10.1128/IAI.00466-12

Everard JD, Everard COR (1993) Leptospirosis in the Caribbean. Rev Med Microbiol 4(2):114

Faine S, Adler B, Bolin C, Perolat P (1999) *Leptospira* and leptospirosis, 2nd edn. MedSci, Melbourne

Gsell OR (1978) Leptospirosis and relapsing fever. In: Handbook of Clinical Neurology. Elsevier, Amsterdam, pp 395–419

Haake DA, Levett PN (2015) Leptospirosis in humans. Curr Top Microbiol Immunol 387:65–97. https://doi.org/10.1007/978-3-662-45059-8_5

Harrison NA, Fitzgerald WR (1988) Leptospirosis: Can it be a sexually transmitted disease? Postgrad Med J 64(748):163–164. https://doi.org/10.1136/pgmj.64.748.163

Hartskeerl RA (2006) Leptospirosis: current status and future trends. Indian J Med Microbiol 24(4): 309. https://doi.org/10.4103/0255-0857.29404

Heath CW Jr, Alexander AD, Galton MM (1965) Leptospirosis in the United States. Analysis of 483 cases in man, 1949, 1961. N Engl J Med 273(17):857–864. https://doi.org/10.1056/NEJM196510212731706

Hill AB (1965) The environment and disease: association or causation? Proc R Soc Med 58(5):295–300

Hintzen RQ, Dale RC, Neuteboom RF, Mar S, Banwell B (2016) Pediatric acquired CNS demyelinating syndromes: features associated with multiple sclerosis. Neurology 87(9 Suppl 2):S67–S73. https://doi.org/10.1212/WNL.0000000000002881

Hoke DE, Egan S, Cullen PA, Adler B (2008) LipL32 is an extracellular matrix-interacting protein of *Leptospira* spp. and *Pseudoalteromonas tunicata*. Infect Immun 76(5):2063–2069. https://doi.org/10.1128/IAI.01643-07

Jacobs RK, Anderson VA, Neale JL, Shield LK, Kornberg AJ (2004) Neuropsychological outcome after acute disseminated encephalomyelitis: impact of age at illness onset. Pediatr Neurol 31(3): 191–197. https://doi.org/10.1016/j.pediatrneurol.2004.03.008

Kassegne K, Hu W, Ojcius DM, Sun D, Ge Y, Zhao J, Yang XF, Li L, Yan J (2014) Identification of collagenase as a critical virulence factor for invasiveness and transmission of pathogenic *Leptospira* species. J Infect Dis 209(7):1105–1115. https://doi.org/10.1093/infdis/jit659

Kędzierska-Mieszkowska S, Arent Z (2020) AAA+ molecular Chaperone ClpB in *Leptospira interrogans*: its role and significance in Leptospiral virulence and pathogenesis of Leptospirosis. Int J Mol Sci 21(18):6645. https://doi.org/10.3390/ijms21186645

King AM, Pretre G, Bartpho T, Sermswan RW, Toma C, Suzuki T, Eshghi A, Picardeau M, Adler B, Murray GL (2014) High-temperature protein G is an essential virulence factor of *Leptospira interrogans*. Infect Immun 82(3):1123–1131. https://doi.org/10.1128/IAI.01546-13

Ko A, Goarant C, Picardeau M (2009) *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol 7:736–747

Koppisch E, Bond WM (1953) The morbid anatomy of human leptospirosis: a report of thirteen fatal cases. In: Symposium on Leptospirosis-Medical Science Publications, Government Printing Office, Washington, DC, pp 83–105

Lambert A, Picardeau M, Haake DA, Sermswan RW, Srikram A, Adler B, Murray GA (2012) FlaA proteins in *Leptospira interrogans* are essential for motility and virulence but are not required for formation of the flagellum sheath. Infect Immun 80(6):2019–2025. https://doi.org/10.1128/IAI.00131-12

Lau CL, Smythe LD, Craig SB, Weinstein P (2010a) Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Trans R Soc Trop Med Hyg 104:631–638

Lau CL, Smythe LD, Weinstein P (2010b) Leptospirosis and emerging disease in travellers. Travel Med Infect Dis 8:33–39

Lelis SS, Fonseca LF, Xavier CC, Horta MB, Cordeiro SS (2009) Acute disseminated encephalomyelitis after leptospirosis. Pediatr Neurol 40(6):471–473. https://doi.org/10.1016/j.pediatrneurol.2008.12.006

Levett PN (2001) Leptospirosis. Clin Microbiol Rev 14(2):296–326. https://doi.org/10.1128/CMR.14.2.296-326.2001

Levett PN, Haake DA (2010) *Leptospira* species (leptospirosis). In: Principles and practice of infectious diseases. Churchill Livingtsone Elsevier, Philadelphia, pp 3059–3065

Lourdault K, Cerqueira GM, Wunder EA Jr, Picardeau M (2011) Inactivation of *clpB* in the pathogen *Leptospira interrogans* reduces virulence and resistance to stress conditions. Infect Immun 79(9):3711–3717. https://doi.org/10.1128/IAI.05168-11

Marshall RB, Scrimgeour G (1978) Schizophreniform psychosis associated with leptospirosis. N Z Med J 88(619):212–213

Matsunaga J, Barocchi MA, Croda J, Young TA, Sanchez Y, Siqueira I, Bolin CA, Reis MG, Riley LW, Haake DA, Ko AI (2003) Pathogenic *Leptospira* species express surface-exposed proteins belonging to the bacterial immunoglobulin superfamily. Mol Microbiol 49(4):929–945. https://doi.org/10.1046/j.1365-2958.2003.03619.x

Merien F, Truccolo J, Rougier Y, Baranton G, Perolat P (1998) In vivo apoptosis of hepatocytes in guinea pigs infected with *Leptospira interrogans* serovar icterohaemorrhagiae. FEMS Microbiol Lett 169(1):95–102. https://doi.org/10.1111/j.1574-6968.1998.tb13304.x

Merien F, Truccolo J, Baranton G, Perolat P (2000) Identification of a 36-kDa fibronectin-binding protein expressed by a virulent variant of Leptospira interrogans serovar icterohaemorrhagiae. FEMS Microbiol Lett 185(1):17–22. https://doi.org/10.1111/j.1574-6968.2000.tb09034.x

Mumford C, Dudley N, Terry H (1990) Leptospirosis presenting as a flaccid paraplegia. Postgrad Med J 66(773):218–220. https://doi.org/10.1136/pgmj.66.773.218

Özbay Y, Brussee P, van der Hoeven JG (2021) *Leptospira*-induced acute disseminated encephalomyelitis. Netherlands J Crit Care, p 31

Panda PK, Sharawat IK, Bolia R (2021) *Leptospira* triggered Anti-N-methyl-d-aspartate receptor encephalitis. J Trop Pediatr 67(1):fmaa067. https://doi.org/10.1093/tropej/fmaa067

Panicker JN, Mammachan R, Jayakumar RV (2001) Primary neuroleptospirosis. Postgrad Med J 77 (911):589–590. https://doi.org/10.1136/pmj.77.911.589

Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N (2008) The globalization of leptospirosis: worldwide incidence trends. Int J Infect Dis 12(4):351–357. https://doi.org/10.1016/j.ijid.2007.09.011

Picardeau M (2013) Diagnosis and epidemiology of leptospirosis. Medecine et Maladies Infectieuses 43(1):1–9. https://doi.org/10.1016/j.medmal.2012.11.005

Picardeau M, Bulach DM, Bouchier C, Zuerner RL, Zidane N, Wilson PJ, Creno S, Kuczek ES, Bommezzadri S, Davis JC, McGrath A, Johnson MJ, Boursaux-Eude C, Seemann T, Rouy Z, Coppel RL, Rood JI, Lajus A, Davies JK, Médigue C, Adler B (2008) Genome sequence of the saprophyte *Leptospira biflexa* provides insights into the evolution of *Leptospira* and the pathogenesis of leptospirosis. PLoS One 3(2):e1607. https://doi.org/10.1371/journal.pone.0001607

Pohl D, Tenembaum S (2012) Treatment of acute disseminated encephalomyelitis. Curr Treat Options Neurol 14(3):264–275. https://doi.org/10.1007/s11940-012-0170-0

Pohl D, Alper G, Van Haren K, Kornberg AJ, Lucchinetti CF, Tenembaum S, Belman AL (2016) Acute disseminated encephalomyelitis: updates on an inflammatory CNS syndrome. Neurology 87(9 Suppl 2):S38–S45. https://doi.org/10.1212/WNL.0000000000002825

Pollak TA, Lennox BR, Müller S, Benros ME, Prüss H, Tebartz van Elst L, Klein H, Steiner J, Frodl T, Bogerts B, Tian L, Groc L, Hasan A, Baune BT, Endres D, Haroon E, Yolken R, Benedetti F, Halaris A, Meyer JH, Bechter K (2020) Autoimmune psychosis: an international consensus on an approach to the diagnosis and management of psychosis of suspected autoimmune origin. Lancet Psychiatry 7(1):93–108. https://doi.org/10.1016/S2215-0366(19)30290-1

Ram P, Karim I (1981) Severe psychiatric disturbance in leptospirosis. Fiji Med J:115–117

Reik L Jr (1987) Spirochaetal infections of the nervous system, Leptospirosis. In: Kennedy PGE, Johnson RT (eds) Infection of the nervous system. Butterworths, London, pp 63–68

Ristow P, Bourhy P, da Cruz McBride FW, Figueira CP, Huerre M, Ave P, Girons IS, Ko AI, Picardeau M (2007) The OmpA-like protein Loa22 is essential for leptospiral virulence. PLoS Pathog 3(7):e97. https://doi.org/10.1371/journal.ppat.0030097

Semiz UB, Turhan V, Basoglu C, Oner O, Ebrinc S, Cetin M (2005) Leptospirosis presenting with mania and psychosis: four consecutive cases seen in a military hospital in Turkey. Int J Psychiatry Med 35(3):299–305. https://doi.org/10.2190/0KUX-JE1J-HV6K-3R9J

Singh P, Gupta A, Saggar K, Kaur M (2011) Acute disseminated encephalomyelitis subsequent to *Leptospira* infection. Ann Trop Med Public Health 4(2):133–133

Solbrig MV, Sher JH, Kula RW (1987) Rhabdomyolysis in leptospirosis (Weil's disease). J Infect Dis 156(4):692–693. https://doi.org/10.1093/infdis/156.4.692

Stevenson B, Choy HA, Pinne M, Rotondi ML, Miller MC, Demoll E, Kraiczy P, Cooley AE, Creamer TP, Suchard MA, Brissette CA, Verma A, Haake DA (2007) *Leptospira interrogans* endostatin-like outer membrane proteins bind host fibronectin, laminin and regulators of complement. PLoS One 2(11):e1188. https://doi.org/10.1371/journal.pone.0001188

Sutliff WD, Shepard R, Dunham WB (1953) Acute *Leptospira pomona* arthritis and myocarditis. Ann Intern Med 39(1):134–140. https://doi.org/10.7326/0003-4819-39-1-134

Teh SH, You RI, Yang YC, Hsu CY, Pang CY (2020) A cohort study: the association between autoimmune disorders and Leptospirosis. Sci Rep 10(1):3276. https://doi.org/10.1038/s41598-020-60267-0

Tenembaum SN (2013) Acute disseminated encephalomyelitis. Handb Clin Neurol 112:1253–1262. https://doi.org/10.1016/B978-0-444-52910-7.00048-9

Tomacruz ID, Sandejas JC, Berba R, Sacdalan DR (2019) Behavioural change: a rare presentation of leptospirosis. BMJ Case Rep 12(8):e230619. https://doi.org/10.1136/bcr-2019-230619

Tu V, Adler B, Faine S (1982) The role of macrophages in the protection of mice against leptospirosis: in vitro and in vivo studies. Pathology 14(4):463–468. https://doi.org/10.3109/00313028209092128

Tulsiani SM, Lau CL, Graham GC, Van Den Hurk AF, Jansen CC, Smythe LD, McKay DB, Craig SB (2011) Emerging tropical diseases in Australia. Part 1. Leptospirosis. Ann Trop Med Parasitol 104(7):543–556. https://doi.org/10.1179/136485910X12851868779867

Victoriano AF, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, Limpakarnjanarat K, Ong BL, Gongal G, Hall J, Coulombe CA, Yanagihara Y, Yoshida S, Adler B (2009) *Leptospirosis* in the Asia Pacific region. BMC Infect Dis 9:147. https://doi.org/10.1186/1471-2334-9-147

Vincent AT, Schiettekatte O, Goarant C, Neela VK, Bernet E, Thibeaux R, Ismail N, Mohd Khalid MKN, Amran F, Masuzawa T, Nakao R, Amara Korba A, Bourhy P, Veyrier FJ, Picardeau M (2019) Revisiting the taxonomy and evolution of pathogenicity of the genus *Leptospira* through the prism of genomics. PLoS Negl Trop Dis 13(5):e0007270. https://doi.org/10.1371/journal.pntd.0007270

Wang B, Sullivan J, Sullivan GW, Mandell GL (1984) Interaction of leptospires with human polymorphonuclear neutrophils. Infect Immun 44(2):459–464. https://doi.org/10.1128/iai.44.2.459-464.1984

Wang N, Han YH, Sung JY, Lee WS, Ou TY (2016) Atypical leptospirosis: an overlooked cause of aseptic meningitis. BMC Res Notes 9:154. https://doi.org/10.1186/s13104-016-1964-z

Watson CJ, Thomas RH, Solomon T, Michael BD, Nicholson TR, Pollak TA (2021) COVID-19 and psychosis risk: real or delusional concern? Neurosci Lett 741:135491. https://doi.org/10.1016/j.neulet.2020.135491

Willcox RR (1976) The epidemiology of the spirochetoses, a worldwide review. In: Biology of Parasitic Spirochaetes. Academic Press, pp 133–155, Cambridge, Massachusetts

World Health Organisation (2003) Human leptospirosis: guidance for diagnosis, surveillance and control. WHO, Geneva

World Health Organisation (2011) Report on the second meeting of the leptospirosis burden epidemiology reference group

World Organisation for Animal Health (2022) Manual of diagnostic tests and vaccines for terrestrial animals. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access

Wunder EA Jr, Figueira CP, Benaroudj N, Hu B, Tong BA, Trajtenberg F, Liu J, Reis MG, Charon NW, Buschiazzo A, Picardeau M, Ko AI (2016) A novel flagellar sheath protein, FcpA, determines filament coiling, translational motility and virulence for the *Leptospira* spirochete. Mol Microbiol 101(3):457–470. https://doi.org/10.1111/mmi.13403

Zhao JF, Chen HH, Ojcius DM, Zhao X, Sun D, Ge YM, Zheng LL, Lin X, Li LJ, Yan J (2013) Identification of *Leptospira interrogans* phospholipase C as a novel virulence factor responsible for intracellular free calcium ion elevation during macrophage death. PLoS One 8(10):e75652. https://doi.org/10.1371/journal.pone.0075652

Glanders and Melioidosis: A Zoonosis and a Sapronosis

43

"Same Same, But Different"

Harjeet Singh Virk, Caoimhe Nic Fhogartaigh, and David A. B. Dance

Contents

H. S. Virk (✉)
Center for Experimental and Molecular Medicine, Amsterdam Infection & Immunity Institute, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands

Division of Microbiology, Portsmouth Hospitals University NHS Trust, Queen Alexandra Hospital, Portsmouth, UK
e-mail: h.s.virk@amsterdamumc.nl

C. N. Fhogartaigh
Lao Oxford Mahosot Hospital Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic

Division of Infection, Barts Health NHS Trust, London, UK
e-mail: caoimhe.nicfhogartaigh@nhs.net

D. A. B. Dance
Lao Oxford Mahosot Hospital Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic

Centre for Tropical Medicine, University of Oxford, Oxford, UK

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
e-mail: david.d@tropmedres.ac

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_35

Abstract

Glanders, caused by infection with *Burkholderia mallei*, primarily causes infection in equines, but may be transmitted to humans, and thus qualifies as a true zoonosis. Melioidosis is caused by *B. pseudomallei*, genetically very similar to *B. mallei*, but which is an environmental saprophyte capable of infecting humans and a wide range of other animals. Good evidence of animal-to-human, or even human-to-human, transmission of melioidosis is lacking, and so it is most appropriately referred to as a sapronosis, or at most a sapro-zoonosis. Although rare in Western countries, both microorganisms have recently gained much interest because of their potential use as bioterrorism agents and widening geographic footprint. The increasing recognition of melioidosis in humans and recent outbreaks of glanders in animals have led to their description as emerging or reemerging diseases, and melioidosis as a neglected tropical disease. Laboratory-associated infections with both organisms have also occurred, resulting in their categorization as Hazard Group 3 pathogens. In this chapter we review the epidemiology of animal and human cases of glanders and melioidosis, the evidence for different modes of transmission, pathogenesis and clinical features, diagnosis and treatment, as well as public health and disease control issues.

Keywords

Glanders · Melioidosis · *Burkholderia mallei* · *Burkholderia pseudomallei* · Equines · Thailand · Australia · Diabetes · Immunosuppression · Sepsis · Gram-negative · Ceftazidime · Co-trimoxazole · Farcy · Sapronosis · Bioterrorism · Intracellular · Pneumonia · Tropical infection · Abscess

43.1 Introduction

Glanders, caused by infection with *Burkholderia mallei*, primarily causes infection in equines, but may be transmitted to humans, and thus qualifies as a true zoonosis. Melioidosis is caused by *B. pseudomallei*, genetically very similar to *B. mallei*, but

which is an environmental saprophyte capable of infecting humans and a wide range of other animals. Good evidence of animal-to-human, or even human-to-human, transmission of melioidosis is lacking, and so it is most appropriately referred to as a sapronosis, or at most a sapro-zoonosis. Although rare in Western countries, both microorganisms have recently gained much interest because of their potential use as bioterrorism agents and widening geographic footprint. The increasing recognition of melioidosis in humans and recent outbreaks of glanders in animals have led to their description as emerging or reemerging diseases, and melioidosis as a neglected tropical disease. Laboratory-associated infections with both organisms have also occurred, resulting in their categorization as Hazard Group 3 pathogens. In this chapter we review the epidemiology of animal and human cases of glanders and melioidosis, the evidence for different modes of transmission, pathogenesis and clinical features, diagnosis and treatment, as well as public health and disease control issues.

43.2 Glanders

43.2.1 History and Epidemiology

Symptoms of glanders in equines were reported as early as 425 BC by Hippocrates; however it was Aristotle who first described it as "μηλις" (malis in Latin, from which *B. mallei* takes its name) in approximately 350 BC (Sharrer 1995). *B. mallei* was first isolated from the liver of an infected horse in 1882 (Boerner 1882). Infection resulted in significant morbidity and mortality in equines worldwide, and was occasionally transmitted to humans in prolonged close contact with horses, such as grooms, coachmen, veterinarians, and butchers, or to other animals through direct or indirect contact.

Glanders has since been eradicated from Western Europe, the USA, and Canada due mainly to the reduction in the use of horses in everyday life, but also to improved animal husbandry and hygiene and strict programs enforcing statutory testing and slaughter of infected animals (Blancou 1994; Derbyshire 2002); however the disease persists in the Middle-East, parts of Asia, and South America. A possible reintroduction occurred in Germany in 2014, which was identified on routine pre-export serological tests despite a lack of epidemiological evidence of contact with *B. mallei* infected horses (Kettle and Wernery 2016). This possibly represented latent disease, which was not detected by standard diagnostics (Kettle and Wernery 2016). Within the last 20 years, increasing numbers of equine cases have been reported from countries including Pakistan, India, Bangladesh, Brazil, Turkey, Iran, Iraq, Afghanistan, Kuwait, Bahrain, UAE, Lebanon, Latvia, Belarus, Mongolia, and China (Rahman et al. 2018; Health 2013; Khan et al. 2013). These are usually sporadic involving single or small numbers of animals, although occasionally larger outbreaks have occurred, such as that in India between 2006 and 2010 involving 8 states and 164 equines (Malik et al. 2012). Sporadic human cases have also been reported from Cameroon, Curaçao, Sri Lanka, and Turkey (Office

International des Épizooties 2011). Laboratory-associated human cases, such as that which occurred in a military research microbiologist in the USA in 2000 (Srinivasan et al. 2001), the first case in the USA for over 50 years, have also been reported occasionally.

Due to its high fatality rates and transmissibility of the disease in animals and humans, glanders has long been considered as a potential biological weapon. When horses were widely used for military purposes, devastating natural outbreaks occurred, for example, during the American Civil War (Sharrer 1995). *B. mallei* was actually used by the Germans in sabotage attempts during World War I (Christopher et al. 1997), and in human experiments by the notorious Japanese Unit 731 in Manchuria in the period leading up to World War II (Darling and Woods 2004). It is reported to have been weaponized by the Soviet Union, and used against the Mujaheddin in Afghanistan in the 1980s (Alibek and Handelman 1999). With resurgent bioterrorism concerns, *B. mallei*, listed as a category B bioterrorism agent and Tier 1 Select Agent by the Centers for Disease Control and Prevention and the US Department of Agriculture, is now being studied in many laboratories throughout the world.

43.2.2 Modes of Transmission

It was initially thought that glanders was transmitted through the air; however in the early eighteenth century it was proposed that transmission took place through direct contact with infected horses, or indirectly through contaminated harnesses and water troughs (Khan et al. 2013; Kinsley 1911). Inoculation or ingestion of infected clinical material was demonstrated to cause infection in horses and other animals in experiments conducted in the late nineteenth and early twentieth centuries, which also confirmed that "glanders" and "farcy" were different manifestations of the same disease (O'Leary 1908; Schutz 1898). Later, nasal discharge and skin exudate from infected animals was shown to contain large numbers of bacteria that could be readily cultured, and it was shown that viable bacteria could survive for at least 4 weeks suspended in water (Miller et al. 1948).

Once it has contaminated harnesses, grooming tools, hoof trimming equipment, water troughs, or hands, *B. mallei* may transmit to new hosts through skin abrasions, mucous membranes, ingestion of water containing infective material, or inhalation of dried infected particles (Carr Gregory 2007). The disease spreads quickly in overcrowded, poorly hygienic, and humid environments (Khan et al. 2013). Occasional cases have been reported in carnivores fed on infected meat (Alibasoglu et al. 1986; Khaki et al. 2012). Vertical transmission has occurred naturally from mare to foal, and from laboratory infected guinea pig sows to their offspring (Loeffler 1886; Rutherford 1906).

Zoonotic transmission to humans appears to be relatively uncommon. During World War II, human infections were rare despite a 30% prevalence in horses in China (Darling and Woods 2004). Disease has occurred almost exclusively in individuals whose occupation involves close and prolonged contact with horses,

but there is often no history or clinical evidence of inoculation or path of infection (Bernstein and Carling 1909). Lethal human glanders has also been documented to occur following a bite by an infected horse (Pilcher 1907). As is the case for melioidosis, diabetes may place humans at greater risk of infection after exposure, although reports of this are remarkably rare, perhaps because of the rarity of human glanders since diabetes became readily treatable (Srinivasan et al. 2001). Human infection by ingestion has not been definitively reported, although it has been seen in carnivores (Alibasoglu et al. 1986; Khaki et al. 2012). In fact even where there is known to have been consumption of glanderous meat, human infection has not occurred (Loeffler 1886). Human-to-human transmission is also rare, but has been reported, and has usually involved close contact with the infected individual either as a family member, a carer, during medical procedures, or postmortem examination (Loeffler 1886; Robins 1906). In a review of 156 reported cases at the turn of the last century, around 10% were believed to have been acquired from human contact (Robins 1906). In the present day, improved living conditions, universal precautions, disinfection, and available treatment make human-to-human transmission much less likely to occur. Recently, a study examining 538 in-contact individuals, including equine handlers, veterinarians/field assistants, and laboratory workers processing *B. mallei* samples, found no seropositive individuals by complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) (Singha et al. 2020a).

In developed countries, laboratory exposure seems to be a greater threat than animal contact, and anecdotal observations suggest that *B. mallei* may be more infectious in this setting than *B. pseudomallei* (Howe and Miller 1947). Some cases have occurred following obvious aerosol exposures during spillage of culture material, or direct inoculation injuries (Howe and Miller 1947) but many did not recall a particular exposure (Srinivasan et al. 2001). It is suspected that most laboratory-acquired cases are a result of inhalation (Carr Gregory 2007).

Although outbreaks continue to occur in the endemic regions listed above, little is known about the ecology and population dynamics of *B. mallei*. A recent study investigated the molecular epidemiology of glanders in Pakistan. Isolates from 15 glanderous horses in the Punjab between 1999 and 2007 underwent variable number of tandem repeat (VNTR) analysis, phylogenetic analysis, and comparison to ten whole-genome-sequenced strains of *B. mallei*. The results confirmed the Punjab strains to be genetically distinct from the sequenced strains, and to form three distinct clades, with the majority belonging to a single clade temporally and geographically spread, suggesting that this is ecologically established in the Punjab region (Hornstra et al. 2009). Together with additional epidemiological data, the authors concluded that human movement of equines contributed to the dispersal of *B. mallei* genotypes and that strains could persist for at least 1.5 years. Similarly, glanders infection in a dromedary in Bahrain was shown to be genetically similar to the Dubai 7 strain, which caused an outbreak in horses in United Arab Emirates in 2004 (Wernery et al. 2011), and it was suggested that the strain was introduced from that region through international horse trade. The first molecular characterization of a Brazilian *B. mallei* strain isolated from a mule in 2016 found that it was most closely related to an Indian strain isolated from a horse in 1932 (Laroucau et al. 2018).

Using 45 *B. mallei* isolates, this study identified three distinct lineages; L1- included only two isolates from Turkey and the United Kingdom, L2- isolates mainly from India, China, and Burma, with some from Hungary, Iran, Pakistan, and the UAE, and L3- mainly Turkish isolates along with some from Brazil, Hungary, India, Iran, Russia, and the USA. Caution is advised due to the paucity of available *B. mallei* genome data, meaning there is likely to be bias in the geographic clustering seen so far (Laroucau et al. 2018). In India, the state of Uttar Pradesh (UP) has become a glanders hotspot. Between 2013 and 2016, ten isolates from horses and mules identified a cluster of five that were linked to UP (Singha et al. 2021). However, further VNTR analysis identified considerable genotypic variability of *B. mallei* isolates from India. These were closely linked to isolates from Pakistan, followed by Turkey, which points to an ancestral clone that disseminated to geographically linked countries via equine movement over time (Singha et al. 2021).

43.2.3 Microbiology

B. mallei is a facultative intracellular, aerobic, nonmotile Gram-negative bacillus. The results of DNA-DNA hybridization (Rogul et al. 1970), multi-locus sequence typing (MLST) (Godoy et al. 2003), and whole genome sequencing (Nierman et al. 2004) have demonstrated unequivocally that *B. mallei* is actually a clone of *B. pseudomallei*, which has undergone a substantial reduction in the size of its genome during the process of adaptation as an equine pathogen (Nierman et al. 2004), differing at only a single nucleotide site in one of seven housekeeping genes studied. Based on these data it should not taxonomically be considered a separate species; however it retains species status due to its specific clinical and epidemiological behavior.

Very few recent clinical isolates are available for study, so knowledge of the characteristics of *B. mallei* is based on historical descriptions and archived strains, which may be laboratory-adapted to varying degrees. The organism often has an irregularly stained appearance on Gram's stain due to the presence of "lipoid granules" (Worley and Young 1945). Miller noted that when the organism was stained in clinical specimens, there was an impression of a capsule; however this is not apparent using common capsule stains (Miller et al. 1948). It is nutritionally versatile, being able to use a wide range of organic compounds as a carbon source, and can oxidize glucose and usually mannitol. It is able to grow on most laboratory media, but requires glycerol for optimum growth (Miller et al. 1948), initially forming shiny and translucent colonies, which tend to become mucoid with age. Selective agars have been developed and may be useful when clinical specimens from non-sterile sites are collected (Kinoshita et al. 2019). Most strains are oxidase positive. The optimal temperature for growth is 37 °C; many strains grow poorly below 25 °C but all will grow at 41 °C. *B. mallei* is unable to survive in dried pus for longer than a few days, or for 24 h when exposed to sunlight, although it can survive in tap water for at least 4 weeks (Miller et al. 1948; Howe and Miller 1947) and for 3–5 weeks in wet, humid, or dark environmental conditions. Following inoculation

onto nonporous laboratory materials (e.g., rubber gloves, glass, and stainless-steel), *B. mallei* China 7 viability decreased to nondetectable levels within 4–7 days. However, it was inactivated on exposure to vapor phase hydrogen peroxide (Rogers et al. 2016).

It grows less luxuriantly on laboratory media than *B. pseudomallei*, from which it may be distinguished by its susceptibility to aminoglycosides and lack of motility. Like *B. pseudomallei*, it is intrinsically resistant to colistin and polymyxin B. *B. mallei* is nonflagellated, despite retaining flagellar genes that are not expressed (Song et al. 2010), whereas *B. pseudomallei* has 2–4 polar flagella per cell.

43.2.4 Pathogenesis

B. mallei has the ability to invade and replicate inside epithelial and phagocytic cells and evade host immune mechanisms, resulting in an acute and fatal course, or a more chronic persistent infection state. In vivo animal models of glanders, in particular hamster and mice models, have provided important data on various pathogenic mechanisms. Nonhuman primate models highlight striking differences in pathological features observed between melioidosis and glanders (Nelson et al. 2014). With *B. pseudomallei* infection, most pathological features were hepato-splenic; multifocal suppurative lesions with areas of variable necrosis, whereas *B. mallei* lesions, although multifocal, were non-necrotic, and more severe in the lungs. All *B. mallei*-challenged animals had multifocal necrotizing pneumonia, but only one-third of *B. pseudomallei*-challenged animals exhibited pneumonia (Nelson et al. 2014).

B. mallei ATCC 23344 genome contains at least two *luxI* and four *luxR* homologues, which are quorum sensing (cell-density)-based regulators of virulence factor expression. When inactivated, reduced bacterial virulence was observed in mice (Ulrich et al. 2004). The genome also encodes a *virAG* two-component regulatory system that is required for virulence in hamsters (Nierman et al. 2004) and overexpression results in transcription of approximately 60 genes (Schell et al. 2007).

Like many pathogenic Gram-negative bacteria, *Burkholderia* spp. use a Type III secretion system (T3SS) to interact with and invade host cells. This system involves secretion of a protein onto the membrane of a host cell, to which the bacteria can subsequently bind, form a pore, and insert effector proteins directly into the host cell cytosol. *B. mallei* contains two T3SS, one of which is the animal pathogen-like Bsa T3SS (T3SSAP), which is required for rupture of endocytic vacuoles, escape into the host cell cytoplasm (Ribot and Ulrich 2006) and actin-based motility to promote spread within and between cells (Ulrich and Deshazer 2004). A type VI secretion system, T6SS-1, part of the *VirAG* regulon, is essential for *B. mallei* virulence in the hamster model of glanders (Schell et al. 2007) and has an important role in growth and actin-based motility following uptake of *B. mallei* by murine macrophages (Burtnick et al. 2010). *B. mallei* also exhibits Bim-A dependent intracellular actin-based motility, similar to that discussed later for *B. pseudomallei*. Multinucleated giant cell (MNGC) formation is characteristic of both organisms and is believed to

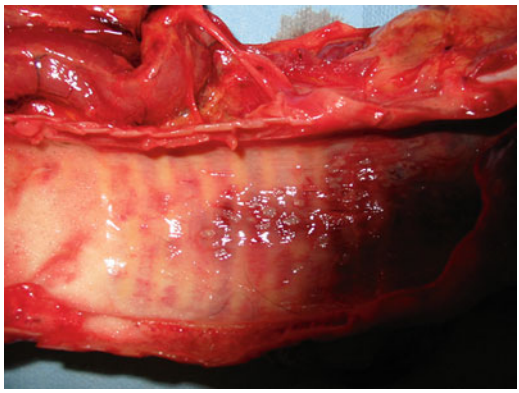

Fig. 1 Tracheal ulcers noted on post-mortem of a glanderous horse. Copyright Prof. D.E. Woods

be involved in establishing persistent infection by facilitating intercellular spread and immune evasion (Duval and White 1907; Burtnick et al. 2011).

In laboratory infected guinea pigs, *B. mallei* has been shown to produce a thick carbohydrate capsule (Popov et al. 1991), the coding sequence of which is 99% identical to that of the *B. pseudomallei* capsule (Deshazer et al. 2001). This enables *B. mallei* to resist macrophage and complement-mediated killing, promoting survival in serum (Burtnick et al. 2002). Furthermore, mutated strains lacking a capsule appear nonpathogenic in mice and hamsters (Deshazer et al. 2001). Lipopolysaccharide has also been shown to be a potent activator of Toll-like receptor-4 in vitro (Brett et al. 2007).

Through the various modes of transmission outlined previously, using these pathogenic mechanisms, *B. mallei* is able to penetrate mucosae, invade lymphatics, and spread hematogenously. Postmortem examinations of glanderous animals have revealed nodules and ulcers of the nasal passages, larynx, lip, and other tissues (Fig. 1); sero-sanguinous fluid in the nasal cavity, paranasal sinuses, and trachea; sub-pleural lung nodules; diffuse, miliary granulomatous nodules with caseo-necrotic centers; pulmonary edema or severe bronchopneumonia; and less frequently, muscle abscesses (Khan et al. 2013). Some ulcerating lesions are believed to be endotoxin mediated (Carr Gregory 2007).

43.2.5 Clinical Presentation in Animals

In addition to the normal equine hosts, glanders has been confirmed in camels, bears, wolves, dogs, and felines (Health 2013; Wernery et al. 2011), and in laboratory experiments guinea-pigs and hamsters appear to be susceptible, whereas cattle, fowl, rats, and pigs appear to be more resistant (Hu et al. 1958, Minnet 1959). Donkeys are particularly susceptible and develop an acute fatal form of infection, whereas horses tend to develop a chronic, more insidious, yet eventually fatal illness. The clinical presentation of equine glanders may be acute or chronic, and it typically manifests as a respiratory illness with pulmonary and nasal

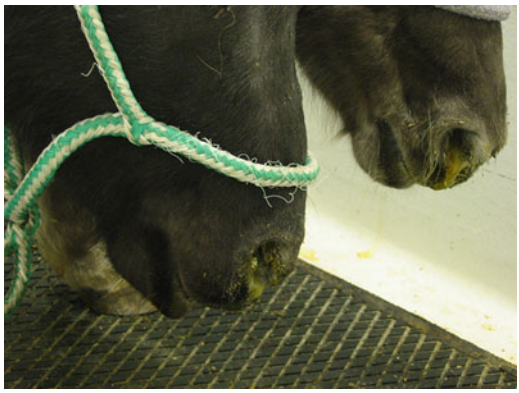

Fig. 2 Typical purulent nasal exudate due to glanders infection in horses. Copyright Prof. D.E. Woods

involvement ("glanders"; Fig. 2) or with cutaneous nodules and lymphangitis ("farcy"; Fig. 3a, b), although these forms often coexist, and pulmonary involvement is almost invariably found at postmortem. The incubation period varies from a few days to several months (Health 2013). The clinical presentation in other susceptible animals appears similar to that in equines. General clinical signs noted may include fever, rough hair coat, breathing difficulty, joint and limb swelling, inappetence, and gradual emaciation (Singha et al. 2020b).

Acute infection in donkeys begins with fever, anorexia, loss of stamina, and respiratory symptoms such as nasal discharge and cough. This is shortly followed by swelling of the nostrils, nodules, and ulceration of the nasal septum, mucopurulent nasal discharge, submaxillary lymphadenopathy (often with suppuration), and increasing shortness of breath (Health 2013; Wernery et al. 2011; Minnet 1959). Death occurs within a few days to weeks as a result of respiratory failure and sepsis.

In horses, glanders generally follows a more chronic course with episodic exacerbations followed by improvement in symptoms. The animal may have intermittent, low-grade fever, and mild respiratory symptoms; however the disease may remain latent for months to years without significant symptoms or signs. As disease progresses, cough, weakness, and signs of wasting develop, and nasal and cutaneous forms ensue with inflammatory nodules and ulceration of the nasal cavity and upper respiratory tract (see Fig. 1), yellow-green purulent nasal discharge (see Fig. 2), and lymphangitis or nodular lymphadenitis particularly affecting the limbs (Fig. 3b). The skin nodules may also ulcerate, and deep lesions are often associated with joint swelling and edema of the hind quarters resulting in lameness. Shortness of breath progresses as lung nodules and abscesses develop, and nodules are often found in the liver and spleen. Neurological involvement has been noted but is rare (Dobberstein 1935). Although chronic cases may survive for many years, the animal will usually become increasingly debilitated and eventually die (Health 2013; Khan et al. 2013; Minnet 1959; Saqib et al. 2012). Chronic and subclinical cases are potential sources of transmission to other animals or humans through shedding of bacteria in respiratory secretions and skin exudate (Wittig et al. 2006). Increased incidence of glanders

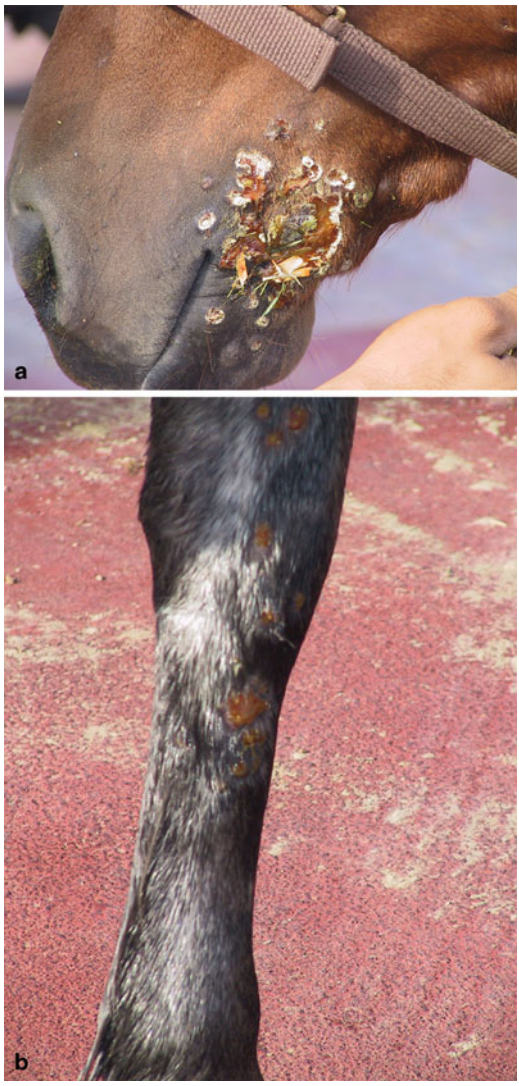

Fig. 3 (**a**) Cutaneous nodules around the jaw and (**b**) lymphangitis of the lower limb representing "fracy" due to glanders in a horse. Copyright of the Central Veterinary Research Laboratory, Dubai, United Arab Emirates

has been recorded during the seasonal transition from spring to summer followed by the humid rainy season, with working stress also playing an important role (Singha et al. 2020b; Fonseca-Rodriguez et al. 2019; Ghori et al. 2017).

43.2.6 Clinical Presentation in Humans

Knowledge of the clinical features of glanders in humans is based on a relatively narrow window in the literature of just over 100 years between the early nineteenth and the early twentieth centuries, and a few more recently published

laboratory-acquired cases (Srinivasan et al. 2001; Robins 1906; Howe and Miller 1947). The clinical manifestations appear to relate to the route of infection, and whether the disease remains localized or disseminates, which probably accounts for the relative frequency of involvement of the head, neck, and upper limbs. Typically, it results in pneumonia, septicemia, and chronic suppurative skin infection. Average incubation is 1–14 days (Nelson et al. 2014). Localized infection typically produces pus-forming, ulcerating nodules and abscesses of the skin, subcutaneous tissues, or mucous membranes, with associated lymphangitis or regional lymphadenopathy. Depending on the site affected, there may be swelling and increased discharge from nasal, ocular, or respiratory mucous membranes. Fever, malaise, headache, myalgia, and gastrointestinal upset are common accompanying features.

Cutaneous inoculation or entry of *B. mallei* via mucous membranes typically results in a localized infection at the site of entry within 1–5 days. Although involvement of the nasal or oral mucosa has been well described, this is by no means invariable and certainly not as prominent as it is in horses, but pustular lesions around the face appear to be common. If untreated, lymphatic or hematogenous spread takes place after 1–4 weeks, resulting in pulmonary, septicemic, or disseminated infection with abscesses in many organs, but particularly the spleen, liver, and lungs (Carr Gregory and D. 2007). Multiple, painful skin and soft tissue nodules and abscesses may be a particularly prominent feature, and these often contain a characteristic oily pus ("farcy oil"). Pulmonary involvement, secondary to aerosol inhalation or as part of disseminated infection, may present with cough, purulent sputum, shortness of breath, and chest pain as a result of pneumonia, lung abscess, or pleural effusion. Pneumonia, abscesses with cavitation, and miliary nodules have been seen on chest radiographs (Carr Gregory 2007). Septicemia may develop immediately or up to 2 weeks after initial exposure or recurrence, and has a poor prognosis. The most recent reported case of human glanders occurred in Brazil in 2020, when an 11-year-old child, who was known to be in constant close contact with families who owned horses, presented with fever, chest pain, and breathing difficulty, going on to develop septic shock. Chest X-rays identified pericardial and pleural effusions with pneumonia. *B. mallei* was subsequently cultured from abscesses, which appeared on his trunk. An abrasion on his left knee was presumed to be the portal of infection (Santos Junior et al. 2020).

Although human glanders was generally fatal over days to weeks before antibiotics were available, a more protracted course of disseminated infection interrupted by latent periods has also been described (Bartlett 1988), as well as cases of localized abscesses, which responded to incision and drainage only (Bernstein 1909). Recent naturally occurring and laboratory-acquired cases have survived with antibiotic treatment similar to that used for melioidosis despite delays in making the diagnosis.

43.2.7 Diagnosis

A definitive diagnosis of glanders, in animals or humans, generally requires isolation and identification of *B. mallei* from clinical samples, although seroconversion following known exposure would also be highly suggestive of infection.

Specimens from suspected or confirmed cases should be handled with appropriate laboratory containment. All suspected cases should have blood and urine culture, together with sputum, pus, exudate from superficial lesions and other samples as available or appropriate. Guidelines for culture and identification of *B. mallei* have been developed (Microbiology 2008). Gram's stain of clinical samples may demonstrate the irregularly stained Gram-negative bacilli. The organisms are difficult to demonstrate in tissue sections where they may have a beaded or encapsulated appearance (Miller et al. 1948). Isolation from non-sterile sites may be optimized by using a selective medium such as *Burkholderia cepacia* agar, although selective media containing aminoglycosides designed for *B. pseudomallei*, such as Ashdown's agar, are inhibitory to the aminoglycoside-susceptible *B. mallei* (Glass et al. 2009). *B. mallei* is often not correctly identified by API 20NE (Amornchai et al. 2007) and other commercial identification systems (Glass and Popovic 2005). Well-resourced laboratories are now using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry for bacterial identification. However, accurate results require good sample preparation and a well-developed database. To ensure highly pathogenic organisms are nonviable and safe for handling, the American Society for Microbiology document "Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases" recommends that laboratories use the tube extraction method followed by filtration through a ≤ 2 μm pore size filter for suspected biothreat agents (Rudrik et al. 2017). Caution is advised with a genus-level identification of *Burkholderia* species by MALDI-TOF MS. All suspected *B. mallei* isolates should be referred to the relevant national reference laboratory for molecular confirmation.

Molecular techniques have been developed to identify *B. mallei* in laboratory culture (Lee et al. 2005; Thibault et al. 2004b; U'ren et al. 2005) and although their use is currently restricted to research and reference laboratories, they could potentially be used to detect the organism in clinical specimens. PCR targeting the flagellin gene of *B. mallei* (fli-P) was used successfully to detect the organism in clinical samples taken during a glanders outbreak in horses (Scholz et al. 2006).

In suspected cases of glanders in animals, the mallein skin test was historically used for diagnostic purposes. The test is based on a hypersensitivity reaction to a protein fraction (mallein) of *B. mallei* following intrapalpebral or subcutaneous injection or administration in eyedrops, leading to marked eyelid swelling, a painful raised lesion, or conjunctivitis, respectively, after 1–2 days, often accompanied by fever (Health 2013). Mallein testing can, however, lead to subsequent false positive results in other serological tests (Hagebock et al. 1993), and may be falsely negative in animals with acute glanders or in the late stages of chronic disease (Neubauer et al. 2005). It is no longer recommended due to animal welfare concerns, but may be used in remote endemic regions where storage and transport of samples for serological testing is problematic (Health 2013).

Numerous serological tests for the diagnosis of glanders in horses exist including complement fixation test (CFT), various enzyme-linked

immunosorbent assay (ELISA), immunoblot (IB), Rose Bengal Test (RBT), indirect hemagglutination assay (IHA), agar-gel immunodiffusion (AGID), indirect fluorescent assay test (IFAT), counter immunoelectrophoresis (CIE), and dot ELISA. Many of these methods are not widely validated, cross-react with other *Burkholderia* species, and, like the mallein test, may give false negative results in acute cases or very debilitated animals. CFT represents the current method of choice for the diagnosis of glanders and is required before international horse trade by the World Organization for Animal Health (OIE) (Health 2013). It is 90–98% sensitive, becoming positive within 1 week of infection, and remaining positive in chronic cases and exacerbations of latent cases (Health 2013). A cELISA has been developed using an uncharacterized anti-lipopolysaccharide monoclonal antibody, and shown to have similar performance characteristics to the CFT (Katz et al. 2000). However, both methods have suboptimal specificity (Neubauer et al. 2005), particularly when testing serum samples from animals in glanders-endemic areas (Khan et al. 2012). A recently optimized BimA protein-based indirect enzyme-linked immunosorbent assay (iELISA) exhibited 96% sensitivity and 91% specificity, with 100% repeatability and minimal decrease in diagnostic efficacy after storage of ELISA plates at room temperature or 37 °C for 90 days (Singh et al. 2018). ELISAs based on recombinant antigens (TssA, TssB, and Hcp1) and semi-purified fractions of *B. mallei* (IDVet) have been shown to have significantly higher specificity, offering a suitable alternative for serological testing of equids (Elschner et al. 2019; Elschner et al. 2021). CFT performance is also significantly affected by the availability of quality reagents and specifically by the *B. mallei* antigen applied. False positive tests can have serious consequences in terms of animal slaughter and financial losses. Performing an immunoblot as a confirmatory test for all positive CFT results has been suggested as a means of overcoming the sensitivity issues (Khan et al. 2012). The serodiagnosis of glanders in animals should only be undertaken and interpreted by specialists with relevant expertise. Ideally, the diagnosis should be confirmed by culture if possible. The European Union Reference Laboratory (EURL) for glanders, nominated in 2008, found good intra-laboratory repeatability of CFT testing; however, a risk of inter-laboratory inconsistency was highlighted, which may misclassify positive samples with low CFT titers (Laroucau et al. 2016). A field deployable recombinase polymerase amplification-lateral flow (RPA-LF) assay, which is highly sensitive (down to 10 fg of *B. mallei* genomic DNA) and specific, shows promise as a tool for use in endemic areas with limited laboratory resources (Saxena et al. 2019).

No validated serological test is currently available for the diagnosis of human glanders, although numerous melioidosis serodiagnostic tests are in use around the world and, given the serological cross-reactivity between *B. mallei* and *B. pseudomallei*, it is likely that these would become positive in many cases of human glanders. Recent molecular and immunological research is leading to the identification of more specific and immunogenic *B. mallei* antigens to optimize serological diagnosis.

43.2.8 Treatment

B. mallei is intrinsically resistant to a range of antimicrobial agents, including early beta-lactams, but unlike *B. pseudomallei*, *B. mallei* remains susceptible to aminoglycosides and macrolides (Kenny et al. 1999; Thibault et al. 2004a). Most strains are susceptible to carbapenems, ceftazidime, amoxycillin–clavulanic acid, piperacillin, doxycycline, and trimethoprim-sulfamethoxazole (Thibault et al. 2004a; Kenny et al. 1999; Al-Izzi and Al-Bassam 1989; Heine et al. 2001). Despite low mean inhibitory concentrations in vitro, certain antimicrobials, including aminoglycosides, may not be effective in vivo due to the intracellular nature of the infection. Doxycycline and fluoroquinolones, such as ciprofloxacin and ofloxacin, with good intracellular and tissue penetration, have demonstrated efficacy when used to treat experimental infection in animals (Batmanov 1991; Iliukhin et al. 1994; Russell et al. 2000); however, in one study relapse occurred in some doxycycline treated animals (Russell et al. 2000). Doxycycline has also been shown to have some efficacy as post-exposure prophylaxis following aerosol and intraperitoneal challenge in animals (Russell et al. 2000; Iliukhin et al. 1994).

Trials to determine optimal treatment for animal and human glanders are lacking. Until recently, glanderous animals, including those that are asymptomatic with positive serological tests, have been euthanized according to strict veterinary public health policies to prevent spread to other domestic animals or humans. In the case of high value animals, such as those in equestrian sports, an expensive treatment regimen may be justified. During an outbreak of culture confirmed glanders in 23 horses at the Lahore Polo Club in Pakistan, a combination of intravenous enrofloxacin and trimethoprim-sulfadiazine for 3 weeks, followed by oral doxycycline for a total of 12 weeks, successfully treated all infections (Saqib et al. 2012).

Recommendations for treatment of human glanders adopt the same antimicrobial regimens as those validated for melioidosis, which are based on clinical trial evidence. This consists of an intensive phase of intravenous antimicrobial therapy (ceftazidime or a carbapenem) for a minimum of 10–14 days, followed by an eradication phase of oral antimicrobial (trimethoprim-sulfamethoxazole) for 12–20 weeks, or longer if there is widespread visceral disease (Lipsitz et al. 2012). Without the latter phase, there is likely to be a high risk of relapse, particularly in those with disseminated infection. Drainage of abscesses where possible is an important adjunct to antimicrobial therapy.

43.2.9 Prevention and Control

Control and eradication of glanders has to date depended on the detection and elimination of infected animals to prevent onward transmission. A requirement for serological testing of animals prior to international transport in order to prevent the introduction of glanders into glanders-free regions, has been recommended by the World Organization for Animal Health (Health 2003).

Attempts to develop vaccines against *B. mallei* have so far been experimental and no vaccine against glanders is yet available for either human or animal use. Intranasally vaccinated BALB/c mice using an iron-acquisition-deficient *B. mallei*$_{\text{tonB}}$ strain had 100% survival on subsequent challenge. However, necropsy and organ colony-forming units (CFU) enumeration showed splenomegaly and abscess formation with persistence of the attenuated *B. mallei,* which poses a significant safety concern (Hatcher et al. 2016). In contrast, a recombinant Parainfluenza virus 5 expressing BatA (autotransporter protein) resulted in 74% survival, with complete bacterial clearance from the lungs and spleen in 78% (Lafontaine et al. 2019). Interestingly, using a double mutant by deletion of tonB and hcp1 genes produced clearance from all organs by 21 days post inoculation, with unremarkable histopathology. Furthermore, serum from these mice was able to inhibit bacterial growth when co-cultured with *B. mallei*. Greatest protection was observed in mice with the highest total IgG titers and IgG2a/IgG1 ratios (markers of Th1-driven immune response and protection). Together, these studies demonstrate that live-attenuated vaccines can elicit a strong humoral response that contributes toward protection (Hatcher et al. 2016). The addition of the Toll-like receptor 9 (TLR9) agonist CpG oligodeoxynucleotide (activating B and NK cells, antibody production, and Th1 cell development) as an adjuvant may yet provide better protection and reduce the number of vaccine doses required (Hatcher et al. 2016). More recent work is taking advantage of genome-wide bio- and immune-informatic analysis to predict highly immunogenic antigens. This led to the development of a nano-glycoconjugate vaccine (containing OmpW, OpcP, and Hemagglutinin protein antigens alongside LPS), which offered complete protection in an inhalational glanders mouse model (Tapia et al. 2020). Vaccine development is gaining momentum and much progress has also been made with melioidosis vaccines (*see below*), which may offer cross-protection.

If animal or human glanders is suspected, the case should be isolated, and personal protective equipment (PPE) worn by any person who must come into contact with the patient or samples. Local and national public health and veterinary authorities must be notified immediately and confirmed cases in animals reported to the World Organization for Animal Health. Any confirmed human glanders case occurring without equine exposure should prompt consideration of a deliberate release of the organism. In human cases, isolation and appropriate infection control precautions (according to the site of infection) should be taken until the patient is culture negative.

Confirmed animal cases and serologically positive animal contacts should be destroyed humanely, with the provision of adequate compensation to owners. Reasonable compensation schemes helped to eradicate glanders in Canada (Derbyshire 2002). In contrast, in some developing countries as little as $1.1 US dollars is paid in compensation for slaughter of a glanderous animal, which may be the basis of the owner's livelihood, thus forcing them to sell the animal and risk onward transmission to other animals and regions (Khan et al. 2013; Saqib et al. 2012). Premises and facilities of infected animals should be quarantined, cleaned, and disinfected. Carcases as well as contaminated bedding, feed, manure, and equipment in the vicinity should be buried or incinerated.

Prevention of laboratory-acquired human infection depends on a full risk assessment, appropriate containment and practices, the use of personal protective equipment, and the institution of appropriate guidelines in the event of accidental laboratory exposure (Lipsitz et al. 2012).

43.3 Melioidosis

43.3.1 History and Epidemiology

Melioidosis was first described by Whitmore and Krishnaswami as a "glanders-like. . . .pyaemic or septicaemic" illness occurring in morphia addicts in Rangoon in 1911 (Whitmore and Krishnaswami 1912) and was documented in 5% of postmortem examinations in Myanmar around this time (Cheng and Currie 2005). Fulminant presentations at autopsy were characterized by widespread caseous consolidation of the lungs and typically abscesses in the liver, spleen, or other organs (Whitmore 1913). The name originates from the Greek "μηλις" (distemper of asses) and "ειδος" (resemblance), and the name was suggested by Stanton and Fletcher in 1921 (Stanton and Fletcher 1921), who went on to report a number of human and animal cases around Kuala Lumpur (Stanton and Fletcher 1932). It was later demonstrated that the causative bacterium, now known as *B. pseudomallei*, was saprophytic and could be cultured from soil and surface water in Vietnam (Chambon 1955) and subsequently from many other parts of Southeast Asia and northern Australia. In Australia, *B. pseudomallei* was first identified in sheep in 1949 (Cottew 1952) and the first human case occurred in a diabetic patient who died of septicemic melioidosis in north Queensland in 1950 (Rimington 1962). Using MLST (Currie et al. 2007), and more recently whole-genome sequencing (Chewapreecha et al. 2017), phylogenetic analysis has suggested that Australian isolates, which demonstrate greater genetic diversity, are ancestral to those found in Southeast Asia (Pearson et al. 2009). This supports the present hypothesis that Australia was the original reservoir for the current *B. pseudomallei* population, which expanded to Southeast Asia, where the Mekong subregion has emerged as a hotspot for *B. pseudomallei* evolution (Chewapreecha et al. 2017). Further dissemination to Africa and Central and South America is thought to have occurred between the seventeenth and nineteenth centuries (Chewapreecha et al. 2017). Gee et al. used a typing scheme for length polymorphisms in the 16S–23S internal transcribed spacer (ITS) of *Burkholderia* spp. and identified ITS type G isolates (containing the *Yersinia*-like fimbrial (YLF) gene) as associated with the Western Hemisphere (Gee et al. 2014). Analysis of single-nucleotide polymorphisms (SNPs) from whole genome sequencing is proving valuable in linking clinical isolates with geographic provenance. For example, a US military veteran who had spent time in Southeast Asia during World War II was initially reported as having the longest latency period (62 years) before developing melioidosis (Ngauy et al. 2005). There was no history of travel to other known endemic regions. However, WGS found the isolate to belong to the Western Hemisphere clade and grouped with genomes from

patient isolates with a travel history to Guatemala, Panama, and Peru. This isolate also belongs to the ITS type G cluster, which suggests that his exposure to *B. pseudomallei* may not actually have occurred during his internment in World War II (Gee et al. 2017). In the Darwin cohort periods of latency were thought to have occurred in 3% of melioidosis cases (Currie et al. 2021).

Melioidosis is endemic in many tropical regions, mainly between latitudes 20°N and 20°S, although *B. pseudomallei* is unevenly distributed in the environment in these areas and the true distribution has not been accurately defined (Dance 2000a). The highest isolation rates have been found in rice paddies, rubber plantations, and other cleared and cultivated areas (Nachiangmai et al. 1985; Strauss et al. 1969) but high rates have also been seen in urban sports fields in Singapore (Thin et al. 1971), and grazing sites of animals with melioidosis in Australia (Thomas et al. 1979). Factors that may influence environmental distribution include temperature, humidity, rainfall, ultra-violet exposure, soil composition, vegetation, fertilizers, and soil disturbance such as excavation or ploughing (Inglis et al. 2001). Recent modeling and epidemiological studies highlighted the underdiagnosis and underreporting of melioidosis, which was estimated to have infected 165,000 people (95% credible interval 68,000–412,000) and caused 89,000 deaths (36,000–227,000) worldwide in 2015 (Limmathurotsakul et al. 2016). This equates to 4.6 million disability adjusted life-years (DALYs), a greater burden than those for dengue and leptospirosis, and suggests that melioidosis should be formally categorized as a reemerging neglected tropical disease (Limmathurotsakul et al. 2016; Birnie et al. 2019; Savelkoel et al. 2021). India, Bangladesh, Vietnam, Nigeria, and Indonesia are predicted to contribute almost three-quarters of the total global disease burden (Birnie et al. 2019). Despite this, only ~1300 cases were reported annually worldwide in 2010, less than 1% of the estimated incidence (Limmathurotsakul et al. 2016). Whether this reflects the inadequacy of current surveillance systems or the inaccuracy of the modeling remains to be determined.

The relatively small numbers of cases reported in endemic areas during the latter half of the twentieth century probably reflects the limited culture facilities in many rural, high risk regions (Dance 1991). This is supported by the fact that western armed forces, with access to high quality laboratory diagnostics, reported at least 100 confirmed cases of melioidosis among French (Rubin et al. 1963) and American (Sanford 1985) soldiers, respectively, during the conflicts in Vietnam in contrast to the numbers of cases identified among the indigenous population. In Thailand, very few cases were reported until the improvement of district microbiology laboratories and increased clinical awareness in the 1980s, which led to in around 800 case reports by 1986 (Leelarasamee and Bovornkitti 1989) and an average of nearly 1800 culture-positive cases annually between 2012 and 2015 (Hantrakun et al. 2019). Sri Lanka has become a case study for uncovering the hidden burden using enhanced surveillance, awareness, and WHO's laboratory capacity building program (Corea et al. 2016). Despite only a handful of cases identified since 1927, and at times even being considered non-endemic for melioidosis (Cheng and Currie 2005), rising annual cases have been identified there since 2006, totaling 250 cases over 10 years in 8 out of 9 provinces (Corea et al. 2018).

Warm climates favor the persistence of *B. pseudomallei* in the environment; however when introduced to a non-endemic area the organism may persist for several years in soil. This apparently occurred during a prolonged outbreak in France in the 1970s, which was thought to have followed the importation of an infected panda (Mollaret 1988). More recently, cases occurring in the USA have been linked to imported tropical fish (see below) and an aromatherapy spray (Dawson et al. 2020; CDC 2021). With increasing movement of humans, animals, and goods around the world, new endemic foci may become established. Sporadic cases have been reported in the Americas, the Caribbean, and sub-Saharan Africa (Cheng and Currie 2005; Dance 1991) although the true incidence in these areas is unclear because of a lack of laboratory facilities and clinical awareness. Ongoing mapping of the distribution of *B. pseudomallei* and melioidosis is available at https://www.melioidosis.info/map.aspx.

Molecular tools have demonstrated that environmental isolates are often identical to epidemiologically related human or animal strains, that there is considerable diversity among isolates persisting in a particular region, and that clonal outbreaks have occurred when the organism is introduced to a non-endemic region (Gee et al. 2017; Cheng and Currie 2005; Currie et al. 1994).

By the year 2000, melioidosis was regarded as an emerging infection due to increasing reports of confirmed cases in endemic regions, particularly Thailand, where it is estimated that more than 2500 culture-positive cases of human melioidosis occur annually, increasing reports of cases from regions where the disease was not known to be endemic (e.g., the Americas and the Caribbean) (Cossaboom et al. 2020; Sanchez-Villamil and Torres 2018), and concerns that it could be spread to non-endemic regions by infected animals (Dance 2000b). Much of the increase has been due to improved diagnostics and clinical awareness, but the increasing prevalence of predisposing medical conditions such as diabetes in populations of endemic areas (Dance 2000b) and possibly climate change and increasing travel and migration have also impacted on melioidosis epidemiology. Analysis of melioidosis among returned travelers in Europe identified Thailand as the main source of infection (53%), with one-fifth of patients being misdiagnosed (Le Tohic et al. 2019). However, even in countries where notification is mandatory, such as the UK, many cases are still going unreported (O'connor et al. 2020).

Currently, the greatest burden of melioidosis is reported in Thailand (especially the northeast) and Northern Australia where annual incidence rates vary between 4 and 50/100,000/year (Parameswaran et al. 2012; Hantrakun et al. 2018). Melioidosis is now the third most common cause of death from infectious disease in northeast Thailand,(Limmathurotsakul et al. 2010) although this has been underrecognized through routine surveillance systems (Hantrakun et al. 2019) and is the commonest cause of fatal community-acquired bacteremic pneumonia in the Northern Territory of Australia (Currie et al. 2000b). The disease is highly seasonal, with 75–85% of cases presenting during the rainy season (Suputtamongkol et al. 1994; Currie et al. 2010), and incidence rates as high as 102.4/100,000 have been recorded in the indigenous Australian population during severe rains (Parameswaran et al. 2012; Currie et al. 2004). Large case series have identified diabetes mellitus as by far

the most important risk factor for infection, with occupational exposure to soil and water, male sex, Aboriginal Australians, alcoholism, chronic lung disease, chronic renal disease, thalassemia, and kava and steroid use all additional risk factors for melioidosis (Cheng and Currie 2005; Suputtamongkol et al. 1994; Currie et al. 2010). The majority of cases have a predisposition, but in around 20% none is identified (Currie et al. 2010). Presence of a single risk factor increases the risk of death from melioidosis by 8.4 times (95% CI 2.7–26.0) (Currie et al. 2021).

With respect to animal infection, *B. pseudomallei* appears to affect a broader range of animal hosts than glanders, with infection in equines being relatively rare, although it may occasionally cause severe infections in horses. Species that have been infected include terrestrial and aquatic mammals, birds, and fish. Goats, sheep, pigs, and camels appear particularly susceptible, whereas dogs, cats, and cattle appear more resistant, but these may develop disease if they become immunocompromised (Choy et al. 2000). Sporadic cases or small outbreaks have been reported in various primates, marsupials, deer, buffalo, camels, llamas, zebras, horses, mules, rabbits, meerkats, rodents, iguanas, parrots, crocodiles, dolphins, and seals (Elschner et al. 2014; Sprague and Neubauer 2004). Animal cases have also been reported in other regions, such as southern and western Australia(Currie et al. 1994; Ketterer et al. 1986), China(Li et al. 1994), Iran (Baharsefat and Amjadi 1970), Saudi Arabia (Barbour et al. 1997), United Arab Emirates (Wernery et al. 1997), South Africa (Van Der Lugt and Henton 1995), and the Americas (Zehnder et al. 2014; Galimand and Dodin 1982). Epizootics have been reported after importation of animals from areas of endemicity. This was believed to be the source of a cluster of infections in sheep, goats, and pigs in Aruba in 1957 (Fournier 1965), an outbreak in a Paris zoo, which spread to other zoos and equestrian clubs in France in the 1970s (Mollaret 1988), and an outbreak in primates in the UK in the 1990s (Dance et al. 1992). More detail on confirmed cases of melioidosis in different animal species worldwide can be found in a review by Sprague and Neubauer (Sprague and Neubauer 2004).

43.3.2 Modes of Transmission

Whitmore's early observations of melioidosis in guinea pigs led him to believe that the infection was transmitted by consumption of food and drink contaminated by urine, sputum, or other secretions containing viable bacteria, from infected persons or animals (Whitmore 1913). In the 1930s, Stanton and Fletcher also proposed that infection occurred by ingestion, although they believed that rodents were a zoonotic reservoir (Stanton and Fletcher 1932). It was subsequently observed that human infections commonly followed exposure to mud and water, and that *B. pseudomallei* could be isolated from mud and surface water (Chambon 1955), leading to the current knowledge that it is an environmental saprophyte. Well-documented modes of transmission include inoculation and aspiration of water during near drowning (such as during the Asian tsunami in 2004), and laboratory-acquired infection (although only two such instances have been reported in the literature). Epidemiological evidence and animal studies also suggest a role for inhalation and ingestion,

although it is often impossible to define precisely how and when infection occurred. Although sporadic cases have been anecdotally associated with infection in animals, there is limited evidence for zoonotic or person-to-person spread (Dance 2000a), and it is equally likely that both humans and animals have acquired infection from the same environmental source. The few suspected human-to-human *B. pseudomallei* transmissions have been in siblings with cystic fibrosis (Holland et al. 2002) and diabetes (Arauz et al. 2020), an American Vietnam veteran diagnosed with *B. pseudomallei*-associated prostatitis and his spouse (Mccormick et al. 1975), and cases of mother-to-child transmission via transplacental, breast, or perinatal routes (Aziz et al. 2020; Rodriguez et al. 2020; Ralph et al. 2004; Kunakorn et al. 1991). Recently, a case of transmission from a breastfeeding mother with mastitis was confirmed using WGS (Aziz et al. 2020).

Inoculation of organisms through penetrating injuries or preexisting skin lesions appears to be the major mode of acquisition, particularly in farmers who are continually exposed while working in the mud and surface water of paddy fields (Suputtamongkol et al. 1994). Twenty five percent of patients in one case series recalled a previous inoculation injury, but often there is no such history (Currie et al. 2000b). *B. pseudomallei* is most abundant in soil depths of >10 cm; however during the rainy season it can rise and concentrate at the surface (Limmathurotsakul et al. 2013a). Inoculation is the method most frequently used to induce infection in animal models, and natural infection in animals occurs in this way by entry of bacteria through minor skin trauma, bite wounds, and scratch injuries. This was the likely mode of infection in a patient from Maryland, USA with no travel history who developed melioidosis in 2019. Isolates of *B. pseudomallei* that were indistinguishable by WGS and clustered with isolates from Southeast Asia were obtained from both the patient and a freshwater aquarium in which all the fish had died. The patient recalled reaching into the aquarium with bare hands and arms a month prior to onset of illness (Dawson et al. 2020). Nosocomial infections have also occasionally been reported, mainly through use of contaminated medical supplies and solutions (Merritt et al. 2016).

Infection after inhalation has been repeatedly demonstrated in laboratory animals (Jeddeloh et al. 2003), and this may be an important and underestimated mode of acquisition in humans. In Australia, *B. pseudomallei* recovered from an air sample was linked to a clinical isolate from a patient with mediastinal melioidosis by WGS (Currie et al. 2015), and the north-easterly winds during the typhoon season in Taiwan were associated with detection of *B. pseudomallei*-specific DNA in aerosols and a hot spot of transmission (Hsueh et al. 2018). It is now established that during periods of very heavy rainfall, increases in pneumonic cases of melioidosis occur, probably as a result of aerosolization of the bacteria (Currie and Jacups 2003). Inhalation was previously thought to be the primary mode of transmission due to the high incidence of melioidosis in US military helicopter crews during and after the Vietnam War.

Ingestion has also been proposed as a mode of infection in both humans and animals. Contaminated water supplies have been implicated by PFGE as the point source of melioidosis outbreaks in Australia (Currie et al. 2001; Inglis et al. 1999).

Suppurative parotitis, a common presentation in children with melioidosis in Southeast Asia, is believed to be due to the ingestion of contaminated water or soil, resulting in the ascent of bacteria from the mouth to the parotid gland (Stoesser et al. 2012). Although not confirmed, an untreated river water supply was implicated in melioidosis outbreaks occurring in intensive piggeries (Ketterer et al. 1986). In these outbreaks, an oral mode of transmission was suspected due to the common finding of infected gastro-hepatic nodes. Feco-oral transmission was felt to be unlikely due to the fact that *B. pseudomallei* was infrequently isolated from fecal samples of infected pigs. However, fecal shedding has been detected from wallabies and wild stock, suggesting it may be a means of expanding the geographical distribution of *B. pseudomallei* (Höger et al. 2016). Recent nonhuman primate models showed that ingestion of $>6 \times 10^6$ cfu resulted in acute-febrile, lethal disease (Nelson et al. 2021). Enteritis was observed in fatal disease with the lungs being the first organ colonized outside of the gastro-intestinal tract. Severe pathological feature in the mesenteric lymph nodes suggests that lymphatic drainage maybe an important route of dissemination post ingestion (Nelson et al. 2021).

Despite early theories, there is relatively little evidence for melioidosis being a true zoonosis. In addition to the aquarium-associated case described above, three anecdotal cases of possible zoonotic infection in Australia were described by Choy et al. (Choy et al. 2000). In one case, *B. pseudomallei* was cultured from a wrist lesion of a meat worker in Darwin; secondly, a vet in rural Queensland developed abscesses on the arm, but this does not appear to have been confirmed as melioidosis by culture; and similarly, a goat farmer had a lesion on his hand resembling a "milker's lesion" for 2 months preceding a diagnosis of melioidosis, which again does not appear to have been culture-confirmed. In Malaysia, a case of suspected sheep-to-human-transmission was reported in a 10-year-old boy (Idris et al. 1998). The evidence for this was entirely circumstantial, and it is more likely that he contracted the illness from soil and water in the environment (from which *B. pseudomallei* was also isolated). Earlier anecdotal evidence of animal-to-human transmission of melioidosis was reported during "L'affaire du Jardin des Plantes," an outbreak of melioidosis that started in a Paris zoo and spread to other zoos and equestrian clubs in France through transport of infected animals and contaminated manure. At least two fatal human cases were said to have occurred during this outbreak, although details were never published (Dodin and Galimand 1986). In none of these cases has there ever been genotypic evidence of the relationship between the human and animal isolates, and so the case for animal-to-human transmission remains unproven. Nonetheless, the potential for zoonotic transmission can lead to significant public health concerns and responses, such as those that occurred following the importation into the USA of a rescue dog from Thailand that was subsequently found to have *B. pseudomallei* urinary tract infection. Fortunately, there were no resultant human infections (Ryan et al. 2018).

There have been concerns that goats, which appear to be particularly susceptible to melioidosis and often develop mastitis as a manifestation of the infection (Fig. 4), could transmit the disease via infected milk. However, small studies of infected goats have found that the organism is only isolated from body fluids in a minority of cases

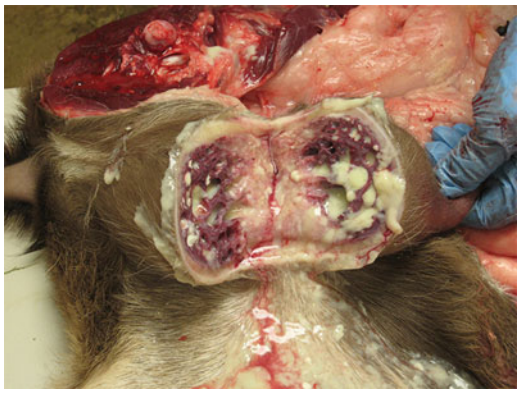

Fig. 4 Mastitis found on post-mortem of a goat who died of melioidosis. Copyright Dr Carl Soffler

(Thomas et al. 1988a). Furthermore, a recent literature review of bacterial infections following animal bites worldwide did not identify any cases of melioidosis, supporting the fact that transmission from body fluids is unlikely (Abrahamian and Goldstein 2011). However, clearly it makes sense in public health terms to avoid drinking milk or eating meat from infected animals.

43.3.3 Microbiology

B. pseudomallei is an irregularly staining, oxidase-positive, motile Gram-negative bacillus, which sometimes exhibits marked bipolarity microscopically. It can be distinguished from *B. mallei* by its motility and usually its resistance to aminoglycosides. It grows readily on most routine culture media, initially forming smooth creamy, nonhemolytic colonies (Day 2), which may become dry and wrinkled (Day 4) with a metallic sheen on prolonged incubation (Fig. 5), sometimes with a zone of hemolysis surrounding confluent growth. Considerable variability in colonial morphology may be seen between, and even within, strains. It is often dismissed as a contaminant or misidentified (e.g., as *Acinetobacter* spp. *Pseudomonas* spp. or *Bacillus* spp.), especially on nonselective agars, where it may be outgrown by other microbial flora. Ideally, selective media (e.g., Ashdown's agar) should be used, particularly where laboratory staff are unfamiliar with its characteristics or when polymicrobial growth is expected, with daily examination of plates for up to 4 days in suspected cases. In respiratory samples from low-incidence settings, this approach improved sensitivity (87.5%) and allowed for quicker identification than routine media (50%) (Subakir et al. 2020). Important characteristics include arginine dihydrolase and gelatinase activity, the inability to assimilate arabinose (distinguishing *B. pseudomallei* from the closely related avirulent *B. thailandensis*), and growth at 42 °C. Its intrinsic resistance to aminoglycosides (although clonal isolates susceptible to gentamicin are common in Sarawak, Malaysia) (Podin et al. 2014), polymyxins and the early beta-lactams, but susceptibility to co-amoxiclav, is

Fig. 5 Typical dry, wrinkled colonies of Burkholderia pseudomallei after 48 hours culture on Ashdown's media

particularly characteristic, and any oxidase positive Gram-negative bacillus with these characteristics should be assumed to be *B. pseudomallei* until proved otherwise (Trinh et al. 2018). The species is antigenically homogeneous, but a number of molecular techniques, most usefully MLST and WGS, can distinguish between isolates.

43.3.4 Pathogenesis

A range of bacterial factors have been associated with virulence, but the relative contributions of individual virulence factors to the disease process have not been fully characterized. A variety of adhesins, in particular type 4 pili, appear to be involved in attachment of bacteria to different eukaryotic cell types, and expression is regulated by the *pilA* gene (Allwood et al. 2011). Capsular polysaccharides also act to inhibit opsonophagocytosis and complement-mediated killing (Egan and Gordon 1996). Like *B. mallei, B. pseudomallei* utilizes up to three T3SS, including Bsa T3SS. In vitro experiments have demonstrated the importance of this system, and its individual components, in host cell invasion, escape from endosomes and intracytoplasmic survival (Stevens et al. 2002). Mutations in components of the T3SS in *B. pseudomallei* have reduced ability to cause disease in animal models (Stevens et al. 2004). Cell-to-cell spread takes place by actin-based motility, which is dependent on the BimA protein (Stevens et al. 2005) (mutations of which have been linked to central nervous system diseases, especially in Australia) and the cluster 1 type VI secretion system (T6SS-1), which mediates endocyte escape and membrane fusion during intracellular spread via VgrG5 spike protein (Toesca et al. 2014). The antiphagocytic polysaccharide capsule, quorum sensing mechanisms, and bacterial components such as lipopolysaccharide, flagella, secreted products (protease, lipase, lecithinase, various toxins), and a siderophore ("malleobactin") also have important roles in environmental protection and adaptation, and host immune system

evasion (Wiersinga et al. 2018; Cheng and Currie 2005). The ability of the organism to survive and grow intracellularly or become metabolically inactive within granulomas probably contributes to the persistent nature of the infection and the risk of relapse.

The clinical outcome after exposure to *B. pseudomallei* in the environment varies from person to person, ranging from asymptomatic seroconversion (the commonest outcome) or localized infection to fulminant sepsis and death, and is dependent on the size and route of the inoculum, the virulence of the infecting strain, and host immune factors. On the host side, innate immune mechanisms, macrophage, and neutrophil function, and both cellular and humoral responses all play a role in defense against the organism, hence the strong associations with immune-suppressing conditions such as diabetes (12-fold increased risk compared with the normal population) (Limmathurotsakul et al. 2010), thalassemia, renal impairment (associated with disease severity in India) (Shaw et al. 2019), and alcohol excess (Cheng and Currie 2005; Currie et al. 2004). In a prospective study of over 1000 patients with melioidosis, a third of whom had diabetes, there was a statistically significant survival advantage in diabetics compared with nondiabetics. However, this was confined only to patients taking glyburide (a second generation sulfonylurea, which acts as K_{ATP}-channel blocker and broad-spectrum ATP-binding cassette (ABC) transporter inhibitor used in treating type 2 diabetes mellitus) (Koh et al. 2011). Subsequent diabetic mice models demonstrated an anti-inflammatory effect of glyburide by reducing IL-1b, diminished cellular influx, and reduced bacterial dissemination to distant organs (Koh et al. 2013).

Interestingly, HIV does not appear to be a risk factor despite murine studies showing a role for the adaptive immune system in control of infection, with increased survival correlated to CD8+ T cell responses in humans (Jenjaroen et al. 2015). An exaggerated host response with high levels of pro-inflammatory cytokines such as TNF-alpha may also have a pathogenic role (Nuntayanuwat et al. 1999), whereas hypofunctional TLR5 has been associated with decreased organ failure, improved survival, and functional cytokine response (Chantratita et al. 2014; West et al. 2014). Antigens and epitopes (e.g., BopE – Type III secreted protein, AhpC – alkyl hydroperoxide reductase, PilO – Type IV pilus biosynthesis protein), immunodominant in survivors, have been identified as immune correlates of protection (Dunachie et al. 2017). Diabetic patients were noted for their impaired response to GroEL proteins (chaperonins that assist in protein folding) during acute infection (Dunachie et al. 2017).

43.3.5 Clinical Presentation in Humans

The majority of infections appear to be subclinical with 60–70% of populations in endemic areas acquiring antibodies to *B. pseudomallei* by the age of 4 years without clinically apparent disease (Wuthiekanun et al. 2006). When disease manifests, it may be localized or disseminated with septicemia. The incubation period varies depending on the mode of acquisition and infecting dose, with most cases occurring

within 3 weeks after an inoculation injury (median 4 days, IQR 3–7 days), (Currie et al. 2021) and as soon as 24 h after a near-drowning event (Currie et al. 2000b; Suputtamongkol et al. 1994). The median age at presentation with melioidosis is 50 years, with 4–10% of patients under 16 years of age (Currie et al. 2010; Mcleod et al. 2015). Pneumonia is the commonest presentation, and is evident in around half of all cases (Currie et al. 2010). Cavitation may occur in the upper zones mimicking tuberculosis. Localized abscesses may occur in any other organ including the skin and soft tissues, lymph nodes, liver, spleen, genitourinary tract (especially the prostate gland in males), parotid gland, bone or joint, and nervous system. Localized disease without bacteremia generally has a good outcome and low mortality. However, in 50–75% of cases the patient is bacteremic, although this is lower in pediatric populations (Turner et al. 2016; Currie et al. 2021), and just over one-fifth of these are in septic shock at presentation, which has a mortality approaching 50% despite optimal treatment (Birnie et al. 2019; Currie et al. 2010; Suputtamongkol et al. 1994). There appear to be geographical variations in manifestations, with hepatosplenic abscesses more common in Asian populations and suppurative parotitis in Asian children, and higher rates of prostatic and neurological melioidosis seen in Australia (Cheng and Currie 2005), although this could be biased by better access to imaging. Recrudescent melioidosis after treatment occurs in up to 5% of cases (Currie et al. 2021). This is due to reactivation of the original strain (relapse) in approximately 75% of cases, which is usually associated with a failure to sterilize deep-seated foci of infection in disseminated disease, but may also be associated with poor adherence to therapy or an insufficient duration of eradication therapy (Currie et al. 2000a). Reinfection with a different strain accounts for ~25% of recurrent infections (Currie et al. 2021).

43.3.6 Clinical Presentation in Animals

As outlined above, a wide range of animal species may be affected by melioidosis, with a range of clinical manifestations and severity. In fact, the disease in animals is usually similar to that in humans, with subclinical infections common and abscesses occurring in any organ, particularly lungs, liver, spleen, and associated lymphatics. The acute form presents as fulminant sepsis with hematogenous dissemination and high mortality, often associated with respiratory distress and diarrhea, and tends to occur in younger animals of susceptible species. The chronic form presents as a more non-specific illness in older animals, with low-grade fever, anorexia, cough, progressive emaciation, and lameness (Choy et al. 2000). In sheep, goats, and horses, nasal, and ocular discharge (similar to that seen in glanders) is common, and central nervous system involvement may contribute to paralysis, convulsions, nystagmus, and blindness (Sprague and Neubauer 2004). Mastitis appears to be a particular feature in goats (Fig. 4), orchitis has been described in rams and boars, and skin lesions, limb edema, lymphangitis, and meningoencephalitis in horses (Sprague and Neubauer 2004). Monkeys are affected in a similar way to horses, but neurological involvement is more unusual (Sprague and Neubauer 2004).

43.3.7 Diagnosis

Melioidosis should be considered in any person or animal who has visited or migrated from an endemic area presenting with septicemia and/or abscesses, especially if they have a predisposing condition such as diabetes. Confirmation of the diagnosis relies on culture of the organism from blood, sputum, pus, or other body fluid indicated by the clinical presentation. Liaison with the microbiology laboratory is of utmost importance if melioidosis is suspected. Firstly, the organism is a hazard group 3 pathogen and must be handled in appropriate laboratory containment in case of transmission to laboratory staff. Secondly, selective media such as Ashdown's or *B. cepacia* media may be used to optimize the isolation of the organism from sites with a normal flora. And thirdly, if not aware of the clinical context, growth in cultures may be dismissed as a contaminant by the unwary.

Culture may take several days, and meanwhile microscopy of pus, sputum, or urine may reveal bipolar or unevenly staining Gram-negative rods, although this appearance is not specific. Immunofluorescent staining of such samples is a useful rapid diagnostic tool but is not widely available (Wuthiekanun et al. 2005). Once cultured, commercial identification kits such as the API 20NE usually identify the organism correctly but may give misleading results (Amornchai et al. 2007), so presumptive isolates should be sent to a Reference Laboratory if in doubt as to the identity. Well-equipped laboratories are increasingly using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry for bacterial identification, provided an appropriate database including hazardous pathogens is available as discussed above (Lasch et al. 2016). A latex agglutination test using a monoclonal antibody to the 200 kDa extracellular polysaccharide is also useful for screening suspect colonies or positive blood culture fluid, with 95% sensitivity and 99.7% specificity (Anuntagool et al. 2000). In low-resource or -incidence settings, selective media followed by real-time PCR has been shown to improve diagnosis of melioidosis at a reasonable cost (Subakir et al. 2020).

Direct detection of *B. pseudomallei* antigens in clinical samples is another approach that might be particularly useful in areas where culture is not available. A lateral flow test using a monoclonal antibody specific for capsular polysaccharide (CPS), with a limit of detection of ~0.2 ng/ml, the Active Melioidosis Detect Lateral Flow Immunoassay (AMD LFI; InBios, USA), is not yet on the market but has undergone evaluation in several settings. Testing against a large panel of *B. pseudomallei* isolates showed an analytical reactivity of 98.7%, with cross-reactivity in only 2.8% of near neighbor species (Houghton et al. 2014). The AMD-LFI was 99% sensitive and 100% specific on turbid blood culture bottles. The high specificity was maintained across all sample types, with relatively high sensitivity in pus and sputum samples but poor sensitivity on serum. In this cohort from Laos, urine samples had a positive predictive value of 94% for diagnosing melioidosis; a potential game changer for diagnostics in resource limited settings (Woods et al. 2018). Similar findings were reproduced from prospective cohorts in India, although highest discordance was demonstrated from serum – 34.1%. This was corroborated in Thailand where sensitivity in serum was 31.3% (Wongsuvan et al. 2018).

There is no standard serological test for melioidosis. An indirect hemagglutination (IHA) test, using a crude mixture of poorly characterized antigens, is most widely used, but it lacks sensitivity and specificity in humans, particularly in endemic areas where background seropositivity rates are high (e.g., 29%, 38%, and 12.8% in studies in India, Thailand, and Australia, respectively) (Vandana et al. 2016; Chaichana et al. 2018; Smith et al. 2018). A rapid immunochromatographic test for IgG appeared to be more sensitive and specific than IHA in populations of endemic areas but is no longer commercially available (Wuthiekanun et al. 2004). Recently, ELISAs employing better characterized antigens such as purified O-polysaccharide (OPS) and hemolysin co-regulated protein (Hcp-1) have been shown to have better performance than the IHA for serodiagnosis of melioidosis in endemic areas (Pumpuang et al. 2017), with IgG antibody levels for both antigens raised from an early stage (Pumpuang et al. 2019). A rapid immunochromatography test (ICT) using Hcp-1 was evaluated in cohorts of melioidosis patients, healthy controls, and patients with other infections, demonstrating an overall sensitivity of 88.3% (Phokrai et al. 2018). However, until kits using more refined, standardized antigens are available, the utility of serological tests is largely limited to non-endemic regions. Despite the limitations, serology continues to be used in veterinary medicine, and a two-step method by screening with IHA followed by confirmation with a complement fixation test was shown to be sensitive and specific in caprine melioidosis (Thomas et al. 1988b). Molecular methods have also been developed but are not yet used for routine diagnostic purposes.

Radiology is an important adjunct to microbiological diagnosis, and may demonstrate diffuse nodular infiltrates, abscess, or cavitating pneumonia on chest radiograph. Liver, splenic, prostatic, or other intra-abdominal abscesses on ultrasound or CT may be suggestive of the diagnosis (Huson et al. 2020).

43.3.8 Treatment

B. pseudomallei is intrinsically resistant to many classes of antibiotics, including some third generation cephalosporins, early penicillins, aminoglycosides, colistin, and polymyxin, and exhibits relative resistance to quinolones and macrolides (Cheng and Currie 2005). Acquired resistance may occur but rarely compromises the choice of antibiotic treatment (Wuthiekanun et al. 2011; Fen et al. 2021), although one recent paper from China reported nearly 13% of isolates as resistant to ceftazidime (Rao et al. 2019).

The treatment of melioidosis may be classified into acute and eradication phases. In the acute phase the aim is to kill bacteria in the circulation and prevent patients dying of overwhelming sepsis, and in the eradication phase the aim is to kill any residual bacteria in abscesses or tissues and prevent relapse of infection. Currently, ceftazidime or a carbapenem for 2 weeks is the treatment of choice for the acute phase, and co-trimoxazole for 12–20 weeks for eradication (Anunnatsiri et al. 2020). Recent updates to Australian guidelines recommend a minimum of 3 weeks intravenous therapy for multi-lobar pneumonia without bacteremia, 4 weeks if

bacteremic, and minimum 3 weeks for those with only single lobar pneumonia with concomitant bacteremia. Even longer courses of treatment (up to 8 weeks intravenously and 6 months orally) are recommended for those with deep-seated foci of infection, including bone and joint, central nervous system, and intravascular involvement (Sullivan et al. 2020).

In a trial of 161 patients in Thailand (65 with confirmed melioidosis, 54 of these septicemic), ceftazidime (120 mg/kg/day) in the acute phase reduced mortality from 74% to 37%, compared with the conventional combination regimen of chloramphenicol, doxycycline, and co-trimoxazole (White et al. 1989). Other cephalosporins, such as cefotaxime and ceftriaxone, were associated with significantly greater mortality compared with ceftazidime in retrospective analyses (Chaowagul et al. 1999). Subsequent trials assessed ceftazidime with and without the addition of co-trimoxazole in the acute phase of melioidosis, and failed to demonstrate any difference in mortality between the monotherapy and combination groups (Chierakul et al. 2005). Median time to defervescence of fever was 9 days (Simpson et al. 1999b). In the Darwin cohort, extending the acute intensive phase to 4 weeks, resulted in a relapse rate of only 1.2% (Pitman et al. 2015).

Carbapenems are the most active drugs in vitro against *B. pseudomallei*, and are more rapidly bactericidal (Smith et al. 1996). A randomized trial comparing ceftazidime (120 mg/kg/day) with imipenem/cilastatin (50 mg/kg/day) for a minimum of 10 days was unfortunately terminated early and therefore underpowered. It showed no difference in mortality between the two groups, but higher rates of treatment failure in the ceftazidime group (41.3% versus 20.3%) (Simpson et al. 1999a). Co-amoxiclav is considered second-line therapy for the acute phase. One study from Malaysia has suggested veterinary cases, especially those involving novel ST 1130 isolates show significantly higher likelihood of resistance to meropenem (Sadiq et al. 2018). *B. pseudomallei* isolates carrying the carbapenemase blaOXA-57 have also been identified, although, >90% of blaOXA-57 carrying isolates were phenotypically susceptible to imipenem (Amladi et al. 2019). The concern is however that IS (insertion sequences) family transposases (carried by these isolates), which facilitate mobilization of extended-spectrum -lactamase (ESBL) and carbapenemase genes, would have been missed had hybrid genome assembly not been performed (Amladi et al. 2019). This highlights the wider threat of AMR and virulence gene acquisition and need for robust surveillance systems globally.

The conventional combination of chloramphenicol, doxycycline, and co-trimoxazole for eradication therapy was extremely poorly tolerated leading to reduced compliance and increased rates of relapse. Omitting chloramphenicol was shown to be beneficial in terms of side effect profile, with no adverse treatment outcomes (Chaowagul et al. 2005). Years of clinical experience in Australia,(Currie et al. 2021) however, suggest that co-trimoxazole monotherapy for 12–20 weeks is probably adequate to prevent relapse. The MERTH trial conducted in Thailand, also supported the use of co-trimoxazole monotherapy on the basis of efficacy, safety, and tolerance by patients (Chetchotisakd et al. 2014). Another recent RCT,which did not meet the primary end point (culture-confirmed recurrent melioidosis), found that

all-cause mortality was significantly lower with a 12-week regimen (0.3%) compared to 20 weeks (3%), meeting the criteria for non-inferiority for the secondary composite end-point (overall recurrent melioidosis and mortality) (Anunnatsiri et al. 2020). In the rare cases of co-trimoxazole resistance (determined by MIC), and where co-trimoxazole is contraindicated, co-amoxiclav is the preferred choice for eradication therapy, although this is associated with increased rates of relapse (Rajchanuvong et al. 1995). In a cohort of >3000 patient isolates, only 0.33% were resistant to co-trimoxazole. Encouragingly, all resistant isolates were susceptible to co-amoxiclav, but only 91% to doxycycline (Saiprom et al. 2015). As mentioned previously, poor compliance also contributes to relapse, and so it is crucial that each patient is counseled in the importance of completing the full treatment course regardless of symptomatic improvement.

Novel agents such as cefiderocol, a siderophore cephalosporin, which inhibits peptidoglycan synthesis and is described as universally stable against β-lactamases (providing greater efficacy than carbapenems, cephalosporins, and other inhibitor combinations), shows promise as it is highly active in vitro against *B. pseudomallei* (Burnard et al. 2021).

Apart from appropriate antibiotic therapy, the management of melioidosis must also incorporate optimal supportive treatment for sepsis, including maintenance of blood pressure, adequate glycemic control, and management of respiratory and acute renal failure. Around one quarter of cases require admission to intensive care (Currie et al. 2021). The drainage of abscesses should also take place where possible. Adjunctive granulocyte colony-stimulating factor (G-CSF) has been used to boost host neutrophils in an attempt to control infection, but despite promising outcomes in a retrospective study, G-CSF did not significantly reduce mortality in a randomized controlled trial (Cheng et al. 2004; Cheng et al. 2007).

Even with appropriate antimicrobial and supportive therapy, mortality remains high for septicemic cases. Poor prognostic factors include shock, absence of fever, leucopenia, abnormal liver function, renal impairment, high level or persistent bacteremia, hypoglycemia, and acidosis (Currie et al. 2021; Limmathurotsakul et al. 2011). However, over the past 5 years, a combination of optimal sepsis management and antibiotic therapy has reduced the overall mortality from melioidosis in Darwin, Australia, to only 6% (Currie et al. 2021).

The efficacy of post-exposure prophylaxis (PEP) in protection against developing melioidosis remains unknown. Animal models show that PEP simply delays the onset of disease, rather than preventing it (Dance et al. 2017). In over 70-years of combined experience in diagnostic laboratories from endemic regions, which handle thousands of *B. pseudomallei* samples at containment levels less stringent than US biosafety level 3, leaders in the melioidosis field have not once been consulted about a case of laboratory-acquired infection (Dance et al. 2017). Exposure of 30 healthcare workers (HCWs) in South Korea, including five high risk exposures to pus/blood through non-intact skin and 25 low risk contacts with blood through intact skin, along with two laboratory staff who opened the lid of an agar plate growing *B. pseudomallei* outside a biological safety cabinet, resulted in no seroconversion or symptoms of melioidosis. The only two well-described laboratory-acquired cases

to date involved sonication outside a safety cabinet and cleaning up a spillage of *B. pseudomallei* culture with bare hands while having an ulcerative lesion on a finger (Jun et al. 2017). A recent study in Queensland (Australia) identified no infections or seroconversions following 1267 instances when *B. pseudomallei* was handled outside a safety cabinet (Gassiep et al. 2021). Using bioaerosol sampling and *B. thailandensis,* as a bioequivalent surrogate in handling experiments, the authors found no evidence of environmental contamination. This suggests the risk of laboratory-acquired melioidosis may be lower than that for glanders or some other hazard group 3 agents. In the rare occasion where a high-risk exposure or low risk exposure in a lab worker with underlying risk factors has occurred, PEP with co-trimoxazole may be considered following a careful discussion of risks and benefits (Peacock et al. 2008). As a caution, of two lab technicians who were exposed to aerosolized *B. pseudomallei* while manipulating cultures outside a biosafety cabinet, neither of whom developed melioidosis or seroconverted, one had to take time off work due to adverse drug reaction (fever, cough, and rash) to co-trimoxazole, so the decision to offer PEP should not be taken lightly (Mitchell et al. 2017).

As is the case for glanders, the long duration of treatment of melioidosis in animals can be expensive and ineffective. In cases where treatment is deemed necessary, such as in animals of economic value, treatment regimens are as for human cases.

43.3.9 Prevention and Control

A cost-benefit analysis has suggested that immunization against melioidosis would be worthwhile if used in high-risk populations, even when only partial protection is assumed (Luangasanatip et al. 2019). A Steering Group on Melioidosis Vaccine Development (SGMVD) was created to advise the scientific community in 2015 (Limmathurotsakul et al. 2015). A key recommendation was that vaccine candidates should also be tested in diabetic animal models. The Defense Threat Reduction Agency (DTRA) recently funded the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) to conduct head-to-head comparisons of promising vaccine candidates in 2020. As recommended by SGMVD, this study should provide concrete evidence for antibody- and cytokine-mediated responses that confer protection and preferably sterilizing immunity (Khakhum et al. 2020). However, although potential candidates are under investigation, as yet there is no licensed human or animal vaccine for melioidosis.

Due to the complex pathogenicity of *B. pseudomallei* and strain heterogeneity, it has become evident that a multicomponent vaccine using a combination of protective antigens will be required for complete protection. Thankfully, intense research has resulted in several multivalent platforms such as glycoconjugates, multivalent subunit preparations, live-attenuated bacteria, nanoparticle platforms, and outer membrane vesicles (OMV) that have proven highly effective in experimental animal models of melioidosis, conferring 40–100% efficacy (Morici et al. 2019). OMVs hold particular promise as they lack any observable toxicity, are self-adjuvanting

(driving dendritic cell maturation), and can contain intracellular stage-specific proteins such as T3SS-3 or T6SS-1 (Baker et al. 2021). A recent study using purified *B. pseudomallei* CPS covalently linked to recombinant CRM197 (a nontoxic mutant of diphtheria toxin) produced opsonizing antibody responses with high IgG titers. Mice vaccinated with a combination of CPS-CRM197 and recombinant Hcp1 showed 100% survival in a lethal inhalational challenge model, with 70% sterilizing immunity (Burtnick et al. 2018). This candidate is planned to be the first melioidosis vaccine used in a human phase 1 clinical trial in diabetic and nondiabetic volunteers.

In the absence of a licensed vaccine, preventive measures must focus on avoidance of contact with *B. pseudomallei* in the environment. A matched case-control study carried out in northeast Thailand found that working in rice fields, walking barefoot, bathing in pond water, exposure to rain, water inhalation, and having an open wound all significantly increased the odds of acquiring melioidosis (Limmathurotsakul et al. 2013b). A lower risk of melioidosis was associated with wearing protective clothing such as long trousers and rubber boots, and washing with clean water after working in the fields. The authors, therefore, recommended avoidance of direct contact with soil and environmental water whenever possible, but wearing protective clothing and washing after exposure if this is unavoidable. Wounds should be kept covered until they have completely healed, and the application of herbal remedies to wounds should be avoided, as this was also associated with an increased risk of melioidosis. Since there was a small but significant risk observed with drinking untreated water, and since *B. pseudomallei* was found in water drunk by 7% of cases and 3% of controls, including borehole, wells, and piped supplies, it was also recommended that only treated water should be drunk in endemic areas (Limmathurotsakul et al. 2013b). It has also been recommended that goat's milk be pasteurized to avoid potential zoonotic transmission by ingestion (Choy et al. 2000), although this has never been reported, but this makes sense in general public health terms.

Very recently, the results of PREMEL, a stepped-wedge cluster-RCT on the effectiveness of a multifaceted prevention program for melioidosis in diabetics from 116 primary care units in northeast Thailand, have been published (Suntornsut et al. 2021). Although rates of culture-confirmed melioidosis were not decreased in participants who had received an intervention in the form of a behavioral support group session, they had a lower incidence of hospital admissions involving infectious diseases and of all-cause mortality. Proposals for modification/addition of behavioral techniques and need for more frequent intervention have been suggested (Suntornsut et al. 2021).

Due to the low but theoretical risk of person-to-person transmission, human cases should be nursed in isolation with contact precautions and care taken when handling any body fluids. People with strongly associated predisposing conditions, such as diabetes, should be informed of their increased risk of melioidosis, and advised to avoid the above high-risk activities. Unfortunately, in rural areas, and during heavy rain and winds, exposure may be unavoidable for many.

It has been recommended that animals be removed from contaminated sources, such as soil or water in endemic regions, to prevent melioidosis outbreaks in herds

(Choy et al. 2000); however infections have still occurred when pigs were reared on artificial, hard surfaces such as concrete (Thomas et al. 1981). Chlorination of water has been shown to eliminate *B. pseudomallei* (Howard and Inglis 2005), but only if pH and concentrations of organic substrates are carefully controlled, and this could prove difficult in water troughs, which may become highly contaminated (Choy et al. 2000). When an animal becomes infected in an endemic area, it has been suggested that strict maintenance of a hygienic environment may prevent a larger outbreak, although supporting evidence is lacking. Regular disinfection with potassium hypochlorite and cresol (to include all surfaces and the lower limbs of the animal), removal of infected excrement several times per day, and the avoidance of large quantities of water were used in an effort to curtail the outbreak in Paris zoos and equestrian clubs in the 1970s (Sprague and Neubauer 2004). Infected carcasses of animals must be condemned and destroyed. Guidelines for handling and disposal are available in the Manual for Meat Inspection in Developing Countries (http://www.fao.org/docrep/003/t0756e/T0756E05.htm#ch4.2.9). There are no mandatory requirements for serological screening for melioidosis in animals that are transported internationally, although it is possible that such animals might react in serological or skin tests to the closely related *B. mallei*. Serological testing of imported primates for melioidosis was used following an outbreak among Cynomolgus monkeys in the United Kingdom in the 1990s but has never been used routinely (Dance et al. 1992).

43.4 Conclusion

Both glanders and melioidosis may be regarded as reemerging infections with the ability to infect both animals and humans, although only glanders is a true zoonosis. Glanders has been eradicated from many countries, whereas melioidosis is widespread across the tropics, particularly South and Southeast Asia and tropical North Australia. Various factors have contributed to their emergence such as increasing awareness and diagnostic capability, increasing prevalence of underlying predisposing conditions, increased transport of animals (and associated contaminated waste and equipment) and other products internationally, human migration patterns, and adventure travel to tropical regions. Subclinically infected human and animal carriers risk further transmission of infection in the case of glanders, as well as persistence in a new environment under the right physical conditions. Global warming may extend the current geographic limitations of melioidosis and place a greater population at risk of exposure. This is particularly of concern as the prevalence of diabetes and other immunosuppressive states increases in many developing countries. Finally, we must be alert to the possible use of these agents in bioterrorism. Increased awareness of these pathogens is important so that early recognition, treatment, and public health action occurs, and so that organisms are handled at the appropriate level of containment to prevent laboratory-associated cases. Further research is required to develop effective vaccines and optimize prevention strategies.

References

Abrahamian FM, Goldstein EJC (2011) Microbiology of animal bite wound infections. Clin Microbiol Rev 24:231–246

Alibasoglu M, Yesildere T, Calislar T, Inal T, Calsikan U (1986) Glanders outbreak in lions in the Istanbul zoological garden. Berl Munch Tierarztl Wochenschr 99:57–63

Alibek, K., and Handelman, S. 1999. Biohazard. Random House, New York

Al-Izzi SA, Al-Bassam LS (1989) In vitro susceptibility of *Pseudomonas mallei* to antimicrobial agents. Comp Immunol Microbiol Infect Dis 12:5–8

Allwood EM, Devenish RJ, Prescott M, Adler B, Boyce JD (2011) Strategies for intracellular survival of *Burkholderia pseudomallei*. Front Microbiol 2:170

Amladi A, Devanga Ragupathi NK, Vasudevan K, Venkatesan M, Anandan S, Veeraraghavan B (2019) First report of *Burkholderia pseudomallei* St412 And St734 clones harbouring blaOXA-57 but susceptible to Imipenem in India. New Microbes New Infect 32:100613

Amornchai P, Chierakul W, Wuthiekanun V, Mahakhunkijcharoen Y, Phetsouvanh R, Currie BJ, Newton PN, Van Vinh Chau N, Wongratanacheewin S, Day NP, Peacock SJ (2007) Accuracy of *Burkholderia pseudomallei* identification using the Api 20ne system and a latex agglutination test. J Clin Microbiol 45:3774–3776

Anunnatsiri S, Chaowagul W, Teparrukkul P, Chetchotisakd P, Tanwisaid K, Khemla S, Narenpitak S, Pattarapongsin M, Kongsawasd W, Pisuttimarn P, Thipmontree W, Mootsikapun P, Chaisuksant S, Chierakul W, Day NPJ, Limmathurotsakul D (2020) A comparison between 12 versus 20 weeks of trimethoprim-sulfamethoxazole as oral eradication treatment for melioidosis (12vs20): an open-label, pragmatic, multicenter, non-inferiority, randomized controlled trial. Clin Infect Dis 73

Anuntagool N, Naigowit P, Petkanchanapong V, Aramsri P, Panichakul T, Sirisinha S (2000) Monoclonal antibody-based rapid identification of *Burkholderia pseudomallei* in blood culture fluid from patients with community-acquired septicaemia. J Med Microbiol 49:1075–1078

Arauz AB, Castillo K, Santiago E, Quintero Y, Adames E, Castillo B, Rodriguez-French A, Henostroza G (2020) Geographic distribution and incidence of melioidosis, Panama(1). Emerg Infect Dis 26:118–121

Aziz A, Currie BJ, Mayo M, Sarovich DS, Price EP (2020) Comparative genomics confirms a rare melioidosis human-to-human transmission event and reveals incorrect phylogenomic reconstruction due to polyclonality. Microb Genom 6

Baharsefat M, Amjadi AR (1970) Equine melioidosis In Iran. Arch Inst Razi 22:209–213

Baker SM, Settles EW, Davitt C, Gellings P, Kikendall N, Hoffmann J, Wang Y, Bitoun J, Lodrigue KR, Sahl JW, Keim P, Roy C, Mclachlan J, Morici LA (2021) *Burkholderia pseudomallei* Omvs derived from infection mimicking conditions elicit similar protection to a live-attenuated vaccine. Npj Vaccines 6:18

Barbour EK, Nabbut NH, Hamadeh SK, Al-Nakhli HM (1997) Bacterial identity and characteristics in healthy and unhealthy respiratory tracts of sheep and calves. Vet Res Commun 21:421–430

Bartlett JG (1988) Glanders. Philadelphia, W.B. Saunders Company

Batmanov VP (1991) Sensitivity of *Pseudomonas mallei* to fluoroquinolones and their efficacy in experimental glanders. Antibiot Khimioter 36:31–34

Bernstein JM (1909) Human glanders. Hospital 45:609–610

Bernstein JM, Carling ER (1909) Observations on human glanders: with a study of six cases and a discussion of the methods of diagnosis. Br Med J 1:319–325

Birnie E, Virk HS, Savelkoel J, Spijker R, Bertherat E, Dance DAB, Limmathurotsakul D, Devleesschauwer B, Haagsma JA, Wiersinga WJ (2019) Global burden of melioidosis in 2015: a systematic review and data synthesis. Lancet Infect Dis 19:892–902

Blancou J (1994) Early methods for the surveillance and control of glanders in Europe. Rev Sci Tech 13:545–557

Boerner P (1882) A preliminary report on work by the Imperial Health care office leading to discovery of the glanders bacillus. Deutsche Medizinische Wochenschrift 2

Brett PJ, Burtnick MN, Snyder DS, Shannon JG, Azadi P, Gherardini FC (2007) *Burkholderia mallei* expresses a unique lipopolysaccharide mixture that is a potent activator of human toll-like receptor 4 complexes. Mol Microbiol 63:379–390

Burnard D, Robertson G, Henderson A, Falconer C, Bauer MJ, Cottrell K, Gassiep I, Norton R, Paterson DL, Harris PNA (2021) *Burkholderia pseudomallei* clinical isolates are highly susceptible in vitro to cefiderocol, a siderophore cephalosporin. Antimicrob Agents Chemother 65: E00685–E00620

Burtnick MN, Brett PJ, Woods DE (2002) Molecular and physical characterization of *Burkholderia mallei* O antigens. J Bacteriol 184:849–852

Burtnick MN, Deshazer D, Nair V, Gherardini FC, Brett PJ (2010) *Burkholderia mallei* cluster 1 type vi secretion mutants exhibit growth and actin polymerization defects in raw 264.7 murine macrophages. Infect Immun 78:88–99

Burtnick MN, Brett PJ, Harding SV, Ngugi SA, Ribot WJ, Chantratita N, Scorpio A, Milne TS, Dean RE, Fritz DL, Peacock SJ, Prior JL, Atkins TP, Deshazer D (2011) The cluster 1 type VI secretion system is a major virulence determinant in *Burkholderia pseudomallei*. Infect Immun 79:1512–1525

Burtnick MN, Shaffer TL, Ross BN, Muruato LA, Sbrana E, Deshazer D, Torres AG, Brett PJ (2018) Development of subunit vaccines that provide high-level protection and sterilizing immunity against acute inhalational melioidosis. Infect Immun 86

Carr Gregory BAWD (2007) Glanders. In: Dembek ZF (ed) Medical aspects of biological warfare. Office Of The Surgeon General At Tmm Publications, Walter Reed Army Medical Centre, Washington DC

Cdc (2021) 2021 Multistate outbreak of melioidosis [Online]. Available: https://www.cdc.gov/melioidosis/outbreak/2021/index.html. Accessed 9 Jan 2022

Chaichana P, Jenjaroen K, Amornchai P, Chumseng S, Langla S, Rongkard P, Sumonwiriya M, Jeeyapant A, Chantratita N, Teparrukkul P, Limmathurotsakul D, Day NPJ, Wuthiekanun V, Dunachie SJ (2018) Antibodies in melioidosis: the role of the indirect hemagglutination assay in evaluating patients and exposed populations. Am J Trop Med Hyg 99:1378–1385

Chambon L (1955) Isolement Du Bacille De Whitmore À Partir Du Milieu Extérieur. Ann Inst Pasteur 89:229–235

Chantratita N, Tandhavanant S, Myers ND, Chierakul W, Robertson JD, Mahavanakul W, Singhasivanon P, Emond MJ, Peacock SJ, West TE (2014) Screen of whole blood responses to Flagellin identifies Tlr5 variation associated with outcome in melioidosis. Genes Immun 15:63–71

Chaowagul W, Simpson AJH, Suputtamongkol Y, White NJ (1999) Empirical cephalosporin treatment of melioidosis. Clin Infect Dis 28:1328

Chaowagul W, Chierakul W, Simpson AJ, Short JM, Stepniewska K, Maharjan B, Rajchanuvong A, Busarawong D, Limmathurotsakul D, Cheng AC, Wuthiekanun V, Newton PN, White NJ, Day NP, Peacock SJ (2005) Open-label randomized trial of oral trimethoprim-sulfamethoxazole, doxycycline, and chloramphenicol compared with trimethoprim-sulfamethoxazole and doxycycline for maintenance therapy of melioidosis. Antimicrob Agents Chemother 49:4020–4025

Cheng AC, Currie BJ (2005) Melioidosis: epidemiology, pathophysiology, and management. Clin Microbiol Rev 18:383–416

Cheng AC, Stephens DP, Anstey NM, Currie BJ (2004) Adjunctive granulocyte Colony-stimulating factor for treatment of septic shock due to melioidosis. Clin Infect Dis 38:32–37

Cheng AC, Limmathurotsakul D, Chierakul W, Getchalarat N, Wuthiekanun V, Stephens DP, Day NP, White NJ, Chaowagul W, Currie BJ, Peacock SJ (2007) A randomized controlled trial of granulocyte Colony-stimulating factor for the treatment of severe sepsis due to melioidosis in Thailand. Clin Infect Dis 45:308–314

Chetchotisakd P, Chierakul W, Chaowagul W, Anunnatsiri S, Phimda K, Mootsikapun P, Chaisuksant S, Pilaikul J, Thinkhamrop B, Phiphitaporn S, Susaengrat W, Toondee C, Wongrattanacheewin S, Wuthiekanun V, Chantratita N, Thaipadungpanit J, Day NP, Limmathurotsakul D, Peacock SJ (2014) Trimethoprim-sulfamethoxazole versus trimethoprim-sulfamethoxazole plus doxycycline as oral eradicative treatment for melioidosis (Merth): a multicentre, double-blind, non-inferiority, randomised controlled trial. Lancet 383: 807–814

Chewapreecha C, Holden MT, Vehkala M, Valimaki N, Yang Z, Harris SR, Mather AE, Tuanyok A, De Smet B, Le Hello S, Bizet C, Mayo M, Wuthiekanun V, Limmathurotsakul D, Phetsouvanh R, Spratt BG, Corander J, Keim P, Dougan G, Dance DAB, Currie BJ, Parkhill J, Peacock SJ (2017) Global and regional dissemination and evolution of *Burkholderia pseudomallei*. Nat Microbiol 2:16263

Chierakul W, Anunnatsiri S, Short JM, Maharjan B, Mootsikapun P, Simpson AJ, Limmathurotsakul D, Cheng AC, Stepniewska K, Newton PN, Chaowagul W, White NJ, Peacock SJ, Day NP, Chetchotisakd P (2005) Two randomized controlled trials of Ceftazidime alone versus Ceftazidime in combination with trimethoprim-sulfamethoxazole for the treatment of severe melioidosis. Clin Infect Dis 41:1105–1113

Choy JL, Mayo M, Janmaat A, Currie BJ (2000) Animal melioidosis in Australia. Acta Trop 74(2–3):153–158

Christopher GW, Cieslak TJ, Pavlin JA, Eitzen EM Jr (1997) Biological warfare a historical perspective. JAMA 278:412–417

Corea EM, Merritt AJ, Ler YH, Thevanesam V, Inglis TJ (2016) Sri Lankan national melioidosis surveillance program uncovers a nationwide distribution of invasive melioidosis. Am J Trop Med Hyg 94:292–298

Corea EM, De Silva AD, Thevanesam V (2018) Melioidosis In Sri Lanka. Trop Med Infect Dis 3

Cossaboom CM, Marinova-Petkova A, Strysko J, Rodriguez G, Maness T, Ocampo J, Gee JE, Elrod MG, Gulvik CA, Liu L, Bower WA, Hoffmaster AR, Blaney DD, Salzer JS, Yoder JS, Mattioli MC, Sidwa TJ, Ringsdorf L, Morrow G, Ledezma E, Kieffer A (2020) Melioidosis in a resident of Texas with no recent travel history, United States. Emerg Infect Dis 26:1295–1299

Cottew GS (1952) Melioidosis in sheep in Queensland: description of an outbreak. Aust Vet J 28: 113–122

Currie BJ, Jacups SP (2003) Intensity of rainfall and severity of melioidosis, Australia. Emerg Infect Dis 9:1538–1542

Currie B, Smith-Vaughan H, Golledge C, Buller N, Sriprakash KS, Kemp DJ (1994) *Pseudomonas pseudomallei* isolates collected over 25 years from a non-tropical endemic focus show clonality on the basis of ribotyping. Epidemiol Infect 113:307–312

Currie BJ, Fisher DA, Anstey NM, Jacups SP (2000a) Melioidosis: acute and chronic disease, relapse and re-activation. Trans R Soc Trop Med Hyg 94:301–304

Currie BJ, Fisher DA, Howard DM, Burrow JN, Selvanayagam S, Snelling PL, Anstey NM, Mayo MJ (2000b) The epidemiology of melioidosis in Australia and Papua New Guinea. Acta Trop 74: 121–127

Currie BJ, Mayo M, Anstey NM, Donohoe P, Haase A, Kemp DJ (2001) A cluster of Melioidosis cases from an endemic region is clonal and is linked to the water supply using molecular typing of *Burkholderia pseudomallei* isolates. Am J Trop Med Hyg 65:177–179

Currie BJ, Jacups SP, Cheng AC, Fisher DA, Anstey NM, Huffam SE, Krause VL (2004) Melioidosis epidemiology and risk factors from a prospective whole-population study in northern Australia. Tropical Med Int Health 9:1167–1174

Currie B, Thomas AD, Godoy D, Dance DAB, Cheng AC, Ward L, Mayo M, Pitt TL, Spratt BG (2007) Australian and Thai isolates of *Burkholderia pseudomallei* are distinct by multilocus sequence typing: revision of a case of mistaken identity. J Clin Microbiol 45:3828–3829

Currie BJ, Ward L, Cheng AC (2010) The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. PLoS Negl Trop Dis 4:E900

Currie BJ, Price EP, Mayo M, Kaestli M, Theobald V, Harrington I, Harrington G, Sarovich DS (2015) Use of whole-genome sequencing to link *Burkholderia pseudomallei* from air sampling to mediastinal melioidosis, Australia. Emerg Infect Dis 21:2052–2054

Currie BJ, Mayo M, Ward LM, Kaestli M, Meumann EM, Webb JR, Woerle C, Baird RW, Price RN, Marshall CS, Ralph AP, Spencer E, Davies J, Huffam SE, Janson S, Lynar S, Markey P, Krause VL, Anstey NM (2021) The Darwin prospective Melioidosis study: A 30-year prospective, observational investigation. Lancet Infect Dis 21:1737–1746

Dance DAB (1991) Melioidosis: the tip of the iceberg? Clin Microbiol Rev 4:52–60

Dance DAB (2000a) Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia* spp. and human-animal hosts. Acta Trop 74:159–168

Dance DAB (2000b) Melioidosis as an emerging global problem. Acta Trop 74(2–3):115–119
Dance DAB, King C, Aucken H, Knott CD, West PG, Pitt TL (1992) An outbreak of melioidosis in imported primates in Britain. Vet Rec 130:525–529
Dance DAB, Limmathurotsakul D, Currie BJ (2017) *Burkholderia pseudomallei*: challenges for the clinical microbiology laboratory-a response from the front line. J Clin Microbiol 55:980–982
Darling, R. G., And Woods J.B. 2004. Usamriid's medical management of biological casualties handbook. Operational Medicine Department, U.S. Army Medical Research Institute Of Infectious Diseases, Fort Detrick, Md, USA
Dawson P, Duwell MM, Thompson RJ, Crum DA, Blythe D, Jacobs R, Elrod MG, Gee JE, Liu L, Blaney DD, Weiner Z, Bower WA, Hoffmaster AR, Salzer JS (2020) 1430. Novel transmission of *Burkholderia pseudomallei* from a freshwater aquarium to a human — Maryland, 2019. Open Forum Infect Dis 7:S721–S721
Derbyshire JB (2002) The eradication of glanders in Canada. Can Vet J. La Revue Veterinaire Canadienne 43:722–726
Deshazer D, Waag DM, Fritz DL, Woods DE (2001) Identification of A *Burkholderia mallei* polysaccharide gene cluster by subtractive hybridization and demonstration that the encoded capsule is an essential virulence determinant. Microb Pathog 30:253–269
Dobberstein J (1935) The pathology of central nervous system of horse. Berlin Schoetz
Dodin A, Galimand M (1986) Origin, course and recession of an infectious disease, melioidosis, in temperate countries. Arch Inst Pasteur Tunis 63:69–73
Dunachie SJ, Jenjaroen K, Reynolds CJ, Quigley KJ, Sergeant R, Sumonwiriya M, Chaichana P, Chumseng S, Ariyaprasert P, Lassaux P, Gourlay L, Promwong C, Teparrukkul P, Limmathurotsakul D, Day NPJ, Altmann DM, Boyton RJ (2017) Infection with *Burkholderia pseudomallei* - immune correlates of survival in acute melioidosis. Sci Rep 7:12143
Duval CW, White PG (1907) The histological lesions of experimental Glanders. J Exp Med 9:352–380
Egan AM, Gordon DL (1996) *Burkholderia pseudomallei* activates complement and is ingested but not killed by polymorphonuclear leukocytes. Infect Immun 64:4952–4959
Elschner MC, Hnizdo J, Stamm I, El-Adawy H, Mertens K, Melzer F (2014) Isolation of the highly pathogenic and zoonotic agent *Burkholderia pseudomallei* from a pet Green iguana in Prague, Czech Republic. BMC Vet Res 10:283
Elschner MC, Laroucau K, Singha H, Tripathi BN, Saqib M, Gardner I, Saini S, Kumar S, El-Adawy H, Melzer F, Khan I, Malik P, Sauter-Louis C, Neubauer H (2019) Evaluation of the comparative accuracy of the complement fixation test, Western blot and five enzyme-linked immunosorbent assays for serodiagnosis of Glanders. PLoS One 14:E0214963
Elschner MC, Melzer F, Singha H, Muhammad S, Gardner I, Neubauer H (2021) Validation of a commercial Glanders ELISA as an alternative to the Cft in international trade of *Equidae*. Front Vet Sci 8:628389
Fen SHY, Tandhavanant S, Phunpang R, Ekchariyawat P, Saiprom N, Chewapreecha C, Seng R, Thiansukhon E, Morakot C, Sangsa N, Chayangsu S, Chuananont S, Tanwisaid K, Silakun W, Buasi N, Chaisuksant S, Hompleum T, Chetchotisakd P, Day NPJ, Chantratita W, Lertmemongkolchai G, West TE, Chantratita N (2021) Antibiotic susceptibility of clinical *Burkholderia pseudomallei* isolates in Northeast Thailand during 2015-2018 and the genomic characterization of Beta-lactam-resistant isolates. Antimicrob Agents Chemother 65:E02230–E02220
Fonseca-Rodriguez O, Pinheiro Junior JW, Mota RA (2019) Spatiotemporal analysis of glanders in Brazil. J Equine Vet Sci 78:14–19
Fournier J (1965) La Mélioïdose Et Le B. De Whitmore. Controverses Épidémiologiques Et Taxonomiques. Bull Soc Pathol Exot Filiales 58:753–765
Galimand M, Dodin A (1982) [Focus on Melioidosis throughout the world]. [French]. Bull De La Soc De Pathol Exotique Et De Ses Filiales 75:375–383
Gassiep I, Bauer MJ, Harris PNA, Chatfield MD, Norton R (2021) Laboratory safety: handling *Burkholderia pseudomallei* isolates without A biosafety cabinet. J Clin Microbiol 59:E0042421
Gee JE, Allender CJ, Tuanyok A, Elrod MG, Hoffmaster AR (2014) *Burkholderia pseudomallei* Type G in Western Hemisphere. Emerg Infect Dis 20:682–684

Gee JE, Gulvik CA, Elrod MG, Batra D, Rowe LA, Sheth M, Hoffmaster AR (2017) Phylogeography of *Burkholderia pseudomallei* isolates, Western hemisphere. Emerg Infect Dis 23:1133–1138

Ghori MT, Khan MS, Khan JA, Rabbani M, Shabbir MZ, Chaudhry HR, Ali MA, Muhammad J, Elschner MC, Jayarao BM (2017) Seroprevalence and risk factors of glanders in working equines – findings of A cross-sectional study in Punjab Province of Pakistan. Acta Trop

Glass MB, Popovic T (2005) Preliminary evaluation of the Api 20ne and rapid Nf plus systems for rapid identification of *Burkholderia pseudomallei* and *B. mallei*. J Clin Microbiol 43:479–483

Glass MB, Beesley CA, Wilkins PP, Hoffmaster AR (2009) Comparison of four selective media for the isolation of *Burkholderia mallei* and *Burkholderia pseudomallei*. Am J Trop Med Hyg 80: 1023–1028

Godoy D, Randle G, Simpson AJ, Aanensen DM, Pitt TL, Kinoshita R, Spratt BG (2003) Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. J Clin Microbiol 41:2068–2079

Hagebock JM, Schlater LK, Frerichs WM, Olson DP (1993) Serologic responses to the Mallein test for glanders in Solipeds. J Vet Diagn Investig 5:97–99

Hantrakun V, Somayaji R, Teparrukkul P, Boonsri C, Rudd K, Day NPJ, West TE, Limmathurotsakul D (2018) Clinical epidemiology and outcomes of community acquired infection and sepsis among hospitalized patients in a resource limited setting in Northeast Thailand: A prospective observational study (Ubon-sepsis). PLoS One 13:E0204509

Hantrakun V, Kongyu S, Klaytong P, Rongsumlee S, Day NPJ, Peacock SJ, Hinjoy S, Limmathurotsakul D (2019) Clinical epidemiology of 7126 melioidosis patients in Thailand and the implications for a National Notifiable Diseases Surveillance System. *Open forum*. Infect Dis 6:Ofz498

Hatcher CL, Mott TM, Muruato LA, Sbrana E, Torres AG (2016) *Burkholderia mallei* Clh001 attenuated vaccine strain is immunogenic and protects against acute respiratory Glanders. Infect Immun 84:2345–2354

Health OIDÉ-WOFA (2003) Glanders. Terrestrial animal Health code, 12th edn. World Organisation For Animal Health

Health OIDÉ-WOFA (2013) Glanders. Oie Terrestrial Manual 2(5):11

Heine HS, England MJ, Waag DM, Byrne WR (2001) In vitro antibiotic susceptibilities of *Burkholderia mallei* (causative agent of glanders) determined by broth microdilution and E-test. Antimicrob Agents Chemother 45:2119–2121

Höger AC, Mayo M, Price EP, Theobald V, Harrington G, Machunter B, Choy JL, Currie BJ, Kaestli M (2016) The Melioidosis agent *Burkholderia pseudomallei* and related opportunistic pathogens detected in faecal matter of wildlife and livestock in Northern Australia. Epidemiol Infect, Firstview:1–9

Holland DJ, Wesley A, Drinkovic D, Currie BJ (2002) Cystic fibrosis and *Burkholderia pseudomallei* infection: an emerging problem? Clin Infect Dis 35:E138–E140

Hornstra H, Pearson T, Georgia S, Liguori A, Dale J, Price E, O'Neill M, Deshazer D, Muhammad G, Saqib M, Naureen A, Keim P (2009) Molecular epidemiology of glanders, Pakistan. Emerg Infect Dis 15:2036–2039

Houghton RL, Reed DE, Hubbard MA, Dillon MJ, Chen H, Currie BJ, Mayo M, Sarovich DS, Theobald V, Limmathurotsakul D, Wongsuvan G, Chantratita N, Peacock SJ, Hoffmaster AR, Duval B, Brett PJ, Burtnick MN, Aucoin DP (2014) Development of a prototype lateral flow immunoassay (Lfi) for the rapid diagnosis of melioidosis. PLoS Negl Trop Dis 8:E2727

Howard K, Inglis TJ (2005) Disinfection of *Burkholderia pseudomallei* in potable water. Water Res 39:1085–1092

Howe C, Miller WR (1947) Human glanders; report of six cases. Ann Intern Med 26:93–115

Hsueh PT, Huang WT, Hsueh HK, Chen YL, Chen YS (2018) Transmission modes of Melioidosis in Taiwan. Trop Med Infect Dis 3

Hu TP, Kung CD, Au BH (1958) Studies on experimental infection of cattle, sheep and goats with Glanders bacilli. Acta Vet Zootech Sinica 8

Huson MAM, Kling K, Chankongsin S, Phongluxa K, Keoluangkhot V, Newton PN, Dance D, Heller T, Neumayr A (2020) Point-of-care ultrasound in the diagnosis of melioidosis in Laos. Am J Trop Med Hyg 103:675–678

Idris A, Rachmat RFN, Ali SMM (1998) Melioidosis: a case of sheep to human transmission. J Vet Malaysia 10:77–79

Iliukhin VI, Alekseev VV, Antonov Iu V, Savchenko ST, Lozovaia NA (1994) Effectiveness of treatment of experimental Glanders after aerogenic infection. Antibiot Khimioter 39:45–48

Inglis TJ, Garrow SC, Adams C, Henderson M, Mayo M, Currie BJ (1999) Acute melioidosis outbreak in Western Australia. Epidemiol Infect 123:437–443

Inglis TJJ, Mee BJ, Chang BJ (2001) The environmental microbiology of melioidosis. Rev Med Microbiol 12:13–20

Jeddeloh JA, Fritz DL, Waag DM, Hartings JM, Andrews GP (2003) Biodefense-driven murine model of pneumonic melioidosis. Infect Immun 71:584–587

Jenjaroen K, Chumseng S, Sumonwiriya M, Ariyaprasert P, Chantratita N, Sunyakumthorn P, Hongsuwan M, Wuthiekanun V, Fletcher HA, Teparrukkul P, Limmathurotsakul D, Day NP, Dunachie SJ (2015) T-cell responses are associated with survival in acute melioidosis patients. PLoS Negl Trop Dis 9:E0004152

Jun JB, Lee T, Jeong J, Chun JH, Shin YW, Jung J (2017) Accidental occupational exposure to *Burkholderia pseudomallei* in South Korea did not result in melioidosis. Infect Control Hosp Epidemiol 38:886–888

Katz J, Dewald R, Nicholson J (2000) Procedurally similar competitive immunoassay systems for the Serodiagnosis of *Babesia equi*, *Babesia caballi*, *Trypanosoma equiperdum*, and *Burkholderia mallei* infection in horses. J Vet Diagn Investig 12:46–50

Kenny DJ, Russell P, Rogers D, Eley SM, Titball RW (1999) In vitro susceptibilities of *Burkholderia mallei* in comparison to those of other pathogenic *Burkholderia* spp. Antimicrob Agents Chemother 43:2773–2775

Ketterer PJ, Webster WR, Shield J, Arthur RJ, Blackall PJ, Thomas AD (1986) Melioidosis in intensive piggeries in south eastern Queensland. Aust Vet J 63:146–149

Kettle ANB, Wernery U (2016) Glanders and the risk for its introduction through the international movement of horses. Equine Vet J 48:654–658

Khakhum N, Chapartegui-Gonzalez I, Torres AG (2020) Combating the great mimicker: latest progress in the development of *Burkholderia pseudomallei* vaccines. Expert Rev Vaccines:1–8

Khaki P, Mosavari N, Khajeh NS, Emam M, Ahouran M, Hashemi S, Taheri MM, Jahanpeyma D, Nikkhah S (2012) Glanders outbreak at Tehran Zoo, Iran. Iran J Microbiol 4:3–7

Khan I, Elschner MC, Melzer F, Gwida M, Wieler LH, Ali R, Saqib M, Neubauer H (2012) Performance of complement fixation test And confirmatory immunoblot as two-cascade testing approach for serodiagnosis of glanders in an endemic region of South East Asia. Berl Munch Tierarztl Wochenschr 125:117–121

Khan I, Wieler LH, Melzer F, Elschner MC, Muhammad G, Ali S, Sprague LD, Neubauer H, Saqib M (2013) Glanders in animals: a review on epidemiology, clinical presentation, diagnosis and countermeasures. Transbound Emerg Dis 60:204–221

Kinoshita Y, Cloutier AK, Rozak DA, Khan MSR, Niwa H, Uchida-Fujii E, Katayama Y, Tuanyok A (2019) A novel selective medium for the isolation of *Burkholderia mallei* from equine specimens. BMC Vet Res [Electronic Res] 15:133

Kinsley AT (1911) Probable influence of open water troughs in the spread of glanders. J Comp Pathol Ther 6

Koh GC, Maude RR, Schreiber MF, Limmathurotsakul D, Wiersinga WJ, Wuthiekanun V, Lee SJ, Mahavanakul W, Chaowagul W, Chierakul W, White NJ, Van Der Poll T, Day NP, Dougan G, Peacock SJ (2011) Glyburide is anti-inflammatory and associated with reduced mortality in melioidosis. Clin Infect Dis 52:717–725

Koh GC, Weehuizen TA, Breitbach K, Krause K, De Jong HK, Kager LM, Hoogendijk AJ, Bast A, Peacock SJ, Van Der Poll T, Steinmetz I, Wiersinga WJ (2013) Glyburide reduces bacterial dissemination in a mouse model of melioidosis. PLoS Negl Trop Dis 7:E2500

Kunakorn M, Jayanetra P, Tanphaichitra D (1991) Man-To-Man transmission of Melioidosis. Lancet 337:1290–1291

Lafontaine ER, Chen Z, Huertas-Diaz MC, Dyke JS, Jelesijevic TP, Michel F, Hogan RJ, He B (2019) The autotransporter protein Bata is a protective antigen against lethal aerosol infection with *Burkholderia mallei* and *Burkholderia pseudomallei*. Vaccine X:1

Laroucau K, Colaneri C, Jay M, Corde Y, Drapeau A, Durand B, Zientara S, Beck C, European Union Laboratories Involved In Glanders, S (2016) Interlaboratory ring trial to evaluate Cft proficiency of European laboratories for diagnosis of glanders in equids. Vet Rec 178:632

Laroucau K, De Assis L, Santana V, Girault G, Martin B, Miranda Da Silveira PP, Brasil Machado M, Joseph M, Wernery R, Wernery U, Zientara S, Madani N (2018) First molecular characterisation of A Brazilian *Burkholderia mallei* strain isolated from a mule in 2016. Infect Genet Evol 57:117–120

Lasch P, Jacob D, Grunow R, Schwecke T, Doellinger J (2016) Matrix-assisted laser desorption/ ionization time-of-flight (Maldi-Tof) mass spectrometry (Ms) for the identification of highly pathogenic bacteria. Trac - Trends Anal Chem 85

Le Tohic S, Montana M, Koch L, Curti C, Vanelle P (2019) A review of melioidosis cases imported into Europe. Eur J Clin Microbiol Infect Dis 38

Lee MA, Wang D, Yap EH (2005) Detection And differentiation of *Burkholderia pseudomallei*, *Burkholderia mallei* and *Burkholderia thailandensis* by multiplex PCR. FEMS Immunol Med Microbiol 43:413–417

Leelarasamee A, Bovornkitti S (1989) Melioidosis: review and update. Rev Infect Dis 11:413–425

Li L, Lu Z, Han O (1994) [Epidemiology of Melioidosis in China]. [Chinese]. Chung-Hua Liu Hsing Ping Hsueh Tsa Chih Chinese. J Epidemiol 15:292–295

Limmathurotsakul D, Wongratanacheewin S, Teerawattanasook N, Wongsuvan G, Chaisuksant S, Chetchotisakd P, Chaowagul W, Day NP, Peacock SJ (2010) Increasing incidence of human melioidosis in Northeast Thailand. Am J Trop Med Hyg 82:1113–1117

Limmathurotsakul D, Wuthiekanun V, Wongsuvan G, Pangmee S, Amornchai P, Teparrakkul P, Teerawattanasook N, Day NP, Peacock SJ (2011) Repeat blood culture positive for *B. pseudomallei* indicates an increased risk of death from melioidosis. Am J Trop Med Hyg 84:858–861

Limmathurotsakul D, Dance DA, Wuthiekanun V, Kaestli M, Mayo M, Warner J, Wagner DM, Tuanyok A, Wertheim H, Yoke Cheng T, Mukhopadhyay C, Puthucheary S, Day NP, Steinmetz I, Currie BJ, Peacock SJ (2013a) Systematic review and consensus guidelines for environmental sampling of *Burkholderia pseudomallei*. PLoS Negl Trop Dis 7:E2105

Limmathurotsakul D, Kanoksil M, Wuthiekanun V, Kitphati R, Destavola B, Day NP, Peacock SJ (2013b) Activities of daily living associated with acquisition of melioidosis in Northeast Thailand: a matched case-control study. PLoS Negl Trop Dis 7:E2072

Limmathurotsakul D, Funnell SG, Torres AG, Morici LA, Brett PJ, Dunachie S, Atkins T, Altmann DM, Bancroft G, Peacock SJ, Steering Group On Melioidosis Vaccine, D (2015) Consensus on the development of vaccines against naturally acquired melioidosis. Emerg Infect Dis 21:E1–E7

Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NP, Peacock SJ, Hay SI (2016) Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol 1:15008

Lipsitz R, Garges S, Aurigemma R, Baccam P, Blaney DD, Cheng AC, Currie BJ, Dance D, Gee JE, Larsen J, Limmathurotsakul D, Morrow MG, Norton R, O'mara E, Peacock SJ, Pesik N, Rogers LP, Schweizer HP, Steinmetz I, Tan G, Tan P, Wiersinga WJ, Wuthiekanun V, Smith TL (2012) Workshop on treatment of and postexposure prophylaxis for *Burkholderia pseudomallei* and *B. mallei* infection, 2010. Emerg Infect Dis 18:E2

Loeffler F (1886) The aetiology of Glanders *Arb Kaiserl Gesundh*, 58

Luangasanatip N, Flasche S, Dance DAB, Limmathurotsakul D, Currie BJ, Mukhopadhyay C, Atkins T, Titball R, Jit M (2019) The global impact and cost-effectiveness of a melioidosis vaccine. BMC Med 17:129

Malik P, Singha H, Khurana SK, Kumar R, Kumar S, Raut AA, Riyesh T, Vaid RK, Virmani N, Singh BK, Pathak SV, Parkale DD, Singh B, Pandey SB, Sharma TR, Chauhan BC, Awasthi V,

Jain S, Singh RK (2012) Emergence and re-emergence of glanders in India: a description of outbreaks from 2006 to 2011. Vet Ital 48:167–178

Mccormick JB, Sexton DJ, Mcmurray JG, Carey E, Hayes P, Feldman RA (1975) Human-To-Human transmission of *Pseudomonas pseudomallei*. Ann Intern Med 83:512–513

Mcleod C, Morris PS, Bauert PA, Kilburn CJ, Ward LM, Baird RW, Currie BJ (2015) Clinical presentation and medical management of melioidosis in children: A 24-year prospective study in the Northern Territory of Australia and review of the literature. Clin Infect Dis 60:21–26

Merritt AJ, Peck M, Gayle D, Levy A, Ler YH, Raby E, Gibbs TM, Inglis TJ (2016) Cutaneous melioidosis cluster caused by contaminated wound irrigation fluid. Emerg Infect Dis 22:1420–1427

Microbiology ASF (2008) *Burkholderia mallei* and *B. pseudomallei*. Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism

Miller WR, Pannell L, Cravitz L, Tanner WA, Ingalls MS (1948) Studies on certain biological characteristics of *Malleomyces mallei* and *Malleomyces pseudomallei*: I. morphology, cultivation, viability, and isolation from contaminated specimens. J Bacteriol 55:115–126

Minnet FC (1959) Glanders and melioidosis. In: Stableforth AWAIAG (ed) Infectious diseases of animals. Butterworths Scientific Publications, London

Mitchell PK, Campbell C, Montgomery MP, Paoline J, Wilbur C, Posivak-Khouly L, Garafalo K, Elrod M, Liu L, Weltman A (2017) Notes from the field: travel-associated melioidosis and resulting laboratory exposures - United States, 2016. MMWR Morb Mortal Wkly Rep 66:1001–1002

Mollaret HH (1988) "L'affaire Du Jardin Des Plantes" Ou Comment La Melioidose Fit Son Apparition En France. Med Et Maladies Infect 18:643–654

Morici L, Torres AG, Titball RW (2019) Novel multi-component vaccine approaches for *Burkholderia pseudomallei*. Clin Exp Immunol 196:178–188

Nachiangmai N, Patamasucon P, Tipayamonthein B, Kongpon A, Nakaviroj S (1985) *Pseudomonas pseudomallei* in southern Thailand. Southeast Asian J Trop Med Public Health 16:83–87

Nelson M, Salguero FJ, Dean RE, Ngugi SA, Smither SJ, Atkins TP, Lever MS (2014) Comparative experimental subcutaneous Glanders and melioidosis in the common marmoset (*Callithrix jacchus*). Int J Exp Pathol 95:378–391

Nelson M, Nunez A, Ngugi SA, Atkins TP (2021) The lymphatic system as a potential mechanism of spread of melioidosis following ingestion of *Burkholderia pseudomallei*. PLoS Negl Trop Dis 15:E0009016

Neubauer H, Sprague LD, Zacharia R, Tomaso H, Al Dahouk S, Wernery R, Wernery U, Scholz HC (2005) Serodiagnosis of *Burkholderia mallei* infections in horses: state-of-the-art and perspectives. J Vet Med B Infect Dis Vet Public Health 52:201–205

Ngauy V, Lemeshev Y, Sadkowski L, Crawford G (2005) Cutaneous melioidosis in a man who was taken as a prisoner of war by the Japanese during World War II. J Clin Microbiol 43:970–972

Nierman WC, Deshazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, Ulrich RL, Ronning CM, Brinkac LM, Daugherty SC, Davidsen TD, Deboy RT, Dimitrov G, Dodson RJ, Durkin AS, Gwinn ML, Haft DH, Khouri H, Kolonay JF, Madupu R, Mohammoud Y, Nelson WC, Radune D, Romero CM, Sarria S, Selengut J, Shamblin C, Sullivan SA, White O, Yu Y, Zafar N, Zhou L, Fraser CM (2004) Structural flexibility in the *Burkholderia mallei* genome. Proc Natl Acad Sci U S A 101:14246–14251

Nuntayanuwat S, Dharakul T, Chaowagul W, Songsivilai S (1999) Polymorphism in the promoter region of tumor necrosis factor- alpha gene is associated with severe meliodosis. HumanImmunology 60:979–983

O'Leary P (1908) Investigations concerning pathogenesis of glanders. Am Vet Rev 3

O'connor C, Kenna D, Walsh A, Zamarreño DV, Dance D (2020) Imported Melioidosis in the United Kingdom: increasing incidence but continued under-reporting. Clin Inf Pract 100051

Office International Des Épizooties, W. O. F. A. H (2011) Handistatus Ii

Parameswaran U, Baird RW, Ward LM, Currie BJ (2012) Melioidosis at Royal Darwin Hospital in the big 2009-2010 wet season: comparison with the preceding 20 years. Med J Aust 196:345–348

Peacock SJ, Schweizer HP, Dance DAB, Smith TL, Gee JE, Wuthiekanun V, Deshazer D, Steinmetz I, Tan P, Currie BJ (2008) Management of accidental laboratory exposure to *Burkholderia pseudomallei* and *B. mallei* Emerg Infect Dis 14:E2

Pearson T, Giffard P, Beckstrom-Sternberg S, Auerbach R, Hornstra H, Tuanyok A, Price EP, Glass MB, Leadem B, Beckstrom-Sternberg JS, Allan GJ, Foster JT, Wagner DM, Okinaka RT, Sim SH, Pearson O, Wu Z, Chang J, Kaul R, Hoffmaster AR, Brettin TS, Robison RA, Mayo M, Gee JE, Tan P, Currie BJ, Keim P (2009) Phylogeographic reconstruction of a bacterial species with high levels of lateral gene transfer. BMC Biol 7:78

Phokrai P, Karoonboonyanan W, Thanapattarapairoj N, Promkong C, Dulsuk A, Koosakulnirand S, Canovali S, Indrawattana N, Jutrakul Y, Wuthiekanun V, Limmathurotsakul D, Brett PJ, Burtnick MN, Lertmemongkolchai G, Chantratita N (2018) A rapid immunochromatography test based on Hcp1 is a potential point-of-care test for serological diagnosis of melioidosis. J Clin Microbiol 56

Pilcher JT (1907) Glanders in the human subject: clinical report of two cases observed in the fourth medical division of Bellevue hospital of New York. Ann Surg 45:444–452

Pitman MC, Luck T, Marshall CS, Anstey NM, Ward L, Currie BJ (2015) Intravenous therapy duration And outcomes in melioidosis: a new treatment paradigm. PLoS Negl Trop Dis 9:E0003586

Podin Y, Sarovich DS, Price EP, Kaestli M, Mayo M, Hii K, Ngian H, Wong S, Wong I, Wong J, Mohan A, Ooi M, Fam T, Wong J, Tuanyok A, Keim P, Giffard PM, Currie BJ (2014) *Burkholderia Pseudomallei* isolates from Sarawak, Malaysian Borneo, are predominantly susceptible to aminoglycosides and macrolides. Antimicrob Agents Chemother 58:162–166

Popov SF, Mel'nikov BI, Lagutin MP, Kurilov V (1991) Capsule formation in the causative agent of glanders. Mikrobiol Zh 53:90–92

Pumpuang A, Dunachie SJ, Phokrai P, Jenjaroen K, Sintiprungrat K, Boonsilp S, Brett PJ, Burtnick MN, Chantratita N (2017) Comparison of O-polysaccharide and Hemolysin co-regulated protein as target antigens for serodiagnosis of melioidosis. PLoS Negl Trop Dis 11:E0005499

Pumpuang A, Phunpang R, Ekchariyawat P, Dulsuk A, Loupha S, Kwawong K, Charoensawat Y, Thiansukhon E, Day NPJ, Burtnick MN, Brett PJ, West TE, Chantratita N (2019) Distinct classes and subclasses of antibodies to Hemolysin co-regulated protein 1 and O-polysaccharide and correlation with clinical characteristics of melioidosis patients. Sci Rep 9:13972

Rahman MS, Bhattacharjee PK, Sarker RR, Parvin MS, Tasnin S, Sarker MAS, Neubauer H, Khatun F, Wares MA, Nishidate I, Elschner MC (2018) Glanders in horses in some selected areas of Bangladesh and comparison between Cft and immunoblot used for the screening of glanders. Indian J Anim Res 54

Rajchanuvong A, Chaowagul W, Suputtamongkol Y, Smith MD, Dance DAB, White NJ (1995) A prospective comparison of co-Amoxiclav and the combination of chloramphenicol, doxycycline, and co-Trimoxazole for the oral maintenance treatment of melioidosis. Trans R Soc Trop Med Hyg 89:546–549

Ralph A, Mcbride J, Currie BJ (2004) Transmission of *Burkholderia pseudomallei* via breast milk in northern Australia. Pediatr Infect Dis J 23:1169–1171

Rao C, Hu Z, Chen J, Tang M, Chen H, Lu X, Cao L, Deng L, Mao X, Li Q (2019) Molecular epidemiology and antibiotic resistance of *Burkholderia pseudomallei* isolates from Hainan, China: a strobe compliant observational study. Medicine 98:E14461

Ribot WJ, Ulrich RL (2006) The animal pathogen-like type III secretion system is required for the intracellular survival of *Burkholderia mallei* within J774.2 macrophages. Infect Immun 74: 4349–4353

Rimington RA (1962) Melioidosis in North Queensland. Med J Aust 49(1):50–53

Robins GD (1906) A study of chronic glanders in man with report of a case

Rodriguez JY, Huertas MG, Rodriguez GJ, Vargas-Otalora S, Benitez-Penuela MA, Esquea K, Rios R, Mendoza LR, Diaz L, Reyes J, Alvarez-Moreno CA (2020) Case report: gestational melioidosis through perinatal transmission. Am J Trop Med Hyg 103:1838–1840

Rogers JV, Lastivka AT, Richter WR (2016) Persistence and inactivation of *Burkholderia mallei* China 7 deposited on nonporous laboratory materials. Appl Biosafe 21:66–70

Rogul M, Brendle JJ, Haapala DK, Alexander AD (1970) Nucleic acid similarities among *Pseudomonas pseudomallei*, *Pseudomonas multivorans*, and *Actinobacillus mallei*. J Bacteriol 101:827–835

Rubin, H. L., Alexander, A. D. & Yager, R. H. 1963. Melioidosis--A Military Medical Problem? Mil Med, 128**,** 538–542

Rudrik JT, Soehnlen MK, Perry MJ, Sullivan MM, Reiter-Kintz W, Lee PA, Pettit D, Tran A, Swaney E (2017) Safety and accuracy of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of highly pathogenic organisms. J Clin Microbiol 55: 3513–3529

Russell P, Eley SM, Ellis J, Green M, Bell DL, Kenny DJ, Titball RW (2000) Comparison of efficacy of ciprofloxacin and doxycycline against experimental melioidosis and glanders. J Antimicrob Chemother 45:813–818

Rutherford JG (1906) Special report on glanders. Veterinary Director-General And Livestock Commissioner, Ottawa

Ryan CW, Bishop K, Blaney DD, Britton SJ, Cantone F, Egan C, Elrod MG, Frye CW, Maxted AM, Perkins G (2018) Public Health response to an imported case of canine melioidosis. Zoonoses Public Health, N/A-N/A

Sadiq MA, Hassan L, Aziz SA, Zakaria Z, Musa HI, Amin MM (2018) The relationship between bacterial sources and genotype to the antimicrobial resistance pattern of *Burkholderia pseudomallei*. Veterinary World 11:1404–1408

Saiprom N, Amornchai P, Wuthiekanun V, Day NP, Limmathurotsakul D, Peacock SJ, Chantratita N (2015) Trimethoprim/sulfamethoxazole resistance in clinical isolates of *Burkholderia pseudomallei* from Thailand. Int J Antimicrob Agents 45:557–559

Sanchez-Villamil JI, Torres AG (2018) Melioidosis in Mexico, Central America, And the Caribbean. Trop Med Infect Dis 3:24

Sanford JP (1985) *Pseudomonas* species (including melioidosis and Glanders). Mandell, Douglas and Bennett's principles and practice of infectious diseases

Santos Junior ELD, Moura JCR, Protasio B, Parente VAS, Veiga M (2020) Clinical repercussions of Glanders (*Burkholderia mallei* infection) in A Brazilian child: A case report. Rev Soc Bras Med Trop 53:E20200054

Saqib M, Muhammad G, Naureen A, Hussain MH, Asi MN, Mansoor MK, Toufeer M, Khan I, Neubauer H, Sprague LD (2012) Effectiveness of an antimicrobial treatment scheme in A confined glanders outbreak. BMC Vet Res 8:214

Savelkoel J, Dance DAB, Currie BJ, Limmathurotsakul D, Wiersinga WJ (2021) A call to action: time to recognise Melioidosis as a neglected tropical disease. Lancet Infect Dis 22

Saxena A, Pal V, Tripathi NK, Goel AK (2019) Development of A rapid and sensitive recombinase polymerase amplification-lateral flow assay for detection of *Burkholderia mallei*. Transbound Emerg Dis 66

Schell MA, Ulrich RL, Ribot WJ, Brueggemann EE, Hines HB, Chen D, Lipscomb L, Kim HS, Mrazek J, Nierman WC, Deshazer D (2007) Type VI secretion is a major virulence determinant in *Burkholderia mallei*. Mol Microbiol 64:1466–1485

Scholz HC, Joseph M, Tomaso H, Al Dahouk S, Witte A, Kinne J, Hagen RM, Wernery R, Wernery U, Neubauer H (2006) Detection of the reemerging agent *Burkholderia mallei* in a recent outbreak of Glanders in the United Arab Emirates by a newly developed Flip-based polymerase chain reaction assay. Diagn Microbiol Infect Dis 54:241–247

Schutz W (1898) A contribution to the subject of glanders. J Comp Pathol Ther 34

Sharrer GT (1995) The great Glanders epizootic, 1861-1866: A civil war legacy. Agric Hist 69:79–97

Shaw T, Tellapragada C, Kamath A, Kalwaje Eshwara V, Mukhopadhyay C (2019) Implications of environmental and pathogen-specific determinants on clinical presentations and disease outcome in melioidosis patients. PLoS Negl Trop Dis 13:E0007312

Simpson AJ, Suputtamongkol Y, Smith MD, Angus BJ, Rajanuwong A, Wuthiekanun V, Howe PA, Walsh AL, Chaowagul W, White NJ (1999a) Comparison of Imipenem and Ceftazidime as therapy for severe melioidosis. Clin Infect Dis 29:381–387

Simpson AJ, Suputtamongkol Y, Smith MD, Angus BJ, Rajanuwong A, Wuthiekanun V, Howe PA, Walsh AL, Chaowagul W, White NJ (1999b) Comparison of Imipenem and Ceftazidime as therapy for severe melioidosis. Clin Inf Dis 29:381–387

Singh S, Dohre SK, Kamthan A, Pal V, Karothia BS, Singha HS, Kumar S (2018) Improvement of recombinant-truncated *Burkholderia* motility protein A (Bima)-based indirect ELISA for equine glanders. J Immunoassay Immunochem 39:565–575

Singha H, Shanmugasundaram K, Saini S, Tripathi BN (2020a) Serological survey of humans exposed to *Burkholderia mallei*-infected equids: a public health approach. Asia Pac J Public Health 1010539520930500

Singha H, Shanmugasundaram K, Tripathi BN, Saini S, Khurana SK, Kanani A, Shah N, Mital A, Kanwar P, Bhatt L, Limaye V, Khasa V, Arora R, Gupta S, Sangha S, Sharma H, Agarwal SK, Tapase J, Parnam S, Dubey P, Baalasundaram SK, Mandal BN, Virmani N, Gulati BR, Malik P (2020b) Serological surveillance and clinical investigation of glanders among indigenous equines in India from 2015 to 2018. Transbound Emerg Dis 67:1336–1348

Singha H, Elschner MC, Malik P, Saini S, Tripathi BN, Mertens-Scholz K, Brangsch H, Melzer F, Singh RK, Neubauer H (2021) Molecular typing of *Burkholderia mallei* isolates from equids with glanders, India. Emerg Infect Dis 27:1745–1748

Smith MD, Wuthiekanun V, Walsh AL, White NJ (1996) In-vitro activity of Carbapenem antibiotics against Beta-lactam susceptible and resistant strains of *Burkholderia pseudomallei*. J Antimicrob Chemother 37:611–615

Smith S, Hanson J, Currie BJ (2018) Melioidosis: an Australian perspective. *Trop med*. Infect Dis 3

Song H, Hwang J, Yi H, Ulrich RL, Yu Y, Nierman WC, Kim HS (2010) The early stage of bacterial genome-reductive evolution in the host. PLoS Pathog 6:E1000922

Sprague LD, Neubauer H (2004) Melioidosis in animals: a review on epizootiology, diagnosis and clinical presentation. J Vet Med B Infect Dis Vet Public Health 51:305–320

Srinivasan A, Kraus CN, Deshazer D, Becker PM, Dick JD, Spacek L, Bartlett JG, Byrne WR, Thomas DL (2001) Glanders in a military research microbiologist. N Engl J Med 345:256–258

Stanton AT, Fletcher W (1921) Melioidosis, a new disease of the tropics. Trans Fourth Congress Far Eastern Assoc Trop Med Batavia 2:196–198

Stanton AT, Fletcher W (1932) *Melioidosis*. John Bale Sons, & Danielsson Ltd, London

Stevens MP, Wood MW, Taylor LA, Monaghan P, Hawes P, Jones PW, Wallis TS, Galyov EE (2002) An Inv/mxi-Spa-like type III protein secretion system in *Burkholderia pseudomallei* modulates intracellular behaviour of the pathogen. Mol Microbiol 46:649–659

Stevens MP, Haque A, Atkins T, Hill J, Wood MW, Easton A, Nelson M, Underwood-Fowler C, Titball RW, Bancroft GJ, Galyov EE (2004) Attenuated virulence and protective efficacy of a *Burkholderia pseudomallei* Bsa type III secretion mutant in murine models of melioidosis. Microbiology 150:2669–2676

Stevens MP, Stevens JM, Jeng RL, Taylor LA, Wood MW, Hawes P, Monaghan P, Welch MD, Galyov EE (2005) Identification of a bacterial factor required for actin-based motility of *Burkholderia pseudomallei*. Mol Microbiol 56:40–53

Stoesser N, Pocock J, Moore CE, Soeng S, Chhat HP, Sar P, Limmathurotsakul D, Day N, Thy V, Sar V, Parry CM (2012) Pediatric Suppurative Parotitis in Cambodia between 2007 and 2011. Pediatr Infect Dis J 31:865–868

Strauss JM, Groves MG, Mariappan M, Ellison DW (1969) Melioidosis in Malaysia. Ii. Distribution of *Pseudomonas pseudomallei* in soil and surface water. Am J Trop Med Hyg 18:698–702

Subakir H, Chong YM, Chan YF, Hasan MS, Jamaluddin MFH, Pang YK, Ponnampalavanar S, Syed Omar SF, Sam IC (2020) Selective media and real-time PCR improves diagnosis of melioidosis in community-acquired pneumonia in a low-incidence setting in Kuala Lumpur, Malaysia. J Med Microbiol 69:49–51

Sullivan RP, Marshall CS, Anstey NM, Ward L, Currie BJ (2020) 2020 review and revision of the 2015 Darwin Melioidosis treatment guideline; paradigm drift not shift. PLoS Negl Trop Dis 14: E0008659

Suntornsut P, Teparrukkul P, Wongsuvan G, Chaowagul W, Michie S, Day NPJ, Limmathurotsakul D (2021) Effectiveness of a multifaceted prevention programme for melioidosis in diabetics (Premel): A stepped-wedge cluster-randomised controlled trial. PLoS Negl Trop Dis 15: E0009060

Suputtamongkol Y, Hall AJ, Dance DAB, Chaowagul W, Rajchanuvong A, Smith MD, White NJ (1994) The epidemiology of melioidosis in Ubon Ratchatani, Northeast Thailand. Int J Epidemiol 23:1082–1090

Tapia D, Sanchez-Villamil JI, Torres AG (2020) Multicomponent gold Nano-Glycoconjugate as a highly immunogenic and protective platform against *Burkholderia mallei*. Npj Vaccines 5

Thibault FM, Hernandez E, Vidal DR, Girardet M, Cavallo JD (2004a) Antibiotic susceptibility of 65 isolates of *Burkholderia pseudomallei* and *Burkholderia mallei* to 35 antimicrobial agents. J Antimicrob Chemother 54:1134–1138

Thibault FM, Valade E, Vidal DR (2004b) Identification and discrimination of *Burkholderia pseudomallei*, *B. mallei*, and *B. thailandensis* by real-time PCR targeting type III secretion system genes. J Clin Microbiol 42:5871–5874

Thin RN, Groves M, Rapmund G, Mariappan M (1971) *Pseudomonas pseudomallei* in the surface water of Singapore. Singap Med J 12:181–182

Thomas AD, Forbes-Faulkner J, Parker M (1979) Isolation of *Pseudomonas pseudomallei* from clay layers at defined depths. Am J Epidemiol 110:515–521

Thomas AD, Norton JH, Forbes-Faulkner JC, Woodland G (1981) Melioidosis in an intensive piggery. Aust Vet J 57:144–145

Thomas AD, Forbes-Faulkner JC, Norton JH, Trueman KF (1988a) Clinical and pathological observations on goats experimentally infected with *Pseudomonas pseudomallei*. Aust Vet J 65:43–46

Thomas AD, Spinks GA, D'arcy TL, Norton JH, Trueman KF (1988b) Evaluation of four serological tests for the diagnosis of caprine melioidosis. Aust Vet J 65:261–264

Toesca IJ, French CT, Miller JF (2014) The type vi secretion system spike protein Vgrg5 mediates membrane fusion during intercellular spread by *Pseudomallei* group *Burkholderia* species. Infect Immun 82:1436–1444

Trinh TT, Hoang TS, Tran DA, Trinh VT, Gohler A, Nguyen TT, Hoang SN, Krumkamp R, Nguyen LTN, May J, Doan PM, Do CD, Que TA, Steinmetz I (2018) A simple laboratory algorithm for diagnosis of melioidosis in resource-constrained areas: a study from north-Central Vietnam. Clin Microbiol Infect 24:84 E1-84 E4

Turner P, Kloprogge S, Miliya T, Soeng S, Tan P, Sar P, Yos P, Moore CE, Wuthiekanun V, Limmathurotsakul D, Turner C, Day NP, Dance DA (2016) A retrospective analysis of melioidosis in Cambodian children, 2009-2013. BMC Infect Dis 16:688

Ulrich RL, Deshazer D (2004) Type III secretion: A virulence factor delivery system essential for the pathogenicity of *Burkholderia mallei*. Infect Immun 72:1150–1154

Ulrich RL, Deshazer D, Hines HB, Jeddeloh JA (2004) Quorum sensing: a transcriptional regulatory system involved in the pathogenicity of *Burkholderia mallei*. Infect Immun 72:6589–6596

U'ren JM, Van Ert MN, Schupp JM, Easterday WR, Simonson TS, Okinaka RT, Pearson T, Keim P (2005) Use of a real-time PCR Taqman assay for rapid identification and differentiation of *Burkholderia pseudomallei* and *Burkholderia mallei*. J Clin Microbiol 43:5771–5774

Van Der Lugt JJ, Henton MM (1995) Melioidosis in A goat. J S Afr Vet Assoc 66:71–73

Vandana KE, Mukhopadhyay C, Tellapragada C, Kamath A, Tipre M, Bhat V, Sathiakumar N (2016) Seroprevalence of *Burkholderia pseudomallei* among adults in coastal areas in Southwestern India. PLoS Negl Trop Dis 10:E0004610

Wernery R, Kinne J, Haydn-Evans J, Ul-Haq A (1997) Melioidosis in a seven year old camel, a new disease in the United Arab Emirates. J Camel Prac Res 4:141–143

Wernery U, Wernery R, Joseph M, Al-Salloom F, Johnson B, Kinne J, Jose S, Jose S, Tappendorf B, Hornstra H, Scholz HC (2011) Natural *Burkholderia mallei* infection in dromedary, Bahrain. Emerg Infect Dis 17:1277–1279

West TE, Myers ND, Chantratita N, Chierakul W, Limmathurotsakul D, Wuthiekanun V, Miao EA, Hajjar AM, Peacock SJ, Liggitt HD, Skerrett SJ (2014) Nlrc4 and Tlr5 each contribute to host defense in respiratory melioidosis. PLoS Negl Trop Dis 8:E3178

White NJ, Dance DA, Chaowagul W, Wattanagoon Y, Wuthiekanun V, Pitakwatchara N (1989) Halving of mortality of severe melioidosis by Ceftazidime. Lancet 2:697–701

Whitmore A (1913) An account of a glanders-like disease occurring in Rangoon. J Hyg (Lond) 13: 1–34

Whitmore A, Krishnaswami CS (1912) An account of the discovery of a hitherto undescribed infective disease occurring among the population of Rangoon. Indian Medical Gazette 47:262–267

Wiersinga WJ, Virk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, Limmathurotsakul D (2018) Melioidosis. Nat Rev Dis Primers 4:17107

Wittig MB, Wohlsein P, Hagen RM, Al Dahouk S, Tomaso H, Scholz HC, Nikolaou K, Wernery R, Wernery U, Kinne J, Elschner M, Neubauer H (2006) Glanders--a Comprehensive Review. Dtsch Tierarztl Wochenschr 113:323–330

Wongsuvan G, Hantrakun V, Teparrukkul P, Imwong M, West TE, Wuthiekanun V, Day NPJ, Aucoin D, Limmathurotsakul D (2018) Sensitivity and specificity of a lateral flow immunoassay (Lfi) in serum samples for diagnosis of melioidosis. Trans R Soc Trop Med Hyg 112:568–570

Woods KL, Boutthasavong L, Nicfhogartaigh C, Lee SJ, Davong V, Aucoin DP, Dance DAB (2018) Evaluation of A rapid diagnostic test for the detection of *Burkholderia pseudomallei* in the Lao People's Democratic Republic. J Clin Microbiol 56:E02002–E02017

Worley G, Young G (1945) The glanders organism with reference to its cell inclusions. J Bacteriol 49:97–100

Wuthiekanun V, Amornchai P, Chierakul W, Cheng AC, White NJ, Peacock SJ, Day NP (2004) Evaluation of immunoglobulin M (Igm) and igg rapid cassette test kits for diagnosis of melioidosis in an area of Endemicity. J Clin Microbiol 42:3435–3437

Wuthiekanun V, Desakorn V, Wongsuvan G, Amornchai P, Cheng AC, Maharjan B, Limmathurotsakul D, Chierakul W, White NJ, Day NP, Peacock SJ (2005) Rapid immunofluorescence microscopy for diagnosis of melioidosis. Clin Diagn Lab Immunol 12:555–556

Wuthiekanun V, Chierakul W, Langa S, Chaowagul W, Panpitpat C, Saipan P, Thoujaikong T, Day NP, Peacock SJ (2006) Development of antibodies to *Burkholderia pseudomallei* during childhood in melioidosis-endemic Northeast Thailand. Am J Trop Med Hyg 74:1074–1075

Wuthiekanun V, Amornchai P, Saiprom N, Chantratita N, Chierakul W, Koh GC, Chaowagul W, Day NP, Limmathurotsakul D, Peacock SJ (2011) Survey of antimicrobial resistance in clinical *Burkholderia pseudomallei* isolates over two decades in Northeast Thailand. Antimicrob Agents Chemother 55:5388–5391

Zehnder AM, Hawkins MG, Koski MA, Lifland B, Byrne BA, Swanson AA, Rood MP, Gee JE, Elrod MG, Beesley CA, Blaney DD, Ventura J, Hoffmaster AR, Beeler ES (2014) *Burkholderia pseudomallei* isolates in 2 pet iguanas, California, USA. Emerg Infect Dis 20:304–306

Part VIII

Emerging and Re-emerging Zoonoses

Control of Zoonotic TB: The Need for Multidisciplinary One Health Framework

44

Adwoa Asante-Poku, Isaac Darko Otchere, Prince Asare, Stephen Osei-Wusu, Eric Koka, and Dorothy Yeboah-Manu

Contents

Abstract

The increasing incidence of human tuberculosis (TB) caused by new mycobacterial strains such as *Mycobacterium orygis* and the syndemic relationship between TB and other endemic diseases have renewed interest in zoonotic TB (zTB) especially in Africa where control strategies and data are very limited. Furthermore, the animal-adapted mycobacterial species are intrinsically resistant to some of the first-line anti-TB medications. The main TB control tool using meat inspection and condemnation of suspected infected meat has not yielded the needed impact to reduce animal-to-human transmissions. There is therefore the need for a renewed energy involving the use of transdisciplinary measures to reduce the risk of the disease in both humans and animals. Such a measure requires scientific, sociocultural, and economic efforts to implement and sustain

A. Asante-Poku · I. D. Otchere (✉) · P. Asare (✉) · S. Osei-Wusu (✉) · D. Yeboah-Manu (✉)
Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana
e-mail: Idotchere@noguchi.ug.edu.gh; Pasare@noguchi.ug.edu.gh; stephenbg128@gmail.com; dyeboah-manu@noguchi.ug.edu.gh

E. Koka (✉)
University of Cape Coast, Cape Coast, Ghana
e-mail: ekoka@ucc.edu.gh

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_61

effective strategies for prevention and control of zoonotic TB transmission. In this chapter, we have elaborated on the importance of using the "One Health" concept in the control of zTB.

Keywords

Zoonotic TB · One health approach · Control

44.1 Introduction

Tuberculosis (TB) remains one of the world's deadliest infectious diseases ever known. Until the coronavirus disease (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent, killing close to two million people annually across many populations in the world. In 2020, 10 million people (range, 8.9–11.0 million) developed TB disease and approximately 1.2 million people (including 208,000 deaths attributed to the TB-HIV syndemics) died from TB (Global TB report 2021). Of the 10 million people diagnosed with active TB, 140,000 (range, 69,800–235,000), that is, 1.4%, were estimated to be new cases of zoonotic TB (zTB) with 8.1% (11,400; range 4470–21,600) deaths. Although TB cases are globally distributed, the burden is skewed heavily toward areas where poverty and high population density overlap (Kibuuka et al. 2021). The WHO Africa region (home to 11% of human population) accounts for one quarter of the world's TB cases, with highest rates of cases and deaths relative to population.

Tuberculosis-causing pathogens are members of *Mycobacterium tuberculosis* complex (MTBC) (Kanabalan et al. 2021). Although genetically similar, they differ in host specificity with occasional cross-species infections. *M. tuberculosis* (Mtb) and *M. africanum* (Maf) cause the majority of human TB. *M. bovis*, primarily a cattle pathogen with the widest host range, is thought to be the main causative agent of zTB (Yeboah-Manu et al. 2016). However, with new evidence from South Asia and Africa, several other mycobacterial species present in animals and environment are now known to cause zTB; these include *M. caprae*, *M. microti*, *M. mungi*, *M. pinnipedii* (Jagielski et al. 2016), and most recently *M. orygis* (members of the Bovidae family) (Brites et al. 2018). These new potential drivers of zTB warrant the urgent assessment of prevalence in order to develop appropriate control tools.

zTB, an infection directly transmissible from animal reservoirs to humans, is potentiated when there is close contact between humans and infected animals or when humans consume unpasteurized infected animal products (Kock et al. 2021). It is considered a major disease that has direct impact on international trade of live animals and animal products and is on the list of notifiable diseases of the World Organisation for Animal Health (OIE) (WHO-FAO 2017). Although zTB is efficiently controlled from commercial animals in the industrialized countries, it regrettably remains poorly controlled in low-income/middle-income countries (LMICs) encumbered with major diagnostic challenges and poor public health surveillance and reporting structures (de Macedo Coutoet et al. 2022). Unfortunately, in these

countries, nearly 85% of cattle and 82% of the human population share the same microenvironment, thence allowing for continuous transmission of zTB (Ayele et al. 2004).

Importantly, an even more troubling possibility particularly in the endemic areas is the prospect of transmission of drug resistance because of reverse zoonosis at the human-animal interface (Rahman et al. 2020). *M. bovis*, the causative agent, is naturally resistant to pyrazinamide, one of the four medications used in the standard first-line anti-TB treatment regimen (Hannan et al. 2001). The potential for MDR-TB strains to acquire an animal reservoir that could then pose a future risk to human TB control is even more troubling (Seung et al. 2015). As most treatment is initiated without drug susceptibility testing, patients with zTB could receive inadequate treatment leading to poorer treatment outcomes and the development of further resistance to other anti-TB drugs (WHO-FAO 2017). Additionally, zTB in humans is often presented as extrapulmonary disease especially among immunocompromised individuals and may be misdiagnosed, and therefore initiation of treatment can be delayed.

Sadly, sub-Saharan Africa, which is home to more than half of the world's cattle population, has been the hardest hit region for various reasons. Firstly, in most African countries, cattle are used to show economic status in society and secondly serve as the main source of income for many natives (Otte & Chilonda 2002). Moreover, countries in Africa are yet to fully implement the test-and-slaughter (TS) policy due to lack of financial commitment on the part of governments to compensate farmers.

Given the insufficient laboratory facilities, lack of accurate diagnostic tools, poor or no surveillance programs leading to underreporting of cases, and lack of financial commitment on the part of governments to compensate farmers with infected animals, the control of zTB is limited and largely ineffective in endemic regions. There is no reliable data to determine if zTB incidence and prevalence is going up or down in many regions. Furthermore, sociocultural practices such as the use of cattle to depict the economic status in the society particularly in countries where zTB is endemic also impede zTB control (Otte & Chilonda 2002).

Current zTB burden and diagnostics are all based on *M. bovis* (Kock et al. 2021). However, the existence of other causative agents such as *M. caprae* and *M. orygis* calls for new laboratory services for accurate identification and speciation to effectively determine the true burden of zTB. Rapid tests are urgently needed to assist veterinarians and farmers to quickly diagnose TB, so infected animals can be separated from the rest of the herd even if the TST strategy cannot be used due to financial limitations (Duffy et al. 2020).

As the world make strides to control TB in humans, it is important to understand the role zTB plays in the human TB burden, especially in LMICs with poor or no animal TB control programs. The "Roadmap for Zoonotic Tuberculosis," which defines milestones for both human and animal health, requires strengthening transdisciplinary and multisectoral collaboration to fight zTB. The implications of zTB go beyond human health. Therefore, an approach that recognizes the interdependence of the human and animal health, the environment, and engagement of relevant

stakeholders in diverse sectors and disciplines is urgently needed. The time is right for a bold and concerted "One Health" approach to strengthen surveillance systems and expand the availability of "appropriate diagnostic tools" that can identify and characterize zTB at points of care for both humans and animals.

44.2 Burden of zTB in Africa

zTB is present in almost all African countries and affects both domestic and wild animals. Until recently in Africa, only 7 countries (Algeria, Burkina Faso, Cameroon, Morocco, Namibia, South Africa, and Zambia) out of 55 had a zTB control program in cattle herds (Carruth et al. 2016). These countries relied mostly on the tuberculin test and post-mortem inspection for the surveillance of this disease. A large proportion of the countries in Africa, i.e., 48 countries, even to date do not have any effective zTB control measures in place (Meiring et al. 2018). Ghana is among the epizootic countries reporting zTB in Africa but has no concerted effective zTB control program with only a few selected dairy farms in the country employing the test-and-slaughter strategy at the expense and convenience of the farm owners (Bonsu et al. 2000; Asante-Poku et al. 2014). However, there is a strong suspicion of underreporting as most cattle herds are reared on a free-range system under the stewardship of nomadic herdsmen. Such animals are hardly screened and might only be screened post-mortem. Many factors account for the failure of developing countries to control and eradicate zTB but they are mostly associated with unavailability of funds to support the globally tested and proven test-and-slaughter strategy. Nevertheless, the impact of insufficient veterinary expertise and communication networks, ineffective collaboration with bordering countries, and smuggling of live animals across state boundaries cannot be understated and need to be taken seriously for better control of zTB in Africa (Etter et al. 2006). Currently, ineffective control programs are implemented by most African countries, partly because of transhumance from areas where the measures are not properly applied to other areas and lack of synergy between countries involved in the fight against zTB.

In the Sahel where most cattle population are reared, cattle breeding is practiced extensively and depends mostly on the availability of natural grazing and water. Their availability is constantly linked to the annual rainfall, often forcing breeders and their livestock to move from arid regions to more humid ones (Sanou et al. 2021). In addition, the emphasis on common enzootic diseases has led to continuous neglect of zTB disease in Africa. Thus, the only information available often comes from suspicions during routine meat inspection at slaughterhouses and slaughter areas.

44.3 Control Efforts and Associated Challenges

One of the effective ways to achieve control of zTB and eventually eradication is through the implementation of an effective surveillance system (de Macedo Couto et al. 2022). With the reclassification of zTB as list B disease by the World

Organisation for Animal Health (OIE), this is of paramount importance. Low zTB prevalence observed in developed countries has been attributed to effective surveillance activities including detection of zTB in live animals, meat inspection, screening of cattle in infected areas, trace back of carcasses suspected of zTB, movement restriction of infected herds, awareness creation on its economic and health implications, and laboratory testing and confirmation techniques (Asseged et al. 2004; Lopes et al. 2016). These, together with the enforcement of regulations such as condemnation of zTB-infected carcasses or organs, periodic test and slaughter or segregation, pasteurization of milk, and restriction of breeding from infected herds, culminated in the reduction of zTB infections (Robinson et al. 2003). And yet, in Africa with regular reports of zTB, the prevalence or actual burden remains unknown with little to no datasets and disease surveillance programs for zTB (Luciano and Roess 2020). This is largely because zTB remains one of the most difficult pathogens to detect. Additionally, maintenance of surveillance activities is very costly and largely not covered by the national budget. Testing technologies are prohibitively expensive and unreliable without state-of-the-art facilities. Moreover, the existing multiple borders and low cattle populations as well as transhumance of cattle within the region with limited tracking systems further aggravate the situation.

Despite these challenges, detection of TB in animal and animal products as well as people at high risk of zTB infection is essential for TB control. While the most widely used method for animals is the test-and-slaughter method (de la Rua-Domemech et al. 2006; Klepp et al. 2019), this is hardly conducted in LMICs due to financial constraint. The routine methods for humans are microscopy (cheap, but with only low sensitivity) in LMIC, PCR mostly in developed countries, and cultivation of bacteria which is time consuming and requires more sophisticated infrastructure absent in most African countries. There are genotyping tools that explore the genetic diversity within the different members of the MTBC, as well as identify closely related strains (Niemann et al. 2000). Several studies have found the sensitivity and specificity of these assays to be 100% (Portillo-Gómez and Sosa-Iglesias 2011). Other gene targets have been evaluated to identify and differentiate between closely related species such as *M. tuberculosis*, *M. bovis*, and *M. caprae*. The gene targets include *katG*, *pncA*, *hupB*, and *gyrB* (Sreevatsan et al. 1996; Mishra et al. 2005; Scorpio & Zhang 1996). However, the major limitations include lack of technical expertise in the characterization of zTB, the expensive instrumentation required, and the lack of these resources in LMICs (Luciano and Roess 2020; Ramos et al. 2015).

Several rapid and automated diagnostic tools have been developed for rapid identification of members of MTBC with emphasis on zTB. Examples of such tools include GeneLEAD VIII (Diagenode, Belgium), Deeplex Myc-TB assay, and GenoType MTBC (Hain Lifescience, Germany) (Bonnet et al. 2021; Jouet et al. 2021). A combination of GeneLEAD and Deeplex Myc-TB assay (diagnostic tool designed according to a 24-plexed amplicon mix) can detect bTB and resistance to 13 anti-TB drugs including pyrazinamide. These combined methods showed promising features of efficiency with sensitivity and specificity of 79.3% and 100%,

respectively (Bonnet et al. 2021; El Achkar et al. 2020). However, these kits are expensive and limited in detecting novel biovars of the MTBC and/or mutations associated with drug resistance.

44.4 Sociocultural and Economic Drivers and Their Potential Impact on zTB

In Africa, the control of zTB still remains a complex process. Livestock systems in Africa are constantly undergoing rapid transition. Changes in market dynamics, environmental factors, and cultural practices are all changing the way people keep livestock, either for food or as sources of income. However, the consequences of these changes on zoonotic disease risk still remain unknown. These changes, be they ecological, political, economic, social, and cultural forces operating at local, national, and regional levels, provide conditions that allow for a selected number of pathogens to expand and adapt to a new niche.

One of the most important drivers of these changes are social and cultural factors (Patz et al. 2000; Daszak et al. 2001; Macpherson 2005). In sub-Saharan Africa, culture, society, and religion influence the kinds of foods people eat, how foods are prepared, and the demand for foods at certain times (Shanklin 1985). In recent times, globalization of food has fostered the taste for foods from other cultures that contain raw meat or fish (e.g., sushi). For example, in some societies, bushmeat which was previously consumed in low quantity is now consumed in large amounts as an inexpensive source of protein or as a sought-after delicacy. An estimated 90 percent of all bushmeat consumed moves through a distinct and well-organized market chain, with numerous nodes along the supply chain where the meat changes hands multiple times between the animal's death and its presence on the dinner table (de Merode and Cowlishaw 2006). The exchangers in this process include, among others, hunters, porters, bicycle traders, wholesalers, market-stall owners, and food preparers. Each person handling the meat or carcasses is exposed to the normal flora as well as any potential pathogens present the animal might carry. In addition, in some societies, parts of the meat including liver, heart, and brain are consumed raw based on ethnic identity, nostalgia, and social memory (Holtzman 2006).

In parts of Africa, animal products are deemed to have medicinal value and, when consumed, play an important role in ethnomedical systems to increase strength as well as enhance virility (Afolayan and Yakubu 2009) or to treat illness in humans and domestic animals (Martin et al. 2001; Mathias and McCorkle 2004; Kakati et al. 2006; Mahawar and Jaroli 2008; Soewu 2008). All these changes increase the chances of zoonotic infection from several different types of diseases (including TB, salmonellosis, *Giardia*, *Cryptosporidium*, toxoplasmosis, or rabies).

The "One Health" approach will have a positive impact on the economic costs related to the management of zoonotic diseases. These economic burdens fall more heavily on emerging countries than on the developed world. Epizootics of disease that can be controlled by vaccination have serious consequences for livestock industries, both upstream (inputs, genetic resources) and downstream (slaughter,

processing, marketing), jobs, income, or market access, and have serious consequences for food security and food safety (Nara et al. 2008). Zoonotic diseases also have negative consequences for livestock production: decreased milk production, reduced fertility, slower growth, animal mortality, as well as market loss for animals and animal products (Lamy et al. 2012; Zinsstag et al. 2008).

Nevertheless, indirect costs of zoonoses are often overlooked. The impact of zoonoses in terms of disability-adjusted life-years (DALYs) can be quantified by using a "One Health" approach (Grace et al. 2012): a cost-benefit analysis of vaccinating livestock in Mongolia for brucellosis found that the estimated costs for vaccination (US$ 8.3 million) were exceeded by the overall benefit (US$ 26.6 million), with an average benefit-cost ratio of 3.2. Cost-benefit analyses have determined that interventions in animal populations to reduce levels of zoonotic diseases were cost effective: control of the animal diseases was less expensive than the costs of disease in humans (Zinsstag et al. 2008). Given the complex nature of the epidemiology of zoonotic TB and the influences of sociological, economic, and ecological factors, "One Health" provides an excellent economical approach for conducting research, as well as development of effective control and prevention programs for zTB.

zTB causes significant economic losses due to increased production costs of infected animals, carcass confiscation, and international trade restrictions (Canto Alarcon et al. 2013). The disease has an important economic impact through reduced meat and milk production, as well as condemnation of carcasses or affected parts that are unfit for human consumption. The drivers of zoonotic diseases, especially zTB, are quite complex – individually, culturally, and socially. Although some of these drivers may be understood in isolation or in their simpler, temporal interactions with each other (e.g., food insecurity for workers in a logging or mining camp in Africa, leading to increased hunting and consumption of bushmeat), the complex ways in which they change over time and how they interact are not well understood, hence the need for a multidisciplinary "One Health" approach to help unravel the complexities associated with the reemergence of zoonotic TB and deal appropriately with them.

44.5 Vaccines for zTB

For an effective control strategy, vaccination provided a promising option in the prevention of human and animal tuberculosis (TB) in the beginning of the twentieth century. Considerable progress has therefore been made in the past years to develop improved vaccines for both humans and animals. *Mycobacterium bovis* bacille Calmette-Guérin (BCG), an experimental vaccine designed to protect cattle from zTB, was administered for the first time to a newborn baby in Paris in 1921. Over the past century, BCG has saved millions of lives and has been given to humans more than any other vaccine. It remains the sole TB vaccine licensed for use in humans. BCG provides long-lasting strong protection against miliary and meningeal TB in children, but it is less effective for the prevention of pulmonary TB, especially in

adults. The legacy of BCG includes fundamental discoveries about TB-specific and non-specific immunity and the demonstration that TB is a vaccine-preventable disease, providing a foundation for new vaccines to hasten TB elimination (Lange et al. 2022). Therefore, a safer and more effective vaccine than BCG is urgently required.

More than a dozen TB vaccine candidates are under active evaluation in clinical trials aimed to prevent infection, disease, and recurrence (Buddle et al. 2013). Candidate vaccines undergoing testing in humans include live mycobacterial vaccines to replace BCG, subunit vaccines (virus vector or protein) to boost BCG, and therapeutic vaccines used as an adjunct to chemotherapy. Encouraging results have been obtained from field trials in cattle using BCG vaccine to protect against natural exposure to *M. bovis*. To date, no subunit TB vaccines have induced improved protection compared with that of BCG, but prime-boost combinations of BCG with DNA, protein, or virus-vectored vaccines have induced better protection than BCG vaccine alone (Buddle et al. 2006, 2013).

Also, development of an oral bait BCG formulation has demonstrated the practicability of delivering TB vaccines to wildlife. Oral BCG preparations have induced protection against experimental challenge of *M. bovis* in possums, badgers, wild boar, and white-tailed deer and against natural exposure to *M. bovis* in possums. Progress in TB vaccine development has provided much impetus for their future use (Buddle et al. 2013).

Achieving dramatic results and vaccination of cattle was discontinued as it compromised the interpretation of the tuberculin skin test. Renewed interest in the use of TB vaccination of domestic livestock rose from the realization of the financial impact of bovine TB on animal health and trade, and the need to identify a cost-effective control measure where test-and-slaughter control programs are not affordable. Strategic use of TB vaccines for cattle or wildlife maintenance species could be applicable where it is not economically feasible to cull the animal serving as the wildlife reservoir or if they are a protected species. Tests differentiating infected from vaccinated animals (DIVA tests) have now been developed, which can differentiate animals vaccinated with BCG from those infected with *M. bovis*. For these tests, antigens from the *M. tuberculosis* complex which are not expressed by BCG are used instead of bovine PPD in skin test or in the whole-blood interferon-gamma release assay (Whelan et al. 2010).

Use of BCG vaccine in cattle is appealing as the vaccine is safe, inexpensive, and commercially produced for human application and DIVA tests are available, although protection may be incomplete. Many of the parameters that may affect the efficacy of BCG for cattle have now been identified. Relatively low doses of 10^4 to 10^6 CFU Pasteur BCG administered subcutaneously to cattle were shown to be effective in inducing protection against experimental challenge with *M. bovis*. Pasteur and Danish strains of BCG have induced similar levels of protection in cattle, although IFN-gamma levels released in antigen-stimulated blood cultures were higher in the Pasteur BCG-vaccinated groups.

Protection of Eurasian badgers (*Meles meles*) from TB after intramuscular vaccination with different doses of BCG was observed in a study in Great Britain

(Lesellier et al. 2011). Vaccinating badgers with BCG has been shown to be efficacious against experimentally induced TB of badgers when administered subcutaneously and orally. As the subcutaneous route is impractical for vaccinating wild badgers and an oral vaccine bait formulation is currently unavailable, the study evaluated the intramuscular (IM) route of BCG administration. It was demonstrated that the IM route is safe in badgers. IM administration has the practical advantage of being relatively easy to perform on trapped wild badgers without recourse to chemical immobilization. Vaccination using BCG Danish strain 1331 at two different doses (high and low) generated a dose-dependent cell-mediated immune response characterized by the production of interferon-γ (IFNγ) and protection against endobronchial challenge with virulent *M. bovis*. Protection, expressed in terms of a significant reduction in the severity of disease, the number of tissues containing acid-fast bacilli, and reduced bacterial excretion, was statistically significant with higher dose only (Lesellier et al. 2011).

In Ethiopia, bTB is prevalent in intensive dairy farms. Vaccination could be an alternative control approach given the socioeconomic challenges of a test-and-slaughter control strategy. An efficacy study using BCG on calves recruited from single intradermal cervical comparative tuberculin (SICCT) test negative herds was carried out in Ethiopian farms with monitoring of immune responses by interferon gamma (IFN-γ) release assay (IGRA), SICCT test, and antibody assay. Vaccinated calves developed strong responses to the SICCT test at the sixth week post-vaccination but did not respond to ESAT-6/CFP-10 peptide antigen-based IGRA. The direct protective efficacy of BCG in protecting against bovine TB in the calves was observed to be low; it was also demonstrated that BCG vaccination reduced the severity and dissemination of lesions and delayed *M. bovis* infection in the vaccinated calves. These impacts of BCG vaccination could contribute to the containment of onward transmission of *M. bovis* from vaccinated animals to other susceptible animals (Bayissa et al. 2021).

Canto Alarcon et al. (2013) in a study showed that the BCG vaccine, alone or in combination with a culture filtrate protein (CFP) boost, has the potential to reduce TB dissemination in cattle by reducing the number of lesions and the bacterial load per lesion (Canto Alarcon et al. 2013). However, to make definitive conclusions about the usefulness of the vaccine in programs against TB in the field, it is necessary to perform long-term field trials.

Also, the use of polymers such as chitosan and poly-lactic-co-glycolic acid (PLGA) improves the immune response against different diseases by improving the interaction of antigens with the cellular immune system and modulating the host immune response (Contreras-Magallanes et al. 2021). In a study on prime vaccination with chitosan-coated Phipps BCG and boosting with CFP-PLGA against tuberculosis in a goat model, it was demonstrated that the prime BCG vaccination, boosted with a culture filtrate protein (CFP) alone or in combination with chitosan and PLGA, has the potential to reduce TB dissemination by reducing the number of animals with lesions, the number of lesions per animal, and the size of the lesions in vaccinated animals, compared with those not vaccinated or those vaccinated with BCG alone. The vaccinated groups showed significantly higher interferon-γ levels in

the blood compared to the control, non-vaccinated group after vaccination, after boosting, and after the challenge with the wild-type *Mycobacterium bovis* strain (Contreras-Magallanes et al. 2021).

44.6 Conclusion and Outlook

The above discussions underpin the need for a concrete and active plan to tackle TB in animals as it has been implemented in humans. Vaccines will protect livestock against TB; as Africa is in quest of becoming self-sufficient, it also thinks about production of vaccines against animal TB. Furthermore, there is a need to intensify surveillance to reduce animal-to-animal as well as animal-to-human transmission and this requires good detection methods. Such method should be simplified and inexpensive and can be done on the farm. A good example is the need for a rapid method such as Xpert MTB/RIF, but this should be able to differentiate between the MTBC. On the other hand, a simple serological method such as a lateral flow assay is also needed.

To design a strategy to include all stakeholders, we employ for more funding to conduct multisectoral formative studies to understand the burden of the problem. Findings from these studies may provide the true picture of global zoonotic TB for all stakeholders to design acceptable One Health interventions.

44.7 Cross-References

- ▶ Bovine Paratuberculosis and Human Crohn's Disease: Is There a Zoonotic Linkage?
- ▶ Giardiasis from a One Health Perspective
- ▶ Influenza from a One Health Perspective: Infection by a Highly Versatile Virus

References

Afolayan AJ, Yakubu MT (2009) Erectile dysfunction management options in Nigeria. J Sex Med 6(4):1090–1102. [PubMed]

Asante-Poku A, Aning KG, Boi-Kikimoto B, Yeboah-Manu D (2014) Prevalence of bovine tuberculosis in a dairy cattle farm and a research farm in Ghana. Onderstepoort j vet res [Internet] [cited 2022 Aug 27] 81(2):1–6. Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0030-24652014000200009&lng=en

Asseged B, Woldesenbet Z, Yimer E, Lemma E (2004) Evaluation of abattoir inspection for the diagnosis of Mycobacterium bovis infection in cattle at Addis Ababa abattoir. Trop Anim Health Prod 36(6):537–546

Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I (2004) Bovine tuberculosis: an old disease but a new threat to Africa. Int J Tuberc Lung Dis 8(8):924–937

Bayissa B, Sirak A, Worku A, Zewude A, Zeleke Y, Chanyalew M, Gumi B, Berg S, Conlan A, Hewinson RG, Wood JLN, Vordermeier HM, Ameni G (2021) Evaluation of the efficacy of BCG in protecting against contact challenge with bovine tuberculosis in Holstein-Friesian and

Zebu Crossbred Calves in Ethiopia. Front Vet Sci 8(July):1–14. https://doi.org/10.3389/fvets.2021.702402

Bonnet I, Enouf V, Morel F, Ok V, Jaffré J, Jarlier V et al (2021) A comprehensive evaluation of GeneLEAD VIII DNA platform combined to Deeplex Myc-TB® assay to detect in 8 days drug resistance to 13 antituberculous drugs and transmission of mycobacterium tuberculosis complex directly from clinical samples. Front Cell Infect Microbiol 11. https://doi.org/10.3389/fcimb.2021.707244

Bonsu OA, Laing E, Akanmori BD (2000) Prevalence of tuberculosis in cattle in the Dangme-West district of Ghana, public health implications. Acta Trop 76(1):9–14. https://doi.org/10.1016/s0001-706x(00)00082-6. PMID: 10913759

Brites D, Loiseau C, Menardo F, Borrell S, Boniotti MB, Warren R et al (2018) A new phylogenetic framework for the animal-adapted mycobacterium tuberculosis complex. Front Microbiol 9 (November):2820

Buddle BM, Wedlock DN, Denis M (2006) Progress in the development of tuberculosis vaccines for cattle and wildlife. Vet Microbiol 112(2–4 SPEC. ISS):191–200. https://doi.org/10.1016/j.vetmic.2005.11.027

Buddle BM, Parlane NA, Wedlock DN, Heiser A (2013) Overview of vaccination trials for control of tuberculosis in cattle, wildlife and humans. Transbound Emerg Dis 60(SUPPL1):136–146. https://doi.org/10.1111/tbed.12092

Canto Alarcon GJ, Rubio Venegas Y, Bojorquez Narvaez L, Pizano Martínez OE, García Casanova L, Sosa Gallegos S, Nava Vargas A, Olvera Ramírez AM, Milian Suazo F (2013) Efficacy of a vaccine formula against tuberculosis in cattle. PLoS One 8(10):1–7. https://doi.org/10.1371/journal.pone.0076418

Carruth L, Roess AA, Mekonnen YT, Melaku SK, Nichter M, Salman M (2016) Zoonotic tuberculosis in Africa: challenges and ways forward. Lancet 388(10059):2460–2461. https://doi.org/10.1016/S0140-6736(16)32186-9. PMID: 27871735; PMCID: PMC7135042

Contreras-Magallanes YG, Durán-Aguilar M, Sosa-Gallegos SL, Álvarez ÁH, Andrade-Santillán FA, Bárcenas-Reyes I, González-Ruíz S, Rodríguez-Hernández E, Cantó-Alarcón GJ, Milián-Suazo F (2021) Prime vaccination with chitosan-coated phipps bcg and boosting with cfp-plga against tuberculosis in a goat model. Animals 11(4):1–16. https://doi.org/10.3390/ani11041046

Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78(2):103–116. [PubMed]

De la Rua-Domenech R, Goodchild A, Vordermeier H, Hewinson R, Christiansen K, Clifton-Hadley RS (2006) Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. Res Vet Sci 81(2):190–210

de Macedo CR, Santana GO, Ranzani OT, Waldman EA (2022) One Health and surveillance of zoonotic tuberculosis in selected low-income, middle-income and high-income countries: a systematic review. PLoS Negl Trop Dis 16(6):e0010428. https://doi.org/10.1371/journal.pntd.0010428. PMID: 35666731; PMCID: PMC9203019

de Merode E, Cowlishaw G (2006) Species protection, the changing informal economy, and the politics of access to the bushmeat trade in the Democratic Republic of Congo. Conserv Biol 20(4):1262–1271. [PubMed]

Duffy SC, Srinivasan S, Schilling MA, Stuber T, Danchuk SN, Michael JS et al (2020) Reconsidering Mycobacterium bovis as a proxy for zoonotic tuberculosis: a molecular epidemiological surveillance study. Lancet Microbe 1(2):e66–e73

El Achkar S, Demanche C, Osman M, Rafei R, Ismail MB, Gaudin C et al (2020) Zoonotic tuberculosis in humans assessed by next-generation sequencing: an 18-month nationwide study in Lebanon. Eur Respir J 55(1):1900513. https://doi.org/10.1183/13993003.00513-2019

Etter E, Donado P, Jori F, Caron A, Goutard F, Roger F (2006) Risk analysis and bovine tuberculosis, a re-emerging zoonosis. Ann N Y Acad Sci 1081:61–73. https://doi.org/10.1196/annals.1373.006

Grace D, Gilbert J, Randolph T, Kang'ethe E. (2012) The multiple burdens of zoonotic disease and an ecohealth approach to their assessment. Trop Anim Health Prod 44:67. https://doi.org/10.1007/s11250-012-0209-y

Hannan MM, Desmond EP, Morlock GP, Mazurek GH, Crawford JT (2001) Pyrazinamide-monoresistant *Mycobacterium tuberculosis* in the United States. J Clin Microbiol 39(2):

647–650. https://doi.org/10.1128/JCM.39.2.647-650.2001. PMID: 11158123; PMCID: PMC87792

Holtzman JD (2006) Food and memory. Annu Rev Anthropol 35(1):361–378

Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Żaczek A et al (2016) Methodological and clinical aspects of the molecular epidemiology of *Mycobacterium tuberculosis* and other mycobacteria. Clin Microbiol Rev 29(2):239–290

Jouet A, Gaudin C, Badalato N, Allix-Béguec C, Duthoy S, Ferré A et al (2021) Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs. Eur Respir J 57(3):2002338. https://doi.org/10.1183/13993003.02338-2020

Kakati LN, Ao B, Doulo V (2006) Indigenous knowledge of zootherapeutic use of vertebrate origin by the Ao tribe of Nagaland. J Hum Ecol 19(3):163–167

Kanabalan RD, Lee LJ, Lee TY, Chong PP, Hassan L, Ismail R, Chin VK (2021) Human tuberculosis and *Mycobacterium tuberculosis* complex: a review on genetic diversity, pathogenesis and omics approaches in host biomarkers discovery. Microbiol Res 246:126674. https://doi.org/10.1016/j.micres.2020.126674. Epub 2021 Jan 29 PMID: 33549960

Kibuuka D, Mpofu C, Neave P, Manda S (2021) A spatial analysis of tuberculosis related mortality in South Africa. Int J Environ Res Public Health 18(22):11865. https://doi.org/10.3390/ijerph182211865. PMID: 34831621; PMCID: PMC8618270

Klepp LI, Eirin ME, Garbaccio S, Soria M, Bigi F, Blanco FC (2019) Identification of bovine tuberculosis biomarkers to detect tuberculin skin test and IFNγ release assay false negative cattle. Res Vet Sci 122:7–14. https://doi.org/10.1016/j.rvsc.2018.10.016

Kock R, Michel AL, Yeboah-Manu D, Azhar EI, Torrelles JB, Cadmus SI, Brunton L, Chakaya JM, Marais B, Mboera L, Rahim Z, Haider N, Zumla A (2021) Zoonotic tuberculosis - the changing landscape. Int J Infect Dis 113 Suppl 1(Suppl 1):S68–S72. https://doi.org/10.1016/j.ijid.2021.02.091. Epub 2021 Mar 10. PMID: 33713812; PMCID: PMC8672060

Lamy E, van Harten S, Sales-Baptista E, Guerra MMM, Almeida AM (2012) Chapter 2: factors influencing livestock productivity. In: Seijan V, Naqvi SMK, Ezeji T, Lakritz J, Lal R (eds) Environmental stress and amelioration in livestock production. Springer-Verlag, Berlin Heidelberg, pp 19–51. https://doi.org/10.1007/978-3-642-29205-7_2

Lange C, Aaby P, Behr MA, Donald PR, Kaufmann SHE, Netea MG, Mandalakas AM (2022) 100 years of *Mycobacterium bovis* bacille Calmette-Guérin. Lancet Infect Dis 22(1):e2–e12. https://doi.org/10.1016/S1473-3099(21)00403-5

Lesellier S, Palmer S, Gowtage-Sequiera S, Ashford R, Dalley D, Davé D, Weyer U, Salguero FJ, Nunez A, Crawshaw T, Corner LAL, Hewinson RG, Chambers MA (2011) Protection of Eurasian badgers (Meles meles) from tuberculosis after intra-muscular vaccination with different doses of BCG. Vaccine 29(21):3782–3790. https://doi.org/10.1016/j.vaccine.2011.03.028

Lopes PH, Akweongo P, Wurapa F, Afari E, Sackey S, Ocansey D, Nyarko KM (2016) Bovine tuberculosis surveillance system evaluation, Greater-Accra Region, Ghana, 2006-2011. Pan Afr Med J 25(Suppl 1):10. https://doi.org/10.11604/pamj.supp.2016.25.1.6181. PMID: 28149435; PMCID: PMC5257018

Luciano SA, Roess A (2020) Human zoonotic tuberculosis and livestock exposure in low- and middle-income countries: a systematic review identifying challenges in laboratory diagnosis. Zoonoses Public Health 67(2):97–111. https://doi.org/10.1111/zph.12684. Epub 2020 Jan 9. PMID: 31919980; PMCID: PMC7027859

Macpherson CNL (2005) Human behaviour and the epidemiology of parasitic zoonoses. Int J Parasitol 35(11–12):1319–1331. [PubMed]

Mahawar MM, Jaroli DP (2008) Traditional zootherapeutic studies in India: a review. J Ethnobiol Ethnomed 4(1):17. [PMC free article] [PubMed]

Martin M, Mathias E, McCorkle CM (2001) Ethnoveterinary medicine: an annotated bibliography of community animal healthcare. ITDG Publishing, London

Mathias E, McCorkle CM (2004) Traditional livestock healers. Rev Sci Tech 23(1):277–284. [PubMed]

Meiring C, van Helden PD, Goosen WJ (2018) TB control in humans and animals in South Africa: a perspective on problems and successes. Front Vet Sci 27(5):298. https://doi.org/10.3389/fvets.2018.00298. PMID: 30542655; PMCID: PMC6277860

Mishra A, Singhal A, Chauhan DS, Katoch VM, Srivastava K, Thakral SS et al (2005) Direct detection and identification of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in bovine samples by a novel nested PCR assay: correlation with conventional techniques. J Clin Microbiol 43(11):5670–5678. https://doi.org/10.1128/JCM.43.11.5670-5678.2005

Nara PL, Nara D, Chaudhuri R, Lin G, Tobin G (2008) Perspectives on advancing preventative medicine through vaccinology at the comparative veterinary, human and conservation medicine interface: not missing the opportunities. Vaccine 26:6200–6211

Niemann S, Richter E, Rüsch-Gerdes S (2000) Differentiation among members of the *Mycobacterium tuberculosis* complex by molecular and biochemical features: evidence for two pyrazinamide-susceptible subtypes of *M. bovis*. J Clin Microbiol 38(1):152–157

Otte MJ, Chilonda P (2002) Cattle and small ruminant production Systems in sub-Saharan Africa. A systematic. Review 32:183

Patz JA, Graczyk TK, Geller N, Vittor AY (2000) Effects of environmental change on emerging parasitic diseases. Int J Parasitol 30(12–13):1395–1405. [PubMed]

Portillo-Gómez L, Sosa-Iglesias EG (2011) Molecular identification of *Mycobacterium bovis* and the importance of zoonotic tuberculosis in Mexican patients. Int J Tubercul Lung Dis 15(10): 1409–1414

Rahman MT, Sobur MA, Islam MS, Ievy S, Hossain MJ, El Zowalaty ME, Rahman AT, Ashour HM (2020) Zoonotic diseases: etiology, impact, and control. Microorganisms 8(9):1405. https://doi.org/10.3390/microorganisms8091405. PMID: 32932606; PMCID: PMC7563794

Ramos D, Silva P, Dellagostin OA (2015) Diagnosis of bovine tuberculosis: review of main techniques. Braz J Biol 75:830–837

Robinson RA, Mainzer H, Chomel B, Bender J (2003) Surveillance methodologies for zoonotic disease at community levels expert consultation on community based public health (VPH) systems. FAO, Rome, pp 27–28

Sanou A, Dicko A, Sow KR, Djibougou A, Kabore A, Diarra B, Ouedraogo AK, Zingue D, Nouctara M, Tarnagda Z (2021) Epidemiology and microscopic diagnosis of tuberculosis in pigs and small ruminants slaughtered at Bobo-Dioulasso abattoir, Burkina Faso Onderstepoort. J Vet Res 88(1):e1–e6. https://doi.org/10.4102/ojvr.v88i1.1908. PMID: 34879685; PMCID: PMC8678950

Scorpio A, Zhang Y (1996) Mutations in *pncA*, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med 2(6): 662–667. https://doi.org/10.1038/nm0696-662

Seung KJ, Keshavjee S, Rich ML (2015) Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med 5(9):a017863. https://doi.org/10.1101/cshperspect.a017863. PMID: 25918181; PMCID: PMC4561400

Shanklin E (1985) Sustenance and symbol: anthropological studies of domesticated animals. Annu Rev Anthropol 14:375–403

Soewu DA (2008) Wild animals in ethnozoological practices among the Yorubas of Southwestern Nigeria and the implications for biodiversity conservation. Afr J Agric Res 3(6):421–427

Sreevatsan S, Escalante P, Pan X, Gillies DA 2nd, Siddiqui S, Khalaf CN et al (1996) Identification of a polymorphic nucleotide in *oxyR* specific for *Mycobacterium bovis*. J Clin Microbiol 34(8): 2007–2010. https://doi.org/10.1128/jcm.34.8.2007-2010.1996

Whelan AO, Clifford D, Upadhyay B, Breadon EL, McNair J, Hewinson GR, Vordermeier MH (2010) Development of a skin test for bovine tuberculosis for differentiating infected from vaccinated animals. J Clin Microbiol 48(9):3176–3181. https://doi.org/10.1128/JCM.00420-10

World Health Organization (WHO), Food and Agriculture Organization of the United Nations: Road Map for Zoonotic Tuberculosis (2017)

Yeboah-Manu D, Asare P, Asante-Poku A, Otchere ID, Osei-Wusu S, Danso E et al (2016) Spatio-temporal distribution of *Mycobacterium tuberculosis* complex strains in Ghana. PLoS One 11(8):e0161892

Zinsstag J, Schelling E, Roth F, Kazwala R (2008) Chapter 9. Economics of bovine tuberculosis. In: Thoen CO, Steele JH, Gilsdorf ML (eds) *Mycobacterium bovis* infection in animals and humans, 2nd edn. Blackwell Publishing, Ames

Hepatitis E Virus Zoonotic Axis

45

HEV, the Master of Hide and Seek

Kush Kumar Yadav and Scott P. Kenney

Contents

Abstract

Viral hepatitis is primarily caused by five unrelated hepatotropic viruses, hepatitis A, B, C, D, and E. While hepatitis A through C are commonly recognized as causing significant liver disease by many average individuals due to successful public health campaigns, hepatitis E is often completely unknown. Despite being unheard of in the general public, hepatitis E virus (HEV) is the leading cause of acute viral hepatitis worldwide with an estimated 20 million cases annually. Hepatotropic viruses are notoriously tricky, utilizing differing mechanisms to avoid detection and elimination by the host organism. While hepatitis B and C infections often produce few symptoms in the host while becoming chronic and spreading silently to new hosts, HEV utilizes a different strategy to continue circulating in its hosts. HEV's long incubation period and ability to self-resolve in many infected individuals coupled with animal reservoirs that show little disease upon infection allow HEV to transmit to humans through

K. K. Yadav (✉) · S. P. Kenney (✉)
Center for Food Animal Health, Department of Veterinary Preventive Medicine, Department of Animal Sciences, The Ohio State University, Wooster, OH, USA
e-mail: yadav.94@osu.edu; kenney.157@osu.edu

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_60

the food chain. Endemic human strains have similar strategies, circulating at low levels within the populace waiting for conditions associated with socioeconomic turmoil when sanitary conditions decrease allowing for massive outbreaks through contaminated water. This virological game of hide and seek ensures the continued survivability and transmission of the pathogen. While most otherwise healthy individuals will be able to self-resolve HEV infections, people with underlying comorbidities, immunocompromised people, and pregnant women in their third trimester are at much greater risk of succumbing to hepatitis E. There is still much work to be done to unravel the nuances of HEV's deadly hide-and-seek game so that humans may rid themselves of this malady.

Keywords

Hepatitis E · Virus · Host · Pregnancy · Animal

45.1 Introduction

Hepatitis E virus (HEV) is the most prevalent cause of acute viral hepatitis, which is mainly transmitted fecal-orally in humans and animals (Meng 2013). In developing countries, the fecal oral route is the predominant route of transmission through contaminated water sources (Khuroo et al. 2016). In developed countries, consumption of contaminated meat, particularly pork products, is known to zoonotically transmit HEV to humans (Capai et al. 2018). While most infections are asymptomatic, they can cause acute hepatitis in patients with preexisting liver disease and in pregnant women, predominantly in developing countries (Wu et al. 2020; Koning et al. 2015). Extra-hepatic manifestations have been described with HEV infection and acute infection can progress to chronicity primarily seen in immunocompromised patients (Horvatits and Pischke 2018).

HEV belongs to the Hepeviridae family, which has two genera: *Piscihepevirus (cutthroat trout virus)* and *Orthohepevirus* (mammalian and avian strains) with four species (pasla-, avi-, roca-, chiro-). *Paslahepevirus* contains HEV variants isolated from human, pig, wild boar, deer, mongoose, rabbit, and camel; *Avihepevirus* from chickens, *Rocahepevirus* from rat, greater bandicoot, Asian musk shrew, ferret, and mink; *Chirohepevirus* from bat (Fig. 1). *Paslahepevirus balayani* is the largest species consisting of at least 8 genotypes (gt) that infect humans (gt1, gt2, gt3,

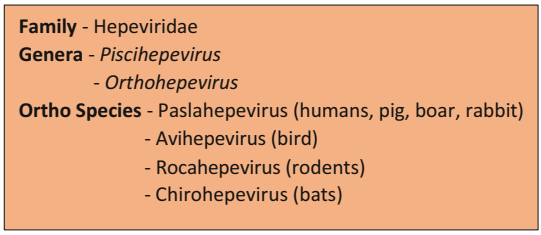

Fig. 1 Taxonomical classification of HEV

gt4, and gt7), pigs (gt3 and gt4), wild boar (gt3, gt4, gt5, and gt6), rabbits (gt3), mongoose (gt3), deer (gt3), yaks (gt4), and camels (gt7 and gt8) (Smith et al. 2014a, 2016). To date, only one serotype has been described (Smith et al. 2014b).

Of the various genotypes infecting humans, gt1 and gt2 are obligate to humans (Meng 2013). Genotype 3 is widely distributed around the world and gt4 is mainly found in Asia. Zoonotic transmission of gt3 and gt4 from pigs, wild boar, deer, and mongoose to humans have been reported (Meng 2013). In addition, rabbit strains that are close to gt3 have been discovered in humans. Camel gt7 was described in a liver transplant recipient who had consumed camel milk and meat (Lee et al. 2016). Recently, *Rocahepevirus ratti* HEV strains have been reported in humans, despite their genetic differences with other human pathogenic strains (Sridhar et al. 2018, 2021; Andonov et al. 2019).

HEV is a single-stranded, positive-sense RNA virus, possessing an ~7.2 kb RNA genome that contains three open reading frames (ORFs), ORF1, ORF2, and ORF3 (Fig. 2). ORF1 encodes a nonstructural protein about 1693 amino acids (aa) long, with several functional domains: methyltransferase, Y domain, cysteine protease, hypervariable region, X domain, helicase, RNA-dependent RNA polymerase (Reyes 1993). It is still unclear whether the ORF1 polyprotein functions as a singular polyprotein or is processed into individual proteins. Recent studies have described the presence of an additional ORF4 in gt1 HEV overlapping with ORF1. Endoplasmic reticulum stress-induced synthesis of ORF4 has been described to play a role on the proper functioning of the HEV RNA polymerase (Nair et al. 2016). Furthermore, ORF2 and ORF3 also partially overlap and are transcribed from the bicistronic subgenomic RNA (Graff et al. 2006). ORF2 protein encodes for 660 aa and is divided into three domains: S (shell), M (middle), and P (protruding) (Yamashita et al. 2009). In addition, ORF2 is known to encode a secreted free form of the capsid protein (ORF2s) that differs from the virion forming capsid protein, ORF2i (for infectious) (Yin et al. 2018). ORF3 encodes for a 113 aa phosphoprotein creating ion channel activity, involved in virus egress from infected cells, and is involved in cell signaling modification (Wang et al. 2014a).

Morphologically, HEV is considered quasi enveloped and presents in two forms: (a) the naked form, excreted in feces where the outer lipid envelope is degraded by the action of bile. (b) enveloped form, circulating in blood (Chapuy-Regaud et al. 2017; Yin et al. 2016) (Fig. 3).

HEV is transmitted mainly by fecal oral route via contaminated water ingestion and the consumption of undercooked pork or wild boar (Geng and Wang 2016). HEV transmission by blood components is becoming more prevalent and has been an increasing concern in European countries and emerging concern in the United States (Ticehurst et al. 2019; Mateos et al. 1998; Harvala et al. 2019). In addition,

Fig. 2 Genomic organization of HEV

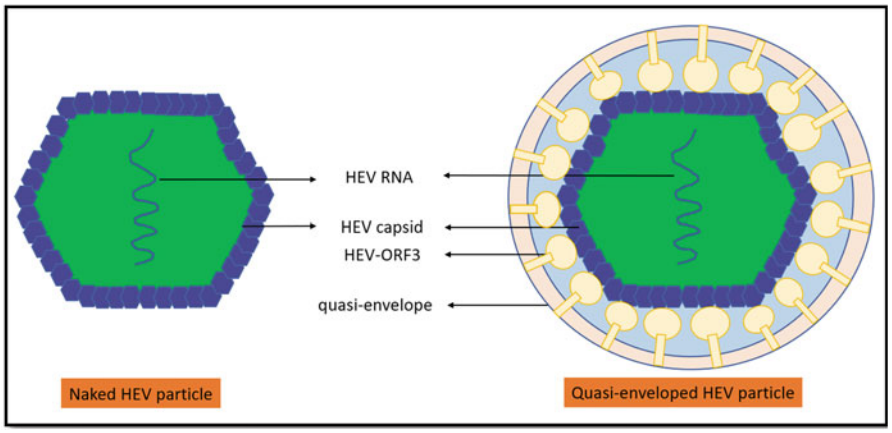

Fig. 3 Morphological forms of HEV

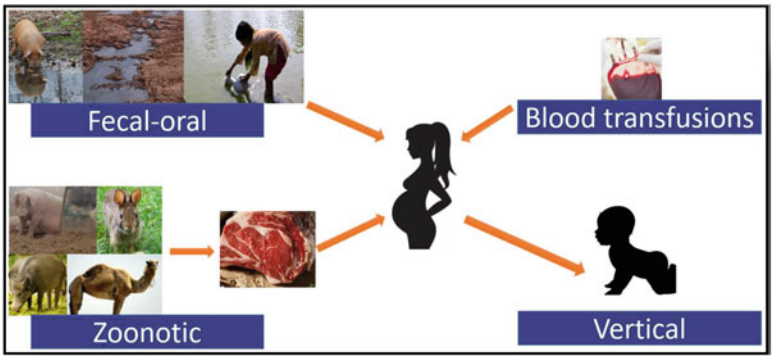

Fig. 4 Transmission route of HEV

vertical transmission from infected mother to fetus has been recognized (Li et al. 2020; Jilani et al. 2007; Gouilly et al. 2018) (Fig. 4). HEV gt1 and gt2 are correlated with poor hygiene and untreated sewage; however, gt3 and gt4 are increasing in the developed countries with high consumption of raw or undercooked pork meat (pig is known as the main reservoir) (Khuroo et al. 2016; King et al. 2018).

45.2 HEV Zoonoses and Cross-Species Infection

HEV is a zoonotic disease capable of transmitting from animals to humans (forward zoonoses) and back to animals from humans (reverse zoonoses). *Paslahepevirus balayani* species are known to infect humans producing severe disease associated with acute liver failure in pregnant women and chronic hepatitis in immunocompromised individuals. gt1 and gt2 HEV within the *Paslahepevirus* species is known to have a limited host range because experimental infection of pigs, rats, and goats was

unsuccessful. However, lambs and Wistar rats were reportedly infected by gt1 human HEV (Usmanov et al. 1994; Maneerat et al. 1996). Although independent confirmation of these reports is still lacking, gt1 and gt2 are considered obligate to humans. Pigs are considered as the major reservoir for HEV and are known to transmit gt3 and gt4 HEV to humans (Kenney and Meng 2019). Pig handlers, farmers, and swine veterinarians in both developing and industrialized countries are at the highest risk for HEV infection. For instance, individuals within the United States from major swine producing states are more likely to be seropositive for HEV antibodies than those from traditionally non-swine states; for example, individuals from Minnesota (major swine state), are approximately five to six times more likely to be seropositive than those from Alabama, which is not a major swine state (Meng et al. 2002). Furthermore, swine veterinarians were 1.9 times more likely to be HEV seropositive than non-swine veterinarians with seroprevalence of 20.8% among Austrian veterinarians (Taus et al. 2019). Interestingly, potential transmission of HEV from a pet cat and a pet pig to human owners were also reported (Renou et al. 2007; Kuno et al. 2003). Recently, *Rocahepevirus* species have been shown to infect humans (Sridhar et al. 2018, 2021; Andonov et al. 2019). Further research is required to understand the cross-species ability of *Rocahepevirus* species in various animals.

The dynamics of HEV spread and adaptation within genotypes allowing for extension of host range are unclear. Since the discovery of the first animal HEV in pigs in 1997, the first avian strain in 1998, and the first rabbit strain in 2009, an abundance of animal species have been identified with distinct circulating HEV strains, many with zoonotic potential. In recent years, it has been demonstrated that several strains causing disease in humans possess a high degree of similarity to strains detected in animals and food products of animal origin. Epidemiological screening studies for HEV via serology demonstrated high (1–53%) antibody prevalence in several countries (high-income and low-income) (Kamar et al. 2012). Furthermore, reports in healthy blood donors suggest that 1 out of 4500 in Germany, and 1 out of 8000 in Sweden, had HEV RNA in their blood at the time of donation (Lapa et al. 2015). Hence, HEV is no longer simply a disease of underdeveloped countries and there are autochthonous sources of HEV present in industrialized countries that cause infections and disease in humans. In addition, some agriculturally important species such as chickens can have reduced productivity due to HEV infection. Infection of poultry with aHEV can display symptoms including hepatitis splenomegaly syndrome, egg drop, regressive ovaries, and acute death reducing the yields of a farmed species that provides the second highest amount of protein to humans globally (Younus et al. 2017). Despite aHEV only infecting poultry, the liver tropism for the disease mimics human HEV infection in some aspects.

(a) **Non-Human Primates (NHPs)**

All four genotypes of HEV can induce infection in rhesus macaques with the development of viremia, fecal shedding, and specific antibody responses. In brief, acute viral hepatitis, viremia, fecal shedding, slight elevation of ALT, and seroconversion was reported in rhesus macaques when infected with gt3 swine HEV and gt4 swine HEV (Meng et al. 1998; Arankalle et al. 2006). Furthermore, rHEV strain developed viremia, fecal shedding along with

jaundice and malaise with elevation of liver enzymes, and seroconversion demonstrating infection in cynomolgus monkeys (Liu et al. 2013). However, avian HEV, rat and ferret strains of HEV could not infect NHPs as evidenced by no detection of viremia, fecal shedding, or seroconversion (Huang et al. 2004; Li et al. 2015a; Purcell et al. 2011). Summarization of the cross-species transmission is described below (Fig. 5).

(b) **Swine**

Specific pathogen-free pigs are susceptible to human HEV gt3 and gt4 but not to gt1 or gt2 as detected via viremia, fecal shedding, and seroconversion (Córdoba et al. 2012; Feagins et al. 2011; Meng 2003). Interestingly, swine can be infected by rabbit HEV demonstrating low levels of viremia, fecal shedding, indicative of active but not robust HEV infection (Cossaboom et al. 2012). Summarization of the cross-species transmission in pigs is described below (Fig. 6).

(c) **Rabbit**

HEV gt3 isolated from rabbit and HEV gt4 isolated from both human and swine origins are infectious to rabbits as indicated by viremia, fecal shedding, elevation of ALT, and histopathologic changes in the liver (Cheng et al. 2012; Han et al. 2014; Ma et al. 2010; Zhang et al. 2015a). Interestingly, even though rHEV strain have been assigned to HEV gt3, human gt3 could not infect SPF rabbits, similar to human gt1 HEV (Cheng et al. 2012; Ma et al. 2010). Summary of the cross-species transmission in rabbits is described below (Fig. 7).

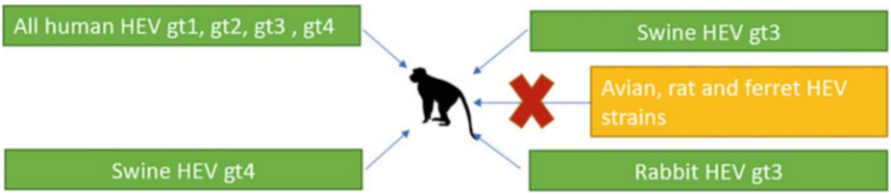

Fig. 5 Cross-species transmission in NHPs

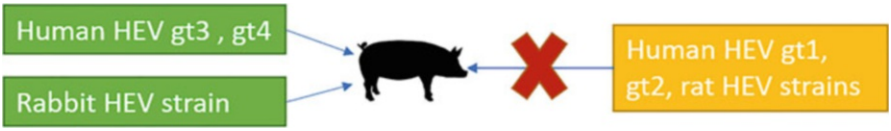

Fig. 6 Cross-species transmission in pigs

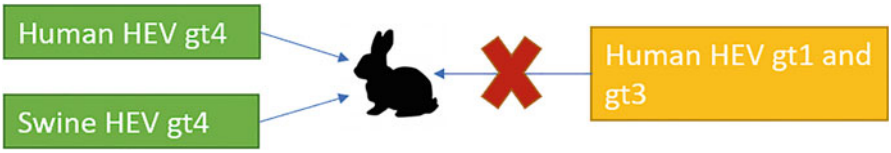

Fig. 7 Cross species transmission in rabbits

45.3 Epidemiology of HEV

HEV is suggested as one of the most common causes of acute hepatitis distributed worldwide (A. R. 2010). Estimated infections around 2.3 million and 70,000 deaths are recognized due to HEV per year (Rein et al. 2012). HEV genotypes, transmission routes, source of virus infection, disease prevalence, clinical characteristics of the disease are known to determine the epidemiological patterns around the globe. However, the HEV epidemiological pattern is mainly based on the predominant genotype in the geographical region and the presence of their respective hosts. Description of these two patterns is summarized in Figs. 8 and 9.

HEV gt1 and gt2 occur as outbreaks and sporadic cases spread via the fecal-oral route through contaminated water in endemic regions (Yugo and Meng 2013). Although sporadic reports of gt1 and gt2 HEV have been observed, circulation of the predominant gt1 and gt2 are primarily seen in Asia and Africa. Furthermore, gt3 and gt4 are frequently reported in Asia (gt4 mainly in China) (WHO 2017; Dai et al. 2013; Minagi et al. 2016). Seroprevalence studies have reported seroconversion for HEV (anti-HEV IgG) between 3% and 27% (Ren et al. 2014; Fierro et al. 2016).

Areas with scarcity of water and compromised sanitary conditions, often seen in low income countries and refugee camps, have been documented with endemic gt1 and gt2 with potential for large-scale outbreaks (A. R. 2010). Africa and Asia are known to be the major geographical regions affected by these two genotypes as reported by the World Health Organization (WHO). Seroprevalence studies have reported seroconversion for HEV (anti-HEV IgG) in Africa between 4.6% and

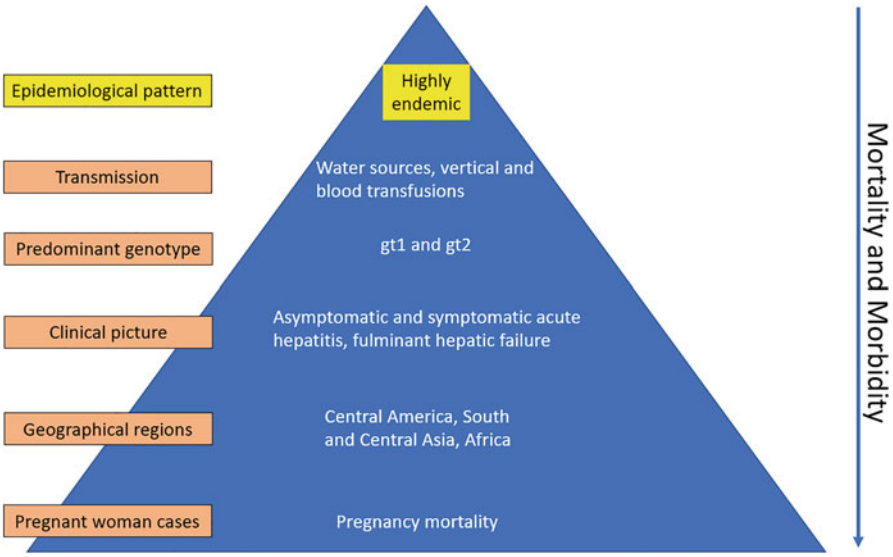

Fig. 8 HEV genotypes, transmission route, and clinical picture in highly endemic region

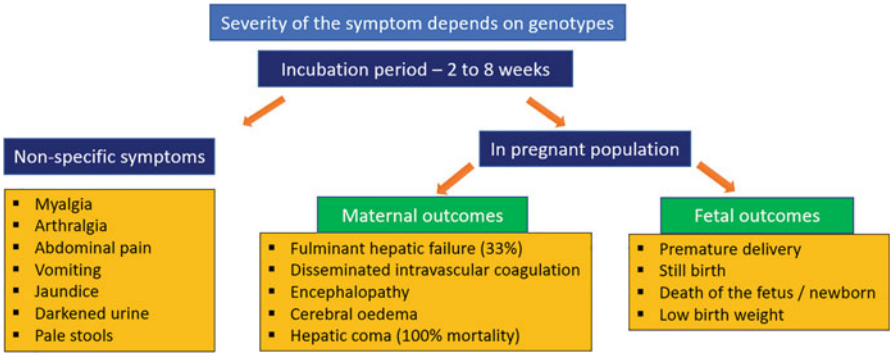

Fig. 9 Symptoms in the HEV infected patient

10.7% and in Asia between 34.8% and 94% (Ren et al. 2014; You et al. 2013; Kang et al. 2017; Korsman et al. 2019; Dagnew et al. 2019).

It has been reported that 15% of the people infected with gt1 and gt2 present signs and symptoms during the HEV gt1 and gt2 outbreaks (Goel and Aggarwal 2016). Infection in children, adolescents, and young adults is often asymptomatic in typical HEV infection. Overall morbidity rates are higher among teenagers and young adults (between 10 and 40 years old) but lower in children and elderly people (A. R. 2017). Infection in men usually outnumbers women, possibly because of greater exposure to contaminated water. However, infection of pregnant woman and patients with preexisting chronic liver disease leads to fulminant hepatitis with higher morbidity and severity of the disease (Kumar Acharya et al. 2007; S. KM 1981). Hepatitis E disease diagnosis is difficult, because clinical and diagnostic enzymatic biochemical panels can be indistinguishable clinically and biochemically from other diseases (Singh et al. 2018).

HEV gt1 and gt2 are also recognized for their ability to transmit via parenteral and vertical routes (Khuroo et al. 1995; Kumar et al. 2001; Arankalle and Chobe 1999). Pregnant women, primarily in the third trimester of pregnancy, demonstrate severe acute liver failure (ALF) with a mortality rate of 15–25% when infected with HEV gt1 (Naidu and Viswanathan 1957; Sharma et al. 2017). Abortion, fetal death, premature delivery, still birth, miscarriage have been reported when infected with HEV gt1 while pregnant (Jilani et al. 2007; Salam et al. 2013) (Fig. 9). Furthermore, anicteric or icteric hepatitis, hypoglycemia, and neonatal death are seen in fetuses and neonates, which is the result of vertical transmission of HEV (Jilani et al. 2007; Sharma et al. 2017). India is reported to have the highest pregnancy mortality cases due to outbreaks of HEV and findings such as HEV viral load, immunological changes, nutrition, and hormonal differences have been correlated with worse outcomes and hence defined as the risk factors associated with HEV in pregnant women (Jilani et al. 2007; Sharma et al. 2017; Salam et al. 2013; Kumar et al. 2014; Bose et al. 2011; Gong et al. 2021).

Low endemic areas for hepatitis E, such as Europe, East Asia (including China), and the Americas are known to have more widespread and better sanitary water processing conditions and well-controlled water supplies ,hence are reported to have

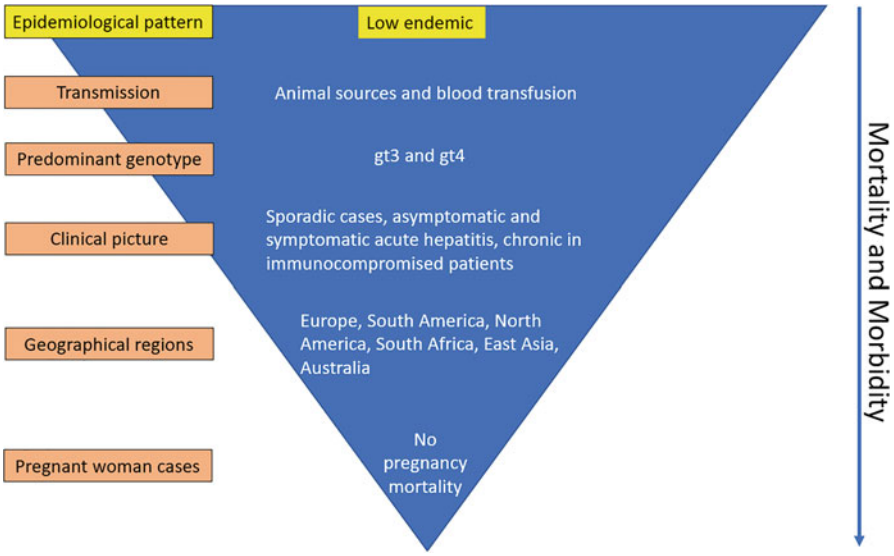

Fig. 10 HEV genotypes, transmission route, and clinical picture in low endemic region

mainly sporadic cases of HEV (Fig. 10) (Fierro et al. 2016; Domanović et al. 2017a; WHO 2017; Panduro et al. 2011).

HEV cases reported in these low endemic regions are autochthonous and are typically zoonotic (forward zoonoses) in nature. The primary infection source is related with the consumption of meat, mainly pork (gt3 and gt4) and its associated products. Hence, ingestion of raw or undercooked meat contaminated with HEV is linked with HEV in low endemic areas (Dalton et al. 2014). For instance, HEV gt3 prevalence in liver samples from France was 2.8% with a level of contamination of up to 1.46×10^8 copies/g (Feurer et al. 2018). Similarly, out of 131 pork products collected from grocery stores and butcher shops between October 2019 and February 2020 in Germany, 10% were positive for HEV RNA. These meats were predominantly pork livers, liver sausage, and liver pates (Pallerla et al. 2021). Similarly, HEV gt3 is present in US swine herds with a small proportion of commercial pork products, such as liver and chitterlings, from US grocery stores contain infectious HEV (Huang et al. 2002; Feagins et al. 2007). Also, in the United States, 15 out of 119 (12.6%) ground pork samples tested positive for HEV RNA and 13 out of 20 packages (65%) contained at least one positive sample. Twenty-five of 56 (45%) of pork liver samples were positive for HEV RNA (Harrison et al. 2021). Two percent of commercial pig livers from local grocery stores in Japan, 4% in Germany, 6.5% in the Netherlands, and 11% in the United States tested positive for zoonotic gt3 HEV RNA. Similarly, 6% of sausages sampled at processing and at the point of sale in Spain were also positive for the zoonotic gt3 HEV RNA (Meng 2016). In France, consumption of pig liver sausage (Figatelli) has been definitively linked to cases of hepatitis E as well and almost 30% of Figatelli in France tested gt3 HEV positive (Colson et al. 2010; Pavio et al. 2014). Beyond the farmed swine industry, cases of HEV have been linked to consumption of wild boar meat, grilled pig

entrails, and raw deer meats in Japan (Takahashi and Okamoto 2014). Hence, prevalence rates of HEV seropositivity in most countries could be explained by pork consumption, as in France and Germany, where the seroprevalence is 17% and 35%, respectively (Lapa et al. 2015), but additional cases may be through other specialty or cultural dishes that are not traditionally pork.

Additionally, iatrogenic transmission of HEV gt3 between humans through infected blood and blood products is reported in Europe. Recently, screening HEV in blood products is gaining importance in developed countries due to transfusion-associated HEV infections reported in Japan (gt3 and gt4), France (gt3), and China (gt1 and gt4) (Domanović et al. 2017b; Satake et al. 2017; Zhang et al. 2017). Such transfusion-transmitted HEV infections occur with red cells, platelets, and fresh-frozen plasma. Currently available inactivation technologies for blood products are unable to prevent transfusion-transmitted HEV infections, which can result in chronic severe hepatitis in immunosuppressed patients (Gallian et al. 2019). For instance, anti-HEV IgG prevalence was reported to be high in blood banks (52%) in France (Bouamra et al. 2014). In American regions, seroprevalence of anti-HEV IgG in United States was found to be 9%, in Brazil 4.2%, and in mixed Caribbean countries 1% (Horvatits et al. 2018). Similarly, gt1, gt2, and gt3 have been reported in humans and animals in Uruguay (gt1 and gt3), Colombia (gt3), Argentina (gt3), Mexico (gt2 and gt3, only humans), Venezuela (gt1 and gt3), Brazil (gt3), and the United States (gt3) (Oxford et al. 2016; Rendon et al. 2016; Lopes Dos Santos et al. 2010; Mirazo et al. 2011; García et al. 2012; Martínez Wassaf et al. 2014; Passos-Castilho et al. 2016; Viera-Segura et al. 2019).

Previously, gt3 and gt4 infection was thought to be self-limiting in humans. However, recent updates have shown chronic infection caused by gt3 in immunosuppressed patients, especially post organ transplant patients (Gérolami et al. 2008). For illustration, a recipient of an HEV-infected liver from a donor with occult HEV infection rapidly developed graft cirrhosis and died from decompensated liver disease (Schlosser et al. 2012). Furthermore, HEV infections were also transmitted via renal grafts given to two recipients (Pourbaix et al. 2017). In addition, preexisting chronic liver patients who acquire HEV gt3 may progress to fulminant hepatitis, as seen in gt1 infected individuals (Dalton et al. 2007). Although nosocomial transmission of HEV infection is unusual, there has been a report of this rare event in the hematology ward in France (Mansuy et al. 2009). In brief, a 33-year-old man receiving treatment for leukemia in a hematological ward developed acute hepatitis. All donor samples were tested and had negative results plus he had no travel history in the HEV endemic areas and had not eaten raw meat or shellfish. Thus, it was demonstrated that his stay in the ward overlapped with that of the other patient with hepatitis E shedding HEV in both blood and stool for a year. Therefore, based on the HEV nucleotide sequence identity between the two patients (97.8–98.6%) and because of the identical insertion in the ORF1 hypervariable region between the strains, it was referred as patient to patient transmission of HEV (Mansuy et al. 2009). Hence, transfusion of blood products, solid organ transplantation, stem cells, and pork-derived products constitute the principal sources of infection, especially for immunosuppressed individuals.

Table 1 Species, host tropism, geographical distribution of zoonotic HEV

Paslahepevirus balayani Genotypes	Host species	Geographical distribution	References
1	Human	Asia, Africa, Central America	Nelson et al. (2016) Kamar et al. (2012, 2014)
2	Human	West Africa, Mexico	Nelson et al. (2016) Kamar et al. (2012, 2014)
3	Human, pig, wild boar, red deer, mongoose, rabbit	Europe, South Africa, East Asia, and Americas	Oxford et al. (2016) Nelson et al. (2016) Kamar et al. (2012, 2014)
4	Human, pig, sheep, cattle	Asia, Europe	Oxford et al. (2016) Nelson et al. (2016) Wang and Ma (2010)
7	Dromedary camel, human	Asia	Sridhar et al. (2017)
Rocahepevirus	Rat, ferret, greater bandicoot, asian musk, shrew, mink, human	Asia, North America	Sridhar et al. (2018, 2021) Andonov et al. (2019)

Interestingly, only *Paslahepevirus* species were thought to infect humans. However, recent reports have suggested that *Rocahepevirus* strains have gained zoonotic potential and is described in two clinical cases: persistent hepatitis in a liver transplant patient in Hongkong and severe acute hepatitis in an immunocompetent patient in Canada (Sridhar et al. 2018; Andonov et al. 2019). Seven additional rat HEV infections have been confirmed recently in Hong Kong (Sridhar et al. 2021) (Table 1).

Disease severity resulting from HEV infection is thought to be dose dependent with higher starting doses leading to more severe disease manifestation. HEV superinfection is known to accelerate disease progression in patients with chronic HBV infection and increases mortality in those with cirrhosis (Tseng et al. 2020). Extrahepatic manifestations including neurological disorders, kidney injury, acute pancreatitis, and hematological abnormalities have been related with acute or chronic HEV infection caused by gt1-gt4. Several neurological disorders such as Guillain-Barre syndrome, brachial neuritis, and meningoencephalitis are known to be rarely seen during viral hepatitis, however, have been noted during HEV infection (Kamar et al. 2016; McLean et al. 2017).

45.4 Epidemiological Information of HEV Pregnancy Mortality

Mortality due to fulminant hepatitis in pregnant women has been reported from developing countries during HEV infection (Fig. 11) (Kar and Sengupta 2019). Underlying mechanisms demonstrating the virulent characteristics of the virus in

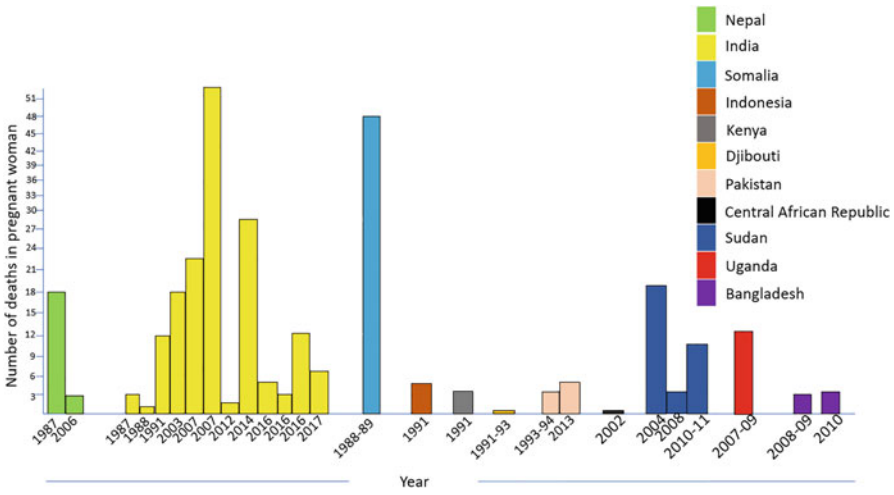

Fig. 11 HEV reported pregnancy mortality in different countries. Numerator = the number of deaths due to HEV during pregnancy. Denominator = the number of infected individuals. 1987, Nepal (18/73); 2006, Nepal (3/19); 1987, India (3/19); 1988, India (2/30); 1991, India (12/48); 2003, India (18/24); 2007, India (23/42); 2007, India (54/132); 2012, India (2/220); 2014, India (29/148); 2016, India (5/36); 2016, India (3/30); 2016, India (10/25); 2017, India (7/32); 1988–1989, Somalia (48/346); 1991, Indonesia (5/19); 1991, Kenya (4/28); 1991–1993, Djibouti (1/3); 1993–1994, Pakistan (4/35); 2013, Pakistan (5/25); 2002, Central African Republic (1/7); 2004, Sudan (19/61); 2008, Sudan (4/6); 2010–2011, Sudan (11/39); 2007–2009, Uganda (13/189); 2008–2009, Bangladesh (4/21); 2010, Bangladesh (3/12)

the pregnant woman is still lacking. Although we have multiple reports of pregnancy-associated deaths in various countries, reasons behind the severity of the disease only in pregnant women is deficient due to inappropriate cell culture model and insufficient animal models (Nimgaonkar et al. 2018).

45.5 Animal Models Demonstrating HEV-Induced Pregnancy Mortality

Various animals naturally susceptible to HEV or experimentally infected with HEV were studied to dissect the mechanism behind HEV-related pregnancy outcomes in humans. However, only few of them somewhat recapitulated the scenario of HEV-induced pregnancy mortality in humans.

(a) **Non-Human Primates**

Chimpanzees, pig-tailed macaques, vervets, owl monkeys, squirrel monkeys, and patas monkeys are known to be susceptible to experimental HEV infection (Yugo et al. 2014; Ticehurst et al. 1992; Tsarev et al. 1993a; Arankalle et al. 1988). However, based on the transmission studies, chimpanzees, rhesus

monkeys, and cynomolgus monkeys were the most susceptible to both human strains of HEV (gt1 to gt4) and animal strains of HEV (gt3 and gt4) (Kenney and Meng 2019; Yugo et al. 2014; Purcell and Emerson 2001). Experimental infection of cynomolgus and rhesus macaques induced clinical signs, coinciding with acute viral hepatitis with occasional excretion of virus like particles (VLPs) in the stool, and detection of antiviral antibodies (Tsarev et al. 1993a, b; Arankalle et al. 1995; Bradley et al. 1987). Previously, infection in pregnant rhesus macaques failed to recapitulate any severe pregnancy outcomes or fulminant hepatitis E observed in pregnant women (Tsarev et al. 1995). However, recently in a study targeted to demonstrate the vertical transmission of HEV gt4, premature delivery and fetal death occurred in one of the HEV-infected pregnant rhesus macaques (Yu et al. 2020).

(b) **Rabbits**

Rabbits are naturally infected with the rabbit strain of HEV (rHEV) (Zhao et al. 2009; Cossaboom et al. 2011; Caruso et al. 2015). In a pathogenesis study, similar clinical manifestations of acute hepatitis E are seen with fecal shedding, viremia, seroconversion, and elevated ALT levels with IV administration of rHEV (Cheng et al. 2012; Ma et al. 2010). In addition, rHEV isolate CHN-BJ-R14 produced infection in six pregnant rabbits with two demonstrating miscarriage and three deaths among the pregnant rabbits. Both positive and negative strands of HEV RNA were detected in the placental tissues of the infected pregnant rabbits, and positive staining for the HEV antigen was observed in placental tissue by immunohistochemistry (Xia et al. 2015). Furthermore, vertical transmission of HEV was also reported with the fecal detection of HEV in the first defecation of the newborn offspring. Seroconversion in the offspring was seen at 3 months of age (Xia et al. 2015). Also, pregnant rabbits administered HEV 239 vaccine and later challenged by rHEV (CHN-BJ-R14) demonstrated prevention against HEV-related adverse outcomes (Li et al. 2019). Furthermore, adverse fetal outcomes with 67–80% fetal mortality in pregnant rabbits experimentally infected with rHEV KOR-Rb-1 was reported (Ahn et al. 2017).

(c) **Mouse**

Pregnant BALB/c mice were experimentally infected in their early, middle, and late stages of pregnancy with genotype 4 swine HEV (KMO1). Miscarriages (7/8, 87.5%) reported in pregnant mice infected with HEV in the middle of pregnancy although no maternal death reported. Vertical transmission was confirmed by the HEV replication in the uterus, placenta, and fetal livers of the newborn mice (Yang et al. 2019) (Fig. 12).

45.6 Cell Culture Propagation Ability of HEV

In vitro studies are the foundation for understanding infectious diseases. Cell culture is utilized to study entry, replication, and pathogenesis mechanism. Due to a history of lacking the ability to cell culture HEV, research has been hindered

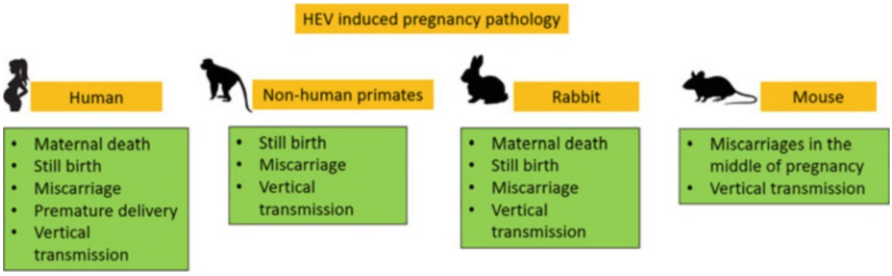

Fig. 12 Summarization of HEV induced pregnancy outcomes in human verses animal models

and focused on screening various cell types for increased HEV replication. Recent improvements in cell culture systems have significantly advanced HEV research. Several years of research led to the development of HEV infectious clones, identification of permissive cells, and optimization of HEV growth because of which the molecular studies of HEV have progressed. These are complemented by efforts in improving not only the hepatocyte systems that are physiologically more important but also differing cell lines origin pertaining to the extra-hepatic manifestations related to HEV. Furthermore, an ideal cell system would recapitulate cell polarity, co-secrete bile acids, and/or other host factors helping to fully mature HEV particles into their non-enveloped form, support virus spread and full lifecycle of HEV, and support infection with the clinical isolates of HEV. Here are some cell lines that are susceptible and permissive to HEV (Table 2).

45.7 Vaccination, Prevention, and Treatment of Zoonotic HEV

(a) **Vaccination**

Public health surveillance in pregnant and non-pregnant women is of utmost importance for limiting the number of sporadic outbreaks, in line with sanitation improvements in endemic countries (Fierro et al. 2016; Domanović et al. 2017a; Clemente-Casares et al. 2009). Due to the lack of in vitro cell culture replication, development of live attenuated or inactivated vaccines for HEV is ceased (Kamili 2011). Recombinant vaccines have been a focus for HEV as active immunization has been found to be effective in experimental animal models and because of the failure of passive immunoprophylaxis (Tsarev et al. 1994).

HEV 239 vaccine (Hecolin, Xiamen Innovax Biotech, China) is the only commercially available vaccine, which has been registered in China since 2011 (Park 2012; Riedmann 2012). However, yet to be approved by the FDA. A special feature of this recombinant vaccine is that it contains 26 aa and is an extension from the N terminal of another peptide, E2, from the HEV capsid protein, which is

Table 2 Different cell lines used for the study of various HEV genotypes

Cell line	HEV genotype	Reference
Hepatoma cell lines		
PLC/PRF/5	Unknown gt4 gt1 gt3	Pillot et al. (1987) Tanaka et al. (2009) Takahashi et al. (2010) Shukla et al. (2011)
HepG2		Okamoto (2011)
HepG2C3A	gt3	Shukla et al. (2011)
Huh7.5	gt3	Shukla et al. (2011)
ORF4 expressing huh7 S10–3	gt3, gt1 Sar55	Yadav et al. (2021)
Non-hepatoma cell lines		
2BS (Human fetal lung diploid fibroblast)	gt1	Huang et al. (1999)
A549 (Human lung epithelial cells)	gt1	Huang et al. (1999)
		Okamoto (2011)
	gt4	Tanaka et al. (2009)
	gt1	Takahashi et al. (2010)
	gt3	Shukla et al. (2012)
LLC-PK1 (Pig kidney cells) LLC-PK1A (Pig kidney cells) SK-RST (Pig kidney cells)	gt3 Kernow-C1	Shukla et al. (2011)
MDCK (Dog kidney cells)	gt3 Kernow-C1	Shukla et al. (2011)
CRFK (Cat kidney cells)	gt3 Kernow-C1	Shukla et al. (2011)
LLC-RK1 (Rabbit kidney)	gt3 Kernow-C1	Shukla et al. (2011)
Caco-2 (Colon carcinoma)	gt1 Sar55	Emerson et al. (2004)
JEG-3 (Human placental cells)	gt1 and gt3	Knegendorf et al. (2018)
BeWo (Human placental cells)	gt1 and gt3	Knegendorf et al. (2018)
MO3.13 (Oligodendrocytic cells)	gt3	Drave et al. (2016)
Ex vivo transplants		
Maternal decidua and fetal placenta	gt1 and gt3	Gouilly et al. (2018)
Primary cells		
Primary human hepatocytes (PHHs)	gt3 and gt4	Oshiro et al. (2014)
Immune competent PHHs	gt3 Kernow-C1 P6	Yin et al. (2017)
Human fetal liver cells	gt3 Kernow-C1 P6	Wu et al. (2018)
Primary mouse neurons	gt3 Kernow-C1 P6	Zhou et al. (2017)

the one major structural protein. The approach was successful, because HEV is antigenically conserved, presenting only one identified serotype observed to be protective for all four HEV genotypes (gt1, gt2, gt3 and gt4) (Anonymous 2015; Wu et al. 2016).

HEV 239 vaccine is proven to be effective in Rhesus macaques, rabbits immunized and challenged with infectious virus strains (gt1 and gt4) (Cheng et al. 2012; Zhang et al. 2009; Li et al. 2015b). In mouse models, a strong T cell-dependent antibody response was observed after vaccination, which was partly attributed to two

T cell epitopes located in the portion of aa 533–552 on the HEV capsid peptide (Khateri et al. 2018).

In humans HEV 239 vaccine is administered as mentioned below.

Vaccine	Route	Volume	Number of dose	Composition	Side effects	Efficacy	Safety in pregnant woman
HEV 239 (Hecolin)	IM	0.5 ml	0, 1, and 6 months	30 μg of the purified protein absorbed to 0.8 mg of aluminum hydroxide suspended in 0.5 ml buffered saline	Well tolerated, no vaccination-related serious adverse events (Zhu et al. 2010)	85.1% for those receiving at least one dose, 93.3% after full three doses (Zhu et al. 2010; Zhang et al. 2015b)	Although contraindicated because of safety concern, preliminary data demonstrated safe and immunogenic among 37 pregnant woman receiving vaccine during pregnancy (Wu et al. 2012)

Furthermore, the cost of Hecolin 239 vaccine from Xiamen Innovax Biotech is around USD 17.60–41.70 per dose (Riedmann 2012; Zhao et al. 2016). This is cost efficient for an effective immunization that can reduce the cost of hospitalization and treatment; hence the implementation of the HEV vaccine would be beneficial to the public from a one health perspective.

(b) **Prevention**

Preventative measures for HEV are almost the same as for other infectious diseases (Nelson et al. 2016; Teshale and Hu 2011; Barnaud et al. 2012; Schielke et al. 2011)

Preventive measures	Procedures
Sanitation and hygiene	Government – treat sewage and water supplies Personal – wash hands, wash vegetables, avoid cross contamination while preparing food, use gloves to prepare food
Surveillance	HEV screening for health workers, live animal, carcass handlers, and in the blood bank
Diagnostic test	HEV test included in the diagnostic list for the hepatitis cases
Virus Inactivation	Cooking food above 71 °C for 20 min (Barnaud et al. 2012) and boiling water above 90 °C Wash fruits and vegetables with chlorine solutions
Vaccination	Perform mass vaccination with a safe vaccine in endemic areas

(c) **Treatment**

The population most at risk for HEV are organ transplant patients, HIV patients, and hemodialysis patients, which have the tendency to develop chronic hepatitis E (Kamar et al. 2014). Treatment drugs prescribed or used in the specific cases while diagnosed with HEV are listed below (Kamar et al. 2015; Pischke et al. 2014).

Clinical representation with HEV	Treatment approach
Liver transplant patients with immunosuppression and hemodialysis, and anemic patients with HEV viremia	Pegylated-interferon monotherapy, although reduction in the immunosuppressive regime leads to self-resolution in many cases
Immunocompromised patients with HEV viremia < 6 months	Ribavirin and pegylated-interferon
Immunocompetent patients with acute infection and immunocompromised patients with HEV viremia > 6 months (chronic infection)	Ribavirin monotherapy
Pregnant woman with HEV	Supportive therapy

Inactivation of RNA polymerase function using antiviral drugs such as zinc salt and nucleoside analogue 2'-C-methylcytidine (2CMC) inhibited HEV replication in human cell lines in vitro (Kaushik et al. 2017; Qu et al. 2017). In addition, sofosbuvir, calcineurin inhibitors, and mTOR inhibitors may help in virus clearance as suggested by in vitro assays, however, testing the efficacy and safety in humans would determine the fate of these drugs (Wang et al. 2014b; Netzler et al. 2019).

Similarly, immunocompetent patients return to their normal physiology within 3–4 weeks of ribavirin treatment due to the short duration of viremia (Péron et al. 2016).

45.8 Difference of HEV with Other Hepatitis-Causing Viruses

Viral hepatitis remains a significant public health problem in the United States, despite advances in antiviral therapy and effective vaccines. CDC reports document 20,000 deaths each year attributed to viral hepatitis, and 5 million people are chronically infected and at risk for serious liver disease and hepatocellular cancer (Loader et al. 2019). Clinical feature of viral hepatitis, including risk for progression to chronic infection with development of cirrhosis, vary considerably and are virus-specific. Differences between various hepatitis viruses are listed below to allow the reader to comprehend HEV with other hepatitis-producing viruses.

Variables	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
Morphology	RNA virus	DNA virus	RNA virus	RNA virus	RNA virus
Transmission routes	Fecal-oral route	Percutaneous or perinatal exposure with HBV-infected blood or body	Infected blood or blood-containing body fluid	Shared needles among drug abusers, contaminated	Fecal-oral route, contaminated pork meat,

(continued)

Variables	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
		fluid, sexual contact		blood, and by sexual contact	and camel milk products
Clinical effects	Incubation Period (IP) – 28 days	I.P – 28 days to 6 months	Asymptomatic	I.P – 1 to 2 months	I.P – 5 to 6 weeks
	Fatigue, abdominal discomfort, vomiting, pruritus, fever, jaundice, dark-colored urine	Fever, fatigue, loss of appetite, vomiting, abdominal pain, dark colored urine, clay-colored stools, arthralgias, jaundice	Chronic infection is associated with hepatic fibrosis, cirrhosis. Extrahepatic manifestation includes cryoglobulinemia vasculitis, membranoproliferative glomerulonephritis, porphyria cutanea tarda, B-cell non-Hodgkin lymphoma	Jaundice, joint pain, abdominal pain, vomiting, loss of appetite, dark urine, fatigue, chronic cases develop cirrhosis	Fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, jaundice, dark urine, clay-colored stool, joint pain
	Acute liver Failure (ALF) – 1%	ALF – 1 to 2%	Cirrhosis – 10 to 20%	Cirrhosis – 4%	Fulminant hepatic failure (FHF) – 30%
Treatment	Supportive	Tenofovir and entecavir	Direct acting antiviral medications, ledipasvir-sofosbuvir, sofosbuvir-velpatasvir, elbasvir-grazoprevir	Supportive	Supportive in pregnant woman. No treatment for acute HEV infections
Vaccine	Havrix (two doses 6 months apart for children 1–2-year-old) and Vaqta (two doses 6 months apart for people traveling to endemic areas)	First dose at birth Heplisav-B (for 18 years and older)	No approved vaccine	No approved vaccine	Hecolin (HEV 239)– only approved in China

45.9 Loopholes in HEV Studies

Inefficiency of the cell culture system for the study of HEV hinders research into this important human pathogen. Thus, finding suitable animal model to dissect the pathogenesis and the virulent adaptation of HEV in the pregnant woman is critically important. Although rhesus macaque, rabbits and humanized mouse model have been used to study HEV, absolute recapitulation of clinical signs, lesions as seen in humans is not found. Various aspects of clinical outcomes are not reproduced in the animal models, including the chronic infection seen in immunocompromised patients and extrahepatic manifestations observed in HEV-infected patients, such as neurological disorders and kidney diseases. Furthermore, scarce knowledge is

present on the key aspects of the viral life cycle – for example, the cellular (co-) factors involved in the viral life cycle and prominent receptors mediating viral entry. In addition, several reports suggesting infection of animal species are based on a small number of serologically positive samples and interpretation needs further analysis. Only serological results are difficult to direct the conclusion as confirmatory assay, a gold standard for HEV, is lacking. There are no established guidelines on the management of HEV and other hepatitis-causing viruses in case of dual infection. Although the HEV vaccine is approved and used in China, worldwide approval and availability need to be prioritized.

45.10 Conclusions

Although HEV is considered as an emerging pathogen, it has been known to produce epidemic outbreaks starting from the 1950s. Current findings suggest that although pigs are the major reservoir spreading the disease to humans, the host range appears to be increasing. The zoonotic axis has gained importance due to the continued reemergence of HEV despite advances in hygiene practices. HEV continues to remain relevant producing new pathology manifestations including chronicity, extrahepatic manifestations, and the continued danger to pregnant women. Technological advances in sequencing, coupled with more thorough sampling will inevitably reveal more novel HEV strains. Continued research will continue unlocking novel treatment options. There is still much work to be done to unravel the nuances of HEV's deadly game of hide and seek so that humans may rid themselves of this malady.

References

A. R. (2010) The global prevalence of hepatitis e virus infection and susceptibility: a systematic review. World Health Organization, Department of Immunization. 300

A. R. (2017) Clinical presentation of hepatitis E. 161:15–22

Ahn H-S, Han S-H, Kim Y-H, Park B-J, Kim D-H, Lee J-B, Park S-Y, Song C-S, Lee S-W, Choi C, Myoung J, Choi I-S (2017) Adverse fetal outcomes in pregnant rabbits experimentally infected with rabbit hepatitis E virus. Virology 512:187–193

Andonov A, Robbins M, Borlang J, Cao J, Hatchette T, Stueck A, Deschambault Y, Murnaghan K, Varga J, Johnston L (2019) Rat hepatitis E virus linked to severe acute hepatitis in an immunocompetent patient. J Infect Dis 220:951–955

Anonymous (2015) Hepatitis E vaccine: WHO position paper, May 2015. Wkly Epidemiol Rec 90: 185–200

Arankalle VA, Chobe LP (1999) Hepatitis E virus: can it be transmitted parenterally? J Viral Hepat 6:161–164

Arankalle VA, Ticehurst J, Sreenivasan MA, Kapikian AZ, Popper H, Pavri KM, Purcell RH (1988) Aetiological association of a virus-like particle with enterically transmitted non-A, non-B hepatitis. Lancet 1:550–554

Arankalle VA, Chadha MS, Chobe LP, Nair R, Banerjee K (1995) Cross-challenge studies in rhesus monkeys employing different Indian isolates of hepatitis E virus. J Med Virol 46:358–363

Arankalle VA, Chobe LP, Chadha MS (2006) Type-IV Indian swine HEV infects rhesus monkeys. J Viral Hepat 13:742–745

Barnaud E, Rogée S, Garry P, Rose N, Pavio N (2012) Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. Appl Environ Microbiol 78:5153–5159

Bose PD, Das BC, Kumar A, Gondal R, Kumar D, Kar P (2011) High viral load and deregulation of the progesterone receptor signaling pathway: association with hepatitis E-related poor pregnancy outcome. J Hepatol 54:1107–1113

Bouamra Y, Gérolami R, Arzouni JP, Grimaud JC, Lafforgue P, Nelli M, Tivoli N, Ferretti A, Motte A, Colson P (2014) Emergence of autochthonous infections with hepatitis E virus of genotype 4 in Europe. Intervirology 57:43–48

Bradley DW, Krawczynski K, Cook EH Jr, McCaustland KA, Humphrey CD, Spelbring JE, Myint H, Maynard JE (1987) Enterically transmitted non-A, non-B hepatitis: serial passage of disease in cynomolgus macaques and tamarins and recovery of disease-associated 27- to 34-nm viruslike particles. Proc Natl Acad Sci U S A 84:6277–6281

Capai L, Charrel R, Falchi A (2018) Hepatitis E in high-income countries: what do we know? and what are the knowledge gaps? Viruses 10:285

Caruso C, Modesto P, Prato R, Scaglione FE, De Marco L, Bollo E, Acutis PL, Masoero L, Peletto S (2015) Hepatitis E virus: first description in a pet house rabbit. A new transmission route for human? Transbound Emerg Dis 62:229–232

Chapuy-Regaud S, Dubois M, Plisson-Chastang C, Bonnefois T, Lhomme S, Bertrand-Michel J, You B, Simoneau S, Gleizes PE, Flan B, Abravanel F, Izopet J (2017) Characterization of the lipid envelope of exosome encapsulated HEV particles protected from the immune response. Biochimie 141:70–79

Cheng X, Wang S, Dai X, Shi C, Wen Y, Zhu M, Zhan S, Meng J (2012) Rabbit as a novel animal model for hepatitis E virus infection and vaccine evaluation. PLoS One 7:e51616

Clemente-Casares P, Rodriguez-Manzano J, Girones R (2009) Hepatitis E virus genotype 3 and sporadically also genotype 1 circulate in the population of Catalonia, Spain. J Water Health 7: 664–673

Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, Heyries L, Raoult D, Gerolami R (2010) Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 202:825–834

Córdoba L, Feagins AR, Opriessnig T, Cossaboom CM, Dryman BA, Huang YW, Meng XJ (2012) Rescue of a genotype 4 human hepatitis E virus from cloned cDNA and characterization of intergenotypic chimeric viruses in cultured human liver cells and in pigs. J Gen Virol 93: 2183–2194

Cossaboom CM, Córdoba L, Dryman BA, Meng XJ (2011) Hepatitis E virus in rabbits, Virginia, USA. Emerg Infect Dis 17:2047–2049

Cossaboom CM, Córdoba L, Sanford BJ, Piñeyro P, Kenney SP, Dryman BA, Wang Y, Meng XJ (2012) Cross-species infection of pigs with a novel rabbit, but not rat, strain of hepatitis E virus isolated in the United States. J Gen Virol 93:1687–1695

Dagnew M, Belachew A, Tiruneh M, Moges F (2019) Hepatitis E virus infection among pregnant women in Africa: systematic review and meta-analysis. BMC Infect Dis 19:519

Dai X, Dong C, Zhou Z, Liang J, Dong M, Yang Y, Fu J, Tian H, Wang S, Fan J, Meng J, Purdy MA (2013) Hepatitis E virus genotype 4, Nanjing, China, 2001–2011. Emerg Infect Dis 19:1528–1530

Dalton HR, Hazeldine S, Banks M, Ijaz S, Bendall R (2007) Locally acquired hepatitis E in chronic liver disease. Lancet 369:1260

Dalton HR, Kamar N, Izopet J (2014) Hepatitis E in developed countries: current status and future perspectives. Future Microbiol 9:1361–1372

Domanović D, Tedder R, Blümel J, Zaaijer H, Gallian P, Niederhauser C, Sauleda Oliveras S, O'Riordan J, Boland F, Harritshøj L, Nascimento MSJ, Ciccaglione AR, Politis C, Adlhoch C, Flan B, Oualikene-Gonin W, Rautmann G, Strengers P, Hewitt P (2017a) Hepatitis E and blood donation safety in selected European countries: a shift to screening? Euro Surveill 22. 22(16): 30514

Domanović D, Tedder R, Blümel J, Zaaijer H, Gallian P, Niederhauser C, Sauleda Oliveras S, O'Riordan J, Boland F, Harritshøj L, Nascimento MSJ, Ciccaglione AR, Politis C, Adlhoch C, Flan B, Oualikene-Gonin W, Rautmann G, Strengers P, Hewitt P (2017b) Hepatitis E and blood donation safety in selected European countries: a shift to screening? Eur Secur 22:30514

Drave SA, Debing Y, Walter S, Todt D, Engelmann M, Friesland M, Wedemeyer H, Neyts J, Behrendt P, Steinmann E (2016) Extra-hepatic replication and infection of hepatitis E virus in neuronal-derived cells. J Viral Hepat 23:512–521

Emerson SU, Nguyen H, Graff J, Stephany DA, Brockington A, Purcell RH (2004) In vitro replication of hepatitis E virus (HEV) genomes and of an HEV replicon expressing green fluorescent protein. J Virol 78:4838–4846

Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ (2007) Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. J Gen Virol 88:912–917

Feagins AR, Córdoba L, Sanford BJ, Dryman BA, Huang YW, LeRoith T, Emerson SU, Meng XJ (2011) Intergenotypic chimeric hepatitis E viruses (HEVs) with the genotype 4 human HEV capsid gene in the backbone of genotype 3 swine HEV are infectious in pigs. Virus Res 156:141–146

Feurer C, Le Roux A, Rossel R, Barnaud E, Dumarest M, Garry P, Pavio N (2018) High load of hepatitis E viral RNA in pork livers but absence in pork muscle at French slaughterhouses. Int J Food Microbiol 264:25–30

Fierro NA, Realpe M, Meraz-Medina T, Roman S, Panduro A (2016) Hepatitis E virus: an ancient hidden enemy in Latin America. World J Gastroenterol 22:2271–2283

Gallian P, Pouchol E, Djoudi R, Lhomme S, Mouna L, Gross S, Bierling P, Assal A, Kamar N, Mallet V, Roque-Afonso A-M, Izopet J, Tiberghien P (2019) Transfusion-transmitted hepatitis E virus infection in France. Transfus Med Rev 33:146–153

García CG, Sánchez D, Villalba MC, Pujol FH, de Los Ángeles Rodríguez Lay L, Pinto B, Chacón EP, Guzmán MG (2012) Molecular characterization of hepatitis E virus in patients with acute hepatitis in Venezuela. J Med Virol 84:1025–1029

Geng Y, Wang Y (2016) Transmission of hepatitis E virus. Adv Exp Med Biol 948:89–112

Gérolami R, Moal V, Colson P (2008) Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. N Engl J Med 358:859–860

Goel A, Aggarwal R (2016) Advances in hepatitis E – II: epidemiology, clinical manifestations, treatment and prevention. Expert Rev Gastroenterol Hepatol 10:1065–1074

Gong S, Hao X, Bi Y, Yang C, Wang W, Mickael HK, Zhang Y, Chen S, Qian Z, Huang F, Wei D, Yu W (2021) Hepatitis E viral infection regulates estrogen signaling pathways: Inhibition of the cAMPK–PKA–CREB and PI3K–AKT–mTOR signaling pathways. J Med Virol 93:3769–3778

Gouilly J, Chen Q, Siewiera J, Cartron G, Levy C, Dubois M, Al-Daccak R, Izopet J, Jabrane-Ferrat N, El Costa H (2018) Genotype specific pathogenicity of hepatitis E virus at the human maternal-fetal interface. Nat Commun 9:4748

Graff J, Torian U, Nguyen H, Emerson SU (2006) A bicistronic subgenomic mRNA encodes both the ORF2 and ORF3 proteins of hepatitis E virus. J Virol 80:5919–5926

Han J, Lei Y, Liu L, Liu P, Xia J, Zhang Y, Zeng H, Wang L, Wang L, Zhuang H (2014) SPF rabbits infected with rabbit hepatitis E virus isolate experimentally showing the chronicity of hepatitis. PLoS One 9:e99861

Harrison L, Ramos TM, Wu X, DiCaprio E (2021) Presence of hepatitis E virus in commercially available pork products. Int J Food Microbiol 339:109033

Harvala H, Hewitt PE, Reynolds C, Pearson C, Haywood B, Tettmar KI, Ushiro-Lumb I, Brailsford SR, Tedder R, Ijaz S (2019) Hepatitis E virus in blood donors in England, 2016 to 2017: from selective to universal screening. Euro Surveill 24:1800386

Horvatits T, Pischke S (2018) Extrahepatic manifestations and HEV, the genotype matters. EBioMedicine 36:3–4

Horvatits T, Ozga AK, Westhölter D, Hartl J, Manthey CF, Lütgehetmann M, Rauch G, Kriston L, Lohse AW, Bendall R, Wedemeyer H, Dalton HR, Pischke S (2018) Hepatitis E seroprevalence in the Americas: a systematic review and meta-analysis. Liver Int 38:1951–1964

Huang R, Li D, Wei S, Li Q, Yuan X, Geng L, Li X, Liu M (1999) Cell culture of sporadic hepatitis E virus in China. Clin Diagn Lab Immunol 6:729–733

Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE, Meng XJ (2002) Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. J Clin Microbiol 40:1326–1332

Huang FF, Sun ZF, Emerson SU, Purcell RH, Shivaprasad HL, Pierson FW, Toth TE, Meng XJ (2004) Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. J Gen Virol 85: 1609–1618

Jilani N, Das BC, Husain SA, Baweja UK, Chattopadhya D, Gupta RK, Sardana S, Kar P (2007) Hepatitis E virus infection and fulminant hepatic failure during pregnancy. J Gastroenterol Hepatol 22:676–682

Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, Dalton HR (2012) Hepatitis E. Lancet 379:2477–2488

Kamar N, Dalton HR, Abravanel F, Izopet J (2014) Hepatitis E virus infection. Clin Microbiol Rev 27:116–138

Kamar N, Lhomme S, Abravanel F, Cointault O, Esposito L, Cardeau-Desangles I, Del Bello A, Dörr G, Lavayssière L, Nogier MB, Guitard J, Ribes D, Goin AL, Broué P, Metsu D, Sauné K, Rostaing L, Izopet J (2015) An early viral response predicts the virological response to ribavirin in hepatitis E virus organ transplant patients. Transplantation 99:2124–2131

Kamar N, Marion O, Abravanel F, Izopet J, Dalton HR (2016) Extrahepatic manifestations of hepatitis E virus. Liver Int 36:467–472

Kamili S (2011) Toward the development of a hepatitis E vaccine. Virus Res 161:93–100

Kang YH, Cong W, Zhang XY, Wang CF, Shan XF, Qian AD (2017) Hepatitis E virus seroprevalence among farmers, veterinarians and control subjects in Jilin province, Shandong province and Inner Mongolia Autonomous Region, China. J Med Virol 89:872–877

Kar P, Sengupta A (2019) A guide to the management of hepatitis E infection during pregnancy. Expert Rev Gastroenterol Hepatol 13:205–211

Kaushik N, Subramani C, Anang S, Muthumohan R, Shalimar NB, Ranjith-Kumar CT, Surjit M (2017) Zinc salts block hepatitis E virus replication by inhibiting the activity of viral RNA-dependent RNA polymerase. J Virol 91

Kenney SP, Meng X-J (2019) Hepatitis E virus: animal models and zoonosis. Annu Rev Anim Biosci 7:427–448

Khateri M, Abdoli A, Motevalli F, Fotouhi F, Bolhassani A, Arashkia A, Jazaeri EO, Shahbazi S, Mehrbod P, Naziri H, Aghasadeghi MR (2018) Evaluation of autophagy induction on HEV 239 vaccine immune response in a mouse model. IUBMB Life 70:207–214

Khuroo MS, Kamili S, Jameel S (1995) Vertical transmission of hepatitis E virus. Lancet 345:1025–1026

Khuroo MS, Khuroo MS, Khuroo NS (2016) Transmission of hepatitis E virus in developing countries. Viruses 8:253

King NJ, Hewitt J, Perchec-Merien AM (2018) Hiding in plain sight? It's time to investigate other possible transmission routes for hepatitis E virus (HEV) in developed countries. Food Environ Virol 10:225–252

Knegendorf L, Drave SA, Dao Thi VL, Debing Y, Brown RJP, Vondran FWR, Resner K, Friesland M, Khera T, Engelmann M, Bremer B, Wedemeyer H, Behrendt P, Neyts J, Pietschmann T, Todt D, Steinmann E (2018) Hepatitis E virus replication and interferon responses in human placental cells. Hepatol Commun 2:173–187

Koning L, Charlton MR, Pas SD, Heimbach JK, Osterhaus AD, Watt KD, Janssen HL, de Knegt RJ, van der Eijk AA (2015) Prevalence and clinical consequences of hepatitis E in patients who underwent liver transplantation for chronic hepatitis C in the United States. BMC Infect Dis 15:371

Korsman S, Hardie D, Kaba M (2019) Hepatitis E virus in patients with acute hepatitis in Cape Town, South Africa, 2011. S Afr Med J 109:582–583

Kumar Acharya S, Kumar Sharma P, Singh R, Kumar Mohanty S, Madan K, Kumar Jha J, Kumar PS (2007) Hepatitis E virus (HEV) infection in patients with cirrhosis is associated with rapid decompensation and death. J Hepatol 46:387–394

Kumar RM, Uduman S, Rana S, Kochiyil JK, Usmani A, Thomas L (2001) Sero-prevalence and mother-to-infant transmission of hepatitis E virus among pregnant women in the United Arab Emirates. Eur J Obstet Gynecol Reprod Biol 100:9–15

Kumar A, Devi SG, Kar P, Agarwal S, Husain SA, Gupta RK, Sharma S (2014) Association of cytokines in hepatitis E with pregnancy outcome. Cytokine 65:95–104

Kuno A, Ido K, Isoda N, Satoh Y, Ono K, Satoh S, Inamori H, Sugano K, Kanai N, Nishizawa T, Okamoto H (2003) Sporadic acute hepatitis E of a 47-year-old man whose pet cat was positive for antibody to hepatitis E virus. Hepatol Res 26:237–242

Lapa D, Capobianchi MR, Garbuglia AR (2015) Epidemiology of hepatitis E virus in European countries. Int J Mol Sci 16:25711–25743

Lee G-H, Tan B-H, Chi-Yuan Teo E, Lim S-G, Dan Y-Y, Wee A, Kim Aw PP, Zhu Y, Hibberd ML, Tan C-K, Purdy MA, Teo C-G (2016) Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. Gastroenterology 150:355–357.e353

Li TC, Yoshizaki S, Ami Y, Suzaki Y, Yang T, Takeda N, Takaji W (2015a) Monkeys and rats are not susceptible to ferret hepatitis E virus infection. Intervirology 58:139–142

Li SW, Zhao Q, Wu T, Chen S, Zhang J, Xia NS (2015b) The development of a recombinant hepatitis E vaccine HEV 239. Hum Vaccin Immunother 11:908–914

Li M, Li S, He Q, Liang Z, Wang L, Wang Q, Wang L (2019) Hepatitis E-related adverse pregnancy outcomes and their prevention by hepatitis E vaccine in a rabbit model. Emerg Microbes Infect 8:1066–1075

Li M, Bu Q, Gong W, Li H, Wang L, Li S, Sridhar S, Cy Woo P, Wang L (2020) Hepatitis E virus infection and its associated adverse feto-maternal outcomes among pregnant women in Qinhuangdao, China. J Matern Fetal Neonatal Med 33:3647–3651

Liu P, Bu QN, Wang L, Han J, Du RJ, Lei YX, Ouyang YQ, Li J, Zhu YH, Lu FM, Zhuang H (2013) Transmission of hepatitis E virus from rabbits to cynomolgus macaques. Emerg Infect Dis 19:559–565

Loader M, Moravek R, Witowski SE, Driscoll LM (2019) A clinical review of viral hepatitis. J Am Acad PAs 32:15–20

Lopes Dos Santos DR, Lewis-Ximenez LL, da Silva MF, de Sousa PS, Gaspar AM, Pinto MA (2010) First report of a human autochthonous hepatitis E virus infection in Brazil. J Clin Virol 47:276–279

Ma H, Zheng L, Liu Y, Zhao C, Harrison TJ, Ma Y, Sun S, Zhang J, Wang Y (2010) Experimental infection of rabbits with rabbit and genotypes 1 and 4 hepatitis E viruses. PLoS One 5:e9160

Maneerat Y, Clayson ET, Myint KS, Young GD, Innis BL (1996) Experimental infection of the laboratory rat with the hepatitis E virus. J Med Virol 48:121–128

Mansuy J-M, Huynh A, Abravanel F, Recher C, Peron JM, Izopet J (2009) Molecular evidence of patient-to-patient transmission of hepatitis E virus in a hematology ward. Clin Infect Dis 48:373–374

Martínez Wassaf MG, Pisano MB, Barril PA, Elbarcha OC, Pinto MA, Mendes de Oliveira J, DiGiusto P, Nates SV, Ré VE (2014) First detection of hepatitis E virus in Central Argentina: environmental and serological survey. J Clin Virol 61:334–339

Mateos ML, Camarero C, Lasa E, Teruel JL, Mir N, Baquero F (1998) Hepatitis E virus: relevance in blood donors and other risk groups. Vox Sang 75:267–269

McLean BN, Gulliver J, Dalton HR (2017) Hepatitis E virus and neurological disorders. Pract Neurol 17:282

Meng XJ (2003) Swine hepatitis E virus: cross-species infection and risk in xenotransplantation. Curr Top Microbiol Immunol 278:185–216

Meng XJ (2013) Zoonotic and foodborne transmission of hepatitis E virus. Semin Liver Dis 33:41–49

Meng X-J (2016) Expanding host range and cross-Species infection of hepatitis E virus. PLoS Pathog 12:e1005695–e1005695

Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU (1998) Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J Virol 72:9714–9721

Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH (2002) Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol 40:117–122

Minagi T, Okamoto H, Ikegawa M, Ideno S, Takahashi K, Sakai K, Hagiwara K, Yunoki M, Wakisaka A (2016) Hepatitis E virus in donor plasma collected in Japan. Vox Sang 111:242–246

Mirazo S, Ramos N, Russi JC, Gagliano G, Arbiza J (2011) Detection and molecular characterization of sporadic cases of acute human hepatitis E virus infection in Uruguay. Arch Virol 156: 1451–1454

Naidu SS, Viswanathan R (1957) Infectious hepatitis in pregnancy during Delhi epidemic. Indian J Med Res 45:71–76

Nair VP, Anang S, Subramani C, Madhvi A, Bakshi K, Srivastava A, Shalimar NB, Ranjith Kumar CT, Surjit M (2016) Endoplasmic reticulum stress induced synthesis of a novel viral factor mediates efficient replication of genotype-1 hepatitis E virus. PLoS Pathog 12:e1005521

Nelson KE, Heaney CD, Labrique AB, Kmush BL, Krain LJ (2016) Hepatitis E: prevention and treatment. Curr Opin Infect Dis 29:478–485

Netzler NE, Enosi Tuipulotu D, Vasudevan SG, Mackenzie JM, White PA (2019) Antiviral candidates for treating hepatitis E virus infection. Antimicrob Agents Chemother 63:e00003–19

Nimgaonkar I, Ding Q, Schwartz RE, Ploss A (2018) Hepatitis E virus: advances and challenges. Nat Rev Gastroenterol Hepatol 15:96–110

Okamoto H (2011) Hepatitis E virus cell culture models. Virus Res 161:65–77

Oshiro Y, Yasue H, Takahashi K, Hattori S, Ideno S, Urayama T, Chiba M, Osari S, Naito T, Takeuchi K, Nagata K, Ohkohchi N (2014) Mode of swine hepatitis E virus infection and replication in primary human hepatocytes. J Gen Virol 95:2677–2682

Oxford JS, Collier LH, Kellam P (2016) Human virology. Oxford University Press, United States. 350

Pallerla SR, Schembecker S, Meyer CG, Linh LTK, Johne R, Wedemeyer H, Bock CT, Kremsner PG, Velavan TP (2021) Hepatitis E virus genome detection in commercial pork livers and pork meat products in Germany. J Viral Hepat 28:196–204

Panduro A, Escobedo Meléndez G, Fierro NA, Ruiz Madrigal B, Zepeda-Carrillo EA, Román S (2011) Epidemiology of viral hepatitis in Mexico. Salud Publica Mex 53(Suppl 1):S37–S45

Park SB (2012) Hepatitis E vaccine debuts. Nature 491:21–22

Passos-Castilho AM, de Sena A, Geraldo A, Spada C, Granato CF (2016) High prevalence of hepatitis E virus antibodies among blood donors in Southern Brazil. J Med Virol 88:361–364

Pavio N, Merbah T, Thébault A (2014) Frequent hepatitis E virus contamination in food containing raw pork liver, France. Emerg Infect Dis 20:1925–1927

Péron JM, Abravanel F, Guillaume M, Gérolami R, Nana J, Anty R, Pariente A, Renou C, Bureau C, Robic MA, Alric L, Vinel JP, Izopet J, Kamar N (2016) Treatment of autochthonous acute hepatitis E with short-term ribavirin: a multicenter retrospective study. Liver Int 36:328–333

Pillot J, Sharma MD, Lazizi Y, Budkowska A, Dauguet C, Galimand M, Sarthou JL (1987) Immunological characterization of a viral agent involved in epidemic and sporadic non-A, non-B hepatitis. Annales de l'Institut Pasteur/Virologie 138:145–158

Pischke S, Greer M, Hardtke S, Bremer B, Gisa A, Lehmann P, Haverich A, Welte T, Manns MP, Wedemeyer H, Gottlieb J (2014) Course and treatment of chronic hepatitis E virus infection in lung transplant recipients. Transpl Infect Dis 16:333–339

Pourbaix A, Ouali N, Soussan P, Roque Afonso AM, Péraldi MN, Rondeau E, Peltier J (2017) Evidence of hepatitis E virus transmission by renal graft. Transpl Infect Dis 19:e12624

Purcell RH, Emerson SU (2001) Animal models of hepatitis A and E. ILAR J 42:161–177

Purcell RH, Engle RE, Rood MP, Kabrane-Lazizi Y, Nguyen HT, Govindarajan S, St Claire M, Emerson SU (2011) Hepatitis E virus in rats, Los Angeles, California, USA. Emerg Infect Dis 17:2216–2222

Qu C, Xu L, Yin Y, Peppelenbosch MP, Pan Q, Wang W (2017) Nucleoside analogue 2′-C-methylcytidine inhibits hepatitis E virus replication but antagonizes ribavirin. Arch Virol 162: 2989–2996

Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST (2012) The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology 55:988–997

Ren F, Zhao C, Wang L, Wang Z, Gong X, Song M, Zhuang H, Huang Y, Shan H, Wang J, Liu Q, Ness P, Nelson KE, Wang Y (2014) Hepatitis E virus seroprevalence and molecular study among blood donors in China. Transfusion 54:910–917

Rendon J, Hoyos MC, di Filippo D, Cortes-Mancera F, Mantilla C, Velasquez MM, Sepulveda ME, Restrepo JC, Jaramillo S, Arbelaez MP, Correa G, Navas MC (2016) Hepatitis E virus genotype

3 in Colombia: survey in patients with clinical diagnosis of viral hepatitis. PLoS One 11: e0148417
Renou C, Cadranel JF, Bourlière M, Halfon P, Ouzan D, Rifflet H, Carenco P, Harafa A, Bertrand JJ, Boutrouille A, Muller P, Igual JP, Decoppet A, Eloit M, Pavio N (2007) Possible zoonotic transmission of hepatitis E from pet pig to its owner. Emerg Infect Dis 13:1094–1096
Reyes GR (1993) Hepatitis E virus (HEV): molecular biology and emerging epidemiology. Prog Liver Dis 11:203–213
Riedmann EM (2012) Chinese biotech partnership brings first hepatitis E vaccine to the market. Hum Vaccin Immunother 8:1743–1744
S. KM, Teli MR, Skidmore S, Sofi MA, Khuroo MI (1981) Incidence and severity of viral hepatitis in pregnancy. Am J Med 2:252–255
Salam GD, Kumar A, Kar P, Aggarwal S, Husain A, Sharma S (2013) Serum tumor necrosis factor-alpha level in hepatitis E virus-related acute viral hepatitis and fulminant hepatic failure in pregnant women. Hepatol Res 43:826–835
Satake M, Matsubayashi K, Hoshi Y, Taira R, Furui Y, Kokudo N, Akamatsu N, Yoshizumi T, Ohkohchi N, Okamoto H, Miyoshi M, Tamura A, Fuse K, Tadokoro K (2017) Unique clinical courses of transfusion-transmitted hepatitis E in patients with immunosuppression. Transfusion 57:280–288
Schielke A, Filter M, Appel B, Johne R (2011) Thermal stability of hepatitis E virus assessed by a molecular biological approach. Virol J 8:487
Schlosser B, Stein A, Neuhaus R, Pahl S, Ramez B, Krüger D, Berg T, Hofmann J (2012) Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. J Hepatol 56:500–502
Sharma S, Kumar A, Kar P, Agarwal S, Ramji S, Husain SA, Prasad S, Sharma S (2017) Risk factors for vertical transmission of hepatitis E virus infection. J Viral Hepat 24:1067–1075
Shukla P, Nguyen HT, Torian U, Engle RE, Faulk K, Dalton HR, Bendall RP, Keane FE, Purcell RH, Emerson SU (2011) Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus–host recombinant. Proc Natl Acad Sci 108:2438
Shukla P, Nguyen HT, Faulk K, Mather K, Torian U, Engle RE, Emerson SU (2012) Adaptation of a genotype 3 hepatitis E virus to efficient growth in cell culture depends on an inserted human gene segment acquired by recombination. J Virol 86:5697–5707
Singh A, Seth R, Gupta A, Shalimar, Nayak B, Acharya SK, Das P (2018) Chronic hepatitis E – an emerging disease in an immunocompromised host. Gastroenterol Rep (Oxf) 6:152–155
Smith DB, Simmonds P, Jameel S, Emerson SU, Harrison TJ, Meng X-J, Okamoto H, Van der Poel WHM, Purdy MA (2014a) Consensus proposals for classification of the family Hepeviridae. J Gen Virol 95(Pt 10):2223–2232 95
Smith DB, Simmonds P, Members of the international committee on the taxonomy of viruses study G, Jameel S, Emerson SU, Harrison TJ, Meng XJ, Okamoto H, der Poel WHM V, Purdy MA (2014b) Consensus proposals for classification of the family Hepeviridae. J Gen Virol 95: 2223–2232
Smith DB, Simmonds P, Izopet J, Oliveira-Filho EF, Ulrich RG, Johne R, Koenig M, Jameel S, Harrison TJ, Meng X-J, Okamoto H, Van der Poel WHM, Purdy MA (2016) Proposed reference sequences for hepatitis E virus subtypes. J Gen Virol 97:537–542
Sridhar S, Teng JLL, Chiu TH, Lau SKP, Woo PCY (2017) Hepatitis E virus genotypes and evolution: emergence of camel hepatitis E variants. Int J Mol Sci 18(4):869
Sridhar S, Yip CCY, Wu S, Cai J, Zhang AJ, Leung KH, Chung TWH, Chan JFW, Chan WM, Teng JLL, Au-Yeung RKH, Cheng VCC, Chen H, Lau SKP, Woo PCY, Xia NS, Lo CM, Yuen KY (2018) Rat hepatitis E virus as cause of persistent hepatitis after liver transplant. Emerg Infect Dis 24:2241–2250
Sridhar S, Yip CC, Wu S, Chew NF, Leung KH, Chan JF, Zhao PS, Chan WM, Poon RW, Tsoi HW, Cai JP, Chan HS, Leung AW, Tse CW, Zee JS, Tsang OT, Cheng VC, Lau SK, Woo PC, Tsang DN, Yuen KY (2021) Transmission of rat hepatitis E virus infection to humans in Hong Kong: a clinical and epidemiological analysis. Hepatology 73:10–22
Takahashi M, Okamoto H (2014) Features of hepatitis E virus infection in humans and animals in Japan. Hepatol Res 44:43–58

Takahashi M, Tanaka T, Takahashi H, Hoshino Y, Nagashima S, Jirintai N, Mizuo H, Yazaki Y, Takagi T, Azuma M, Kusano E, Isoda N, Sugano K, Okamoto H (2010) Hepatitis E virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: characterization of HEV virions in blood circulation. J Clin Microbiol 48:1112–1125

Tanaka T, Takahashi M, Takahashi H, Ichiyama K, Hoshino Y, Nagashima S, Mizuo H, Okamoto H (2009) Development and characterization of a genotype 4 hepatitis E virus cell culture system using a HE-JF5/15F strain recovered from a fulminant hepatitis patient. J Clin Microbiol 47: 1906–1910

Taus K, Schmoll F, El-Khatib Z, Auer H, Holzmann H, Aberle S, Pekard-Amenitsch S, Monschein S, Sattler T, Steinparzer R, Allerberger F, Schmid D (2019) Occupational swine exposure and Hepatitis E virus, Leptospira, Ascaris suum seropositivity and MRSA colonization in Austrian veterinarians, 2017-2018-A cross-sectional study. Zoonoses Public Health 66:842–851

Teshale EH, Hu DJ (2011) Hepatitis E: epidemiology and prevention. World J Hepatol 3:285–291

Ticehurst J, Rhodes LL Jr, Krawczynski K, Asher LV, Engler WF, Mensing TL, Caudill JD, Sjogren MH, Hoke CH Jr, LeDuc JW et al (1992) Infection of owl monkeys (Aotus trivirgatus) and cynomolgus monkeys (Macaca fascicularis) with hepatitis E virus from Mexico. J Infect Dis 165:835–845

Ticehurst JR, Pisanic N, Forman MS, Ordak C, Heaney CD, Ong E, Linnen JM, Ness PM, Guo N, Shan H, Nelson KE (2019) Probable transmission of hepatitis E virus (HEV) via transfusion in the United States. Transfusion 59:1024–1034

Tsarev SA, Tsareva TS, Emerson SU, Kapikian AZ, Ticehurst J, London W, Purcell RH (1993a) ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: identification of HEV infection in primates. J Infect Dis 168:369–378

Tsarev SA, Emerson SU, Tsareva TS, Yarbough PO, Lewis M, Govindarajan S, Reyes GR, Shapiro M, Purcell RH (1993b) Variation in course of hepatitis E in experimentally infected cynomolgus monkeys. J Infect Dis 167:1302–1306

Tsarev SA, Tsareva TS, Emerson SU, Govindarajan S, Shapiro M, Gerin JL, Purcell RH (1994) Successful passive and active immunization of cynomolgus monkeys against hepatitis E. Proc Natl Acad Sci U S A 91:10198–10202

Tsarev SA, Tsareva TS, Emerson SU, Rippy MK, Zack P, Shapiro M, Purcell RH (1995) Experimental hepatitis E in pregnant rhesus monkeys: failure to transmit hepatitis E virus (HEV) to offspring and evidence of naturally acquired antibodies to HEV. J Infect Dis 172:31–37

Tseng TC, Liu CJ, Chang CT, Su TH, Yang WT, Tsai CH, Chen CL, Yang HC, Liu CH, Chen PJ, Chen DS, Kao JH (2020) HEV superinfection accelerates disease progression in patients with chronic HBV infection and increases mortality in those with cirrhosis. J Hepatol 72:1105–1111

Usmanov RK, Balaian MS, Dvoĭnikova OV, Alymbaeva DB, Zamiatina NA, Kazachkov Iu A, Belov VI (1994) An experimental infection in lambs by the hepatitis E virus. Vopr Virusol 39:165–168

Viera-Segura O, Realpe-Quintero M, Panduro A, Roman S, Jose-Abrego A, Gonzalez-Aldaco K, Trujillo-Ochoa JL, Fierro NA (2019) First detection of hepatitis E virus genotype 3 as a common infectious agent in patients with chronic liver damage in Mexico. Ann Hepatol 18:571–577

Wang Y, Ma X (2010) Detection and sequences analysis of sheep hepatitis E virus RNA in Xinjiang autonomous region. Wei sheng wu xue bao= Acta Microbiol Sin 50:937–941

Wang C, Guo L, Yu D, Hua X, Yang Z, Yuan C, Cui L (2014a) HEV-ORF3 encoding phosphoprotein interacts with hepsin. Hepat Mon 14:e13902

Wang Y, Zhou X, Debing Y, Chen K, Van Der Laan LJW, Neyts J, Janssen HLA, Metselaar HJ, Peppelenbosch MP, Pan Q (2014b) Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. Gastroenterology 146:1775–1783

WHO (2017) Hepatitis E. WHO, Geneva

Wu T, Zhu F-C, Huang S-J, Zhang X-F, Wang Z-Z, Zhang J, Xia N-S (2012) Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis. Hepatology 55:2038–2038

Wu X, Chen P, Lin H, Hao X, Liang Z (2016) Hepatitis E virus: current epidemiology and vaccine. Hum Vaccin Immunother 12:2603–2610

Wu X, Dao Thi VL, Liu P, Takacs CN, Xiang K, Andrus L, Gouttenoire J, Moradpour D, Rice CM (2018) Pan-genotype hepatitis E virus replication in stem cell-derived hepatocellular systems. Gastroenterology 154:663–674.e667

Wu C, Wu X, Xia J (2020) Hepatitis E virus infection during pregnancy. Virol J 17:73
Xia J, Liu L, Wang L, Zhang Y, Zeng H, Liu P, Zou Q, Wang L, Zhuang H (2015) Experimental infection of pregnant rabbits with hepatitis E virus demonstrating high mortality and vertical transmission. J Viral Hepat 22:850–857
Yadav KK, Boley PA, Fritts Z, Kenney SP (2021) Ectopic expression of genotype 1 hepatitis E virus ORF4 increases genotype 3 HEV viral replication in cell culture. Viruses 13:75
Yamashita T, Mori Y, Miyazaki N, Cheng RH, Yoshimura M, Unno H, Shima R, Moriishi K, Tsukihara T, Li TC, Takeda N, Miyamura T, Matsuura Y (2009) Biological and immunological characteristics of hepatitis E virus-like particles based on the crystal structure. Proc Natl Acad Sci 106:12986
Yang C, Hao X, Li Y, Long F, He Q, Huang F, Yu W (2019) Successful establishment of hepatitis E virus infection in pregnant BALB/c mice. Viruses 11:451
Yin X, Li X, Feng Z (2016) Role of envelopment in the HEV life cycle. Viruses 8:229
Yin X, Li X, Ambardekar C, Hu Z, Lhomme S, Feng Z (2017) Hepatitis E virus persists in the presence of a type III interferon response. PLoS Pathog 13:e1006417
Yin X, Ying D, Lhomme S, Tang Z, Walker CM, Xia N, Zheng Z, Feng Z (2018) Origin, antigenicity, and function of a secreted form of ORF2 in hepatitis E virus infection. Proc Natl Acad Sci U S A 115:4773–4778
You S, Rong Y, Zhu B, Zhang A, Zang H, Liu H, Li D, Wan Z, Xin S (2013) Changing etiology of liver failure in 3,916 patients from northern China: a 10-year survey. Hepatol Int 7:714–720
Younus M, Nisa Q, Munir MT, Jamil T, Kaboudi K, Rehman ZU, Umar S, Shah MA (2017) Viral hepatitis in chicken and turkeys. Worlds Poult Sci J 73:379–394
Yu W, Hao X, Li Y, Yang C, Li Y, He Z, Huang F (2020) Vertical transmission of hepatitis E virus in pregnant rhesus macaques. Sci Rep 10:17517
Yugo DM, Meng XJ (2013) Hepatitis E virus: foodborne, waterborne and zoonotic transmission. Int J Environ Res Public Health 10:4507–4533
Yugo DM, Cossaboom CM, Meng XJ (2014) Naturally occurring animal models of human hepatitis E virus infection. ILAR J 55:187–199
Zhang J, Liu CB, Li RC, Li YM, Zheng YJ, Li YP, Luo D, Pan BB, Nong Y, Ge SX, Xiong JH, Shih JW, Ng MH, Xia NS (2009) Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 27:1869–1874
Zhang Y, Zeng H, Liu P, Liu L, Xia J, Wang L, Zou Q, Wang L, Zhuang H (2015a) Hepatitis E vaccine immunization for rabbits to prevent animal HEV infection and zoonotic transmission. Vaccine 33:4922–4928
Zhang J, Zhang X-F, Huang S-J, Wu T, Hu Y-M, Wang Z-Z, Wang H, Jiang H-M, Wang Y-J, Yan Q, Guo M, Liu X-H, Li J-X, Yang C-L, Tang Q, Jiang R-J, Pan H-R, Li Y-M, Shih JW-K, Ng M-H, Zhu F-C, Xia N-S (2015b) Long-term efficacy of a hepatitis E vaccine. N Engl J Med 372:914–922
Zhang L, Jiao S, Yang Z, Xu L, Liu L, Feng Q, Zhang X, Hou Y, He S, Saldanha J, Wang S, Wang B (2017) Prevalence of hepatitis E virus infection among blood donors in mainland China: a meta-analysis. Transfusion 57:248–257
Zhao C, Ma Z, Harrison TJ, Feng R, Zhang C, Qiao Z, Fan J, Ma H, Li M, Song A, Wang Y (2009) A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. J Med Virol 81: 1371–1379
Zhao Y, Zhang X, Zhu F, Jin H, Wang B (2016) A preliminary cost-effectiveness analysis of hepatitis E vaccination among pregnant women in epidemic regions. Hum Vaccin Immunother 12:2003–2009
Zhou X, Huang F, Xu L, Lin Z, de Vrij FMS, Ayo-Martin AC, van der Kroeg M, Zhao M, Yin Y, Wang W, Cao W, Wang Y, Kushner SA, Marie Peron J, Alric L, de Man RA, Jacobs BC, van Eijk JJ, Aronica EMA, Sprengers D, Metselaar HJ, de Zeeuw CI, Dalton HR, Kamar N, Peppelenbosch MP, Pan Q (2017) Hepatitis E virus infects neurons and brains. J Infect Dis 215:1197–1206
Zhu F-C, Zhang J, Zhang X-F, Zhou C, Wang Z-Z, Huang S-J, Wang H, Yang C-L, Jiang H-M, Cai J-P (2010) Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 376:895–902

West Nile Virus: From Africa to Europe, America, and Beyond

46

Lyle R. Petersen and Randall J. Nett

Contents

L. R. Petersen (✉) · R. J. Nett
CDC, Fort Collins, CO, USA
e-mail: lxp2@cdc.gov; GGE5@CDC.GOV

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_38

Abstract

Since the mid-1990s, West Nile virus (WNV) outbreaks of increased severity first appeared in Africa, then in Europe, and finally in North America. These outbreaks were due to related lineage 1 viruses of apparent increased pathogenicity. More recently lineage 2 strains also of apparent increased pathogenicity have caused outbreaks in Europe. Some of the implicated lineage 1 strains and the lineage 2 strains have a mutation in the NS3 helicase gene, which confers increased viral pathogenesis in birds. The recent pattern of sporadic cases and outbreaks of WNV that has emerged in Europe and North America shows no signs of abating. While broad areas of high risk can be identified, the sporadic, local, and regional outbreaks that occur within these areas remain elusively unpredictable. Fewer than 1% of persons infected develop neuroinvasive disease, characterized by meningitis, encephalitis, or acute flaccid paralysis. Increasing age is a risk factor for neuroinvasive disease, both in humans and horses. Four WNV vaccines are currently marketed for horses in the United States. Although clinical trials have been conducted for six human vaccines, no vaccines are currently approved for human use. Treatment is supportive.

Keywords

West Nile Virus · West Nile Virus Infection · West Nile Fever · West Nile Virus Transmission · West Nile Virus Strain

46.1 Introduction

The last three decades have witnessed the global emergence or reemergence of the West Nile virus. The virus was introduced to and spread throughout the Americas and outbreaks resulting in significant morbidity are now commonplace in North America and Europe. This chapter discusses the geographic emergence of this virus, the virology and biology behind this emergence, the virus's clinical and diagnostic aspects, and treatment and control measures.

46.2 Virology

West Nile virus (WNV) is one of more than 70 viruses of the family *Flaviviridae* of the genus Flavivirus (Heinz et al. 2000). The flaviviruses comprise some of the most medically important arthropod-borne viruses, including the yellow fever, Japanese

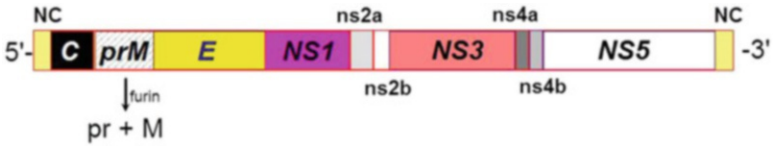

Fig. 1 WNV genome

encephalitis, dengue, and tick-borne encephalitis viruses (Mackenzie and Williams 2009). Like other flaviviruses, WNV is an enveloped spherical-shaped virion encompassing a single-stranded RNA molecule of positive polarity of approximately 11-kb. The genomic RNA of WNV encodes three 5′ structural proteins (C-prM-E) and seven nonstructural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5) at the 3′ end, which are flanked by 5′ and 3′ untranslated regions involved in transcription and translation (Fig. 1). Both structural and nonstructural proteins are translated as a single polyprotein that is cleaved into the respective viral proteins by host and viral proteases both co- and post-translationally (Chambers et al. 1990).

Serologically, WNV is a member of the Japanese encephalitis serocomplex, which includes the Japanese encephalitis, Murray Valley encephalitis, and St. Louis encephalitis viruses (Calisher 1988). Sequence analyses suggest that WNVs can be categorized into at least five phylogenetic lineages (May et al. 2011), although up to nine genetic lineages have been suggested (De Filette et al. 2012; Mackenzie and Williams 2009; Pachler et al. 2014; Vazquez et al. 2010). Only lineage 1 and 2 WNVs have been associated with significant disease outbreaks in humans.

The lineage 1 viruses can be further subdivided into three sublineages (a–c): isolates from the Western Hemisphere, Africa, the Middle East, and Europe constitute lineage 1a; Kunjin virus from Australasia represents lineage 1b; and lineage 1c consists of viruses from India (Bondre et al. 2007; May et al. 2011). Distribution of lineage 1 most likely occurred during the past 300 years, possibly resulting from increased trade between Africa and India and Australia. The lineage 1a viruses are the most widely dispersed and epidemiologically important, having caused large outbreaks in Europe, Russia, and North America during the past three decades. Lineage 1a can be further subdivided phylogenetically into several clusters, each with a relatively distinct geographic focus of circulation (May et al. 2011). Nevertheless, all but one cluster contains isolates from Africa, suggesting frequent gene flow from Africa to Europe and Russia, most likely by migrating birds.

WNV was first discovered in the Western Hemisphere during simultaneous human epidemics and avian epizootics in the New York City area in 1999 (Mostashari et al. 2001). The means and origin of the WNV brought into North America are unknown. A Middle Eastern origin was first hypothesized based on the fact that the initial North American isolates (East Coast genotype) from 1999 were most closely related to a lineage 1a WNV isolated from Israel in 1998

(Lanciotti et al. 1999). However, more recent analysis suggests that the Israel outbreak was not the progenitor of the North American outbreak, but rather that both outbreaks were initiated by the introduction of strains from the same independent location, probably Africa (May et al. 2011). The WNV strain imported into North America contains a single nucleotide change in the NS3 gene (T249P), which confers avian virulence in otherwise nonvirulent strains (Brault et al. 2007). Since approximately 2002, the East Coast genotype has largely been displaced by a new genotype (WN02) encompassing several conserved amino acid substitutions (Davis et al. 2005). This displacement may have resulted from a temperature-dependent increased efficiency and rapidity of dissemination and transmission of the WN02 virus in North American mosquito vectors (Ebel et al. 2004; Kilpatrick et al. 2008).

Only lineage 1 and 3 WNV strains had been found in Europe until 2004, when a lineage 2 strain pathogenic to birds of prey was identified in Hungary (Bakonyi et al. 2006). Before then, lineage 2 WNVs were isolated almost exclusively from African transmission cycles and were considered to be of low pathogenicity (Murgue et al. 2002). Subsequently, highly pathogenic lineage 2 WNV strains have caused both human and animal disease in South Africa (Venter 2009; Zaayman and Venter 2012) and Europe (Bakonyi et al. 2013; Papa 2012). Isolates from lineage 2 viruses generally have histidine at the 249 locus of the NS3 gene. While isolates that have caused large outbreaks in Greece since 2010 have contained a proline substitution at this locus (Barzon et al. 2013b; Papa et al. 2011a, 2013), experimental data indicate that this residue does not cause increased viral replication or virulence in European birds (Del Amo et al. 2014; Sotelo et al. 2011a). Like lineage 1 viruses, phylogenetic analysis suggests multiple introductions of lineage 2 viruses into Europe from Africa (Ciccozzi et al. 2013; McMullen et al. 2013). Two subclades of lineage 2 viruses circulate in Europe. The Central European/Hungarian subclade present in central Europe and the eastern Mediterranean region now causes most seasonal outbreaks in Europe (Chaintoutis et al. 2019). The Eastern European/Russian subclade has caused outbreaks in Eastern Europe and Russia.

46.3 Transmission Cycle

WNV exists in a bird-mosquito-bird transmission cycle (Fig. 2). Following mating, female *Culex spp*. mosquitoes obtain a blood meal from a vertebrate host to obtain protein to make eggs. She can become infected from a blood meal from a vertebrate host with sufficient viremia levels to efficiently infect mosquitoes. Viremia in vertebrate hosts generally lasts about 5–7 days (Komar et al. 2003). After depositing her eggs, she seeks another vertebrate host to feed upon. Sufficient time is required after the initial blood meal for the virus to replicate within the mosquito and spread to its salivary glands (extrinsic incubation period), at which time it can transmit the virus to this and subsequent vertebrate hosts during feeding.

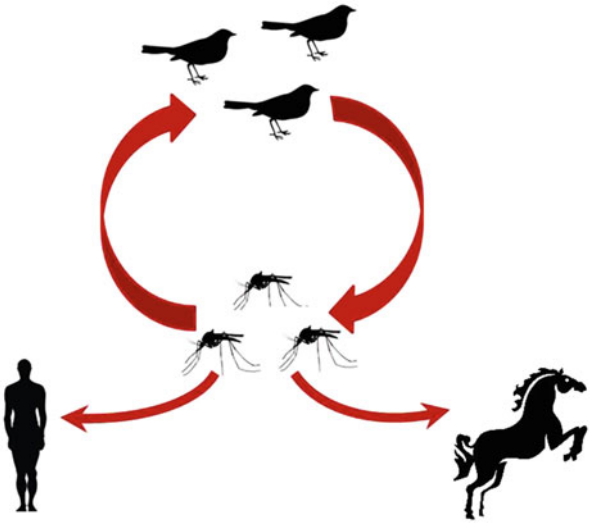

Fig. 2 WNV transmission cycle

46.3.1 Mosquitoes

WNV has been detected in 65 different mosquito species in the United States (Centers for Disease Control and Prevention). However, most of these detections used molecular methods without virus isolation, thus confirming the presence of WNV but not necessarily the ability to transmit the virus (Rochlin et al. 2019). Further, mosquito host preference is complex and driven by multiple intrinsic (e.g., preference for birds) and extrinsic (e.g., host abundance) determinants (Takken and Verhulst 2013). Only a few ornithophilic *Culex* mosquito species drive epizootic and epidemic transmission: *Culex pipiens* in the northern half of the United States; the closely-related species *Culex quinquefasciatus* in the southern and western United States; and *Culex tarsalis* in many areas that overlap with the distribution of *Culex pipiens* and *Culex quinquefasciatus* (Andreadis 2012; Andreadis et al. 2004; Godsey Jr. et al. 2012; Kilpatrick et al. 2006b). *Culex pipiens* and *Culex quinquefasciatus* typically use man-made habitats for laying eggs (oviposition) and larval development, including peridomestic containers and above- and below-ground waste water systems (Reisen 2012; Reisen et al. 1990; Rochlin et al. 2019). *Culex tarsalis* is often associated with irrigated farmland (Reisen 2012), but can use diverse sources for breeding such as abandoned swimming pools (Reisen et al. 2008). The ubiquitous sources for Culex mosquito breeding make larval control a formidable challenge. In Europe, *Culex pipiens* is considered the most important vector (Reiter 2010; Vogels et al. 2017). The major mosquito vector in Africa and the Middle East is *Culex univittatus*, with other Culex species important in some areas (Hubalek and Halouzka 1999; Ochieng et al. 2013; Solomon 2004).

Several other mosquito species can play important roles in certain circumstances. For example, *Culex restuans*, an ornithophilic mosquito present in high numbers early in the transmission season and commonly found infected with WNV, can play an important role in early amplification of enzootic transmission in the northeastern United States (Andreadis and Armstrong 2007). *Culex salinarius*, a mosquito found in high numbers during August and September in coastal areas of the northeastern United States, is frequently found to have high WNV infection rates. Because this mosquito feeds indiscriminantly on both birds and mammals and readily bites humans, it can be an important bridge vector to humans (Andreadis 2012).

In temperate climates, WNV overwinters in hibernating (diapause) adult female Culex mosquitoes, probably in some birds and possibly in rodents (Nasci et al. 2001; Platt et al. 2008; Reisen et al. 2006a). Although the means by which pre-hibernating Culex females become infected is not entirely clear, it has been conclusively demonstrated that vertically infected female *Culex pipiens* that enter diapause in the fall are able to initiate infection in the spring (Anderson and Main 2006). In semi-tropical environments, such as found in the southeastern United States, transmission occurs throughout the year, albeit at a very low level during cooler periods (Tesh et al. 2004).

46.3.2 Vertebrate Hosts

The extensive distribution of WNV throughout Africa, the Middle East, southern Europe, western Russia, southwestern Asia, and Australia (Kunjin subtype) derives in part from its ability to infect numerous bird species. Infection has been documented in at least 326 bird species in the United States alone (Centers for Disease Control and Prevention). Numerous passerine birds develop extremely high viremias and thus are competent amplifier hosts (Komar et al. 2003; Weingartl et al. 2004). Nevertheless, a relatively small subset of the bird community can significantly influence WNV transmission dynamics (McKenzie and Goulet 2010); high avian species diversity, particularly if it is rich in non-passerine species, can dampen WNV transmission (Ezenwa et al. 2006). For example, the American robin (*Turdus migratorius*) can be an important amplifier host despite its low abundance relative to other WNV susceptible birds (Kilpatrick et al. 2006a; Savage et al. 2007). Kilpatrick has hypothesized that WNV outbreaks are promoted by mosquito feeding shifts from American robins to humans coincident with late-season robin dispersal (Kilpatrick et al. 2006b); however, others have failed to demonstrate a shift of feeding preference from robins to mammals (Savage et al. 2007).

WNV transmission might be influenced by the spatial interactions of birds in several ways. One study examining American robins indicated that communal roosts can form important WNV amplification foci (Diuk-Wasser et al. 2010) while another indicated that *Culex quinquefasciatus* more frequently fed on species that roost communally, including the American robin, finch, and sparrow (Hannon et al. 2019). A potential mechanism is the CO_2 generated from many birds congregating at night, which attracts host-seeking mosquitoes (Janousek et al. 2014). In contrast,

one study demonstrated that roosts are not necessarily important for WNV amplification (Benson et al. 2012). Cloacal and oral shedding of WNV is common in infected birds (Komar et al. 2003) and direct bird-to-bird transmission has been documented in birds housed together (Banet-Noach et al. 2003; Komar et al. 2003; Weingartl et al. 2004). The influence of non-mosquito, direct bird-to-bird transmission on the amplification cycle is unknown, although one mathematical model indicated that it can play an important role in some circumstances (Naowarat and Tang 2004).

Other factors can potentially influence avian-related WNV transmission and increase the risk for epidemics. American robins that were food-deprived for 48 h before WNV infection were found to develop higher viral titers and had a longer infectious period compared with robins fed normally (Owen et al. 2021). Another study demonstrated that exposure to artificial light at night increased the duration of potentially infectious titers in house sparrows without greater WNV-induced mortality (Kernbach et al. 2019).

The role of non-avian vertebrate hosts for maintaining or amplifying the virus is unknown. Humans and horses generally develop insufficient WNV titers in the blood to infect mosquitoes, but squirrels, chipmunks, and rabbits, and potentially opossum, can develop sufficiently high viremia to infect mosquitoes, raising the possibility that small mammals might contribute to WNV transmission cycles (Platt et al. 2007, 2008; Reisen and Brault 2007; Root and Bosco-Lauth 2019). Snakes could play a possible role in the WNV transmission cycle (Dahlin et al. 2016). Alligators might also serve as competent reservoirs in the southeastern United States and have been shown experimentally to be capable of WNV amplification and transmission to mosquitoes, thus indicating the potential for maintaining WNV outside of the bird-mosquito transmission cycle (Byas et al. 2022; Klenk et al. 2004).

46.3.3 Determinants of Human Disease Incidence and Outbreaks

As with most mosquito-borne arboviral diseases, WNV incidence in humans exhibits considerable annual and geographic variation. Nevertheless, outbreaks in temperate climates tend to occur during mid- to late summer after sufficient viral amplification in the bird-mosquito-bird transmission cycle has produced enough infected mosquitoes to present a human infection risk (Fig. 3). Certain areas also tend to have consistently higher human disease incidence, indicating an underlying permissive ecology that promotes WNV amplification. Factors such as urban and agricultural land covers (Bowden et al. 2011), rural irrigated landscapes (DeGroote and Sugumaran 2012), orchard habitats (Crowder et al. 2013), farming activities as determined by total crop sales (Miramontes Jr. et al. 2006) and several socioeconomic factors such as housing age and community drainage patterns (Ruiz et al. 2007), per capita income (DeGroote and Sugumaran 2012), and even neglected swimming pool density (Harrigan et al. 2010; Reisen et al. 2008) relate to higher WNV incidence in some locations. Overall, persons living in rural areas in the United States and Canada seem to be at higher risk of acquiring WNV infection

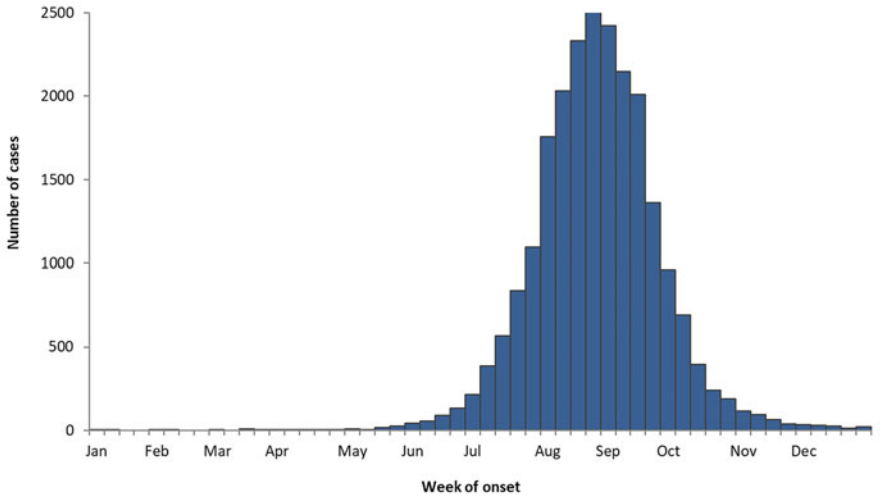

Fig. 3 Cumulative incidence of WNV neuroinvasive disease, by week of symptom onset, United States, 1999–2019. Source: US Centers for Disease Control and Prevention

than those living in urban settings. A United States nationwide study comparing viremic to uninfected blood donors illustrated that residents of rural areas were 3.4 times more likely to be infected than residents of surburban or urban locations (Orton et al. 2006). In Saskatchewan, Canada, residents of rural areas were approximately six times more likely than urban dwellers to have WNV antibodies (Schellenberg et al. 2006).

Weather has an enormous impact on WNV amplification in the bird-mosquito-bird transmission cycle. Increasing ambient temperature shortens the viral extrinsic incubation period and increases viral replication in mosquitoes, both of which greatly increase the probability of viral transmission to susceptible hosts (Cornel et al. 1993; Dohm et al. 2002; Kilpatrick et al. 2008; Reisen et al. 2006b; Richards et al. 2007). However, changes in ambient temperature influence the abundance of vector mosquitoes, host survival and distribution, and human behavior in unpredictable ways differentially influenced by the underlying local ecology. Rainfall and humidity variably influence mosquito abundance and survival, distribution of hosts, and human behavior. Given the infinite possible combinations of temperature and rainfall events and their variable effects on arboviral transmission parameters, combined with other parameters that influence transmission, such as underlying immunity in birds, no models have been able to predict when and where WNV outbreaks will occur nationally (Petersen and Fischer 2012). Nevertheless, human disease incidence over broad areas in temperate climates has correlated with increased temperatures (Hartley et al. 2012; Soverow et al. 2009; Watts et al. 2021; Wimberly et al. 2008) as well as increased (Landesman et al. 2007; Soverow et al. 2009) or decreased rainfall (Landesman et al. 2007; Mavrakis et al. 2021; Wang

et al. 2010). A substantial need exists to develop short-term planning models over small geographic areas that can assist mosquito control districts and public health authorities in surveillance, vector control, and public health messaging (Keyel et al. 2021).

46.4 Transmission to Humans

Nearly all humans acquire WNV infection from mosquito bites. Persons with extensive outdoor exposure are likely at higher risk for infection. One study in the United States found that children were more likely than adults to spend time outdoors and less likely to practice personal protective behaviors (LaBeaud et al. 2007). People of lower socioeconomic status and particularly those experiencing homelessness might be at higher risk of WNV infection (Gujral et al. 2007; Meyer et al. 2007).

Transfusion-associated WNV transmission was first detected during the 2002 WNV epidemic in the United States when 23 transfusion recipients were infected through receipt of platelets, red blood cells, or fresh frozen plasma from 16 viremic blood donors (Pealer et al. 2003). Mathematical models indicated the risk of transfusion-associated WNV transmission during the 2002 epidemic ranged from 2.1 to 4.7 per 10,000 donors in high incidence areas (Biggerstaff and Petersen 2003). These findings prompted screening of the United States and Canadian blood supplies using WNV nucleic acid amplification (NAT) tests since 2003. Blood centers conduct NAT testing on minipools of 6 to 16 specimens, depending on test kit manufacturer. While universal pooled blood donation screening has nearly eliminated WNV transfusion transmission, some "breakthrough" transmissions have occurred from donations with virus levels below the limit of detection by minipool screening (Centers for Disease Control and Prevention 2007; Groves et al. 2017; Petersen and Epstein 2005). To minimize the risk of "breakthrough" transmissions, blood centers switch to individual donation testing in areas experiencing outbreaks; however, one transmission occurred from a donor with viremia below levels detected by individual unit testing (Centers for Disease and Prevention 2013).

In 2002, transmission via donated organs was first documented when four recipients of organs from a common donor developed WNV infection (Iwamoto et al. 2003). Since then, seven additional transmission clusters in the United States (Nett et al. 2012; Soto et al. 2022) and two in Italy (Inojosa et al. 2012; Morelli et al. 2010) have been documented and published. Interestingly, archived serum from the donors of four of these nine clusters had negative NAT results, indicating that viable WNV can be sequestered in organs for a short time after viremia has cleared. The urgency of procuring and transplanting organs has precluded most organ procurement organizations from routinely conducting WNV screening of organ donors (Nett et al. 2012; Theodoropoulos et al. 2021).

Intrauterine transmission of WNV was first documented in 2002 when a woman with WNV encephalitis during the 27th week of pregnancy delivered a term infant with chorioretinitis, cerebral lesions, and laboratory evidence of congenitally

acquired WNV infection (Alpert et al. 2003; Centers for Disease Control and Prevention 2002a). Fortunately, intrauterine transmission appears uncommon. A follow-up study of 71 women infected with WNV during pregnancy demonstrated that none of their 72 live infants had malformations linked to WNV infection or had conclusive laboratory evidence of congenital WNV infection (O'Leary et al. 2006). However, three infants born to women with illness occurring within 3 weeks prepartum had evidence of WNV infection that could have been congenitally acquired or acquired at the time of birth: one had WNV meningitis at 10 days of age, one had a neonatal rash and was positive for anti-WNV IgM at 1 month of age, and one had WNV encephalitis with underlying lissencephaly detected at 17 days of age (O'Leary et al. 2006). A study of 549 infants born to mothers who were pregnant during a community WNV outbreak found evidence of WNV infection in 4% of the mothers and in none of the infants (Paisley et al. 2006). Similar birth outcomes were noted among infants born to mothers with and without evidence of WNV infection during pregnancy (Paisley et al. 2006). A small prospective study that compared 28 pregnant women who had serologically confirmed WNV illness with 26 WNV-uninfected pregnant women found that none of the infants born to the WNV-infected mothers were born with clinical evidence of infection and none had neurologic deficits at birth (Pridjian et al. 2016). Further, infants born to the WNV-infected mothers were no more likely to have congenital malformations or developmental delays.

WNV transmission has also been reported through percutaneous exposure and inhalation in laboratories, conjunctival exposure while handling dead birds, in a dialysis center by unidentified means, and at a turkey farm, possibly by aerosol (Centers for Disease Control and Prevention 2002b, 2003, 2004; Fonseca et al. 2005; Nir et al. 1965). Transmission via breast milk has also been reported, but appears uncommon (Hinckley et al. 2007).

46.5 Global Epidemiology

46.5.1 Africa

WNV lineages 1, 2, 7 (now classified as a separate virus, Koutango virus), and 8 have been identified in Africa (Fall et al. 2017). WNV lineage 1 is mainly found in Central and Northern Africa; whereas, lineage 2 is found in Southern Africa (Mencattelli et al. 2022). Phylogenetic analysis suggests that all European lineage 1 and 2 strains originated from Africa (Bakonyi et al. 2006, 2013; Charrel et al. 2003; May et al. 2011). Consistent with this hypothesis, serological studies in humans and animals conducted in several African countries indicate widespread viral circulation (Mencattelli et al. 2022; Murgue et al. 2002). Seroprevalence ranges markedly by location, even within the same country. For example, a serological study of Egyptian university students showed that WNV antibody prevalence ranged from 28% in Cairo to 74% in Upper Egypt (Darwish and Ibrahim 1971). Similarly, a serological survey of randomly selected household members demonstrated WNV antibody

prevalences ranging from 2% in the Northern Sinai to 35% in Upper Egypt (Soliman et al. 2010). Consistent with an endemic pattern, antibody seroprevalence increased by age. A similar pattern of increasing seroprevalence with age was noted in a serological survey of humans in Kenya (Sutherland et al. 2011). While serological data suggest frequent WNV exposure in Africa, these data must be interpreted cautiously because of differing testing methodologies and the considerable serological cross-reactivity among the flaviviruses.

In contrast to the apparent frequent exposure to WNV throughout much of Africa, human and animal illness has been reported infrequently. Nevertheless, sporadic cases of mild illness undoubtedly occur much more commonly than reports indicate. The virus was first recognized in a febrile woman in Uganda in 1937 (Smithburn et al. 1940). WN viral nucleic acid has confirmed WNV circulation in humans in eight countries between 1983 and 2020 and serological surveys have suggested viral exposure to humans in 23 countries (Mencattelli et al. 2022). The most notable outbreak occurred in South Africa in 1974 following heavy rains and above normal temperatures in early summer. Despite an estimated 18,000 cases, neuroinvasive disease or mortality was not recorded (Jupp 2001). More recently, a new pattern of outbreaks of unusual severity in northern Africa has occurred. An outbreak of approximately 50 human cases with neurological disease occurred in Algeria in 1994 (Le Guenno et al. 1996), which was followed in 1997 by an outbreak involving 173 patients (Triki et al. 2001). An outbreak associated with severe neurological disease in a military camp in the Democratic Republic of Congo was noted in 1998 (Nur et al. 1999). In Sudan in 2002, at least 31 cases of encephalitis occurred during an outbreak in children (Depoortere et al. 2004). In addition, outbreaks of severe neurological disease in equines in Morocco in 1996 and 2003 were associated with 94 and 7 equines, respectively (Schuffenecker et al. 2005). These outbreaks were associated with viral strains of apparent increased virulence and were closely related to the lineage 1 WNV strains that caused large human outbreaks in Romania, Russia, Israel, and the United States (Lanciotti et al. 1999; Schuffenecker et al. 2005). In recent years, sporadic cases of neurologic disease from lineage 2 WNV strains have been identified in South Africa (Venter 2009; Venter and Swanepoel 2010; Zaayman and Venter 2012).

46.5.2 Europe

WNV strains are likely transported between sub-Saharan Africa and Europe by migratory birds. This hypothesis is supported by the relatively high prevalence of WNV antibodies in migratory birds noted in Russia, Israel, and several European countries (Lelli et al. 2012; Valiakos et al. 2012), as well as by phylogenetic analyses of WNV strains suggesting multiple WNV introductions to Europe in recent years (Parreira et al. 2007). Thus, the likely long co-evolution of virus and hosts in Europe may account for the relative paucity of widespread mortality in European birds compared to what has occurred among many North American bird species (Komar et al. 2003; LaDeau et al. 2007). Nevertheless, lineage I WNV strains genetically

related to the strain imported into New York City in 1999 (East Coast strain) resulted in clinical illness in white storks (*Cicona ciconia*) migrating from central Europe through Israel in 1998 (Malkinson et al. 2002) and an epizootic of encephalitis in Hungarian geese (*Anser anser domesticus*) in 2003 (Bakonyi et al. 2006). In 2004 and 2005, several deaths in goshawks (*Accipiter gentilis*) and a sparrow hawk (*Accipiter nisus*) from the same region of Hungary were attributed to a central African lineage 2 virus, which was the first report of a lineage 2 virus outside of Africa (Erdelyi et al. 2007).

From the 1960s through the 1980s, WNV isolates were obtained infrequently in southern and central Europe from mosquitoes, humans, birds, and horses, although serological surveys conducted in humans, birds, and other animals suggested more widespread viral exposure (Hubalek and Halouzka 1999; Linke et al. 2007). Human or equine illness was sporadic, with isolated human cases of WN fever identified in France, Spain, Romania, Belarus, and Czechland (Hubalek and Halouzka 1999). An outbreak in the Camargue region of southern France from 1962 to 1965 resulted in 15 virologically confirmed human cases with 1 death and approximately 80 equine cases with 25–30% mortality (Murgue et al. 2001a).

The first large human outbreak in Europe occurred in Romania with 352 cases of neuroinvasive disease in 1996 (Tsai et al. 1998). An equine outbreak with 14 cases occurred in Italy in 1998 and another with 76 cases in the Camargue region in 2000 (Autorino et al. 2002; Murgue et al. 2001b). Four humans with WNV-related illness were noted in the Camargue region in 2003, which represented the first human WNV-related illnesses documented there since 1965 (Del Giudice et al. 2004).

WNV outbreaks and sporadic cases subsequently have increased in frequency and geographic distribution in Europe. Human cases and dead birds have been associated with both lineage 1 and 2 WNV strains (Barzon et al. 2012a, b, c; Magurano et al. 2012; Savini et al. 2012) and strains from both virus lineages have been identified in *Culex pipiens* mosquitoes (Savini et al. 2012), indicating co-circulation of both viruses. Lineage 2, first detected in Hungary in 2004, subsequently spread across central Europe and the eastern Mediterranean region, causing major outbreaks in Greece in 2010 and the largest outbreak recorded in Europe in 2018. The 2010 Greek outbreak resulted in 262 laboratory-confirmed cases and 35 deaths while the 2018 outbreak involved 1993 cases and 207 deaths mainly in Greece, Hungary, Italy, and Romania (Fig.4) (Young et al. 2021). The totals from 2018 exceeded those of the previous 7 years combined. It is hypothesized that the 2018 outbreak was precipitated by environmental factors including an exceptionally hot summer. In recent years, WNV lineage 2 has extended its northern bounds (Bakonyi and Haussig 2020). The virus was first detected in birds and horses in Germany for the first time in 2018 (Ziegler et al. 2019), which preceded the first human cases in that country in 2019 (Ziegler et al. 2020). In 2020, WNV was reported in birds, mosquitoes, and humans for the first time in the Netherlands (Vlaskamp et al. 2020). In addition, WNV lineage 1 has spread westward, causing an outbreak of 71 humans in Andalusia, Spain in 2020 (Casimiro-Soriguer et al. 2021).

Fig. 4 WNV disease cases in the European Union, 2018. Source: European Centers for Disease Control

46.5.3 Americas

WNV spread rapidly throughout the Americas following its discovery in the New York City area in 1999 (Nash et al. 2001). It spread across the United States and reached the Pacific Coast by 2003 (Lindsey et al. 2010). The virus was first detected in Canada in 2001. By 2001 WNV had spread south to islands in the Caribbean Sea (Komar and Clark 2006); by 2003 to El Salvador, Guatemala, and Belize; by 2004 to Colombia and Venezuela (Bosch et al. 2007; Mattar et al. 2005); and by 2005 to Argentina (Adrian Diaz et al. 2008). Despite the virus' apparent extensive distribution in Latin America and the Caribbean, only isolated instances of human illness have been reported (Gubler 2007; Hunsperger et al. 2009; Komar and Clark 2006; Pupo et al. 2006). The explanation for this discrepancy is unknown; however, it is noteworthy that this same pattern is observed in tropical Africa. Perhaps the continuous avian host availability for ornithophilic mosquitoes in tropical areas might decrease the likelihood that infected mosquitoes feed on humans.

The virus is now endemic throughout much of the United States and southern Canada (Lindsey et al. 2010; Petersen et al. 2013a). Through 2019, 51,801 persons have been reported with WNV disease in the United States, including 25,290 patients with neuroinvasive disease and 1549 deaths; 6423 patients with WNV disease were reported in Canada. In the United States, sporadic human cases occur each year throughout the country along with focal or regional outbreaks of varying

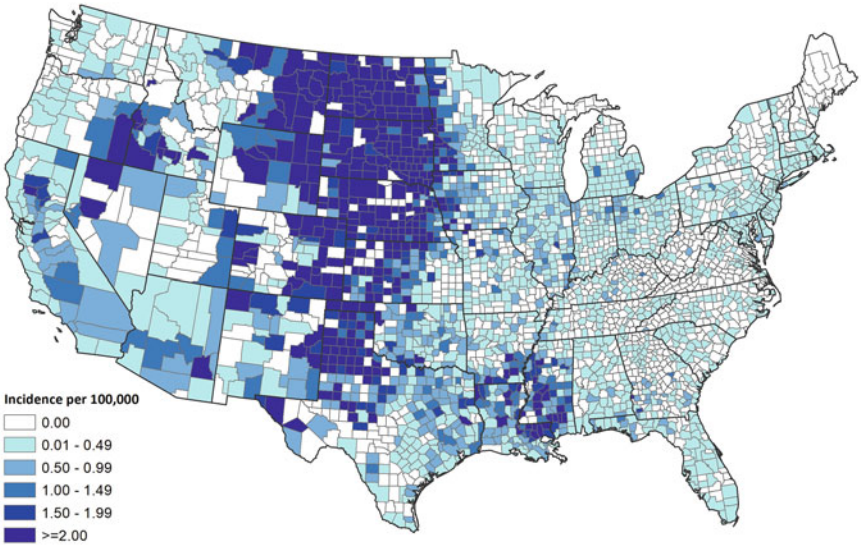

Fig. 5 Average annual incidence of WNV neuroinvasive disease, by county, United States, 1999–2019. Source: US Centers for Disease Control and Prevention

sizes. Large regional outbreaks, each involving nearly 3000 patients with neuroinvasive disease, occurred in 2002, 2003, and 2012. Despite the sporadic nature of outbreaks, certain areas appear to be at higher overall risk than others (Fig. 5). Nearly 90% of cases have onsets from July through September (Fig. 3) (McDonald et al. 2021).

46.5.4 Israel, Asia, Russia

The first reported WNV human epidemic in Israel occurred in 1951–1952 (Bernkopf et al. 1953), and was followed by a series of human outbreaks throughout the 1950s and in 1980. Illnesses were generally mild, although neuroinvasive disease was prominent during an outbreak among the elderly in 1957 (Murgue et al. 2002). Little subsequent WNV activity occurred in Israel until 1997 and 1998 when WNV was identified from dying migrating storks from Europe as well as other bird species, including domestic geese (Malkinson and Banet 2002; Malkinson et al. 2002). Two human fatalities occurred in 1999, followed by an outbreak with 417 cases and 35 deaths the following year (Chowers et al. 2001; Weinberger et al. 2001). Since then, outbreaks have occurred every few years, including a lineage 1 outbreak involving at least 139 cases in 2018 (Lustig et al. 2019).

Since 1963, WNV has been isolated from ticks, birds, and mosquitoes in the southern area of European Russia and western Siberia, and in adjacent republics of the former Soviet Union. Serological surveys of healthy humans indicated up to 8%

anti-WNV IgG antibody seroprevalence (Platonov 2001). Yet outbreaks were uncommon until 1999 when a large outbreak of severe neurological disease involved 318 cases and 40 deaths in Volgograd (Platonov et al. 2001). This outbreak occurred during an unusually hot and dry summer. The lineage 1 virus that caused the 1999 outbreak was genetically related to that which caused the 1996 Romanian outbreak (Lanciotti et al. 2002). Sporadic cases and outbreaks have subsequently occurred from both lineage 1 and 2 viruses, mainly in areas near the Volga River (Fig. 4). Nevertheless, the distribution of the virus may be extensive in Russia, as suggested by a 2004 report of WNV in patients in Novosibirsk in southwestern Siberia and by a serosurvey showing a 15% seroprevalence of WNV neutralizing antibodies in birds in far-Eastern Siberia (Murata et al. 2011).

Although WNV illness is infrequent elsewhere in the Middle East and South Asia, Turkey began experiencing large numbers of human cases throughout the country in 2010 and 2011, concurrent with many cases occurring in Greece, Russia, and Israel (Kalaycioglu et al. 2012). Serological surveys in humans and horses conducted in Iran since 1967 indicate circulation of the virus, although human illness has been uncommonly reported (Chinikar et al. 2012, 2013; Kalantari et al. 2019; Naficy and Saidi 1970). Serological evidence of WNV antibodies in humans in India was first identified in 1952 and the virus was identified since then in mosquitoes and in ill humans in several areas (George et al. 1984; Paul et al. 1970). A study of samples collected throughout India from 1992 to 2001 identified 88 ill persons with WNV-specific antibodies, suggesting that WNV-related illness incidence may be higher than that currently recognized (Thakare et al. 2002). Several recent outbreaks occurring concurrently with other mosquito-borne illness have been noted in India (Khan et al. 2011; Shukla et al. 2012). A WNV outbreak occurred in Xinjiang, China in 2004 (Li et al. 2013).

46.5.5 Australia

Kunjin virus, a lineage I WNV variant (1b) (May et al. 2011), is endemic throughout most of tropical Australia and eastern Queensland. Phylogenetic analysis suggests a single successful introduction of a virus originating from Africa sometime within the last 300 years (May et al. 2011). Documented illness is rare, and cases occur in infrequent small outbreaks or sporadically. Illness is typically mild, non-encephalogenic, and non-life threatening (Knope et al. 2013).

46.6 Clinical Aspects

46.6.1 Humans

Approximately 25% of humans infected with WNV become ill (Zou et al. 2010). The incubation period for clinical illness generally ranges from 2 to 14 days, but immunocompromised patients may experience prolonged incubation periods up to

21 days (Pealer et al. 2003; Rhee et al. 2011). Most of those who become ill develop West Nile fever, characterized by the sudden onset of symptoms such as headache, malaise, fever, myalgia, chills, vomiting, rash, and eye pain (Pacenti et al. 2020; Zou et al. 2010). Fever may be low-grade or absent (Landry et al. 2019). A rash, which often appears around the time of defervescence, tends to be morbilliform, maculopapular, and non-pruritic and predominates over the torso and extremities, sparing the palms and soles (Anderson et al. 2004; Del Giudice et al. 2005; Ferguson et al. 2005; Gorsche and Tilley 2005). Persistent fatigue, headaches, and difficulties concentrating may continue for weeks or months and can be quite debilitating (Patel et al. 2015).

Risk factors for developing West Nile fever are not well defined. A follow-up study of asymptomatic, viremic blood donors indicated that increasing viral load and female gender, but not age, subsequently increased the risk of developing West Nile fever (Zou et al. 2010). A smaller follow-up study of viremic blood donors suggested that younger persons were more likely to develop West Nile fever (Brown et al. 2007).

Approximately one in 150 to 250 persons infected with WNV develop neuroinvasive disease (Mostashari et al. 2001; Petersen et al. 2012), which is manifested by meningitis, encephalitis, or acute flaccid paralysis. Advancing age profoundly increases the risk of neuroinvasive disease, particularly encephalitis (Carson et al. 2012; Lindsey et al. 2010). The risk may approach 1 in 50 among persons aged at least 65 years, a rate 16 times higher than that for persons aged 16–24 years (Carson et al. 2012). In addition, a history of cancer, diabetes, hypertension, alcohol abuse, renal disease, immunosuppressive medications, and *CCR5* deficiency as well as male gender may increase the risk of neuroinvasive disease (Bode et al. 2006; Carson et al. 2012; Cho and Diamond 2012; Lindsey et al. 2010, 2012b; Murray et al. 2006). Persons infected through transplantation of infected organs are at extreme risk of developing neuroinvasive disease (Nett et al. 2012); however, conflicting data exist regarding risk among previous organ recipients infected via a mosquito bite (Freifeld et al. 2010; Kumar et al. 2004).

West Nile meningitis is clinically like that of other viral meningitides with abrupt onset of fever and headache along with meningeal signs and photophobia. Headache may be severe, requiring hospitalization for pain control; associated gastrointestinal disturbance may result in dehydration (Sejvar et al. 2008). West Nile encephalitis ranges in severity from a mild, self-limited confusional state to severe encephalopathy, coma, and death. Extrapyramidal disorders are frequently observed (Burton et al. 2004; Pepperell et al. 2003; Sayao et al. 2004; Sejvar et al. 2003a). Increased intracranial pressure, cerebral edema, and seizures are infrequent (Doron et al. 2003). Patients with West Nile encephalitis frequently develop a coarse tremor, particularly in the upper extremities. The tremor tends to be postural, and may have a kinetic component (Burton et al. 2004; Emig and Apple 2004; Sayao et al. 2004; Sejvar et al. 2003a). Myoclonus, predominantly of the upper extremities and facial muscles, may occur, and may be present during sleep. Features of Parkinsonism may be seen (Robinson et al. 2003; Sejvar et al. 2003a) and cerebellar ataxia has been described (Burton et al. 2004; Kanagarajan et al. 2003; Sayao et al. 2004).

WNV-associated paralysis most commonly results from destruction of the anterior horn cells of the spinal cord (Glass et al. 2002; Jeha et al. 2003; Leis et al. 2002; Li et al. 2003; Sejvar et al. 2003b, 2005). Asymmetric weakness usually develops rapidly within the first 48 h after symptom onset, although patients with extensive spinal cord involvement develop a more symmetric dense quadriplegia. Central facial weakness, frequently bilateral, may occur (Jeha et al. 2003). Respiratory failure requiring emergent endotracheal intubation may result from diaphragmatic and intercostal muscle paralysis (Fan et al. 2004; Sejvar et al. 2005). Sensory loss or numbness is generally absent though some patients experience intense pain in the affected limbs just before or during the onset of weakness (Sejvar et al. 2005). Other causes of weakness associated with WNV infection include Guillian-Barré syndrome and other demyelinating neuropathies, transverse myelitis, motor axonopathy, axonal polyneuropathy, involvement of ventral spinal roots, myasthenia gravis, and brachial plexopathies (Leis and Stokic 2012).

Other manifestations described with WNV infection include myocarditis, pancreatitis, fulminant hepatitis, rhabdomyolysis, stiff-person syndrome, and autonomic instability (Petersen and Hayes 2008). Chorioretinitis is the most common ocular manifestation, occurring in approximately 80% of persons with neuroinvasive disease (Rousseau et al. 2020). Other reported ocular manifestations include anterior uveitis, occlusive retinal vasculitis, and optic neuritis (Rousseau et al. 2020).

Full recovery is the norm for patients with uncomplicated West Nile fever or meningitis; however, initial symptoms, particularly extreme fatigue, may be prolonged (Patel et al. 2015; Watson et al. 2004). West Nile fever may precipitate death among a few persons of advanced age or with underlying medical conditions (Sejvar et al. 2011). Outcomes of West Nile encephalitis are variable and may not correlate with severity of initial illness. Patients hospitalized with WNV encephalitis frequently require assistance with daily activities following acute care discharge (Emig and Apple 2004; Pepperell et al. 2003) and often report substantial functional and cognitive difficulties for up to a year following acute infection (Patel et al. 2015).

While some studies have documented neurocognitive deficits on standardized testing as long as 1 year after acute illness (Haaland et al. 2006; Sadek et al. 2010), others have failed to confirm this finding (Sejvar et al. 2008). Nevertheless, self-reported fatigue, somatic, and cognitive complaints lasting months or years are common among persons recovering from WNV illness (Anastasiadou et al. 2013; Carson et al. 2006; Klee et al. 2004; Patel et al. 2015). Neuropsychiatric symptoms, including depression, anxiety, agitation, and apathy, have been reported (Berg et al. 2010; Loeb et al. 2008; Murray et al. 2007; Patel et al. 2015; Sejvar et al. 2003a). One investigator found WNV RNA in urine in patients up to 7 years following acute illness and implied an association with chronic renal failure (Murray et al. 2010); however, three other studies failed to substantiate persistent WNV RNA in urine (Barzon et al. 2013a; Baty et al. 2012; Gibney et al. 2011). Among patients with acute flaccid paralysis due to poliomyelitis-like syndrome, one-third recover

regarding their strength to near baseline, one-third have some improvement, and one-third have little or no improvement (Sejvar et al. 2006).

Case fatality rates among patients with neuroinvasive disease generally are around 10% (Lindsey et al. 2010), with persons developing encephalitis or acute flaccid paralysis having a higher case fatality rate (14% and 13%, respectively) than persons with meningitis (2%) (McDonald et al. 2021). Advanced age is the most important risk factor for death, ranging from 0.8% among those aged less than 40 years to 17% aged at least 70 years (Lindsey et al. 2010). Encephalitis with severe muscle weakness, change in the level of consciousness, diabetes, cardiovascular disease, hepatitis C virus infection, and immunosuppression are possible risk factors for death (Lindsey et al. 2010; Murray et al. 2006; Nash et al. 2001; Popescu et al. 2020). Long-term, all-cause mortality is two to three times higher among survivors of acute WNV illness (Green et al. 2005; Lindsey et al. 2012a).

46.6.2 Birds

Serologic studies demonstrate that numerous bird species are exposed to WNV during outbreaks, with antibody prevalence as high as 70% noted in some species (Gibbs et al. 2006; Komar et al. 2001a, b; Ringia et al. 2004; Savage et al. 1999; Valiakos et al. 2012). In the United States, avian mortality has been noted in more than 300 species (http://www.cdc.gov/westnile/faq/deadBirds.html), with corvids particularly impacted (Foppa et al. 2011; LaDeau et al. 2007; Nemeth et al. 2007). Consistent with these findings, mortality following experimental infection of eight species of North American birds ranged from 33% to 100% (Komar et al. 2003). However, surveillance in recent years in the United States has recorded fewer WNV-related dead bird reports. Whether this reflects surveillance fatigue, decreases in susceptible populations, or decreased avian mortality following infection is not known. One analysis suggested increasing survival in American crows (*Corvus brachyrhynchos*) following WNV infection (Reed et al. 2009). Fortunately, available data indicates WNV has had low to moderate impact on most avian populations long term (Kilpatrick and Wheeler 2019).

One particular area of concern has been the impact of WNV on raptors (Gancz et al. 2006; Harris and Sleeman 2007; Saito et al. 2007). Experimental infection via the oral route of several raptor species, including great horned owls (*Bubo virginianus*) and American kestrels (*Falco sparverius*), has been recorded (Nemeth et al. 2006), suggesting that oral ingestion in addition to mosquito exposure may be important routes of infection in at least some raptor species.

While considerably less avian mortality has been noted in Europe compared to the United States, avian mortality has been occasionally reported in several species, particularly the Northern goshawk (*Accipiter gentilis*) (Bakonyi et al. 2013; Feyer et al. 2021; Glavits et al. 2005; Hubalek et al. 2019; Malkinson et al. 2002; Ziegler et al. 2019). The reason for this difference is unknown but may be related to the long-standing exposure of European birds to WNV strains imported from Africa. Nevertheless, experimental infection of several European species, including red-legged

partridges (Sotelo et al. 2011a), falcons (Ziegler et al. 2013), and Carrion crows (*Corvus corone*) (Dridi et al. 2013), with WNV strains circulating in Europe in recent years result in considerable mortality.

46.6.3 Equines

Serologic studies demonstrate that a high proportion of horses living in endemic areas may be exposed to WNV (Epp et al. 2007; Gardner et al. 2007; Metz et al. 2021; Olufemi et al. 2021; Schmidt and Elmansoury 1963). However, studies of experimentally infected horses confirm that most infections are clinically unapparent and produce a transient low-level viremia of approximately 1 week in duration (Bunning et al. 2002; Schmidt and Elmansoury 1963). These low-level viremias are inadequate to infect mosquitoes, confirming that horses are incidental hosts (Bunning et al. 2002). Reported symptoms and signs include fever, anorexia, incoordination, weakness or ataxia, muscle rigidity, fasciculations, tremors, and cranial nerve dysfunction, depression, and recumbency (Bertram et al. 2020; Cantile et al. 2000; Porter et al. 2003; Ward et al. 2004). The mean duration of illness is approximately 22 days (Salazar et al. 2004) and reported survival rates are in the range of 20–30% (Porter et al. 2003; Salazar et al. 2004; Ward et al. 2004). Animals that become recumbent and unable to rise are nearly 80 times more likely to die than those able to rise (Salazar et al. 2004). Residual symptoms are common among surviving horses.

46.6.4 Other Animals

Numerous other animals may become infected with WNV, mostly resulting in minimal or no symptoms. Species without reported clinical disease but with WNV antibodies detected during serologic surveys include bats, deer, raccoons, opossums, and small rodents. Experimental infection of several species as diverse as fox squirrels (*Sciurus niger*) (Root et al. 2006), American alligators (*Alligator mississippiensis*) (Klenk et al. 2004), dogs (Blackburn et al. 1989), cats (Austgen et al. 2004), Eastern chipmunks (*Tamias striatus*) (Platt et al. 2007), monkeys (*Macaca mulatta*) (Pogodina et al. 1983), and common garter snakes (*Thamnophis sirtalis*) (Steinman et al. 2006) exhibit transient viremia and some morbidity and mortality. Viremia in some species such as American alligators and Eastern chipmunks was high enough to infect mosquitoes, suggesting a possible role in WNV transmission in some settings. Isolated reports of morbidity and mortality due to WNV in numerous wild or captive animal species exist. These include squirrels, harbor seals, macaques, elk, sheep, crocodiles, and alpacas. Experimental evidence suggests that WNV infection can persist in some species. Virus could be isolated from the organs of rhesus monkeys at least 5 months after inoculation, and interestingly, virus isolates obtained at least 2 months after inoculation were non-pathogenic when inoculated into white mice (Pogodina

et al. 1983). WNV could be cultured from urine in hamsters up to 242 days following inoculation (Tesh et al. 2005).

46.7 Pathogenesis

Mosquito salivary components introduced at the site of infection modulate the initial immune response by target cells including keratinocytes (Lim et al. 2011) and dendritic cells through several mechanisms including focalized suppression of immune effector cell trafficking to the site of inoculation (Schneider and Higgs 2008; Styer et al. 2011). Infected dendritic cells or keratinocytes migrate to the draining lymph node from which a serum viremia is generated that relays infection to visceral organs and potentially to the central nervous system (CNS). Additional mosquitoes that feed on avian amplification hosts exhibiting high-level viremias during this viremic phase become infected. Given the low-level serum viremias observed in humans and horses, they are unlikely to infect probing mosquitoes and as such are considered "dead end" hosts despite the potential for development of severe neurological disease.

West Nile virus is capable of replicating and eliciting pathology in the brain (i.e., neurovirulent); however, a critical perquisite to generate neurological disease manifestations in humans is the virus' capacity to gain access to the CNS (i.e., neuroinvasiveness). Postulated WNV neuroinvasive mechanisms based on small rodent models include: (i) direct viral crossing of the blood–brain barrier due to cytokine-mediated increased vascular permeability (Kong et al. 2008; Wang et al. 2004), (ii) a Trojan horse mechanism in which infected tissue macrophages are trafficked across the blood–brain barrier (Bai et al. 2010; Verma et al. 2009), (iii) direct infection and passage through the endothelium of the blood–brain barrier (Dropulic and Masters 1990), and (iv) retrograde axonal transport of the virus to the CNS via the infection of olfactory or peripheral neurons (Hunsperger and Roehrig 2006; Wang et al. 2009). Regardless of how WNV enters the CNS, additional studies in murine models have indicated that viral replication can persist in various tissues, including the CNS, thus shedding additional light on the potential etiology for long-term neurological sequelae observed in neuroinvasive disease patients (Appler et al. 2010).

Many of the clinical features of CNS infection in both humans and animals are accounted for by the predilection of WNV to certain areas of the CNS, such as the basal ganglia, thalami, brain stem, cerebellum, and anterior horn cells (Cantile et al. 2000, 2001; Guarner et al. 2004; Kelley et al. 2003; Leis et al. 2003; Li et al. 2003). For example, asymmetrical paralysis is associated with destruction of anterior horn cells and Parkinsonian symptoms with involvement of the basal ganglia (Sejvar and Marfin 2006). Histopathologic changes include microglial nodules composed of lymphocytes and histiocytes, perivascular inflammation consisting predominantly of CD8 T-lymphocytes, and leptomeningeal mononuclear inflammatory infiltrates when meningitis is present (Cantile et al. 2000; Sejvar and Marfin 2006).

46.8 Diagnosis

Numerous experimental studies in animals as well as observations in humans indicate that viremia usually develops 1–2 days following infection, which then lasts approximately 1 week. Viremia is cleared about the time WNV-specific IgM antibodies can be detected, with IgG antibodies developing shortly thereafter (Busch et al. 2008). One unusual feature is that IgM antibodies persist in many humans for 1 year or longer (Busch et al. 2008; Murray et al. 2013; Roehrig et al. 2003). WNV can persist in organs and tissues of humans and animals after acute viremia has cleared. Immunocompromised patients can exhibit prolonged viremia with delayed or absent development of IgM and IgG antibodies (Pealer et al. 2003). IgG and neutralizing antibodies probably can be detected for life in immunocompetent individuals following natural infection.

46.8.1 Human Diagnosis

Detection of IgM antibodies in CSF or serum forms the cornerstone of laboratory diagnosis in most clinical settings. Because IgM antibody does not pass the blood–brain barrier, presence of IgM antibodies in CSF is indicative of CNS infection. IgM antibodies are present in at least 90% of patients with encephalitis or meningitis within 8 days of symptom onset. IgM or IgG antibodies may not be present at clinical presentation, particularly among patients with West Nile fever (Anastasiadou et al. 2011; Papa et al. 2011c). One study showed that only 58% of patients with West Nile fever had a positive MAC-ELISA result (Tilley et al. 2006). Nevertheless, IgM antibody testing of acute- and convalescent-phase sera should provide a definitive diagnosis.

Considerable cross-reactivity of serologic tests among the flaviviruses can complicate serological diagnosis. Recent vaccination with yellow fever or Japanese encephalitis vaccines or recent infection with a related flavivirus such as St. Louis encephalitis or dengue viruses can produce a false-positive IgM antibody result. The plaque reduction neutralization test can help distinguish the cross-reactions among the flaviviruses when the infecting flavivirus is the first flavivirus exposure. However, neutralization test results for WNV infected patients with previous flavivirus exposure are usually inconclusive; often the highest neutralizing antibody titer is to the first infecting flavivirus rather than to WNV ("original antigenic sin" phenomenon) (Johnson et al. 2005b). In addition, the persistence of detectable IgM antibodies (Papa et al. 2011b) – in one study in 17% of patients after 1 year (Busch et al. 2008) – means that a positive WNV IgM antibody result may be unrelated to the current illness.

Identification of WNV RNA by RT-PCR in human cerebrospinal fluid, serum, or other tissues has diagnostic utility in certain clinical settings as an adjunct to IgM antibody testing. A combined approach using nucleic acid and IgM antibody testing increased the sensitivity of testing from 58% using serology alone to 94% among patients with West Nile fever (Tilley et al. 2006). IgM antibody development may be

delayed in immunocompromised patients and in these instances RT-PCR testing may be diagnostic (Goates et al. 2017; Pacenti et al. 2020; Pealer et al. 2003). Immunohistochemistry can detect WNV in formalin-fixed tissue.

46.8.2 Diagnosis in Non-Human Vertebrates

The IgM antibody capture ELISA has been developed for use in horses and can be readily adapted to other animal species where anti-IgM antibody reagents are commercially available. Alternatively, seroconversion for IgG, neutralizing antibodies, and hemagglutinin inhibition (HAI) assays in acute- and convalescent-phase serum samples collected 2–3 weeks apart can be used as screening assays. The latter two approaches do not require species-specific reagents and thus have broad applicability. The ELISA format may be used when employed as inhibition or competition ELISAs, which avoid the use of species-specific reagents. Blocking ELISAs have been applied to a variety of vertebrate species with very high specificity and sensitivity (Blitvich et al. 2003; Sotelo et al. 2011b). Similarly, the microsphere immunoassay, when used comparatively with WNV antigen-coated beads and SLEV antigen-coated beads, performs with high specificity and sensitivity (Johnson et al. 2005a). As with human diagnostics, the PRNT is used to confirm serology in non-human vertebrates. The same caveats regarding the "original antigenic sin" phenomenon apply both to human and non-human vertebrate diagnostics. Deceased animals can be tested for viral nucleic acid or antigen (Nemeth et al. 2007).

46.9 Prevention

In the absence of an approved WNV vaccine for humans, preventing human cases of WNV disease is accomplished by preventing infected mosquitoes from biting people. Equine cases of WNV disease can be prevented through vaccination and preventing mosquitoes from biting susceptible horses. Preventing bites from infected mosquitoes is accomplished by reducing mosquito numbers using an integrated mosquito management approach and by use of personal protection measures such as application of mosquito repellents. The integrated mosquito management approach is guided by timely surveillance to monitor the level of risk to humans.

46.9.1 Surveillance

Integrated mosquito management is guided by regular monitoring of vector mosquito populations and WNV activity levels in humans and other vertebrates to determine if, when, and where interventions are needed to keep mosquito numbers below levels that produce risk of human disease. Mosquito surveillance is accomplished by either larval or adult monitoring. Larval surveillance identifies and samples aquatic habitats where vector mosquitoes can breed. Adult mosquito

surveillance identifies the abundance of adult vector mosquitoes and monitors viral infection rates in mosquitoes. Adult mosquito surveillance for WNV is mostly accomplished using CO_2-baited light traps or gravid traps. CO_2-baited light traps monitor host-seeking mosquitoes, whereas gravid traps capture females seeking a location to deposit eggs. *Culex* species are differentially attracted to each of these types of traps. Gravid traps are most likely to capture infected mosquitoes because they have already taken a blood meal. A consistent approach is required from year to year to establish levels of mosquito activity that equate to increased human risk.

Different approaches to assessing human risk from vector mosquitoes have differing sensitivities and specificities, which should be balanced by available resources, including funding, labor, and equipment (Caillouet and Robertson 2016). The vector index is considered the most useful of the available WNV surveillance indicators and is roughly defined as the number of infected vector mosquitoes multiplied by the infection rate in those mosquitoes, and might be the best measure of impending human risk in urban settings (Chung et al. 2013). The vector index requires personnel, facilities, and equipment that allow for species identification, pathogen testing, and statistical analyses (Caillouet and Robertson 2016).

WNV activity in non-human vertebrates can be measured by WNV-related avian mortality, seroconversion to WNV in sentinel chickens or other sentinel animals, seroprevalence in wild birds, and cases of WNV illness in animals, primarily horses. Unfortunately, all of these approaches have significant limitations: there is little avian mortality in Europe and Africa, avian mortality appears to be decreasing in North America, sentinel chickens are expensive to maintain, seroprevalence in wild birds is difficult to monitor consistently and might not correlate highly with future risk of transmission, and mortality in horses has been dramatically reduced because of equine vaccination (Gardner et al. 2007). It is also difficult to correlate activity in non-human vertebrates with human risk.

Monitoring human neuroinvasive disease cases provides the best measure of the overall scope of a WNV outbreak given neuroinvasive disease cases are most consistently recognized and reported because of disease severity (Weber et al. 2011; Zou et al. 2010). It is estimated approximately 30–70 nonneuroinvasive disease cases occur for every case of WNV neuroinvasive disease (Petersen et al. 2013b). The total number of human infections can be estimated by multiplying the number of neuroinvasive disease cases by the ratio of infections to neuroinvasive disease cases. Only a small fraction of West Nile fever cases is captured by surveillance, with estimates varying between 1 neuroinvasive disease case in 140 WNV infections to 1 in 256–353 infections (Busch et al. 2006; Mostashari et al. 2001). However, West Nile fever cases can be the first indication of the occurrence of human infections in an area. Infections in blood donors can be monitored in areas where blood donor screening is conducted. The main limitation of human surveillance is that several weeks can occur between the date of infection and when illness is reported to health authorities (Chung et al. 2013).

46.9.2 Mosquito Control

Community-based mosquito control programs using integrated mosquito management principles include several methods to reduce mosquito populations below levels that increase human risk (Reisen and Brault 2007). These include eliminating mosquito breeding sites (source reduction) and applying larvicides to aquatic habitats where mosquitoes might breed. When adult mosquito density becomes high, adulticides can be applied through ultra-low volume (ULV) spraying applied by ground-based or aerial mounted sprayers. A modeling analysis using mosquito surveillance data collected in Sacramento and Yolo counties in California during 2006–2017 demonstrated reductions in *Cx. pipiens* and *Cx. tarsalis* at 1-week post-insecticide treatments (Holcomb et al. 2021). Although the effectiveness of this approach cannot be readily assessed because of the highly focal and sporadic nature of WNV illness, a few well-controlled studies do exist. In response to surveillance findings indicating increasing human risk, early-season control of adult mosquitoes using ULV applications of insecticides in a populated agricultural area in California decreased subsequent WNV transmission (Lothrop et al. 2008). In addition, ULV pesticide application decreased WNV infected mosquito abundance and reduced human WNV case incidence during a WNV outbreak in another populated area of California (Carney et al. 2008; Macedo et al. 2010).

Human health risks associated with ULV organophosphate or synthetic pyrethroid pesticide use appear to be negligible, largely because the timing of application and low volume of pesticide used result in minimal human exposure (Centers for Disease Control and Prevention 2005; Chung et al. 2013; Duprey et al. 2008). Further, an assessment of the correlation between ULV application of pyrethrin insecticide and emergency department visits in Sacramento, California, demonstrated that ULV pyrethrin applications were not correlated with types of diagnoses known to be associated with pyrethrin exposures (Geraghty et al. 2013).

46.9.3 Personal Protection

Personal protection measures include application of mosquito repellents and wearing permethrin-treated clothing. However, the effectiveness of these measures is difficult to assess. A Canadian study comparing people who practiced at least two personal protective strategies (wearing repellent, wearing protective clothing, or avoiding outdoor exposure to mosquitoes) with those who did not demonstrated that personal protective strategies halved the risk of WNV infection (Loeb et al. 2005). A study comparing two adjacent communities in the United States found that incidence of WNV disease was better correlated ecologically with the practice of personal protection strategies than with the level of local mosquito control efforts (Gujral et al. 2007).

Commercially available insect repellents containing DEET, IR3535, oil of lemon eucalyptus, and picaradin are effective in reducing or preventing mosquito biting (Fradin and Day 2002). Unfortunately, regular repellent use is not widespread even during well-publicized WNV outbreaks (Gibney et al. 2012; McCarthy et al. 2001).

However, a study in Maryland demonstrated that adults aged ≥60 years who expressed worry about WNV disease were over three times more likely to report insect repellent use in the previous 90 days, indicating a potential benefit for using targeted prevention messages for adults at increased risk of severe WNV disease (Mitchell et al. 2018).

46.9.4 Vaccines

The introduction of a vaccine against WNV for use in horses has substantially reduced the incidence of equine WNV disease in the United States (Bosco-Lauth and Bowen 2019; Gardner et al. 2007; Petersen and Roehrig 2007). Two licensed live, attenuated equine vaccines and two licensed inactivated vaccines are available in the United States or the European Union (De Filette et al. 2012; Long et al. 2007a, b; Seino et al. 2007; Siger et al. 2006); a DNA vaccine has also been licensed in the United States, but is not commercially available (Davis et al. 2001) (Table 1). Vaccination of horses with a WNV Lineage 1 vaccine appears to confer protection against WNV Lineage 2 infection (Bowen et al. 2014). Although the routine use of vaccines have reduced the incidence of WNV disease in horses, the available

Table 1 West Nile virus vaccines for horses approved in the United States and the European Union

Vaccine	Viral antigen	Status	Comment
West Nile Innovator® in U.S. and Equip® WNV in E.U. (Zoetis)	Formalin inactivated whole virus	Approved in US/EU	Two doses, booster annually
Vetera® WNV vaccine (Boehringer Ingelheim)	Inactivated virus	Approved in US.	Two doses, booster annually
Recombitek® West Nile Virus and Proteq® West Nile in E.U. (Merial)	prM and E proteins expressed in canarypox virus	Approved in US/EU	Two doses, booster annually
Equi-Nile® (Merck)	Inactivated chimeric virus, WNV prM/E proteins in yellow fever 17D backbone	Approved in US	Two doses, booster annually
PreveNile® (Intervet)	Live chimeric virus, WNV prM/E proteins in yellow fever 17D backbone	Approved in US; recalled in 2010 because of adverse events	
West Nile-Innovator DNA® (Fort Dodge Animal Health)	Plasmid DNA coding for prM and E proteins	Approved in US; currently not commercially available	

vaccines do have limitations, including the need for 2-dose initial series and annual or bi-annual boosters (Bosco-Lauth and Bowen 2019; Saiz 2020).

Several human WNV vaccine constructs employing various strategies have been developed; six have reached human clinical trials, but none have advanced past phase 2 (De Filette et al. 2012; Ulbert 2019). Chimeric vaccines inserting WNV PrM and E protein genes into attenuated yellow fever and dengue 4 virus backbones have undergone successful phase 2 and 1 clinical trials, respectively (Biedenbender et al. 2011; Dayan et al. 2012; Durbin et al. 2013). A successful phase 1 WNV DNA vaccine trial has been completed (Martin et al. 2007). An inactivated whole virus WNV vaccine candidate underwent a phase-1/2 clinical trial and demonstrated an immune response without related serious adverse events (Barrett et al. 2017). Phase-3 efficacy trials have not been attempted because of the unknown market potential of a WNV vaccine and logistical difficulties in conducting phase-3 clinical trials for this sporadic and widely dispersed disease (Barrett et al. 2017; Beasley 2011; Martina et al. 2010).

Initial simulations concluded that a universal WNV vaccine would not result in societal cost savings unless WNV incidence increased substantially or the estimated cost of the vaccination was less than $12 USD per person vaccinated (Zohrabian et al. 2006). A subsequent analysis demonstrated a WNV vaccine strategy targeting older adults would be more cost-effective than a universal program (Shankar et al. 2017). A more recent analysis highlighted that an age-based and incidence-based WNV strategy was more cost-effective than an age-based strategy alone (Curren et al. 2021).

46.10 Treatment

Treatment is supportive. Multiple drug discovery studies have evaluated anti-flaviviral drug candidates, but these studies have not advanced past the preclinical stage with most only tested in vitro (Sinigaglia et al. 2020). Several investigated therapeutic approaches in humans include immune γ-globulin, WNV-specific neutralizing monoclonal antibodies, corticosteroids, ribavirin, interferon α-2b, and antisense oligomers (Beasley 2011; Chowers et al. 2001; Diamond 2009; Gnann Jr et al. 2019). No study has documented efficacy, largely because of difficulty in recruiting enough patients. Case reports suggesting efficacy should be interpreted with extreme caution because of WNV's highly variable clinical course. No clinical studies have been conducted in horses.

46.11 Future Directions

The pattern of sporadic cases and outbreaks of WNV that has emerged in Europe and North America shows no signs of abating. While broad areas of high risk can be identified, the sporadic, local and regional outbreaks that occur within these areas remain elusively unpredictable. In populated areas within high-risk zones,

establishing effective surveillance and response capacity, and assessing the effectiveness of these activities are priorities. Further evaluation of target populations and cost-efficacy of a human WNV vaccine will help determine the need for continued human vaccine development. Practical regulatory pathways and paradigms for testing and approval of WNV vaccines and therapeutics adapted to the sporadic outbreak nature of WNV are required.

46.12 Conclusion

Within the last three decades, the West Nile virus has become a formidable public health problem in North America and Europe. Many mysteries remain. For example, although the virus has been detected in East Asia and Latin America and the Caribbean, significant clinically apparent human infection remains uncommon in those locations. Prediction and control of outbreaks remain problematic; thus, outbreaks will continue largely unabated for the foreseeable future.

References

Adrian Diaz L, Komar N, Visintin A, Dantur Juri MJ, Stein M, Lobo Allende R et al (2008) West Nile virus in birds, Argentina. Emerg Infect Dis 14(4):689–691. https://doi.org/10.3201/eid1404.071257

Alpert SG, Fergerson J, Noel LP (2003) Intrauterine West Nile virus: ocular and systemic findings. Am J Ophthalmol 136(4):733–735

Anastasiadou A, Economopoulou A, Kakoulidis I, Zilidou R, Butel D, Zorpidou D et al (2011) Non-neuroinvasive West Nile virus infections during the outbreak in Greece. Clin Microbiol Infect 17(11):1681–1683. https://doi.org/10.1111/j.1469-0691.2011.03642.x

Anastasiadou A, Kakoulidis I, Butel D, Kehagia E, Papa A (2013) Follow-up study of Greek patients with West Nile virus neuroinvasive disease. Int J Infect Dis 17(7):e494–e497. https://doi.org/10.1016/j.ijid.2012.12.006

Anderson JF, Main AJ (2006) Importance of vertical and horizontal transmission of West Nile virus by Culex pipiens in the northeastern United States. J Infect Dis 194(11):1577–1579. https://doi.org/10.1086/508754

Anderson RC, Horn KB, Hoang MP, Gottlieb E, Bennin B (2004) Punctate exanthem of West Nile virus infection: report of 3 cases. J Am Acad Dermatol 51(5):820–823

Andreadis TG (2012) The contribution of *Culex pipiens* complex mosquitoes to transmission and persistence of West Nile virus in North America. J Am Mosq Control Assoc 28(4 Suppl):137–151

Andreadis TG, Armstrong PM (2007) A two-year evaluation of elevated canopy trapping for Culex mosquitoes and West Nile virus in an operational surveillance program in the northeastern United States. J Am Mosq Control Assoc 23(2):137–148

Andreadis TG, Anderson JF, Vossbrinck CR, Main AJ (2004) Epidemiology of West Nile virus in Connecticut: a five-year analysis of mosquito data 1999–2003. Vector Borne Zoonotic Dis 4(4):360–378

Appler KK, Brown AN, Stewart BS, Behr MJ, Demarest VL, Wong SJ et al (2010) Persistence of West Nile virus in the central nervous system and periphery of mice. PLoS One 5(5):e10649. https://doi.org/10.1371/journal.pone.0010649

Austgen LE, Bowen RA, Bunning ML, Davis BS, Mitchell CJ, Chang GJ (2004) Experimental infection of cats and dogs with West Nile virus. Emerg Infect Dis 10(1):82–86

Autorino GL, Battisti A, Deubel V, Ferrari G, Forletta R, Giovannini A et al (2002) West Nile virus epidemic in horses, Tuscany region, Italy. Emerg Infect Dis 8(12):1372–1378
Bai F, Kong KF, Dai J, Qian F, Zhang L, Brown CR et al (2010) A paradoxical role for neutrophils in the pathogenesis of West Nile virus. J Infect Dis 202(12):1804–1812. https://doi.org/10.1086/657416
Bakonyi T, Haussig JM (2020) West Nile virus keeps on moving up in Europe. Euro Surveill 25(46). https://doi.org/10.2807/1560-7917.ES.2020.25.46.2001938
Bakonyi T, Ivanics E, Erdelyi K, Ursu K, Ferenczi E, Weissenbock H et al (2006) Lineage 1 and 2 strains of encephalitic West Nile virus, Central Europe. Emerg Infect Dis 12(4):618–623. https://doi.org/10.3201/eid1204.051379
Bakonyi T, Ferenczi E, Erdelyi K, Kutasi O, Csorgo T, Seidel B et al (2013) Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. Vet Microbiol 165(1–2): 61–70. https://doi.org/10.1016/j.vetmic.2013.03.005
Banet-Noach C, Simanov L, Malkinson M (2003) Direct (non-vector) transmission of West Nile virus in geese. Avian Pathol 32(5):489–494
Barrett PN, Terpening SJ, Snow D, Cobb RR, Kistner O (2017) Vero cell technology for rapid development of inactivated whole virus vaccines for emerging viral diseases. Expert Rev Vaccines 16(9):883–894. https://doi.org/10.1080/14760584.2017.1357471
Barzon L, Pacenti M, Franchin E, Lavezzo E, Martello T, Squarzon L et al (2012a) New endemic West Nile virus lineage 1a in northern Italy, July 2012. Euro Surveill 17(31)
Barzon L, Pacenti M, Franchin E, Martello T, Lavezzo E, Squarzon L et al (2012b) Clinical and virological findings in the ongoing outbreak of West Nile virus Livenza strain in northern Italy, July to September 2012. Euro Surveill 17(36):20260
Barzon L, Pacenti M, Franchin E, Squarzon L, Lavezzo E, Toppo S et al (2012c) Novel West Nile virus lineage 1a full genome sequences from human cases of infection in North-Eastern Italy, 2011. Clin Microbiol Infect 18(12):E541–E544. https://doi.org/10.1111/1469-0691.12001
Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M et al (2013a) Excretion of West Nile virus in urine during acute infection. J Infect Dis 208(7):1086–1092. https://doi.org/10.1093/infdis/jit290
Barzon L, Papa A, Pacenti M, Franchin E, Lavezzo E, Squarzon L et al (2013b) Genome sequencing of West Nile virus from human cases in Greece, 2012. Viruses 5(9):2311–2319. https://doi.org/10.3390/v5092311
Baty SA, Gibney KB, Staples JE, Patterson AB, Levy C, Lehman J et al (2012) Evaluation for West Nile virus (WNV) RNA in urine of patients within 5 months of WNV infection. J Infect Dis 205(9):1476–1477. https://doi.org/10.1093/infdis/jis221
Beasley DW (2011) Vaccines and immunotherapeutics for the prevention and treatment of infections with West Nile virus. Immunotherapy 3(2):269–285. https://doi.org/10.2217/imt.10.93
Benson TJ, Ward MP, Lampman RL, Raim A, Weatherhead PJ (2012) Implications of spatial patterns of roosting and movements of American robins for West Nile virus transmission. Vector Borne Zoonotic Dis 12(10):877–885. https://doi.org/10.1089/vbz.2011.0902
Berg PJ, Smallfield S, Svien L (2010) An investigation of depression and fatigue post West Nile virus infection. S D Med 63(4):127–129. 31-3
Bernkopf H, Levine S, Nerson R (1953) Isolation of West Nile virus in Israel. J Infect Dis 93(3):207–218
Bertram FM, Thompson PN, Venter M (2020) Epidemiology and clinical presentation of West Nile virus infection in rorses in South Africa, 2016–2017. Pathogens 10(1). https://doi.org/10.3390/pathogens10010020
Biedenbender R, Bevilacqua J, Gregg AM, Watson M, Dayan G (2011) Phase II, randomized, double-blind, placebo-controlled, multicenter study to investigate the immunogenicity and safety of a West Nile virus vaccine in healthy adults. J Infect Dis 203(1):75–84. https://doi.org/10.1093/infdis/jiq003
Biggerstaff BJ, Petersen LR (2003) Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 43(8):1007–1017
Blackburn NK, Reyers F, Berry WL, Shepherd AJ (1989) Susceptibility of dogs to West Nile virus: a survey and pathogenicity trial. J Comp Pathol 100(1):59–66

Blitvich BJ, Bowen RA, Marlenee NL, Hall RA, Bunning ML, Beaty BJ (2003) Epitope-blocking enzyme-linked immunosorbent assays for detection of West Nile virus antibodies in domestic mammals. J Clin Microbiol 41(6):2676–2679

Bode AV, Sejvar JJ, Pape WJ, Campbell GL, Marfin AA (2006) West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. Clin Infect Dis 42(9):1234–1240

Bondre VP, Jadi RS, Mishra AC, Yergolkar PN, Arankalle VA (2007) West Nile virus isolates from India: evidence for a distinct genetic lineage. J Gen Virol 88(Pt 3):875–884. https://doi.org/10.1099/vir.0.82403-0

Bosch I, Herrera F, Navarro JC, Lentino M, Dupuis A, Maffei J et al (2007) West Nile virus, Venezuela. Emerg Infect Dis 13(4):651–653. https://doi.org/10.3201/eid1304.061383

Bosco-Lauth AM, Bowen RA (2019) West Nile virus: veterinary health and vaccine development. J Med Entomol 56(6):1463–1466. https://doi.org/10.1093/jme/tjz125

Bowden SE, Magori K, Drake JM (2011) Regional differences in the association between land cover and West Nile virus disease incidence in humans in the United States. Am J Trop Med Hyg 84(2):234–238. https://doi.org/10.4269/ajtmh.2011.10-0134

Bowen RA, Bosco-Lauth A, Syvrud K, Thomas A, Meinert TR, Ludlow DR et al (2014) Protection of horses from West Nile virus lineage 2 challenge following immunization with a whole, inactivated WNV lineage 1 vaccine. Vaccine 32(42):5455–5459. https://doi.org/10.1016/j.vaccine.2014.07.093

Brault AC, Huang CY, Langevin SA, Kinney RM, Bowen RA, Ramey WN et al (2007) A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat Genet 39(9):1162–1166

Brown JA, Factor DL, Tkachenko N, Templeton SM, Crall ND, Pape WJ et al (2007) West Nile viremic blood donors and risk factors for subsequent West Nile fever. Vector Borne Zoonotic Dis 7(4):479–488. https://doi.org/10.1089/vbz.2006.0611

Bunning ML, Bowen RA, Cropp CB, Sullivan KG, Davis BS, Komar N et al (2002) Experimental infection of horses with West Nile virus. Emerg Infect Dis 8(4):380–386

Burton JM, Kern RZ, Halliday W, Mikulis D, Brunton J, Fearon M et al (2004) Neurological manifestations of West Nile virus infection. Can J Neurol Sci 31(2):185–193

Busch MP, Wright DJ, Custer B, Tobler LH, Stramer SL, Kleinman SH et al (2006) West Nile virus infections projected from blood donor screening data, United States, 2003. Emerg Infect Dis 12(3):395–402

Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I et al (2008) Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis 198(7):984–993. https://doi.org/10.1086/591467

Byas AD, Gallichotte EN, Hartwig AE, Porter SM, Gordy PW, Felix TA et al (2022) American alligators are capable of West Nile virus amplification, mosquito infection and transmission. Virology 568:49–55. https://doi.org/10.1016/j.virol.2022.01.009

Caillouet KA, Robertson S (2016) In: Colpitts TM (ed) West Nile virus – methods and protocols. Springer Science, New York, pp 221–234

Calisher CH (1988) Antigenic classification and taxonomy of flaviviruses (family Flaviviridae) emphasizing a universal system for the taxonomy of viruses causing tick-borne encephalitis. Acta Virol 32(5):469–478

Cantile C, Di Guardo G, Eleni C, Arispici M (2000) Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. Equine Vet J 32(1):31–35

Cantile C, Del Piero F, Di Guardo G, Arispici M (2001) Pathologic and immunohistochemical findings in naturally occuring West Nile virus infection in horses. Vet Pathol 38(4):414–421

Carney RM, Husted S, Jean C, Glaser C, Kramer V (2008) Efficacy of aerial spraying of mosquito adulticide in reducing incidence of West Nile virus, California, 2005. Emerg Infect Dis 14(5):747–754

Carson PJ, Konewko P, Wold KS, Mariani P, Goli S, Bergloff P et al (2006) Long-term clinical and neuropsychological outcomes of West Nile virus infection. Clin Infect Dis 43(6):723–730

Carson PJ, Borchardt SM, Custer B, Prince HE, Dunn-Williams J, Winkelman V et al (2012) Neuroinvasive disease and West Nile virus infection, North Dakota, USA, 1999–2008. Emerg Infect Dis 18(4):684–686. https://doi.org/10.3201/eid1804.111313

Casimiro-Soriguer CS, Perez-Florido J, Fernandez-Rueda JL, Pedrosa-Corral I, Guillot-Sulay V, Lorusso N et al (2021) Phylogenetic analysis of the 2020 West Nile virus (WNV) outbreak in Andalusia (Spain). Viruses 13(5). https://doi.org/10.3390/v13050836

Centers for Disease C, Prevention (2013) Fatal West Nile virus infection after probable transfusion-associated transmission--Colorado, 2012. MMWR Morb Mortal Wkly Rep 62(31):622–624

Centers for Disease Control and Prevention (2002a) Intrauterine West Nile virus infection--New York, 2002. MMWR Morb Mortal Wkly Rep 51(50):1135–1136

Centers for Disease Control and Prevention (2002b) Laboratory-acquired West Nile virus infections--United States, 2002. MMWR Morb Mortal Wkly Rep 51(50):1133–1135

Centers for Disease Control and Prevention (2003) West Nile virus infection among Turkey breeder farm workers--Wisconsin, 2002. MMWR Morb Mortal Wkly Rep 52(42):1017–1019

Centers for Disease Control and Prevention (2004) Possible dialysis-related west nile virus transmission--Georgia, 2003. MMWR Morb Mortal Wkly Rep 53(32):738–739

Centers for Disease Control and Prevention (2005) Human exposure to mosquito-control pesticides--Mississippi, North Carolina, and Virginia, 2002 and 2003. MMWR Morb Mortal Wkly Rep 54(21):529–532

Centers for Disease Control and Prevention (2007) West Nile virus transmission through blood transfusion--South Dakota, 2006. MMWR Morb Mortal Wkly Rep 56(4):76–79

Chaintoutis SC, Papa A, Pervanidou D, Dovas CI (2019) Evolutionary dynamics of lineage 2 West Nile virus in Europe, 2004–2018: phylogeny, selection pressure and phylogeography. Mol Phylogenet Evol 141:106617. https://doi.org/10.1016/j.ympev.2019.106617

Chambers TJ, Hahn CS, Galler R, Rice CM (1990) Flavivirus genome organization, expression, and replication. Annu Rev Microbiol 44:649–688. https://doi.org/10.1146/annurev.mi.44.100190.003245

Charrel RN, Brault AC, Gallian P, Lemasson JJ, Murgue B, Murri S et al (2003) Evolutionary relationship between Old World West Nile virus strains. Evidence for viral gene flow between Africa, the Middle East, and Europe. Virology 315(2):381–388

Chinikar S, Javadi A, Ataei B, Shakeri H, Moradi M, Mostafavi E et al (2012) Detection of West Nile virus genome and specific antibodies in Iranian encephalitis patients. Epidemiol Infect 140(8):1525–1529. https://doi.org/10.1017/S0950268811002056

Chinikar S, Shah-Hosseini N, Mostafavi E, Moradi M, Khakifirouz S, Jalali T et al (2013) Seroprevalence of West Nile virus in Iran. Vector Borne Zoonotic Dis 13(8):586–589. https://doi.org/10.1089/vbz.2012.1207

Cho H, Diamond MS (2012) Immune responses to West Nile virus infection in the central nervous system. Viruses 4(12):3812–3830. https://doi.org/10.3390/v4123812

Chowers MY, Lang R, Nassar F, Ben-David D, Giladi M, Rubinshtein E et al (2001) Clinical characteristics of the West Nile fever outbreak, Israel, 2000. Emerg Infect Dis 7(4):675–678

Chung WM, Buseman CM, Joyner SN, Hughes SM, Fomby TB, Luby JP et al (2013) The 2012 West Nile encephalitis epidemic in Dallas, Texas. JAMA 310(3):297–307. https://doi.org/10.1001/jama.2013.8267

Ciccozzi M, Peletto S, Cella E, Giovanetti M, Lai A, Gabanelli E et al (2013) Epidemiological history and phylogeography of West Nile virus lineage 2. Infect Genet Evol 17:46–50. https://doi.org/10.1016/j.meegid.2013.03.034

Cornel AJ, Jupp PG, Blackburn NK (1993) Environmental temperature on the vector competence of Culex univittatus (Diptera: Culicidae) for West Nile virus. J Med Entomol 30(2):449–456

Crowder DW, Dykstra EA, Brauner JM, Duffy A, Reed C, Martin E et al (2013) West nile virus prevalence across landscapes is mediated by local effects of agriculture on vector and host communities. PLoS One 8(1):e55006. https://doi.org/10.1371/journal.pone.0055006

Curren EJ, Shankar MB, Fischer M, Meltzer MI, Erin Staples J, Gould CV (2021) Cost-effectiveness and impact of a targeted age- and incidence-based West Nile virus vaccine strategy. Clin Infect Dis 73(9):1565–1570. https://doi.org/10.1093/cid/ciab540

Dahlin CR, Hughes DF, Meshaka WE Jr, Coleman C, Henning JD (2016) Wild snakes harbor West Nile virus. One Health 2:136–138. https://doi.org/10.1016/j.onehlt.2016.09.003
Darwish MA, Ibrahim AH (1971) Survey for antibodies to arboviruses in Egyptian sera. I. West Nile virus antihemagglutinins in human and animal sera. J Egypt Public Health Assoc 46(2):61–70
Davis BS, Chang GJ, Cropp B, Roehrig JT, Martin DA, Mitchell CJ et al (2001) West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. J Virol 75(9):4040–4047. https://doi.org/10.1128/JVI.75.9.4040-4047.2001
Davis CT, Ebel GD, Lanciotti RS, Brault AC, Guzman H, Siirin M et al (2005) Phylogenetic analysis of North American West Nile virus isolates, 2001–2004: evidence for the emergence of a dominant genotype. Virology 342(2):252–265
Dayan GH, Bevilacqua J, Coleman D, Buldo A, Risi G (2012) Phase II, dose ranging study of the safety and immunogenicity of single dose West Nile vaccine in healthy adults ≥ 50 years of age. Vaccine 30(47):6656–6664. https://doi.org/10.1016/j.vaccine.2012.08.063
De Filette M, Ulbert S, Diamond M, Sanders NN (2012) Recent progress in West Nile virus diagnosis and vaccination. Vet Res 43(1):16. https://doi.org/10.1186/1297-9716-43-16
DeGroote JP, Sugumaran R (2012) National and regional associations between human West Nile virus incidence and demographic, landscape, and land use conditions in the coterminous United States. Vector Borne Zoonotic Dis 12(8):657–665. https://doi.org/10.1089/vbz.2011.0786
Del Amo J, Llorente F, Figuerola J, Soriguer RC, Moreno AM, Cordioli P et al (2014) Experimental infection of house sparrows (Passer domesticus) with West Nile virus isolates of Euro-Mediterranean and North American origins. Vet Res 45:33. https://doi.org/10.1186/1297-9716-45-33
Del Giudice P, Schuffenecker I, Vandenbos F, Counillon E, Zeller H (2004) Human West Nile virus, France. Emerg Infect Dis 10(10):1885–1886
Del Giudice P, Schuffenecker I, Zeller H, Grelier M, Vandenbos F, Dellamonica P et al (2005) Skin manifestations of West Nile virus infection. Dermatology 211(4):348–350
Depoortere E, Kavle J, Keus K, Zeller H, Murri S, Legros D (2004) Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002. Tropical Med Int Health 9(6):730–736
Diamond MS (2009) Progress on the development of therapeutics against West Nile virus. Antivir Res 83(3):214–227. https://doi.org/10.1016/j.antiviral.2009.05.006
Diuk-Wasser MA, Molaei G, Simpson JE, Folsom-O'Keefe CM, Armstrong PM, Andreadis TG (2010) Avian communal roosts as amplification foci for West Nile virus in urban areas in northeastern United States. Am J Trop Med Hyg 82(2):337–343. https://doi.org/10.4269/ajtmh.2010.09-0506
Dohm DJ, O'Guinn ML, Turell MJ (2002) Effect of environmental temperature on the ability of Culex pipiens (Diptera: Culicidae) to transmit West Nile virus. J Med Entomol 39(1):221–225
Doron SI, Dashe JF, Adelman LS, Brown WF, Werner BG, Hadley S (2003) Histopathologically proven poliomyelitis with quadriplegia and loss of brainstem function due to West Nile virus infection. Clin Infect Dis 37(5):e74–e77
Dridi M, Vangeluwe D, Lecollinet S, van den Berg T, Lambrecht B (2013) Experimental infection of carrion crows (Corvus corone) with two European West Nile virus (WNV) strains. Vet Microbiol 165(1–2):160–166. https://doi.org/10.1016/j.vetmic.2012.12.043
Dropulic B, Masters CL (1990) Entry of neurotropic arboviruses into the central nervous system: an in vitro study using mouse brain endothelium. J Infect Dis 161(4):685–691
Duprey Z, Rivers S, Luber G, Becker A, Blackmore C, Barr D et al (2008) Community aerial mosquito control and naled exposure. J Am Mosq Control Assoc 24(1):42–46
Durbin AP, Wright PF, Cox A, Kagucia W, Elwood D, Henderson S et al (2013) The live attenuated chimeric vaccine rWN/DEN4Delta30 is well-tolerated and immunogenic in healthy flavivirus-naive adult volunteers. Vaccine 31:5772–5777. https://doi.org/10.1016/j.vaccine.2013.07.064
Ebel GD, Carricaburu J, Young D, Bernard KA, Kramer LD (2004) Genetic and phenotypic variation of West Nile virus in New York, 2000–2003. Am J Trop Med Hyg 71(4):493–500
Emig M, Apple DJ (2004) Severe West Nile virus disease in healthy adults. Clin Infect Dis 38(2):289–292

Epp T, Waldner C, Leighton FA, Berke O, Townsend HG (2007) Seroprevalence and risk factors for infection with West Nile virus in Saskatchewan horses, 2003. Can J Vet Res 71(4): 256–263

Erdelyi K, Ursu K, Ferenczi E, Szeredi L, Ratz F, Skare J et al (2007) Clinical and pathologic features of lineage 2 West Nile virus infections in birds of prey in Hungary. Vector Borne Zoonotic Dis 7(2):181–188

Ezenwa VO, Godsey MS, King RJ, Guptill SC (2006) Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. Proc Biol Sci 273(1582):109–117. https://doi.org/10.1098/rspb.2005.3284

Fall G, Di Paola N, Faye M, Dia M, Freire CCM, Loucoubar C et al (2017) Biological and phylogenetic characteristics of West African lineages of West Nile virus. PLoS Negl Trop Dis 11(11):e0006078. https://doi.org/10.1371/journal.pntd.0006078

Fan E, Needham DM, Brunton J, Kern RZ, Stewart TE (2004) West Nile virus infection in the intensive care unit: a case series and literature review. Can Respir J 11(5):354–358

Ferguson DD, Gershman K, LeBailly A, Petersen LR (2005) Characteristics of the rash associated with West Nile virus fever. Clin Infect Dis 41(8):1204–1207

Feyer S, Bartenschlager F, Bertram CA, Ziegler U, Fast C, Klopfleisch R et al (2021) Clinical, pathological and virological aspects of fatal West Nile virus infections in ten free-ranging goshawks (*Accipiter gentilis*) in Germany. Transbound Emerg Dis 68(2):907–919. https://doi.org/10.1111/tbed.13759

Fonseca K, Prince GD, Bratvold J, Fox JD, Pybus M, Preksaitis JK et al (2005) West Nile virus infection and conjunctival exposure. Emerg Infect Dis 11(10):1648–1649

Foppa IM, Beard RH, Mendenhall IH (2011) The impact of West Nile virus on the abundance of selected North American birds. BMC Vet Res 7:43. https://doi.org/10.1186/1746-6148-7-43

Fradin MS, Day JF (2002) Comparative efficacy of insect repellents against mosquito bites. N Engl J Med 347(1):13–18. https://doi.org/10.1056/NEJMoa011699

Freifeld AG, Meza J, Schweitzer B, Shafer L, Kalil AC, Sambol AR (2010) Seroprevalence of West Nile virus infection in solid organ transplant recipients. Transpl Infect Dis 12(2):120–126. https://doi.org/10.1111/j.1399-3062.2009.00464.x

Gancz AY, Smith DA, Barker IK, Lindsay R, Hunter B (2006) Pathology and tissue distribution of West Nile virus in North American owls (family: Strigidae). Avian Pathol 35(1):17–29. https://doi.org/10.1080/03079450500465676

Gardner IA, Wong SJ, Ferraro GL, Balasuriya UB, Hullinger PJ, Wilson WD et al (2007) Incidence and effects of West Nile virus infection in vaccinated and unvaccinated horses in California. Vet Res 38(1):109–116

George S, Gourie-Devi M, Rao JA, Prasad SR, Pavri KM (1984) Isolation of West Nile virus from the brains of children who had died of encephalitis. Bull World Health Organ 62(6):879–882

Geraghty EM, Margolis HG, Kjemtrup A, Reisen W, Franks P (2013) Correlation between aerial insecticide spraying to interrupt West Nile virus transmission and emergency department visits in Sacramento County, California. Public Health Rep 128(3):221–230. https://doi.org/10.1177/003335491312800312

Gibbs SE, Allison AB, Yabsley MJ, Mead DG, Wilcox BR, Stallknecht DE (2006) West Nile virus antibodies in avian species of Georgia, USA: 2000–2004. Vector Borne Zoonotic Dis 6(1):57–72. https://doi.org/10.1089/vbz.2006.6.57

Gibney KB, Lanciotti RS, Sejvar JJ, Nugent CT, Linnen JM, Delorey MJ et al (2011) West Nile virus RNA not detected in urine of 40 people tested 6 years after acute West Nile virus disease. J Infect Dis 203(3):344–347. https://doi.org/10.1093/infdis/jiq057

Gibney KB, Colborn J, Baty S, Bunko Patterson AM, Sylvester T, Briggs G et al (2012) Modifiable risk factors for West Nile virus infection during an outbreak--Arizona, 2010. Am J Trop Med Hyg 86(5):895–901. https://doi.org/10.4269/ajtmh.2012.11-0502

Glass JD, Samuels O, Rich MM (2002) Poliomyelitis due to West Nile virus. N Engl J Med 347(16): 1280–1281

Glavits R, Ferenczi E, Ivanics E, Bakonyi T, Mato T, Zarka P et al (2005) Co-occurrence of West Nile fever and circovirus infection in a goose flock in Hungary. Avian Pathol 34(5):408–414. https://doi.org/10.1080/03079450500268039

Gnann JW Jr, Agrawal A, Hart J, Buitrago M, Carson P, Hanfelt-Goade D et al (2019) Lack of efficacy of high-titered immunoglobulin in patients with West Nile virus central nervous system disease. Emerg Infect Dis 25(11):2064–2073. https://doi.org/10.3201/eid2511.190537

Goates C, Tsuha S, Working S, Carey J, Spivak ES (2017) Seronegative West Nile virus infection in a patient treated with rituximab for rheumatoid arthritis. Am J Med 130(6):e257–e2e8. https://doi.org/10.1016/j.amjmed.2017.01.014

Godsey MS Jr, Burkhalter K, Young G, Delorey M, Smith K, Townsend J et al (2012) Entomologic investigations during an outbreak of West Nile virus disease in Maricopa County, Arizona, 2010. Am J Trop Med Hyg 87(6):1125–1131. https://doi.org/10.4269/ajtmh.2012.11-0700

Gorsche R, Tilley P (2005) The rash of West Nile virus infection. CMAJ 172(11):1440

Green MS, Weinberger M, Ben-Ezer J, Bin H, Mendelson E, Gandacu D et al (2005) Long-term death rates, West Nile virus epidemic, Israel, 2000. Emerg Infect Dis 11(11):1754–1757. https://doi.org/10.3201/eid1111.040941

Groves JA, Shafi H, Nomura JH, Herron RM, Baez D, Dodd RY et al (2017) A probable case of West Nile virus transfusion transmission. Transfusion 57(3pt2):850–856. https://doi.org/10.1111/trf.14018

Guarner J, Shieh WJ, Hunter S, Paddock CD, Morken T, Campbell GL et al (2004) Clinicopathologic study and laboratory diagnosis of 23 cases with West Nile virus encephalomyelitis. Hum Pathol 35(8):983–990

Gubler DJ (2007) The continuing spread of West Nile virus in the Western Hemisphere. Clin Infect Dis 45(8):1039–1046

Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R (2007) Behavioral risks for West Nile virus disease, northern Colorado, 2003. Emerg Infect Dis 13(3):419–425

Haaland KYSJ, Pergam S, Echevarria LA, Davis LE, Goade D, Harnar J, Nfchissey RA, Sewel CM, Ettestad P (2006) Mental status after West Nile virus infection. Emerg Infect Dis 12(8):1260–1262

Hannon ER, Jackson KC, Biggerstaff BJ, Raman V, Komar N (2019) Bloodmeal host selection of Culex quinquefasciatus (Diptera: Culicidae) in Las Vegas, Nevada, United States. J Med Entomol 56(3):603–608. https://doi.org/10.1093/jme/tjy237

Harrigan RJ, Thomassen HA, Buermann W, Cummings RF, Kahn ME, Smith TB (2010) Economic conditions predict prevalence of West Nile virus. PLoS One 5(11):e15437. https://doi.org/10.1371/journal.pone.0015437

Harris MC, Sleeman JM (2007) Morbidity and mortality of bald eagles (*Haliaeetus leucocephalus*) and peregrine falcons (Falco peregrinus) admitted to the Wildlife Center of Virginia, 1993-2003. J Zoo Wildl Med 38(1):62–66

Hartley DM, Barker CM, Le Menach A, Niu T, Gaff HD, Reisen WK (2012) Effects of temperature on emergence and seasonality of West Nile virus in California. Am J Trop Med Hyg 86(5):884–894. https://doi.org/10.4269/ajtmh.2012.11-0342

Heinz F, Collet M, Purcell R, Gould E, Howeard C, Houghton M (2000) Family Flaviviridae. In: Van Regenmortel M (ed) Virus taxonomy: classification and nomenclature of viruses. Academic Press, San Diego, pp 859–878

Hinckley AF, O'Leary DR, Hayes EB (2007) Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics 119(3):e666–e671

Holcomb KM, Reiner RC, Barker CM (2021) Spatio-temporal impacts of aerial adulticide applications on populations of West Nile virus vector mosquitoes. Parasit Vectors 14(1):120. https://doi.org/10.1186/s13071-021-04616-6

Hubalek Z, Halouzka J (1999) West Nile fever--a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis 5(5):643–650

Hubalek Z, Tomesek M, Kosina M, Sikutova S, Strakova P, Rudolf I (2019) West Nile virus outbreak in captive and wild raptors, Czech Republic, 2018. Zoonoses Public Health 66(8):978–981. https://doi.org/10.1111/zph.12638

Hunsperger EA, Roehrig JT (2006) Temporal analyses of the neuropathogenesis of a West Nile virus infection in mice. J Neurovirol 12(2):129–139

Hunsperger EA, McElroy KL, Bessoff K, Colon C, Barrera R, Munoz-Jordan JL (2009) West Nile virus from blood donors, vertebrates, and mosquitoes, Puerto Rico, 2007. Emerg Infect Dis 15(8):1298–1300. https://doi.org/10.3201/eid1508.090333

Inojosa WO, Scotton PG, Fuser R, Giobbia M, Paolin A, Maresca MC et al (2012) West Nile virus transmission through organ transplantation in North-Eastern Italy: a case report and implications for pre-procurement screening. Infection 40(5):557–562. https://doi.org/10.1007/s15010-012-0263-4

Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC et al (2003) Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med 348(22):2196–2203

Janousek WM, Marra PP, Kilpatrick AM (2014) Avian roosting behavior influences vector-host interactions for West Nile virus hosts. Parasit Vectors 7:399. https://doi.org/10.1186/1756-3305-7-399

Jeha LE, Sila CA, Lederman RJ, Prayson RA, Isada CM, Gordon SM (2003) West Nile virus infection: a new acute paralytic illness. Neurology 61(1):55–59

Johnson AJ, Noga AJ, Kosoy O, Lanciotti RS, Johnson AA, Biggerstaff BJ (2005a) Duplex microsphere-based immunoassay for detection of anti-West Nile virus and anti-St. Louis encephalitis virus immunoglobulin m antibodies. Clin Diagn Lab Immunol 12(5):566–574. https://doi.org/10.1128/CDLI.12.5.566-574.2005

Johnson BW, Kosoy O, Martin DA, Noga AJ, Russell BJ, Johnson AA et al (2005b) West Nile virus infection and serologic response among persons previously vaccinated against yellow fever and Japanese encephalitis viruses. Vector Borne Zoonotic Dis 5(2):137–145

Jupp PG (2001) The ecology of West Nile virus in South Africa and the occurrence of outbreaks in humans. Ann N Y Acad Sci 951:143–152

Kalantari M, Salehi-Vaziri M, Pouriayevali MH, Baniasadi V, Salmanzadeh S, Kharat M et al (2019) Seroprevalence of West Nile virus in Khuzestan province, southwestern Iran, 2016–2017. J Vector Borne Dis 56(3):263–267. https://doi.org/10.4103/0972-9062.289391

Kalaycioglu H, Korukluoglu G, Ozkul A, Oncul O, Tosun S, Karabay O et al (2012) Emergence of West Nile virus infections in humans in Turkey, 2010 to 2011. Euro Surveill 17(21)

Kanagarajan K, Ganesh S, Alakhras M, Go ES, Recco RA, Zaman MM (2003) West Nile virus infection presenting as cerebellar ataxia and fever: case report. South Med J 96(6):600–601

Kelley TW, Prayson RA, Ruiz AI, Isada CM, Gordon SM (2003) The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. Am J Clin Pathol 119(5):749–753

Kernbach ME, Newhouse DJ, Miller JM, Hall RJ, Gibbons J, Oberstaller J et al (2019) Light pollution increases West Nile virus competence of a ubiquitous passerine reservoir species. Proc Biol Sci 286(1907):20191051. https://doi.org/10.1098/rspb.2019.1051

Keyel AC, Gorris ME, Rochlin I, Uelmen JA, Chaves LF, Hamer GL et al (2021) A proposed framework for the development and qualitative evaluation of West Nile virus models and their application to local public health decision-making. PLoS Negl Trop Dis 15(9):e0009653. https://doi.org/10.1371/journal.pntd.0009653

Khan SA, Dutta P, Khan AM, Chowdhury P, Borah J, Doloi P et al (2011) West Nile virus infection, Assam, India. Emerg Infect Dis 17(5):947–948. https://doi.org/10.3201/eid1705.100479

Kilpatrick AM, Wheeler SS (2019) Impact of West Nile virus on bird populations: limited lasting effects, evidence for recovery, and gaps in our understanding of impacts on ecosystems. J Med Entomol 56(6):1491–1497. https://doi.org/10.1093/jme/tjz149

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD (2006a) Host heterogeneity dominates West Nile virus transmission. Proc Biol Sci 273(1599):2327–2333. https://doi.org/10.1098/rspb.2006.3575

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P (2006b) West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol 4(4):e82. https://doi.org/10.1371/journal.pbio.0040082

Kilpatrick AM, Meola MA, Moudy RM, Kramer LD (2008) Temperature, viral genetics, and the transmission of West Nile virus by Culex pipiens mosquitoes. PLoS Pathog 4(6):e1000092. https://doi.org/10.1371/journal.ppat.1000092

Klee AL, Maidin B, Edwin B, Poshni I, Mostashari F, Fine A et al (2004) Long-term prognosis for clinical West Nile virus infection. Emerg Infect Dis 10(8):1405–1411

Klenk K, Snow J, Morgan K, Bowen R, Stephens M, Foster F et al (2004) Alligators as West Nile virus amplifiers. Emerg Infect Dis 10(12):2150–2155

Knope K, Whelan P, Smith D, Johansen C, Moran R, Doggett S et al (2013) Arboviral diseases and malaria in Australia, 2010–11: annual report of the National Arbovirus and Malaria Advisory Committee. Commun Dis Intell Q Rep 37(1):E1–E20

Komar N, Clark GG (2006) West Nile virus activity in Latin America and the Caribbean. Rev Panam Salud Publica 19(2):112–117

Komar N, Burns J, Dean C, Panella NA, Dusza S, Cherry B (2001a) Serologic evidence for West Nile virus infection in birds in Staten Island, New York, after an outbreak in 2000. Vector Borne Zoonotic Dis 1(3):191–196

Komar N, Panella NA, Burns JE, Dusza SW, Mascarenhas TM, Talbot TO (2001b) Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg Infect Dis 7(4):621–625

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D et al (2003) Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis 9(3):311–322

Kong KF, Delroux K, Wang X, Qian F, Arjona A, Malawista SE et al (2008) Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. J Virol 82(15):7613–7623. https://doi.org/10.1128/jvi.00618-08

Kumar D, Drebot MA, Wong SJ, Lim G, Artsob H, Buck P et al (2004) A seroprevalence study of West Nile virus infection in solid organ transplant recipients. Am J Transplant 4(11):1883–1888

LaBeaud AD, Kile JR, Kippes C, King CH, Mandalakas AM (2007) Exposure to West Nile virus during the 2002 epidemic in Cuyahoga County, Ohio: a comparison of pediatric and adult behaviors. Public Health Rep 122(3):356–361

LaDeau SL, Kilpatrick AM, Marra PP (2007) West Nile virus emergence and large-scale declines of North American bird populations. Nature 447(7145):710–713

Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K et al (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286 (5448):2333–2337

Lanciotti RS, Ebel GD, Deubel V, Kerst AJ, Murri S, Meyer R et al (2002) Complete genome sequences and phylogenetic analysis of West Nile virus strains isolated from the United States, Europe, and the Middle East. Virology 298(1):96–105

Landesman WJ, Allan BF, Langerhans RB, Knight TM, Chase JM (2007) Inter-annual associations between precipitation and human incidence of West Nile virus in the United States. Vector Borne Zoonotic Dis 7(3):337–343. https://doi.org/10.1089/vbz.2006.0590

Landry K, Rabe IB, Messenger SL, Hacker JK, Salas ML, Scott-Waldron C et al (2019) Patients with laboratory evidence of West Nile virus disease without reported fever. Epidemiol Infect 147:e219. https://doi.org/10.1017/S0950268819001079

Le Guenno B, Bougermouh A, Azzam T, Bouakaz R (1996) West Nile: a deadly virus? Lancet 348 (9037):1315

Leis AA, Stokic DS (2012) Neuromuscular manifestations of West Nile virus infection. Front Neurol 3(Article 37):1–10. https://doi.org/10.3389/fneur.2012.00037

Leis AA, Stokic DS, Polk JL, Dostrow V, Winkelmann M (2002) A poliomyelitis-like syndrome from West Nile virus infection. N Engl J Med 347(16):1279–1280

Leis AA, Stokic DS, Webb RM, Slavinski SA, Fratkin J (2003) Clinical spectrum of muscle weakness in human West Nile virus infection. Muscle Nerve 28(3):302–308

Lelli R, Calistri P, Bruno R, Monaco F, Savini G, Di Sabatino D et al (2012) West Nile transmission in resident birds in Italy. Transbound Emerg Dis 59(5):421–428. https://doi.org/10.1111/j.1865-1682.2011.01287.x

Li J, Loeb JA, Shy ME, Shah AK, Tselis AC, Kupski WJ et al (2003) Asymmetric flaccid paralysis: a neuromuscular presentation of West Nile virus infection. Ann Neurol 53(6):703–710

Li XL, Fu SH, Liu WB, Wang HY, Lu Z, Tong SX et al (2013) West Nile virus infection in Xinjiang, China. Vector Borne Zoonotic Dis 13(2):131–133. https://doi.org/10.1089/vbz.2012.0995

Lim PY, Behr MJ, Chadwick CM, Shi PY, Bernard KA (2011) Keratinocytes are cell targets of West Nile virus in vivo. J Virol. https://doi.org/10.1128/jvi.02692-10

Lindsey NP, Staples JE, Lehman JA, Fischer M (2010) Centers for disease C, prevention. Surveillance for human West Nile virus disease – United States, 1999–2008. MMWR Morb Mortal Wkly Rep Surveill Summ 59(2):1–17

Lindsey NP, Sejvar JJ, Bode AV, Pape WJ, Campbell GL (2012a) Delayed mortality in a cohort of persons hospitalized with West Nile virus disease in Colorado in 2003. Vector Borne Zoonotic Dis 12(3):230–235. https://doi.org/10.1089/vbz.2011.0721

Lindsey NP, Staples JE, Lehman JA, Fischer M (2012b) Medical risk factors for severe West Nile virus disease, United States, 2008–2010. Am J Trop Med Hyg 87(1):179–184. https://doi.org/10.4269/ajtmh.2012.12-0113

Linke S, Niedrig M, Kaiser A, Ellerbrok H, Muller K, Muller T et al (2007) Serologic evidence of West Nile virus infections in wild birds captured in Germany. Am J Trop Med Hyg 77(2):358–364

Loeb M, Elliott SJ, Gibson B, Fearon M, Nosal R, Drebot M et al (2005) Protective behavior and West Nile virus risk. Emerg Infect Dis 11(9):1433–1436

Loeb M, Hanna S, Nicolle L, Eyles J, Elliott S, Rathbone M et al (2008) Prognosis after West Nile virus infection. Ann Intern Med 149(4):232–241

Long MT, Gibbs EP, Mellencamp MW, Bowen RA, Seino KK, Zhang S et al (2007a) Efficacy, duration, and onset of immunogenicity of a West Nile virus vaccine, live Flavivirus chimera, in horses with a clinical disease challenge model. Equine Vet J 39(6):491–497. https://doi.org/10.2746/042516407X217416

Long MT, Gibbs EP, Mellencamp MW, Zhang S, Barnett DC, Seino KK et al (2007b) Safety of an attenuated West Nile virus vaccine, live Flavivirus chimera in horses. Equine Vet J 39(6):486–490. https://doi.org/10.2746/042516407X214473

Lothrop HD, Lothrop BB, Gomsi DE, Reisen WK (2008) Intensive early season adulticide applications decrease arbovirus transmission throughout the Coachella Valley, Riverside County, California. Vector Borne Zoonotic Dis 8(4):475–489. https://doi.org/10.1089/vbz.2007.0238

Lustig Y, Gosinov R, Zuckerman N, Glazer Y, Orshan L, Sofer D et al (2019) Epidemiologic and phylogenetic analysis of the 2018 West Nile virus (WNV) outbreak in Israel demonstrates human infection of WNV lineage I. Euro Surveill 24(1). https://doi.org/10.2807/1560-7917.ES.2019.24.1.1800662

Macedo PA, Schleier JJ 3rd, Reed M, Kelley K, Goodman GW, Brown DA et al (2010) Evaluation of efficacy and human health risk of aerial ultra-low volume applications of pyrethrins and piperonyl butoxide for adult mosquito management in response to West Nile virus activity in Sacramento County, California. J Am Mosq Control Assoc 26(1):57–66

Mackenzie JS, Williams DT (2009) The zoonotic flaviviruses of southern, south-eastern and eastern Asia, and Australasia: the potential for emergent viruses. Zoonoses Public Health 56(6–7):338–356. https://doi.org/10.1111/j.1863-2378.2008.01208.x

Magurano F, Remoli ME, Baggieri M, Fortuna C, Marchi A, Fiorentini C et al (2012) Circulation of West Nile virus lineage 1 and 2 during an outbreak in Italy. Clin Microbiol Infect 18(12):E545–E547. https://doi.org/10.1111/1469-0691.12018

Malkinson M, Banet C (2002) The role of birds in the ecology of West Nile virus in Europe and Africa. Curr Top Microbiol Immunol 267:309–322

Malkinson M, Banet C, Weisman Y, Pokamunski S, King R, Drouet MT et al (2002) Introduction of West Nile virus in the Middle East by migrating white storks. Emerg Infect Dis 8(4):392–397

Martin JE, Pierson TC, Habka S, Rucker S, Gordon IJ, Enama ME et al (2007) A West Nile virus DNA vaccine induces neutralizing antibody in healthy adults in a phase one clinical trial. J Infect Dis 196:1732–1740

Martina BE, Koraka P, Osterhaus AD (2010) West Nile virus: is a vaccine needed? Curr Opin Investig Drugs 11(2):139–146

Mattar S, Edwards E, Laguado J, Gonzalez M, Alvarez J, Komar N (2005) West Nile virus antibodies in Colombian horses. Emerg Infect Dis 11(9):1497–1498. https://doi.org/10.3201/eid1109.050426

Mavrakis A, Papavasileiou C, Alexakis D, Papakitsos EC, Salvati L (2021) Meteorological patterns and the evolution of West Nile virus in an environmentally stressed Mediterranean area. Environ Monit Assess 193(4):227. https://doi.org/10.1007/s10661-021-09011-3

May FJ, Davis CT, Tesh RB, Barrett AD (2011) Phylogeography of West Nile virus: from the cradle of evolution in Africa to Eurasia, Australia, and the Americas. J Virol 85(6):2964–2974. https://doi.org/10.1128/JVI.01963-10

McCarthy TA, Hadler JL, Julian K, Walsh SJ, Biggerstaff BJ, Hinten SR et al (2001) West Nile virus serosurvey and assessment of personal prevention efforts in an area with intense epizootic activity: Connecticut, 2000. Ann N Y Acad Sci 951:307–316

McDonald E, Mathis S, Martin SW, Staples JE, Fischer M, Lindsey NP (2021) Surveillance for West Nile virus disease – United States, 2009–2018. MMWR Surveill Summ 70(1):1–15. https://doi.org/10.15585/mmwr.ss7001a1

McKenzie VJ, Goulet NE (2010) Bird community composition linked to human West Nile virus cases along the Colorado front range. EcoHealth 7(4):439–447. https://doi.org/10.1007/s10393-010-0360-8

McMullen AR, Albayrak H, May FJ, Davis CT, Beasley DW, Barrett AD (2013) Molecular evolution of lineage 2 West Nile virus. J Gen Virol 94(Pt 2):318–325. https://doi.org/10.1099/vir.0.046888-0

Mencattelli G, Ndione MHD, Rosa R, Marini G, Diagne CT, Diagne MM et al (2022) Epidemiology of West Nile virus in Africa: an underestimated threat. PLoS Negl Trop Dis 16(1):e0010075. https://doi.org/10.1371/journal.pntd.0010075

Metz MBC, Olufemi OT, Daly JM, Barba M (2021) Systematic review and meta-analysis of seroprevalence studies of West Nile virus in equids in Europe between 2001 and 2018. Transbound Emerg Dis 68(4):1814–1823. https://doi.org/10.1111/tbed.13866

Meyer TE, Bull LM, Cain Holmes K, Pascua RF, Travassos da Rosa A, Gutierrez CR et al (2007) West Nile virus infection among the homeless, Houston, Texas. Emerg Infect Dis 13(10):1500–1503

Miramontes R Jr, Lafferty WE, Lind BK, Oberle MW (2006) Is agricultural activity linked to the incidence of human West Nile virus? Am J Prev Med 30(2):160–163

Mitchell KC, Ryan P, Howard DE, Feldman KA (2018) Understanding knowledge, attitudes, and behaviors toward West Nile virus prevention: a survey of high-risk adults in Maryland. Vector Borne Zoonotic Dis 18(3):173–180. https://doi.org/10.1089/vbz.2017.2188

Morelli MC, Sambri V, Grazi GL, Gaibani P, Pierro A, Cescon M et al (2010) Absence of neuroinvasive disease in a liver transplant recipient who acquired West Nile virus (WNV) infection from the organ donor and who received WNV antibodies prophylactically. Clin Infect Dis 51(4):e34–e37. https://doi.org/10.1086/655146

Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ et al (2001) Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet 358(9278):261–264

Murata R, Hashiguchi K, Yoshii K, Kariwa H, Nakajima K, Ivanov LI et al (2011) Seroprevalence of West Nile virus in wild birds in far eastern Russia using a focus reduction neutralization test. Am J Trop Med Hyg 84(3):461–465. https://doi.org/10.4269/ajtmh.2011.09-0714

Murgue B, Murri S, Triki H, Deubel V, Zeller HG (2001a) West Nile in the Mediterranean basin: 1950–2000. Ann N Y Acad Sci 951:117–126

Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H (2001b) West Nile outbreak in horses in southern France, 2000: the return after 35 years. Emerg Infect Dis 7(4):692–696

Murgue B, Zeller H, Deubel V (2002) The ecology and epidemiology of West Nile virus in Africa, Europe and Asia. Curr Top Microbiol Immunol 267:195–221

Murray K, Baraniuk S, Resnick M, Arafat R, Kilborn C, Cain K et al (2006) Risk factors for encephalitis and death from West Nile virus infection. Epidemiol Infect 134(6):1325–1332

Murray KO, Resnick M, Miller V (2007) Depression after infection with West Nile virus. Emerg Infect Dis 13(3):479–481

Murray K, Walker C, Herrington E, Lewis JA, McCormick J, Beasley DW et al (2010) Persistent infection with West Nile virus years after initial infection. J Infect Dis 201(1):2–4. https://doi.org/10.1086/648731

Murray KO, Garcia MN, Yan C, Gorchakov R (2013) Persistence of detectable immunoglobulin M antibodies up to 8 years after infection with West Nile virus. Am J Trop Med Hyg 89(5):996–1000. https://doi.org/10.4269/ajtmh.13-0232

Naficy K, Saidi S (1970) Serological survey on viral antibodies in Iran. Trop Geogr Med 22(2):183–188

Naowarat S, Tang IM (2004) Effect of bird-to-bird transmission of the West Nile virus on the dynamics of the transmission of this disease. Southeast Asian J Trop Med Public Health 35(1): 162–166

Nasci RS, Savage HM, White DJ, Miller JR, Cropp BC, Godsey MS et al (2001) West Nile virus in overwintering Culex mosquitoes, New York City, 2000. Emerg Infect Dis 7(4):742–744

Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K et al (2001) The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med 344(24):1807–1814

Nemeth N, Gould D, Bowen R, Komar N (2006) Natural and experimental West Nile virus infection in five raptor species. J Wildl Dis 42(1):1–13

Nemeth NM, Beckett S, Edwards E, Klenk K, Komar N (2007) Avian mortality surveillance for West Nile virus in Colorado. Am J Trop Med Hyg 76(3):431–437

Nett RJ, Kuehnert MJ, Ison MG, Orlowski JP, Fischer M, Staples JE (2012) Current practices and evaluation of screening solid organ donors for West Nile virus. Transpl Infect Dis 14(3):268–277. https://doi.org/10.1111/j.1399-3062.2012.00743.x

Nir Y, Beemer A, Goldwasser RA (1965) West Nile virus infection in mice following exposure to a viral aerosol. Br J Exp Pathol 46(4):443–449

Nur YA, Groen J, Heuvelmans H, Tuynman W, Copra C, Osterhaus AD (1999) An outbreak of West Nile fever among migrants in Kisangani, Democratic Republic of Congo. Am J Trop Med Hyg 61(6):885–888

Ochieng C, Lutomiah J, Makio A, Koka H, Chepkorir E, Yalwala S et al (2013) Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007–2012. Virol J 10(1):140. https://doi.org/10.1186/1743-422x-10-140

O'Leary DR, Kuhn S, Kniss KL, Hinckley AF, Rasmussen SA, Pape WJ et al (2006) Birth outcomes following West Nile virus infection of pregnant women in the United States: 2003–2004. Pediatrics 117(3):e537–e545

Olufemi OT, Barba M, Daly JM (2021) A scoping review of West Nile virus seroprevalence studies among African equids. Pathogens 10(7). https://doi.org/10.3390/pathogens10070899

Orton SL, Stramer SL, Dodd RY (2006) Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors. Transfusion 46(2):272–277

Owen JC, Landwerlen HR, Dupuis AP 2nd, Belsare AV, Sharma DB, Wang S et al (2021) Reservoir hosts experiencing food stress alter transmission dynamics for a zoonotic pathogen. Proc Biol Sci 288(1956):20210881. https://doi.org/10.1098/rspb.2021.0881

Pacenti M, Sinigaglia A, Franchin E, Pagni S, Lavezzo E, Montarsi F et al (2020) Human West Nile virus lineage 2 infection: epidemiological, clinical, and virological findings. Viruses 12(4). https://doi.org/10.3390/v12040458

Pachler K, Lebl K, Berer D, Rudolf I, Hubalek Z, Nowotny N (2014) Putative new West Nile virus lineage in *Uranotaenia unguiculata* mosquitoes, Austria, 2013. Emerg Infect Dis 20(12):2119–2122. https://doi.org/10.3201/eid2012.140921

Paisley JE, Hinckley AF, O'Leary DR, Kramer WC, Lanciotti RS, Campbell GL et al (2006) West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004. Pediatrics 117(3):814–820

Papa A (2012) West Nile virus infections in Greece: an update. Expert Rev Anti-Infect Ther 10(7): 743–750. https://doi.org/10.1586/eri.12.59

Papa A, Bakonyi T, Xanthopoulou K, Vazquez A, Tenorio A, Nowotny N (2011a) Genetic characterization of West Nile virus lineage 2, Greece, 2010. Emerg Infect Dis 17(5):920–922. https://doi.org/10.3201/eid1705.101759

Papa A, Danis K, Athanasiadou A, Delianidou M, Panagiotopoulos T (2011b) Persistence of West Nile virus immunoglobulin M antibodies, Greece. J Med Virol 83(10):1857–1860. https://doi.org/10.1002/jmv.22190

Papa A, Danis K, Tsergouli K, Tsioka K, Gavana E (2011c) Development time of IgG antibodies to West Nile virus. Arch Virol 156(9):1661–1663. https://doi.org/10.1007/s00705-011-1014-z

Papa A, Papadopoulou E, Gavana E, Kalaitzopoulou S, Mourelatos S (2013) Detection of West Nile virus lineage 2 in *Culex* mosquitoes, Greece, 2012. Vector Borne Zoonotic Dis 13(9):682–684. https://doi.org/10.1089/vbz.2012.1212

Parreira R, Severino P, Freitas F, Piedade J, Almeida AP, Esteves A (2007) Two distinct introductions of the West Nile virus in Portugal disclosed by phylogenetic analysis of genomic sequences. Vector Borne Zoonotic Dis 7(3):344–352. https://doi.org/10.1089/vbz.2006.0632

Patel H, Sander B, Nelder MP (2015) Long-term sequelae of West Nile virus-related illness: a systematic review. Lancet Infect Dis 15(8):951–959. https://doi.org/10.1016/S1473-3099(15)00134-6

Paul SD, Murthy DP, Das M (1970) Isolation of West Nile virus from a human case of febrile illness. Indian J Med Res 58(9):1177–1179

Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL et al (2003) Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 349(13):1236–1245

Pepperell C, Rau N, Krajden S, Kern R, Humar A, Mederski B et al (2003) West Nile virus infection in 2002: morbidity and mortality among patients admitted to hospital in southcentral Ontario. CMAJ 168(11):1399–1405

Petersen LR, Epstein JS (2005) Problem solved? West Nile virus and transfusion safety. N Engl J Med 353(5):516–517

Petersen LR, Fischer M (2012) Unpredictable and difficult to control--the adolescence of West Nile Virus. N Engl J Med 367(14):1281–1284. https://doi.org/10.1056/NEJMp1210537

Petersen LR, Hayes EB (2008) West Nile virus in the Americas. Med Clin North Am 92(6):1307–1322. ix

Petersen LR, Roehrig JT (2007) Flavivirus DNA vaccines – good science, uncertain future. J Infect Dis 196(12):1721–1723. https://doi.org/10.1086/523655

Petersen LR, Carson PJ, Biggerstaff BJ, Custer B, Borchardt SM, Busch MP (2012) Estimated cumulative incidence of West Nile virus infection in US adults, 1999–2010. Epidemiol Infect:1–5. https://doi.org/10.1017/S0950268812001070

Petersen LR, Brault AC, Nasci RS (2013a) West Nile virus: review of the literature. JAMA 310(3):308–315. https://doi.org/10.1001/jama.2013.8042

Petersen LR, Carson PJ, Biggerstaff BJ, Custer B, Borchardt SM, Busch MP (2013b) Estimated cumulative incidence of West Nile virus infection in US adults, 1999-2010. Epidemiol Infect 141(3):591–595. https://doi.org/10.1017/S0950268812001070

Platonov AE (2001) West Nile encephalitis in Russia 1999–2001: were we ready? Are we ready? Ann N Y Acad Sci 951:102–116

Platonov AE, Shipulin GA, Shipulina OY, Tyutyunnik EN, Frolochkina TI, Lanciotti RS et al (2001) Outbreak of West Nile virus infection, Volgograd region, Russia, 1999. Emerg Infect Dis 7(1):128–132

Platt KB, Tucker BJ, Halbur PG, Tiawsirisup S, Blitvich BJ, Fabiosa FG et al (2007) West Nile virus viremia in eastern chipmunks (*Tamias striatus*) sufficient for infecting different mosquitoes. Emerg Infect Dis 13(6):831–837. https://doi.org/10.3201/eid1306.061008

Platt KB, Tucker BJ, Halbur PG, Blitvich BJ, Fabiosa FG, Mullin K et al (2008) Fox squirrels (*Sciurus niger*) develop West Nile virus viremias sufficient for infecting select mosquito species. Vector Borne Zoonotic Dis 8(2):225–233. https://doi.org/10.1089/vbz.2007.0182

Pogodina VV, Frolova MP, Malenko GV, Fokina GI, Koreshkova GV, Kiseleva LL et al (1983) Study on West Nile virus persistence in monkeys. Arch Virol 75(1–2):71–86

Popescu CP, Florescu SA, Hasbun R, Harxhi A, Evendar R, Kahraman H et al (2020) Prediction of unfavorable outcomes in West Nile virus neuroinvasive infection – result of a multinational ID-IRI study. J Clin Virol 122:104213. https://doi.org/10.1016/j.jcv.2019.104213

Porter MB, Long MT, Getman LM, Giguere S, MacKay RJ, Lester GD et al (2003) West Nile virus encephalomyelitis in horses: 46 cases (2001). J Am Vet Med Assoc 222(9):1241–1247

Pridjian G, Sirois PA, McRae S, Hinckley AF, Rasmussen SA, Kissinger P et al (2016) Prospective study of pregnancy and newborn outcomes in mothers with west nile illness during pregnancy. Birth Defects Res A Clin Mol Teratol 106(8):716–723. https://doi.org/10.1002/bdra.23523

Pupo M, Guzman MG, Fernandez R, Llop A, Dickinson FO, Perez D et al (2006) West Nile Virus infection in humans and horses, Cuba. Emerg Infect Dis 12(6):1022–1024

Reed LM, Johansson MA, Panella N, McLean R, Creekmore T, Puelle R et al (2009) Declining mortality in American crow (*Corvus brachyrhynchos*) following natural West Nile virus infection. Avian Dis 53(3):458–461

Reisen WK (2012) The contrasting bionomics of Culex mosquitoes in western North America. J Am Mosq Control Assoc 28(4 Suppl):82–91

Reisen W, Brault AC (2007) West Nile virus in North America: perspectives on epidemiology and intervention. Pest Manag Sci 63(7):641–646. https://doi.org/10.1002/ps.1325

Reisen WK, Meyer RP, Tempelis CH, Spoehel JJ (1990) Mosquito abundance and bionomics in residential communities in Orange and Los Angeles Counties, California. J Med Entomol 27(3): 356–367

Reisen WK, Fang Y, Lothrop HD, Martinez VM, Wilson J, Oconnor P et al (2006a) Overwintering of West Nile virus in Southern California. J Med Entomol 43(2):344–355

Reisen WK, Fang Y, Martinez VM (2006b) Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). J Med Entomol 43(2):309–317

Reisen WK, Takahashi RM, Carroll BD, Quiring R (2008) Delinquent mortgages, neglected swimming pools, and West Nile virus, California. Emerg Infect Dis 14(11):1747–1749. https://doi.org/10.3201/eid1411.080719

Reiter P (2010) West Nile virus in Europe: understanding the present to gauge the future. Euro Surveill 15(10):19508

Rhee C, Eaton EF, Concepcion W, Blackburn BG (2011) West Nile virus encephalitis acquired via liver transplantation and clinical response to intravenous immunoglobulin: case report and review of the literature. Transpl Infect Dis 13(3):312–317. https://doi.org/10.1111/j.1399-3062.2010.00595.x

Richards SL, Mores CN, Lord CC, Tabachnick WJ (2007) Impact of extrinsic incubation temperature and virus exposure on vector competence of *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae) for West Nile virus. Vector Borne Zoonotic Dis 7(4):629–636. https://doi.org/10.1089/vbz.2007.0101

Ringia AM, Blitvich BJ, Koo HY, Van de Wyngaerde M, Brawn JD, Novak RJ (2004) Antibody prevalence of West Nile virus in birds, Illinois, 2002. Emerg Infect Dis 10(6):1120–1124

Robinson RL, Shahida S, Madan N, Rao S, Khardori N (2003) Transient parkinsonism in West Nile virus encephalitis. Am J Med 115(3):252–253

Rochlin I, Faraji A, Healy K, Andreadis TG (2019) West Nile Virus mosquito vectors in North America. J Med Entomol 56(6):1475–1490. https://doi.org/10.1093/jme/tjz146

Roehrig JT, Nash D, Maldin B, Labowitz A, Martin DA, Lanciotti RS et al (2003) Persistence of virus-reactive serum immunoglobulin m antibody in confirmed West Nile Virus encephalitis cases. Emerg Infect Dis 9(3):376–379

Root JJ, Bosco-Lauth AM (2019) West Nile Virus Associations in wild mammals: an update. Viruses 11(5). https://doi.org/10.3390/v11050459

Root JJ, Oesterle PT, Nemeth NM, Klenk K, Gould DH, McLean RG et al (2006) Experimental infection of fox squirrels (Sciurus niger) with West Nile virus. Am J Trop Med Hyg 75(4): 697–701

Rousseau A, Haigh O, Ksiaa I, Khairallah M, Labetoulle M (2020) Ocular manifestations of West Nile virus. Vaccines (Basel) 8(4). https://doi.org/10.3390/vaccines8040641

Ruiz MO, Walker ED, Foster ES, Haramis LD, Kitron UD (2007) Association of West Nile virus illness and urban landscapes in Chicago and Detroit. Int J Health Geogr 6:10

Sadek JR, Pergam SA, Harrington JA, Echevarria LA, Davis LE, Goade D et al (2010) Persistent neuropsychological impairment associated with West Nile virus infection. J Clin Exp Neuropsychol 32(1):81–87. https://doi.org/10.1080/13803390902881918

Saito EK, Sileo L, Green DE, Meteyer CU, McLaughlin GS, Converse KA et al (2007) Raptor mortality due to West Nile virus in the United States, 2002. J Wildl Dis 43(2):206–213

Saiz JC (2020) Animal and human vaccines against West Nile Virus. Pathogens 9(12). https://doi.org/10.3390/pathogens9121073

Salazar P, Traub-Dargatz JL, Morley PS, Wilmot DD, Steffen DJ, Cunningham WE et al (2004) Outcome of equids with clinical signs of West Nile virus infection and factors associated with death. J Am Vet Med Assoc 225(2):267–274

Savage HM, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R, Vladimirescu A et al (1999) Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. Am J Trop Med Hyg 61(4):600–611

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK et al (2007) Host choice and West Nile virus infection rates in blood-fed mosquitoes, including members of the Culex pipiens complex, from Memphis and Shelby County, Tennessee, 2002–2003. Vector Borne Zoonotic Dis 7(3):365–386. https://doi.org/10.1089/vbz.2006.0602

Savini G, Capelli G, Monaco F, Polci A, Russo F, Di Gennaro A et al (2012) Evidence of West Nile virus lineage 2 circulation in Northern Italy. Vet Microbiol 158(3–4):267–273. https://doi.org/10.1016/j.vetmic.2012.02.018

Sayao AL, Suchowersky O, Al-Khathaami A, Klassen B, Katz NR, Sevick R et al (2004) Calgary experience with West Nile virus neurological syndrome during the late summer of 2003. Can J Neurol Sci 31(2):194–203

Schellenberg TL, Anderson ME, Drebot MA, Vooght MT, Findlater AR, Curry PS et al (2006) Seroprevalence of West Nile virus in Saskatchewan's five hills health region, 2003. Can J Public Health 97(5):369–373

Schmidt JR, Elmansoury HK (1963) Natural and experimental infection of Egyptian equines with West Nile virus. Ann Trop Med Parasitol 57:415–427

Schneider BS, Higgs S (2008) The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. Trans R Soc Trop Med Hyg 102(5):400–408. https://doi.org/10.1016/j.trstmh.2008.01.024

Schuffenecker I, Peyrefitte CN, el Harrak M, Murri S, Leblond A, Zeller HG (2005) West Nile virus in Morocco, 2003. Emerg Infect Dis 11(2):306–309

Seino KK, Long MT, Gibbs EP, Bowen RA, Beachboard SE, Humphrey PP et al (2007) Comparative efficacies of three commercially available vaccines against West Nile Virus (WNV) in a short-duration challenge trial involving an equine WNV encephalitis model. Clin Vaccine Immunol 14(11):1465–1471. https://doi.org/10.1128/CVI.00249-07

Sejvar JJ, Marfin AA (2006) Manifestations of West Nile neuroinvasive disease. Rev Med Virol 16(4):209–224

Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, Marfin AA, Van Gerpen JA et al (2003a) Neurologic manifestations and outcome of West Nile virus infection. JAMA 290(4):511–515

Sejvar JJ, Leis AA, Stokic DS, Van Gerpen JA, Marfin AA, Webb R et al (2003b) Acute flaccid paralysis and West Nile virus infection. Emerg Infect Dis 9(7):788–793

Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Ewing D, Mazowiecki M et al (2005) West Nile virus-associated flaccid paralysis. Emerg Infect Dis 11(7):1021–1027

Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Pape J, Biggerstaff BJ et al (2006) West Nile virus-associated flaccid paralysis outcome. Emerg Infect Dis 12(3):514–516

Sejvar JJ, Curns AT, Welburg L, Jones JF, Lundgren LM, Capuron L et al (2008) Neurocognitive and functional outcomes in persons recovering from West Nile virus illness. J Neuropsychol 2 (Pt 2):477–499

Sejvar JJ, Lindsey NP, Campbell GL (2011) Primary causes of death in reported cases of fatal West Nile fever, United States, 2002–2006. Vector Borne Zoonotic Dis 11(2):161–164. https://doi.org/10.1089/vbz.2009.0086

Shankar MB, Staples JE, Meltzer MI, Fischer M (2017) Cost effectiveness of a targeted age-based West Nile virus vaccination program. Vaccine 35(23):3143–3151. https://doi.org/10.1016/j.vaccine.2016.11.078

Shukla J, Saxena D, Rathinam S, Lalitha P, Joseph CR, Sharma S et al (2012) Molecular detection and characterization of West Nile virus associated with multifocal retinitis in patients from southern India. Int J Infect Dis 16(1):e53–e59. https://doi.org/10.1016/j.ijid.2011.09.020

Siger L, Bowen R, Karaca K, Murray M, Jagannatha S, Echols B et al (2006) Evaluation of the efficacy provided by a recombinant canarypox-vectored equine West Nile virus vaccine against an experimental West Nile virus intrathecal challenge in horses. Vet Ther 7(3):249–256

Sinigaglia A, Peta E, Riccetti S, Barzon L (2020) New avenues for therapeutic discovery against West Nile virus. Expert Opin Drug Discovery 15(3):333–348. https://doi.org/10.1080/17460441.2020.1714586

Smithburn KC, Hughes TP, Burke AW, Paul JH (1940) A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 20:470–492

Soliman A, Mohareb E, Salman D, Saad M, Salama S, Fayez C et al (2010) Studies on West Nile virus infection in Egypt. J Infect Public Health 3(2):54–59. https://doi.org/10.1016/j.jiph.2009.11.002

Solomon T (2004) Flavivirus encephalitis. N Engl J Med 351(4):370–378

Sotelo E, Gutierrez-Guzman AV, Del Amo J, Llorente F, El-Harrak M, Perez-Ramirez E et al (2011a) Pathogenicity of two recent Western Mediterranean West Nile virus isolates in a wild bird species indigenous to Southern Europe: the red-legged partridge. Vet Res 42(1):11. https://doi.org/10.1186/1297-9716-42-11

Sotelo E, Llorente F, Rebollo B, Camunas A, Venteo A, Gallardo C et al (2011b) Development and evaluation of a new epitope-blocking ELISA for universal detection of antibodies to West Nile virus. J Virol Methods 174(1–2):35–41. https://doi.org/10.1016/j.jviromet.2011.03.015

Soto RA, McDonald E, Annambhotla P, Velez JO, Laven J, Panella AJ et al (2022) West Nile virus transmission by solid organ transplantation and considerations for organ donor screening practices, United States. Emerg Infect Dis 28(2):403–406. https://doi.org/10.3201/eid2802.211697

Soverow JE, Wellenius GA, Fisman DN, Mittleman MA (2009) Infectious disease in a warming world: how weather influenced West Nile virus in the United States (2001–2005). Environ Health Perspect 117(7):1049–1052

Steinman A, Banet-Noach C, Simanov L, Grinfeld N, Aizenberg Z, Levi O et al (2006) Experimental infection of common garter snakes (*Thamnophis sirtalis*) with West Nile virus. Vector Borne Zoonotic Dis 6(4):361–368. https://doi.org/10.1089/vbz.2006.6.361

Styer LM, Lim PY, Louie KL, Albright RG, Kramer LD, Bernard KA (2011) Mosquito saliva causes enhancement of West Nile virus infection in mice. J Virol 85(4):1517–1527. https://doi.org/10.1128/JVI.01112-10

Sutherland LJ, Cash AA, Huang YJ, Sang RC, Malhotra I, Moormann AM et al (2011) Serologic evidence of arboviral infections among humans in Kenya. Am J Trop Med Hyg 85(1):158–161. https://doi.org/10.4269/ajtmh.2011.10-0203

Takken W, Verhulst NO (2013) Host preferences of blood-feeding mosquitoes. Annu Rev Entomol 58:433–453. https://doi.org/10.1146/annurev-ento-120811-153618

Tesh RB, Parsons R, Siirin M, Randle Y, Sargent C, Guzman H et al (2004) Year-round West Nile virus activity, Gulf Coast region, Texas and Louisiana. Emerg Infect Dis 10(9):1649–1652. https://doi.org/10.3201/eid1009.040203

Tesh RB, Siirin M, Guzman H, Travassos da Rosa AP, Wu X, Duan T et al (2005) Persistent West Nile virus infection in the golden hamster: studies on its mechanism and possible implications for other flavivirus infections. J Infect Dis 192(2):287–295. https://doi.org/10.1086/431153

Thakare JP, Rao TL, Padbidri VS (2002) Prevalence of West Nile virus infection in India. Southeast Asian J Trop Med Public Health 33(4):801–805

Theodoropoulos NM, Greenwald MA, Chin-Hong P, Ison MG (2021) Testing deceased organ donors for infections: an organ procurement organization survey. Am J Transplant 21(5):1924–1930. https://doi.org/10.1111/ajt.16552

Tilley PA, Fox JD, Jayaraman GC, Preiksaitis JK (2006) Nucleic acid testing for West Nile virus RNA in plasma enhances rapid diagnosis of acute infection in symptomatic patients. J Infect Dis 193(10):1361–1364

Triki H, Murri S, Le Guenno B, Bahri O, Hili K, Sidhom M et al (2001) West Nile viral meningoencephalitis in Tunisia. Med Trop (Mars) 61(6):487–490

Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI (1998) West Nile encephalitis epidemic in southeastern Romania. Lancet 352(9130):767–771

Ulbert S (2019) West Nile virus vaccines – current situation and future directions. Hum Vaccin Immunother 15(10):2337–2342. https://doi.org/10.1080/21645515.2019.1621149

Valiakos G, Touloudi A, Athanasiou LV, Giannakopoulos A, Iacovakis C, Birtsas P et al (2012) Serological and molecular investigation into the role of wild birds in the epidemiology of West Nile virus in Greece. Virol J 9:266. https://doi.org/10.1186/1743-422x-9-266

Vazquez A, Sanchez-Seco MP, Ruiz S, Molero F, Hernandez L, Moreno J et al (2010) Putative new lineage of West Nile virus, Spain. Emerg Infect Dis 16(3):549–552

Venter M (2009) Lineage 2 West Nile virus as cause of fatal neurologic disease in horses, South Africa. Emerg Infect Dis 15(6):659–664

Venter M, Swanepoel R (2010) West Nile virus lineage 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. Vector Borne Zoonotic Dis 10(7):659–664. https://doi.org/10.1089/vbz.2009.0230

Verma S, Lo Y, Chapagain M, Lum S, Kumar M, Gurjav U et al (2009) West Nile virus infection modulates human brain microvascular endothelial cells tight junction proteins and cell adhesion molecules: transmigration across the in vitro blood-brain barrier. Virology 385(2):425–433. https://doi.org/10.1016/j.virol.2008.11.047

Vlaskamp DR, Thijsen SF, Reimerink J, Hilkens P, Bouvy WH, Bantjes SE et al (2020) First autochthonous human West Nile virus infections in the Netherlands, July to August 2020. Euro Surveill 25(46). https://doi.org/10.2807/1560-7917.ES.2020.25.46.2001904

Vogels CB, Goertz GP, Pijlman GP, Koenraadt CJ (2017) Vector competence of European mosquitoes for West Nile virus. Emerg Microbes Infect 6(11):e96. https://doi.org/10.1038/emi.2017.82

Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA (2004) Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med 10(12):1366–1373

Wang H, Siddharthan V, Hall JO, Morrey JD (2009) West Nile virus preferentially transports along motor neuron axons after sciatic nerve injection of hamsters. J Neurovirol:1–7. https://doi.org/10.1080/13550280902973978

Wang G, Minnis RB, Belant JL, Wax CL (2010) Dry weather induces outbreaks of human West Nile virus infections. BMC Infect Dis 10:38. https://doi.org/10.1186/1471-2334-10-38

Ward MP, Levy M, Thacker HL, Ash M, Norman SK, Moore GE et al (2004) Investigation of an outbreak of encephalomyelitis caused by West Nile virus in 136 horses. J Am Vet Med Assoc 225(1):84–89

Watson JT, Pertel PE, Jones RC, Siston AM, Paul WS, Austin CC et al (2004) Clinical characteristics and functional outcomes of West Nile fever. Ann Intern Med 141(5):360–365

Watts MJ, Sarto IMV, Mortyn PG, Kotsila P (2021) The rise of West Nile Virus in Southern and Southeastern Europe: a spatial-temporal analysis investigating the combined effects of climate, land use and economic changes. One Health 13:100315. https://doi.org/10.1016/j.onehlt.2021.100315

Weber IB, Lindsey NP, Bunko-Patterson AM, Briggs G, Wadleigh TJ, Sylvester TL et al (2011) Completeness of West Nile virus testing in patients with meningitis and encephalitis during an outbreak in Arizona, USA. Epidemiol Infect 140(9):1632–1636. https://doi.org/10.1017/S0950268811002494

Weinberger M, Pitlik SD, Gandacu D, Lang R, Nassar F, Ben David D et al (2001) West Nile fever outbreak, Israel, 2000: epidemiologic aspects. Emerg Infect Dis 7(4):686–691

Weingartl HM, Neufeld JL, Copps J, Marszal P (2004) Experimental West Nile virus infection in blue jays (*Cyanocitta cristata*) and crows (*Corvus brachyrhynchos*). Vet Pathol 41(4):362–370

Wimberly MC, Hildreth MB, Boyte SP, Lindquist E, Kightlinger L (2008) Ecological niche of the 2003 West Nile virus epidemic in the northern great plains of the United States. PLoS One 3(12):e3744. https://doi.org/10.1371/journal.pone.0003744

Young JJ, Haussig JM, Aberle SW, Pervanidou D, Riccardo F, Sekulic N et al (2021) Epidemiology of human West Nile virus infections in the European Union and European Union enlargement

countries, 2010 to 2018. Euro Surveill 26(19). https://doi.org/10.2807/1560-7917.ES.2021.26.19.2001095

Zaayman D, Venter M (2012) West Nile virus neurologic disease in humans, South Africa, September 2008–May 2009. Emerg Infect Dis 18(12):2051–2054. https://doi.org/10.3201/eid1812.111208

Ziegler U, Angenvoort J, Fischer D, Fast C, Eiden M, Rodriguez AV et al (2013) Pathogenesis of West Nile virus lineage 1 and 2 in experimentally infected large falcons. Vet Microbiol 161(3–4):263–273. https://doi.org/10.1016/j.vetmic.2012.07.041

Ziegler U, Luhken R, Keller M, Cadar D, van der Grinten E, Michel F et al (2019) West Nile virus epizootic in Germany, 2018. Antivir Res 162:39–43. https://doi.org/10.1016/j.antiviral.2018.12.005

Ziegler U, Santos PD, Groschup MH, Hattendorf C, Eiden M, Hoper D et al (2020) West Nile virus epidemic in Germany triggered by epizootic emergence, 2019. Viruses 12(4). https://doi.org/10.3390/v12040448

Zohrabian A, Hayes EB, Petersen LR (2006) Cost-effectiveness of West Nile virus vaccination. Emerg Infect Dis 12(3):375–380

Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL (2010) West Nile fever characteristics among viremic persons identified through blood donor screening. J Infect Dis 202(9):1354–1361. https://doi.org/10.1086/656602

Crimean-Congo Hemorrhagic Fever Virus: An Emerging and Re-emerging Pathogen of Public Health Concern

47

Felicity Jane Burt and Dominique Goedhals

Contents

F. J. Burt (✉)
Division of Virology, National Health Laboratory Service, Bloemfontein, South Africa

Division of Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa
e-mail: burtfj@ufs.ac.za

D. Goedhals
Division of Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

PathCare Vermaak, Pretoria, South Africa
e-mail: gnvrdg@ufs.ac.za

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_39

Abstract

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne zoonosis distributed in Africa, Asia, Eastern Europe, and the Balkans. Humans become infected by tick-bite or from contact with infected blood or other tissues of livestock or human patients. Human infection is usually characterized by febrile illness, which can progress to a hemorrhagic state with a fatal outcome. The virus has the propensity to cause nosocomial infections, however implementation of molecular assays has facilitated rapid and safe diagnosis, especially in regions where high containment access is limited. Early diagnosis contributes toward protection of healthcare workers. The absence of any specific anti-viral treatment or approved efficacious vaccines contributes toward the public health concern regarding emergence, re-emergence, and spread of CCHFV. While the immune correlates of protection remain unclear, available data indicate that both humoral and cellular responses are required. Vaccine development has, however, in recent years been facilitated by the availability of novel animal models.

The distribution of CCHFV correlates with that of the primary vector of the virus, ticks belonging to the genus *Hyalomma*. The importance of the tick-vertebrate-tick cycle in maintaining CCHFV transmission is well established and sero-surveillance studies have contributed toward understanding the role of wild and domestic animals and birds as reservoirs and amplifying agents. The distribution of these ticks has, in recent years, expanded to regions where conditions are favorable for the species to establish endemnicity. Hence, there is growing concern that this virus has the potential to emerge and spread to new geographic regions.

Keywords

CCHFV · Arbovirus · Emerging and re-emerging pathogen · Orthonairovirus · Tick-borne virus · Emerging pathogen · Public health concern · Global concern · Biosafety level 4 pathogen

47.1 Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne viral zoonosis distributed in Africa, Asia, Eastern Europe, and the Balkans. The broad geographic range correlates with that of the principal vector of the virus, ticks belonging to the genus *Hyalomma* (Hoogstraal 1979). The virus is a member of the *Orthonairovirus* genus of the family *Nairoviridae* (Adams et al. 2017; Hoogstraal 1979). A disease with symptoms suggestive of CCHFV infection was described as early as the twelfth century in regions of Eastern Europe and Asia. In 1944, a disease was described among peasants that became ill following exposure to tick-bites while harvesting crops on the Crimean Peninsula and was given the name Crimean hemorrhagic fever (CHF). The disease was subsequently shown to be caused by a filterable agent

present in suspensions prepared from certain tick species and in human blood samples collected from patients during the early stages of disease. It was only in 1967 that the etiologic agent was isolated in a laboratory using newborn mice. The availability of a laboratory host made it possible to characterize the virus, and it was shown in 1969 that isolates of CHF were antigenically identical to an African virus, named Congo virus, isolated from a febrile child in Stanleyville in the Belgian Congo (now referred to as Kisangani in the Democratic Republic of the Congo). Studies on physicochemical characteristics, morphology, and morphogenesis showed that the viruses were indistinguishable (Casals 1969; Chumakov et al. 1970; Hoogstraal 1979; Korolev et al. 1976; Simpson et al. 1967). The names were subsequently combined and the virus referred to as Crimean-Congo hemorrhagic fever virus (CCHFV).

Humans become infected by tick-bite or from contact with infected blood or other tissues of livestock or human patients. Human infection is usually characterized by febrile illness with headache, myalgia, and petechial rash, which can progress to a hemorrhagic state with a fatal outcome. In livestock, including ostriches, and in potential reservoir hosts, usually small mammals such as hares and ground frequenting birds, infection is either asymptomatic or causes mild fever which is frequently associated with a short period of viraemia playing a role in transmission as well as amplification of the virus in nature.

The distribution of CCHFV correlates with that of the primary vector of the virus, ticks belonging to the genus *Hyalomma*. The distribution of these ticks has, in recent years, expanded to regions where conditions are favorable for the species to establish endemnicity. Hence, there is growing concern that this virus has the potential to emerge and spread to new geographic regions. The absence of any specific anti-viral treatment or approved efficacious vaccines contributes toward the public health concern regarding emergence, re-emergence, and spread of CCHFV.

47.2 Crimean-Congo Hemorrhagic Fever Virus, an Emerging and Re-Emerging Pathogen

47.2.1 The Virus

CCHFV is classified as a member of the genus *Orthonairovirus*, within the family *Nairoviridae* (Adams et al. 2017; Calisher 1991; Casals 1969; Garrison et al. 2020; Karabatsos 1985). Nairoviruses are classified into three genera: *Orthonairovirus*, *Shaspivirus*, and *Striwavirus* (Garrison et al. 2020). The *Orthonairovirus* genus currently includes 54 viruses, and 14 species. Within the genus, CCHFV is known to be a significant human pathogen associated with fatalities. The only other members of the genus associated with disease in humans are Dugbe virus, Nairobi sheep disease virus, and, possibly, Erve virus, which have all been reported as causing mild, non-lethal disease in humans (Garrison et al. 2020). Although the classification of the Orthonairoviruses was originally based on antigenic

relationships, the groupings have subsequently been substantiated using molecular analyses to determine genetic relationships between the viruses (Calisher and Karabatsos 1989; Casals and Tignor 1980; Garrison et al. 2020).

Members of the genus *Orthonairovirus* are spherical structures, 90–120 nm in diameter (Donets et al. 1977). As for all the orthonairoviruses, the genome of CCHFV is comprised of three single-stranded RNA segments in a negative-sense orientation. The segments, designated small (S), medium (M), and large (L), encode for the viral nucleoprotein (NP), glycoprotein precursor (GPC), and RNA dependent RNA polymerase (RdRp) proteins, respectively (Schmaljohn and Patterson 1990). Each of the three RNA segments is encapsulated in the viral encoded nucleoprotein to form ribonucleoprotein particles (RNP) within the virion (Clerx et al. 1981). Each segment comprises a non-translated region (NTR) at the 3′ and 5′ termini flanking a single transcriptional unit. The NTRs have genus specific sequences at the termini, with cis-acting signals for RNA synthesis and segment packaging, and internal regions that are neither genus specific nor conserved between segments within a species, which are likely associated with genome replication (Barr et al. 2003). The 3′ and 5′ NTRs have significant nucleotide complementarity, and base pairing of these regions leads to formation of panhandles resulting in circular conformation of each segment. The base-pairing structure likely provides a functional promotor region for the viral polymerase, RdRp (Flick et al. 2002).

The L segment of CCHFV is approximately 12,000 bases in length and has a single ORF encoding for a large, approximately 450 kDa, protein (Honig et al. 2004; Kinsella et al. 2004). The L protein of CCHFV, and other orthonairoviruses, is significantly larger than the RdRps of other orthonairoviruses and comparison of sequence data led to the identification of an ovarian tumor (OTU) like protease motif in the amino terminal region of the L protein (L-OTU) of CCHFV followed by a zinc finger motif and helicase domain (Honig et al. 2004). The OTU domains are a superfamily of proteases. Viral OTU domains specifically have ubiquitin (UB) deconjugating activity. Conjugation of UB and ubiquitin like (UBL) molecules to specifically targeted proteins plays a role in the regulation of innate immune responses (Frias-Staheli et al. 2007). Deconjugation of these molecules has an inhibitory effect on antiviral pathways dependent on UB and UBL activation. The size of the polyprotein and the identification of amino terminal domains led to the proposal that the L protein is potentially a polyprotein that is cleaved auto-proteolytically. Although in the absence of L-OTU activity the RdRp has been shown to function, and viral replication is unaffected; to date there is no evidence of proteolytic processing of the L protein and the function of the L-OTU has yet to be fully clarified (Bergeron et al. 2010). The protease could likely have a role in evasion of the host immune response by suppression of the innate immunity activated by UB and UBL molecules (Bergeron et al. 2010; Frias-Staheli et al. 2007).

The synthesis of orthonairovirus glycoproteins, encoded on M segment RNA, appears to involve a precursor polypeptide, a coding strategy which is quite distinct from that used by other genera of orthonairoviruses. The M segment of CCHFV is approximately 5400 bases and has one ORF, which encodes a precursor polypeptide with a highly variable amino-terminal domain and a fairly conserved carboxyl-terminal region. The two mature glycoproteins, Gn (37 kDa) and Gc (75 kDa), are

derived by signalase cleavage of two precursors, designated preGn (140 kDa) and preGc (85 kDa) (Sanchez et al. 2002). Post-translational cleavage of the precursors by SKI-1 and SKI-1 like proteases generates mature Gn and Gc and a mucin-like and GP38 domain (Sanchez et al. 2006). Formation of infectious virus has been shown to be dependent on the presence of cellular serine endoprotease (Bergeron et al. 2007). Further processing of the mucin like GP38 domain by furin/proprotein convertases generates three secreted glycoproteins GP160, GP85, and GP38 of unknown function. More recently, a non-structural (NSm) protein has been identified although its function is currently unknown (Altamura et al. 2007).

By analogy with other genera of the *Nairoviridae*, it can be assumed that glycoproteins are responsible for recognition of receptor sites on susceptible cells and consequently cell tropism and pathogenicity of the virus in humans, for the induction of protective immune response, and probably play a role in tick host selection. Monoclonal antibodies directed against Gc have been shown to prevent CCHFV infection using in vitro neutralization assays, although not all protected mice against lethal infection in passive immunization experiments (Bertolotti-Ciarlet et al. 2005). In contrast, non-neutralizing monoclonal antibodies directed against Gn protected mice against lethal challenge suggesting a role for antibody dependent cell-mediated cytotoxicity in viral clearance (Bertolotti-Ciarlet et al. 2005). The exact immune correlates of protection have yet to be determined.

The CCHFV S segment is approximately 1600 bases in length with a single open reading frame which encodes a 482 amino acid NP (approximately 54 kDa), the major structural protein of the virus (Marriott and Nuttall 1992). Viral RNA is encapsidated by NP to form the ribonucleoprotein (RNP) complex. The exact mechanism of this interaction is unknown; complex formation, however, is essential for RNA synthesis and segment packaging.

CCHFV is classified as a class four pathogen because it has the propensity for human-to-human transmission, can be responsible for laboratory infections, and causes severe human disease with possible fatal outcome. This dictates that culture of the virus is permitted only in biosafety level four, maximum-security, laboratories.

47.2.2 CCHFV in Nature

The importance of the tick-vertebrate-tick cycle in maintaining CCHFV transmission is well established. Although the virus has been isolated from at least 31 species of ticks of 7 genera, including 29 ixodids and 2 argasids, in most instances the isolations likely resulted from recent ingestion of a blood meal from a viraemic host and are therefore no definite proof that these ticks can act as competent vectors in all these cases (Hoogstraal 1979; Watts et al. 1989). However, the distribution of human cases, serological evidence, and virus isolations from ticks correlate exactly with that of the ticks belonging to the genus *Hyalomma*, providing strong evidence that these ticks are the principal vectors associated with CCHFV (Hoogstraal 1979; Watts et al. 1989). Transtadial transmission has been shown for members of three genera of ixodid ticks, *Hyalomma*, *Dermacentor*, and *Rhipicephalus* (Hoogstraal 1979; Watts

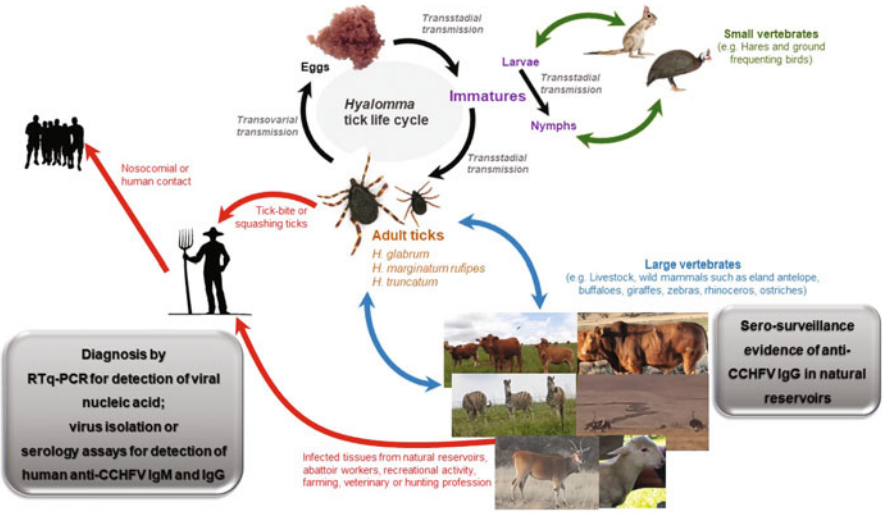

Fig. 1 The natural cycle of Crimean-Congo hemorrhagic fever orthonairovirus in a two host tick

et al. 1989). Similarly, transovarial transmission has been shown to occur within some species from these genera (Gonzalez et al. 1992; Gordon et al. 1993; Logan et al. 1989; Wilson et al. 1991).

Hyalomma ticks are referred to as two-host-ticks with regards to their life cycle in nature. Each stage in the tick life cycle attaches to a vertebrate host and takes a blood meal before molting to the next instar. The immature ticks, larvae, and nymphs, attach to small vertebrate hosts and feed before molting to the next instar. Infection acquired by the larvae feeding on a viremic host can be transmitted after the molt to the next instar. Similarly, virus acquired by the immature stages can be transmitted to adult ticks. Transovarial transmission of CCHFV to larvae, and the ability of larvae to transmit infection to vertebrates, was found to occur but at low levels considered below a threshold that would be adequate to perpetuate the virus in the absence of amplification in mammalian hosts (Hoogstraal 1979; Watts et al. 1989). An illustration of the natural cycle of CCHFV in ticks is shown in Fig. 1.

47.2.3 Zoonotic Hosts and Their Role in Transmission

Small mammals are considered important amplifying hosts of CCHFV. Viremia has been demonstrated in several small vertebrates such as little susliks, hedgehogs, and scrub hares (Shepherd et al. 1989a; Watts et al. 1989) and in some instances it has been shown that these hosts are capable of infecting ticks (Hoogstraal 1979; Shepherd et al. 1989a, 1991; Watts et al. 1989). Domestic livestock show mild or no clinical signs of illness but develop a short period of viremia, lasting up to a week, during which the virus can be transmitted to humans and to naïve ticks. Humans acquire infection through broken skin from infected blood or tissues while

performing procedures on animals such as castration or during slaughtering. Hence, there is an occupational risk associated with employment in the livestock industry among farmers, farm workers, abattoir workers and veterinarians. Within abattoirs, infection tends to occur among workers involved in handling animals during the initial bleeding stages of the slaughter process or the workers handling animal hides that are likely tick infested. Virus infectivity is likely reduced as the pH of the tissues decreases after time reducing the risk to workers at later stages in the slaughter process and to meat consumers (Swanepoel et al. 1998).

Immature *Hyalomma* ticks feed on small mammals and ground-frequenting birds, and this plays a significant role in maintenance of the virus in nature (Hoogstraal 1979; Watts et al. 1989). The role of large vertebrates in the cycle of CCHFV in nature is limited by the low frequency of transovarial transmission from adult ticks. Acquisition of infection by immature ticks on small vertebrates likely constitutes the most important amplifying mechanism, which ensures perpetuation of the virus (Watts et al. 1989).

Birds tend to be refractory to CCHF infection, with the exception of ostriches, which, in the absence of clinical disease, develop a high-level viremia demonstrable for 4 days and a strong antibody response following experimental infection (Hoogstraal 1979; Shepherd et al. 1987; Swanepoel et al. 1998). However, an additional mechanism of transmission referred to as "non-viraemic" transmission has been demonstrated in birds in which infected ticks are able to transfer infection to uninfected ticks during feeding on a non-viremic host. Non-viremic transmission of infection between ticks is believed to be facilitated by factors present in tick saliva (Jones et al. 1987) and has been demonstrated for CCHFV using infected adult and non-infected immature *H. truncatum*, and *H. impeltatum* ticks fed together on non-viremic mammals.

Serological surveillance of domestic livestock, birds, and wild animals have detected anti-CCHFV antibody in a wide range of species (Spengler et al. 2016). With the exception of humans, CCHFV is not known to be pathogenic in other species and infections are asymptomatic. The role of many of these species as reservoirs and/or amplifying hosts is not always clear, as some may be incidental hosts. Infected domestic livestock are, however, an important source of transmission and antibody detection in these animals has provided one of the most important markers for determining the presence and prevalence of CCHFV within regions.

47.2.4 Epidemiology and Genetic Diversity

CCHFV has the most extensive worldwide distribution of any of the arboviruses. Prior to 2002, cases of naturally acquired human infection had been documented in the former Soviet Union, China, Bulgaria, Yugoslavia, Albania, Kosovo (formerly Yugoslavia), Afghanistan, Pakistan, Iran, Iraq, United Arab Emirates, Saudi Arabia, Oman, Tanzania, Central African Republic, Democratic Republic of the Congo (formerly Zaire), Uganda, Kenya, Mauritania, Burkina Faso, South Africa, and Namibia (Al Tikriti et al. 1981; Burney et al. 1980; Drosten et al. 2002a, b; Dunster et al. 2002; El Azazy and Scrimgeour 1997; Gear et al. 1982; Hassanein et al. 1997; Hoogstraal 1979; Khan et al. 1997; Papa et al. 2002b; Saluzzo et al. 1984, 1985;

Schwarz et al. 1995; Suleiman et al. 1980; Tantawi et al. 1980; Watts et al. 1989; Yen et al. 1985). More recently, reports of human infections have been documented in India, Sudan, Senegal, Greece, Nigeria, and Georgia (Aradaib et al. 2010; Bukbuk et al. 2016; Mishra et al. 2011; Nabeth et al. 2004; Papa et al. 2010; Zakhashvilli et al. 2010).

Evidence for the presence of CCHFV either from isolation of the virus or detection of viral nucleic acid from ticks or non-human mammals has been documented in Egypt, Madagascar, Senegal, Nigeria, Central African Republic, Ethiopia, and, more recently, Morocco (Bendary et al. 2022; Palomar et al. 2013; Watts et al. 1989). Serological evidence alone either in humans or livestock has been reported from Cameroon, Mozambique, Egypt, Zimbabwe, Benin, Kuwait, Portugal, and France (Muianga et al. 2017; Watts et al. 1989).

In most countries, cases occur sporadically; however, cases in Turkey occur far more frequently for reasons that are not known. The virus was first identified in Turkey in 2002 and the country now has the highest number of laboratory confirmed cases globally with over 10,000 cases reported and a fatality rate of approximately 5% (Leblebicioglu et al. 2016; Maltezou et al. 2010).

Whereas recent identification of the virus in African countries is more likely due to increased awareness rather than emergence and spread, in Europe the virus appears to have spread to new endemic regions. Serological evidence of CCHFV was recently detected in Romania (Ceianu et al. 2012) and in 2010 virus was detected in adult *Hyalomma lusitanicum* ticks collected from red deer (*Cervus elaphus*) in Spain (Estrada-Peña et al. 2012). Phylogenetic analysis showed the virus was genetically similar to strains circulating in Africa. CCHFV detected in ticks collected from migratory birds in Morocco showed 100% identity with isolates from Sudan and Mauritania and 98.9% identity with the isolate from Spain (Palomar et al. 2013). The first autochthonous case of CCHF in south western Europe was reported from Spain in 2016 with additional cases reported in 2018 and 2020 (European Centre for Disease Prevention and Control 2022; Negredo et al. 2017; Portillo et al. 2021). Phylogenetic analysis placed the human isolate in genotype III (Africa III) confirming the genetic similarity with previous isolates from ticks collected in Spain and Morocco (de Arellano et al. 2017).

The emergence of CCHFV from 2002 in several countries in the Balkans and re-emergence in southwestern regions of the Russian Federation in 1999 after a 27 year absence had previously raised concerns that this virus could expand its current geographic distribution and establish new endemic foci (Maltezou et al. 2010). Serological evidence of the virus circulating in livestock in Bosnia and Herzogovina and human cases identified in Spain have confirmed the distribution of this virus extends from southwestern Europe to the western most region of the Balkans (Satrovic et al. 2022).

The reasons for reemergence are likely multi-factorial and include global warming with changes in weather patterns that influence tick populations, increased animal movement as a result of livestock trade, as well as human activities such as changes in farming practices and land development (Maltezou et al. 2010; Maltezou and Papa 2010; Randolph and Rigers 2007).

Molecular methods have facilitated the identification and differentiation of genotypes of CCHFV. Partial and complete genome sequence data have been used to determine the genetic relationship between strains of CCHFV within specific geographic locations and between geographically distinct regions (Burt and Swanepoel 2005; Chinikar et al. 2004; Deyde et al. 2006; Drosten et al. 2002a, b; Hewson et al. 2004a, 2004b; Papa et al. 2002a, b, c, 2004, 2005; Seregin et al. 2004; Tonbak et al. 2006; Yashina et al. 2003). The studies concur that a high degree of nucleotide diversity exists although amino acid diversity is less, particularly within the NP, which could account for the serological cross-reactivity between geographically distinct isolates of the virus. Analysis of global diversity has shown that CCHFV isolates group within at least six genotypes, designated genotypes I, II, and III endemic within the African continent, genotype IV (Asia), V (eastern Europe or Europe 1), VI (Europe 2 or AP-92 like). More recently, genotype VI viruses, which include the AP-92 like isolates from Greece, have been reclassified into a novel orthonairovirus species *Congoid orthonairovirus*, distinct from *CCHF orthonairovirus* species. The AP-92 like viruses, now referred to as Aigai virus (AIGV), originally considered apathogenic for humans, have been associated with acute febrile disease (Papa et al. 2022).

Previous analysis of tree topologies for each segment has shown incongruencies in groupings, particularly for the M segment, providing evidence for the natural occurrence of segment reassortment (Burt et al. 2009; Deyde et al. 2006; Hewson et al. 2004b). There appears to be a higher frequency of reassortment events for the M segment. Alternatively, M segment switching may result in a more viable virus compared with other segment reassortment. RNA viruses have the ability to reassort when dual infection occurs. It has been proposed that these events are more likely to occur within vectors rather than vertebrate hosts as ticks remain infected for longer periods and are exposed to multiple hosts potentially infected by different strains of CCHFV (Deyde et al. 2006; Hewson et al. 2004b; Morikawa et al. 2007). Reassortment events provide a mechanism for genetic diversity and, although relatively rare, genetic recombinantion events have also been shown to occur for CCHFV contributing to genetic variability (Deyde et al. 2006).

The same genotypes can be found in geographically distinct locations and different genotypes can be located in similar regions. CCHFV appears to circulate within and between continents with phylogeny studies supporting the proposed mechanisms for dispersal of the virus. Genetic diversity within regions has likely resulted from movement and trade in livestock and bird migration with resultant introduction of multiple lineages from carriage of infected ticks. In addition, reassortment and recombination provide additional mechanisms for the generation of genetic diversity.

47.3 Disease in Humans

The incubation period of CCHFV in humans following infection from a tick bite is usually 1 to 3 days (maximum 9) and 3 to 6 days (maximum 13) from exposure to infected blood or tissues of animals or humans including secondary infections in a

nosocomial setting. The onset of illness is often sudden, with non-specific symptoms including headache, dizziness, sore throat, sore eyes, and photophobia. Myalgia and malaise are also common with backache and leg pains. High fever and rigors often become apparent at this stage and the fever may be intermittent. Gastrointestinal symptoms may include nausea, vomiting, diarrhea, and abdominal pain. During the early stages of illness, lassitude, depression, and somnolence may be noted as well as neuropsychiatric changes such as confusion and aggression. Other signs include hyperemia of the face, neck, and chest; injected conjunctivae; and chemosis. Hepatomegaly with right hypochondrial pain, tachycardia, and hypovolemia are often present. On days 3 to 6 of illness, a petechial rash often appears especially on the trunk and limbs. This may progress to large ecchymoses and bruising (Hoogstraal 1979; Kilinc et al. 2016; Swanepoel et al. 1987). Other skin presentations include a macular or maculopapular rash, and facial rash (Akyol et al. 2010; Duygu et al. 2018; Ergönül et al. 2004). When present, hemorrhagic manifestions appear at day 4 to 5 including hematemesis, melena, hematuria, epistaxis, vaginal and gingival bleeding. Subconjunctival and retinal hemorrhages have also been described (Engin et al. 2009). In other cases, the bleeding tendency may be limited to leakage or oozing from injection or venipuncture sites. Jaundice may be present during the second week of illness (Hoogstraal 1979; Swanepoel et al. 1987). Isolated cases of epididymo-orchitis, parotitis, peritoneal and pleural effusions, acalculous cholecystitis, intraabdominal abscesses, compartment syndrome, and ascending paralysis have been described during the acute stage of disease (Aijazi et al. 2020; Aksoy et al. 2010; Guner et al. 2011; Kaya et al. 2012; Kerget et al. 2021; Prasad et al. 2020; Sahin et al. 2016; Şensoy et al. 2011; Ture et al. 2016). Although uncommon, hemophagocytic lymphohistiocytosis has also been described in both adult and pediatric patients (Gayretli Aydin et al. 2021; Yakut et al. 2021).

Fatalities are usually due to multiorgan failure or hemorrhagic complications such as intracranial hemorrhage, occurring mostly on days 5 to 14 of illness. The mortality rate is reported to be 5–50% with higher mortality rates following nosocomial infections than for infection via tick bites (Gozalan et al. 2007; Hoogstraal 1979). Distinct geographical variations in mortality rates are noted, with rates in Turkey described at 1–5% compared to approximately 30% in South Africa (Ergönül et al. 2006a; National Institute for Communicable Diseases 2020; Swanepoel et al. 1987). Differences observed in mortality rates may be influenced by the significantly higher numbers of cases occurring in eastern Europe compared to South Africa, as well as likely circulation of strains of low virulence identified in Greece and Turkey (Elevli et al. 2010; Midilli et al. 2009; Ozkaya et al. 2010). Recovery usually begins by day 9 or 10 of illness, although conjunctivitis, weakness, confusion, and amnesia may persist beyond a month. Other residual symptoms and signs may include polyneuritis, headache, dizziness, nausea, anorexia, alopecia, vision and hearing loss, and poor memory (Hoogstraal 1979; Swanepoel et al. 1987). Post-traumatic stress disorder and impaired health-related quality of life have been described in the long-term follow up of CCHF patients, with intensive care admission, bleeding, and administration of blood products identified as risk factors (Gul et al. 2012). Similar findings have been described in survivors of other acute, life-threatening

conditions and it seems likely that this is related to the severity of the illness rather than the infectious organism.

The clinical picture of CCHFV infection in children is similar to that in adults, although tonsillopharyngitis and gastrointestinal complaints are more common in this age group. While initial reports which included relatively small number of cases identified in children suggested a tendency toward milder disease, reported mortality rates have varied widely (Aslani et al. 2017; Dilber et al. 2009; Tezer et al. 2010; Tuygun et al. 2012). Intrauterine or perinatal infection of infants has been described following maternal infection with CCHFV during pregnancy and resulting in abortion or hemorrhagic manifestation at birth, with high rates of maternal and fetal/neonatal deaths reported (Ergönül et al. 2010; Pshenichnaya et al. 2017). Transmission by breastfeeding has not been detected in exposed infants (Erbay et al. 2008).

Abnormal clinical pathology values in patients with CCHF include elevated aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatise (ALP), γ-glutamyltransferase (GGT), lactic dehydrogenase (LDH), creatine kinase (CK), bilirubin, and creatinine levels, respectively. These elevations are marked in patients with fatal infections. Leukocyte levels may be elevated or decreased, with leukocytosis more common in fatal cases. Thrombocytopenia is found in all patients with CCHF; low thrombocyte counts at an early stage of illness are associated with an increased mortality. In addition, markedly abnormal prothrombin ratio (PR), activated partial thromboplastin time (APPT), thrombin time (TT), fibrinogen, and fibrin degradation products (FDP) are found early in patients with a fatal outcome, with milder abnormalities in nonfatal cases. The hemoglobin levels often decline even in the absence of overt bleeding (Ergönül et al. 2006a; Swanepoel et al. 1989).

47.4 Pathogenesis

Many similarities exist between the pathogenesis of CCHF and other viral hemorrhagic fevers. Following inoculation, the virus replicates in local cells before spreading to regional lymph nodes. Local cells which may act as early targets for viral infection include macrophages, dendritic cells, and Langerhans cells, which have been shown to be susceptible and permissive to CCHFV infection (Connolly-Andersen et al. 2009; Peyrefitte et al. 2010; Rodriguez et al. 2018; Welch et al. 2019). The virus then disseminates hematogenously to various tissues and organs both in lymph and blood monocytes. Early in infection, impairment of the innate immune response by CCHFV may delay activation of the adaptive immune response, which prevents viral clearance and therefore facilitates viral replication, particularly in the liver and spleen, and dissemination (Bente et al. 2010; Peyrefitte et al. 2010; Saksida et al. 2010). High levels of viral replication in organs such as the liver and adrenal glands contribute to the clinical picture by decreasing synthesis of coagulation and plasma proteins and dysregulation of blood pressure homeostasis (Geisbert and Jahrling 2004). This is supported by histopathological findings of coagulative necrosis in the liver, kidneys, and adrenal glands (Burt et al. 1997). The

liver injury which occurs during CCHFV infection occurs due to the direct effects of viral replication, but also due to the activation of extrinsic death receptor signaling pathways (Lindquist et al. 2018). The effect of CCHFV on endothelial cells resulting in capillary leakage appears to be exerted chiefly by immunologically mediated mechanisms including immune complex deposition and complement activation, although direct viral replication in endothelial cells has been demonstrated (Connolly-Andersen et al. 2011; Joubert et al. 1985). Release of inflammatory mediators such as tumor necrosis factor alpha (TNF-α) also increases endothelial permeability. Increased levels of TNF-α are significantly associated with severe CCHF disease (Ergönül et al. 2006b; Papa et al. 2006). TNF-α is known to be associated with macrophage activation leading to hemophagocytosis and also stimulates vasodilating substances and antifibrinolytic activity. Interleukin-6 (IL-6), which is released by Kupffer cells due to liver injury, is increased in all patients with CCHF including both mild and severe disease presentations (Ergönül et al. 2006b; Papa et al. 2006). Both TNF-α and IL-6 are Th1 cytokines which stimulate activation of monocytes and contribute to hemophagocytosis. Hemophagocytosis has been described in both adults and children with CCHFV infection and may contribute to the cytopenias observed (Dilber et al. 2009; Fisgin et al. 2008; Karti et al. 2004). High levels of interleukin-10 (IL-10) and interferon gamma (IFN-γ) are also associated with a fatal outcome (Ergönül et al. 2006b). It seems likely that high levels of IL-10 released early in infection result in a degree of immunosuppression, which allows high levels of viral replication. This in turn stimulates release of IFN-γ and TNF-α (Saksida et al. 2010). These findings are also supported by studies in mouse models (Bente et al. 2010). RIG-1 has been identified as a pattern recognition receptor for CCHFV, which results in activation of type 1 interferon and other proinflammatory cytokines that play a role in the antiviral response (Spengler et al. 2015). The pathogenesis of CCHF also includes disseminated intravascular coagulopathy (DIC) early in infection (Swanepoel et al. 1989).

47.5 Laboratory Diagnosis

The recent emergence and re-emergence of CCHFV in Eastern Europe, southwestern Europe, and the Balkans and the threat of spread to new geographic locations where competent vectors are emerging, emphasizes the importance of increasing diagnostic capacity and developing standardized, rapid, and sensitive assays. Although human cases of infection with CCHFV can be identified based on clinical and laboratory criteria, laboratory confirmation is essential for distinguishing CCHF from conditions with similar clinical features (Ergönül 2006; Swanepoel et al. 1987, 1989). Classification of the virus as a biosafety level four pathogen determines that the virus can only be cultured within the confines of a biosafety level four laboratory and that laboratories with less sophisticated biosafety levels must inactivate clinical samples prior to testing.

Virus can be isolated in a variety of susceptible mammalian cell cultures, although Vero cells are most frequently used. The virus seldomly induces cytopathic

effects and infection must be confirmed using IF tests. Alternatively, the virus can be isolated by intracerebral inoculation of day-old mice (Clerx et al. 1981; Hoogstraaal 1979; Watts et al. 1989). Isolation of the virus in cell cultures can take 1–6 days whereas mice need 5–10 days to succumb to infection (Shepherd et al. 1986). Although isolation in cells is more rapid, mouse inoculation is a more sensitive technique.

During the acute stage of illness, viral nucleic acid can be readily amplified and detected using reverse transcription polymerase chain (RT-PCR) assays. Viral RNA is extracted from clinical samples and as negative sense RNA is not infectious, the amplification can be performed without the requirement of a biosafety level four laboratory. Diagnostic RT-PCR is based on amplification of a conserved region of the genome.

The first diagnostic RT-PCR for CCHFV was based on two nested primer pairs designed by an alignment of the S segment from seven geographically distinct isolates (Burt et al. 1998; Rodriguez et al. 1997; Schwarz et al. 1995). Subsequently, there has been significant development and implementation of diagnostic real time RT-PCR assays. Amplicons can be detected using an intercalating dye and the use of a melt curve analysis to detect specific amplified products or sequence specific probes in which probes are hybridized to complementary regions of the genome (Burt et al. 1998; Drosten et al. 2002a, b; Duh et al. 2006, 2007; Garrison et al. 2007; Kondiah et al. 2010; Papa et al. 2007; Rodriguez et al. 1997; Schwarz et al. 1995; Wölfel et al. 2007; Wölfel et al. 2009; Yapar et al. 2005). Real time molecular assays can be designed to determine viral load. Quantification of viral load using real time RT-PCR has been used as a prognostic indicator with reports that a viral load greater than $1x10^8$ RNA copies/ml plasma can be considered to predict a fatal outcome (Cevik et al. 2007; Duh et al. 2006; Garrison et al. 2007; Kondiah et al. 2010; Wölfel et al. 2007). The considerable genetic diversity of the various genotypes must be taken into consideration when developing molecular assays. Hence, most molecular assays have targeted conserved regions of the gene encoding the nucleoprotein. To facilitate diagnosis and eliminate the need for sophisticated laboratory equipment, nucleic acid tests (NAAT) have been described that do not require thermal cyclers, such as reverse transcription loop–mediated isothermal amplification (RT-LAMP) and recombinase polymerase amplification assay (Bonney et al. 2017; Kumar et al. 2022; Osman et al. 2013). These assays targeted conserved regions of the S gene and were shown to have good correlation with conventional assays with regard to sensitivity. Rapid isothermal assays have potential application in field settings.

Enzyme linked immunosorbent assays (ELISA) have been described for detection of viral antigen. Although having the advantage that sophisticated laboratory equipment is not required, they lack the sensitivity of molecular amplification or viral isolation and are not frequently used for routine diagnosis (Saijo et al. 2005a; Shepherd et al. 1988).

Serological assays have an important diagnostic role during the convalescent stage of infection. Infection is confirmed based on demonstration of seroconversion, a fourfold or greater increase in IgG antibody activity in paired serum samples, or IgM activity in a single specimen. In contrast, indirect immunofluorescence

(IF) assays and ELISA are frequently employed as diagnostic tools. These assays can distinguish between IgG and IgM responses and are rapid and sensitive techniques for detecting an early immune response (Burt et al. 1994; Shepherd et al. 1989b). Although traditionally most reagents were prepared in house requiring culture of the virus in maximum containment laboratories with subsequent inactivation of the reagents, there are now commercially available ELISA and IF assays. In addition, recombinant antigens have been developed for use as diagnostic and surveillance tools (Samudzi et al. 2012).

In fatal cases, antigen and viral nucleic acid can be detected in post mortem tissues. Histopathologic features are not pathognomonic and definitive diagnosis requires virological assays or antigen detection in formalin fixed tissue samples using immunohistochemistry (Burt et al. 1997).

To date no commercial rapid lateral flow assays (LFA) are available for point of care testing. Development of LFA for detection of CCHFV antigen, and for detection of IgG and IgM antibody, would have important public health implications particularly in low resource countries.

47.6 Kinetics of Viremia and Antibody Responses

CCHFV is most frequently isolated from sera collected from patients on days 1 to 6 after onset of illness when virus titers are highest, although virus has also been isolated from samples collected from days 1 to 12 (Shepherd et al. 1986). Viral nucleic acid has been detected in samples collected up to 18 days after onset of illness; the diagnostic sensitivity, however, decreases with the presence of an antibody response. The development of molecular assays has significantly improved diagnostic capability during the acute stage of illness when patients lack immune markers and virus isolation is dependent on severity of illness and levels of viremia. In non-fatal infections, the ability to isolate virus decreases from days 7 to 12. Although IgG and IgM antibodies have been detected as early as day 3 of illness, they are more frequently detected from day 5 onwards (Burt et al. 1994; Saijo et al. 2005b; Shepherd et al. 1989b; Tang et al. 2003). Patients with a fatal outcome frequently do not develop a detectable antibody response. IgG antibodies remain detectable at least 10 to 12 years after illness and possibly for longer, whereas IgM antibodies decline to undetectable levels in most patients 4–6 months post-infection (Burt et al. 1994; Shepherd et al. 1989b).

To date it is unclear what facilitates clearance of the virus. Appearance of a humoral antibody response does not always correlate with clearance of CCHFV. Conserved immunogenic epitopic regions in the G_C, G_N, GP38, and mucin like domain have been described; however, although immunogenic, these epitopes are unlikely to be involved in inducing neutralizing responses (Fritzen et al. 2018; Goedhals et al. 2015). Although survivors develop neutralizing antibody responses, the levels of neutralizing antibody remain low (Shepherd et al. 1989b). The role and mechanism of non-neutralizing antibodies is still to be determined. In an investigation to try and elucidate the effect of antibodies on viral load, it was deduced that the detection of IgM had no influence on survival or viral load. While IgG levels

appeared to be inversely related to viral load, the virus titers decreased in non-fatal infections independent of detectable antibodies (Duh et al. 2007; Wölfel et al. 2007). The results indicate a role for innate or cellular immune responses in viral clearance.

In summary, during the acute phase of illness confirmation of infection is achieved by isolation of the virus, detection of viral nucleic acid using molecular techniques, or detection of viral antigen in ELISA. During convalescent stages antibody responses are detected using ELISA or indirect IF assays. Accurate interpretation of the results is facilitated by an accurate history of date of onset of illness and consideration of the kinetics of viremia and antibody responses.

47.7 Differential Diagnosis

A number of conditions must be considered in the differential diagnosis of CCHFV infection. The geographic location and travel history of patients presenting with a compatible clinical picture can assist in excluding unlikely infectious conditions. Conditions which should be considered include other tick-borne infections such as rickettsiosis (especially by *Rickettsia conori* and *Rickettsia africae*), Q fever (*Coxiella burnetti*), ehrlichiosis, babesiosis, borreliosis, and severe fever with thrombocytopenia syndrome virus. Other viral hemorrhagic fevers must be considered dependent on the geographic location, e.g., Ebola virus, Marburg virus, Lassa virus, and Lujo virus in Africa, Rift Valley fever virus in Africa and the Middle East, yellow fever virus in Africa, South and Central America, and dengue virus in tropical and subtropical regions. In addition, other infectious conditions should be ruled out such as bacterial sepsis (including meningococcaemia), malaria, leptospirosis, salmonellosis, brucellosis, viral hepatitis, and disseminated herpes simplex virus infection (Burt 2011; Ergönül 2006). Non-infectious conditions may include hematological diseases and malignancies, drugs, auto-immune diseases, and HELLP syndrome (hemolytic anaemia, elevated liver enzymes, low platelet count) in pregnancy (Ergönül et al. 2010; van Eeden et al. 1985).

47.8 Treatment

Strict barrier nursing should be implemented when managing CCHF patients to prevent nosocomial transmission. Supportive therapy should include maintenance of fluid and electrolyte balance and administration of platelets, fresh frozen plasma, and red cell preparations as needed (Ergönül 2008).

Limited information is available regarding the use of specific CCHFV immunoglobulin or monoclonal antibody preparations. Randomized controlled trials demonstrating efficacy in a clinical setting for both treatment and post-exposure prophylaxis are lacking (Keshtkar-Jahromi et al. 2011).

Similarly, the use of oral or intravenous ribavirin for the treatment of CCHF remains controversial. Although the majority of systematic reviews and meta-analyses of the available randomized trial and observational studies showed insufficient evidence for a clear benefit to using ribavirin in the treatment of CCHFV infection, this

antiviral agent is included in the World Health Organization Model List for Essential Medicines for treatment of viral hemorrhagic fevers (Ascioglu et al. 2011; Johnson et al. 2018; Soares-Weiser et al. 2010; World Health Organization 2021). In studies where a reduced risk of death was noted with ribavirin use, the timing of administration was an important consideration, with early initiation within the first 2–4 days of disease onset being required (Arab-Bafrani et al. 2019; Ergönül et al. 2018). Adverse drug effects are uncommon, but may include mild hemolytic anemia and thrombocytosis (Ergönül et al. 2004; Fisher-Hoch et al. 1995; Ozkurt et al. 2006).

Favipiravir (T-705) is a nucleoside analogue, which is approved in Japan for the treatment of influenza virus infections and has been shown to have activity against a number of RNA viruses, including Lassa virus (Shiraki and Daikoku 2020). Favipiravir has shown benefit in both mouse and non-human primate models for CCHF; however, clinical data are lacking (Dülger et al. 2020; Hawman et al. 2018, 2020; Oestereich et al. 2014). A novel nucleoside analogue, H44, was able to protect mice against lethal challenge and may warrant further studies (Wang et al. 2022).

Therapeutic plasma exchange (TPE) is another therapeutic modality that has been investigated in critically ill patients with CCHF. While a lower mortality rate was noted following TPE, patients receiving standard supportive therapy had a shorter duration of hospitalization and shorter time to recovery of platelet counts (Beştepe Dursun et al. 2021). A role for TPE in the management of CCHF thus remains unclear at present.

47.9 Developments in Vaccine Developments

Currently, there is one vaccine for human use that is only available in Bulgaria. The vaccine is an inactivated suckling mouse brain derived preparation (Papa et al. 2011). The vaccine elicits both T-cell responses and high levels of antibodies, but multiple doses are needed to induce antibodies with neutralizing activity (Mousavi-Jazi et al. 2012). Widespread use of the vaccine has been limited by the lack of clinical trials and efficacy data and due to concerns regarding myelin basic protein induced auto-immune and allergic responses (Dowall et al. 2017).

While the immune correlates of protection remain unclear, available data indicate that both humoral and cellular responses are required. Vaccine development has been facilitated by the availability of animal models including interferon α/β receptor knockout mice (IFNAR−/−), STAT-1 knockout mice, transiently immune-suppressed (IS) mice, and cynomolgous macaques. Vaccines targeting the viral nucleoprotein and/or glycoproteins currently under investigation, using various approaches including inactivated vaccines, subunit vaccines, virus-like replicon particles, viral vectored vaccines, DNA vaccines, mRNA, and plant-based vaccines (Aligholipour Farzani et al. 2019a, 2019b; Appelberg et al. 2022; Berber et al. 2021; Buttigieg et al. 2014; Canakoglu et al. 2015; Dowall et al. 2016; Garrison et al. 2017; Ghiasi et al. 2011; Hawman et al. 2021; Hinkula et al. 2017; Kortekaas et al. 2015; Pavel et al. 2020; Rodriguez et al. 2019; Scholte et al. 2019;

Spengler et al. 2019, 2021; Spik et al. 2006; Suschak et al. 2021; Tipih et al. 2021; Zivcec et al. 2018). A number of these vaccines have been shown to be both immunogenic and protective in animal models, thus warranting further investigation.

47.10 Prevention and Control

Widespread control of ticks using acaricides is an impractical approach to infection control. Prevention of infection and awareness of the disease is a more practical method of reducing the risk of infection and the number of cases. Exposure to tick bites should be minimized through the use of tick repellents such as pyrethroids and protective clothing by individuals who are at risk of CCHF through occupational or recreational activities. The correct removal of ticks should also be implemented. Anti-tick vaccines for use in animal amplifying hosts are a novel approach to the prevention of CCHF, which are still in the early stages of development (Manjunathachar et al. 2019; Shrivastava et al. 2020).

Although the use of ribavirin as post-exposure prophylaxis is controversial, it has been recommended for administration to high-risk contacts. High-risk cases include those with direct contact with infectious fluids, contamination of mucosae with infectious fluids, and needle stick injuries (de la Calle-Prieto et al. 2018; Ergönül et al. 2018). Ribavirin is usually well tolerated in this context, but side effects described include anemia, myalgia, allergic skin reactions, gastrointestinal symptoms (nausea, vomiting diarrhea), hemolysis, and mildly elevated liver functions (Guven et al. 2017).

47.11 Conclusions

CCHFV remains a pathogen of significant public health concern with the propensity to cause nosocomial infections and outbreaks among occupationally exposed workers, and people at risk of exposure to tick-bite due to recreational activities or residing in rural areas. Molecular assays based on amplification of conserved genome regions have facilitated a more rapid diagnosis, allowing laboratory confirmation to occur in the absence of high containment laboratories. However, in the absence of sophisticated equipment and skilled technicians, the development of rapid lateral flow assays and point of care assays are essential, especially for lower resource countries and to facilitate early detection to protect health care workers. The spread and emergence of this virus has been confirmed in recent years and this threat will continue with climate change and expansion of vector populations into new endemic regions. Vaccine development has traditionally been hampered by lack of a suitable animal model for challenge studies; however, several animal models have been described for CCHFV in recent and this has advanced development of vaccines. Further understanding of the immune correlates of protection will contribute toward development of an efficacious vaccine for a virus with potential to cause significant human disease. In the absence of vaccines or treatment, raising awareness of CCHFV as a tick-borne zoonosis is crucial.

References

Adams MJ, Lefkowitz EJ, King AMQ et al (2017) Changes to taxonomy and the international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses. Arch Virol 162:2505–2538. https://doi.org/10.1007/s00705-017-3358-5

Aijazi I, Al Shama FMA, Shandala Y et al (2020) Crimean-Congo haemorrhagic fever presenting with acute compartment syndrome of the extremities (think beyond normal infections). BMJ Case Rep 13:e232323. https://doi.org/10.1136/bcr-2019-232323

Aksoy HZ, Yilmaz G, Aksoy F et al (2010) Crimean-Congo haemorrhagic fever presenting as epididymo-orchitis. J Clin Virol 48:282–284

Akyol M, Özçelik S, Engin A et al (2010) Cutaneous manifestations of Crimean-Congo haemorrhagic fever: morbilliform eruptions may reflect a favourable outcome and not low platelet levels. Eur J Dermatol 20:523–524

Al Tikriti SK, Al Ani F, Jurji FJ et al (1981) Congo/Crimean haemorrhagic fever in Iraq. Bull WHO 59:85–90

Aligholipour Farzani T, Hanifehnezhad A, Földes K et al (2019a) Co-delivery effect of CD24 on the immunogenicity and lethal challenge protection of a DNA vector expressing nucleocapsid protein of Crimean Congo hemorrhagic fever virus. Viruses 11:75. https://doi.org/10.3390/v11010075

Aligholipour Farzani T, Földes K, Ergünay K et al (2019b) Immunological analysis of a CCHFV mRNA vaccine candidate in mouse models. Vaccine 7:115. https://doi.org/10.3390/vaccines7030115

Altamura LA, Bertolotti-Ciarlet A, Teogler J (2007) Identification of a novel C-terminal cleavage of Crimean-Congo hemorrhagic fever virus PreGN that leads to generation of an NSM protein. J Virol 81:6632–6642

Appelberg S, John L, Pardi N et al (2022) Nucleoside-modified mRNA vaccines protect IFNAR$^{-/-}$ mice against Crimean-Congo hemorrhagic fever virus infection. J Virol 96:e0156821. https://doi.org/10.1128/JVI.01568-21

Arab-Bafrani Z, Jabbari A, Hashemi MM et al (2019) Identification of the crucial parameters regarding the efficacy of ribavirin therapy in Crimean-Congo haemorrhagic fever (CCHF) patients: a systematic review and meta-analysis. J Antimicrob Chemother 74:3432–3439. https://doi.org/10.1093/jac/dkz328

Aradaib IE, Erickson BR, Mustafa ME et al (2010) Nosocomial outbreak of Crimean-Congo hemorrhagic fever. Sudan Emerg Infect Dis 16(5):837–839. https://doi.org/10.3201/eid1605.091815

Ascioglu S, Leblebicioglu H, Vahaboglu H et al (2011) Ribavirin for patients with Crimean-Congo haemorrhagic fever: a systematic review and meta-analysis. J Antimicrob Chemother 66:1215–1222

Aslani D, Salehi-Vaziri M, Baniasadi V et al (2017) Crimean-Congo hemorrhagic fever among children in Iran. Arch Virol 162:721–725

Barr JN, Elliott RM, Dunn EF et al (2003) Segment specific terminal sequences of Bunyamwera bunyavirus regulate genome replication. Virology 311:326–338

Bendary HA, Rasslan F, Wainwright M et al (2022) Crimean-Congo hemorrhagic fever virus in ticks collected from imported camels in Egypt. Saudi J Biol Sci 29(4):2597–2603. https://doi.org/10.1016/j.sjbs.2021.12.043

Bente DA, Alimonti JB, Wun-Ju S et al (2010) Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. J Virol 84:11089–11100

Berber E, Çanakoğlu N, Tonbak Ş et al (2021) Development of a protective inactivated vaccine against Crimean-Congo hemorrhagic fever infection. Heliyon 7:e08161. https://doi.org/10.1016/j.heliyon.2021.e08161

Bergeron E, Vincent MJ, Nichol ST (2007) Crimean-Congo hemorrhagic fever virus glycoprotein processing by the endoprotease SKI-1/S1P is critical for virus infectivity. J Virol 81:13271–13276

Bergeron E, Albarino CG, Khristova ML et al (2010) Crimean-Congo hemorrhagic fever virus-encoded ovarian tumor protease activity is dispensable for virus RNA polymerase function. J Virol 84:216–226

Bertolotti-Ciarlet A, Smith J, Strecker K et al (2005) Cellular localization and antigenic characterization of Crimean-Congo hemorrhagic fever virus glycoproteins. J Virol 79:6152–6162

Beştepe Dursun Z, Korkmaz S, Türe Z et al (2021) Efficacy of therapeutic plasma exchange in patients with Crimean-Congo hemorrhagic fever. J Clin Apher 36:390–397. https://doi.org/10.1002/jca.21875

Bonney LC, Watson RJ, Afrough B et al (2017) A recombinase polymerase amplification assay for rapid detection of Crimean-Congo haemorrhagic fever virus infection. PLoS Negl Trop Dis 11(10):e0006013. https://doi.org/10.1371/journal.pntd.0006013

Bukbuk DN, Dowall SD, Lewandowski K et al (2016) Serological and virological evidence of Crimean-Congo haemorrhagic fever virus circulation in the human population of Borno state, northeastern Nigeria. PLoS Negl Trop Dis 10(12):e0005126. https://doi.org/10.1371/journal.pntd.0005126

Burney MI, Ghafoor A, Saleen M et al (1980) Nosocomial outbreak of viral hemorrhagic fever caused by Crimean hemorrhagic fever-Congo virus in Pakistan, January 1976. Am J Trop Med Hyg 29:941–947

Burt FJ (2011) Laboratory diagnosis of Crimean-Congo hemorrhagic fever virus infections. Future Virol 6:831–841

Burt FJ, Swanepoel R (2005) Molecular epidemiology of African and Asian Crimean-Congo haemorrhagic fever isolates. Epidemiol Infect 133:659–666

Burt FJ, Leman PA, Abbott JC et al (1994) Serodiagnosis of Crimean-Congo haemorrhagic fever. Epidemiol Infect 113:551–562

Burt FJ, Swanepoel R, Shieh WJ et al (1997) Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. Arch Pathol Lab Med 121:839–846

Burt FJ, Leman PA, Smith JF et al (1998) The use of a reverse transcription-polymerase chain reaction for the detection of viral nucleic acid in the diagnosis of Crimean-Congo haemorrhagic fever. J Virol Methods 70:129–137

Burt FJ, Paweska JT, Ashkettle B et al (2009) Genetic relationship among southern African Crimean-Congo haemorrhagic fever virus isolates and evidence for occurrence of reassortment. Epidemiol Infect 137:1302–1308

Buttigieg KR, Dowall SD, Findlay-Wilson S et al (2014) A novel vaccine against Crimean-Congo haemorrhagic fever protects 100% of animals against lethal challenge in a mouse model. PLoS One 9:e91516. https://doi.org/10.1371/journal.pone.0091516

Calisher CH (1991) Bunyaviridae. In: RJB R, Fauquet CM, Knidson DL, Brown F (eds) Classification of viruses. Fifth report of the international committee on taxonomy of viruses, Arch Virol, vol 121, pp S273–S283

Calisher CH, Karabatsos N (1989) Arbovirus serogroups: definition and geographic distribution. In: Monath TP (ed) The arboviruses: epidemiology and ecology, vol 1. CRC Press, Florida, pp 19–57

Canakoglu N, Berber E, Tonbak S et al (2015) Immunization of knock-out α/β interferon receptor mice against high lethal dose of Crimean-Congo hemorrhagic fever virus with a cell culture based vaccine. PLoS Negl Trop Dis 9:e0003579. https://doi.org/10.1371/journal.pntd.0003579

Casals J (1969) Antigenic similarity between the virus causing Crimean hemorrhagic fever and Congo virus. Proc Soc Exp Biol Med 131:233–236

Casals J, Tignor GH (1980) The Nairovirus genus: serological relationships. Intervirology 14:144–147

Ceianu CS, Panculescu-Gatej RI, Coudrier D et al (2012) First serologic evidence for the circulation of Crimean-Congo hemorrhagic fever virus in Romania. Vector Borne Zoonotic Dis 12:718–721

Cevik MA, Erbay A, Bodur H et al (2007) Viral load as a predictor of outcome in Crimean-Congo hemorrhagic fever. Clin Infect Dis 45:96–100

Chinikar S, Persson SM, Johansson M et al (2004) Genetic analysis of Crimean-Congo hemorrhagic fever virus in Iran. J Med Virol 73:404–411

Chumakov MP, Smirnova SE, Tkachenko EA (1970) Relationship between strains of Crimean haemorrhagic fever and Congo viruses. Acta Virol 14:82–85

Clerx JP, Casals J, Bishop DH (1981) Structural characteristics of nairoviruses (genus Nairovirus, Bunyaviridae). J Gen Virol 55:165–178
Connolly-Andersen AM, Douagi I, Kraus AA et al (2009) Crimean Congo hemorrhagic fever virus infects human monocyte-derived dendritic cells. Virology 390:157–162. https://doi.org/10.1016/j.virol.2009.06.010
Connolly-Andersen AM, Moll G, Andersson C et al (2011) Crimean-Congo hemorrhagic fever virus activates endothelial cells. J Virol 85:7766–7774
de Arellano ER, Hernández L, Goyanes MJ et al (2017) Phylogenetic characterization of Crimean-Congo hemorrhagic fever virus, Spain. Emerg Infect Dis 23(12):2078–2080. https://doi.org/10.3201/eid2312.171002
de la Calle-Prieto F, Martín-Quirós A, Trigo E et al (2018) Therapeutic management of Crimean-Congo haemorrhagic fever. Enferm Infecc Microbiol Clin (Engl Ed) 36(8):517–522. https://doi.org/10.1016/j.eimc.2017.04.007
Deyde VM, Khristova ML, Rollin PE et al (2006) Crimean-Congo hemorrhagic fever virus genomics and global diversity. J Virol 80:8834–8842
Dilber E, Cakir M, Acar EA et al (2009) Crimean-Congo haemorrhagic fever among children in North-Eastern Turkey. Ann Trop Paediatr 29:23–28
Donets MA, Chumakov MP, Korolev MB et al (1977) Physicochemical characteristics, morphology and morphogenesis of virions of the causative agent of Crimean hemorrhagic fever. Intervirology 8:294–308
Dowall SD, Buttigieg KR, Findlay-Wilson SJ et al (2016) A Crimean-Congo hemorrhagic fever (CCHF) viral vaccine expressing nucleoprotein is immunogenic but fails to confer protection against lethal disease. Hum Vaccin Immunother 12:519–527. https://doi.org/10.1080/21645515.2015.1078045
Dowall SD, Carroll MW, Hewson R (2017) Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine 35:6015–6023. https://doi.org/10.1016/j.vaccine.2017.05.031
Drosten CS, Gottig S, Schilling M et al (2002a) Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. J Clin Microbiol 40:2323–2330
Drosten S, Minnak D, Emmerich P et al (2002b) Crimean-Congo hemorrhagic fever in Kosovo. J Clin Microbiol 40(3):1122–1123. https://doi.org/10.1128/JCM.40.3.1122-1123.2002
Duh D, Saksida A, Petrovec M et al (2006) Novel one-step real-time RT-PCR assay for rapid and specific diagnosis of Crimean-Congo hemorrhagic fever encountered in the Balkans. J Virol Methods 133:175–179
Duh D, Saksida A, Petrovec M et al (2007) Viral load as predictor of Crimean-Congo hemorrhagic fever outcome. Emerg Infect Dis 13:1769–1772
Dülger AC, Yakarişik M, Uzun YE et al (2020) Treatment of Crimean-Congo haemorrhagic fever by favipiravir in a patient with novel coronavirus co-infection. Eur J Case Rep Intern Med 7:002042. https://doi.org/10.12890/2020_002042
Dunster L, Dunster M, Ofula V et al (2002) First documentation of human Crimean-Congo hemorrhagic fever, Kenya. Emerg Infect Dis 8:1005–1006
Duygu F, Sari T, Gunal O et al (2018) Cutaneous findings of Crimean-Congo hemorrhagic fever: a study of 269 cases. Jpn J Infect Dis 71:408–412. https://doi.org/10.7883/yoken.JJID.2018.005
El Azazy OM, Scrimgeour EM (1997) Crimean-Congo haemorrhagic fever virus infection in the western province of Saudi Arabia. Trans R Soc Trop Med Hyg 91:275–278
Elevli M, Ozkul AA, Civilibal M et al (2010) A newly identified Crimean-Congo hemorrhagic fever virus strain in Turkey. Int J Infect Dis 14:e213–e216
Engin A, Erdogan H, Ozec AV et al (2009) Ocular findings in patients with Crimean-Congo hemorrhagic fever. Am J Ophthalmol 147:634–638
Erbay A, Çevik MA, Önguru P et al (2008) Breastfeeding in Crimean-Congo haemorrhagic fever. Scand J Infect Dis 40:186–188
Ergönül Ö (2006) Crimean-Congo haemorrhagic fever. Lancet Inf Dis 6:203–214
Ergönül Ö (2008) Treatment of Crimean-Congo hemorrhagic fever. Antivir Res 78:125–131

Ergönül O, Çelikbaş A, Dokuzoğuz B et al (2004) Characteristics of patients with Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and impact of oral ribavirin therapy. Clin Infect Dis 39:284–287

Ergönül Ö, Celikbas A, Baykam N et al (2006a) Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. Clin Microbiol Infect 12:551–554

Ergönül Ö, Tuncbilek S, Baykam N et al (2006b) Evaluation of serum levels of interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha in patients with Crimean-Congo hemorrhagic fever. J Infect Dis 193:941–944

Ergönül Ö, Celikbas A, Yildrim U et al (2010) Pregnancy and Crimean-Congo haemorrhagic fever. Clin Microbiol Infect 16:647–650

Ergönül Ö, Keske Ş, Çeldir MG et al (2018) Systematic review and meta-analysis of postexposure prophylaxis for Crimean-Congo hemorrhagic fever virus among healthcare workers. Emerg Infect Dis 24:1642–1648. https://doi.org/10.3201/eid2409.171709

Estrada-Peña A, Palomar AM, Santibáñez P et al (2012) Crimean-Congo hemorrhagic fever virus in ticks, Southwestern Europe, 2010. Emerg Infect Dis 18:179–180

European Centre for Disease Prevention and Control (2022) Cases of Crimean–Congo haemorrhagic fever in the EU/EEA, 2013–present [Internet]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/surveillance/cases-eu-since-2013

Fisgin NT, Fisgin T, Tanyel E et al (2008) Crimean-Congo hemorrhagic fever: five patients with hemophagocytic syndrome. Am J Hematol 83:73–76

Fisher-Hoch SP, Khan JA, Rehman S et al (1995) Crimean Congo-haemorrhagic fever treated with oral ribavirin. Lancet 346:472–475

Flick R, Elgh F, Pettersson RF (2002) Mutational analysis of the Uukuniemei virus (Bunyaviridae family) promoter reveals two elements of functional importance. J Virol 76:10849–10860

Frias-Staheli N, Giannakopoulos NV, Kikkert M et al (2007) Ovarian tumor (OTU)-domain containing viral proteases evade ubiquitin- and ISG15-dependent innate immune responses. Cell Host Microbe 2:404–416

Fritzen A, Risinger C, Korukluoglu G et al (2018) Epitope-mapping of the glycoprotein from Crimean-Congo hemorrhagic fever virus using a microarray approach. PLoS Negl Trop Dis 12(7):e0006598. https://doi.org/10.1371/journal.pntd.0006598

Garrison AR, Alakbarova S, Kulesh DA et al (2007) Development of a TaqMan minor groove binding protein assay for the detection and quantification of Crimean-Congo hemorrhagic fever virus. Am J Trop Med Hyg 77:514–520

Garrison AR, Shoemaker CJ, Golden JW et al (2017) A DNA vaccine for Crimean-Congo hemorrhagic fever protects against disease and death in two lethal mouse models. PLoS Negl Trop Dis 11:e0005908. https://doi.org/10.1371/journal.pntd.0005908

Garrison AR, Alkhovsky SV, Avšič-Županc T et al (2020) ICTV virus taxonomy profile: *Nairoviridae*. J Gen Virol 101(8):798–799. https://doi.org/10.1099/jgv.0.001485

Gayretli Aydin ZG, Yesilbas O, Reis GP et al (2021) The first pediatric case of hemophagocytic lymphohistiocytosis secondary to Crimean-Congo haemorrhagic fever successfully treated with therapeutic plasma exchange accompanying ribavirin and intravenous immunoglobulin. J Clin Apher 36:780–784. https://doi.org/10.1002/jca.21915

Gear JH, Thomson PD, Hopp M et al (1982) Congo-Crimean haemorrhagic fever in South Africa. Report of a fatal case in the Transvaal. S Afr Med J 62:576–580

Geisbert TW, Jahrling PB (2004) Exotic emerging viral diseases: progress and challenges. Nat Med 10:S110–S121

Ghiasi SM, Salmanian AH, Chinikar S et al (2011) Mice orally immunized with a transgenic plant expressing the glycoprotein of Crimean-Congo hemorrhagic fever virus. Clin Vaccine Immunol 18:2031–2037. https://doi.org/10.1128/CVI.05352-11

Goedhals D, Paweska JT, Burt FJ (2015) Identification of human linear B-cell epitope sites on the envelope glycoproteins of Crimean-Congo haemorrhagic fever virus. Epidemiol Infect 143(7):1451–1456. https://doi.org/10.1017/S0950268814002271

Gonzalez J-P, Camicas JL, Cornet JP et al (1992) Sexual and transovarian transmission of Crimean-Congo haemorrhagic fever in *Hyalomma truncatum* ticks. Res Virol 142:23–28

Gordon SW, Linthicum KJ, Moulton JR (1993) Transmission of Crimean-Congo hemorrhagic fever virus in two species of *Hyalomma* ticks from infected adults to cofeeding immature forms. Am J Trop Med Hyg 48:576–580

Gozalan A, Esen B, Fitzner J et al (2007) Crimean-Congo haemorrhagic fever cases in Turkey. Scand J Infect Dis 39:332–336

Gul S, Gul EU, Yesilyurt M et al (2012) Health-related quality of life and the prevalence of post-traumatic stress disorder among Crimean-Congo hemorrhagic fever survivors. Jpn J Infect Dis 65:392–395

Guner R, Hasanoglu I, Yapar D et al (2011) A case of Crimean Congo hemorrhagic fever complicated with acalculous cholecystitis and intraabdominal abscess. J Clin Virol 50:162–163

Guven G, Talan L, Altintas ND et al (2017) An unexpected fatal CCHF case and management of exposed health care workers. Int J Infect Dis 55:118–121. https://doi.org/10.1016/j.ijid.2016.12.026

Hassanein KM, El Azazy OM, Yousef HM (1997) Detection of Crimean-Congo haemorrhagic fever virus antibodies in humans and imported livestock in Saudi Arabia. Trans R Soc Trop Med Hyg 91:536–537

Hawman DW, Haddock E, Meade-White K et al (2018) Favipiravir (T-705) but not ribavirin is effective against two distinct strains of Crimean-Congo hemorrhagic fever virus in mice. Antivir Res 157:18–26. https://doi.org/10.1016/j.antiviral.2018.06.013

Hawman DW, Haddock E, Meade-White K et al (2020) Efficacy of favipiravir (T-705) against Crimean-Congo hemorrhagic fever virus infection in cynomolgus macaques. Antivir Res 181:104858. https://doi.org/10.1016/j.antiviral.2020.104858

Hawman DW, Ahlén G, Appelberg KS et al (2021) A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a *Cynomolgus* macaque model. Nat Microbiol 6:187–195. https://doi.org/10.1038/s41564-020-00815-6

Hewson R, Chamberlain J, Mioulet V et al (2004a) Crimean-Congo haemorrhagic fever virus: sequence analysis of the small RNA segments from a collection of viruses worldwide. Virus Res 102:185–189

Hewson R, Gmyl A, Gmyl L et al (2004b) Evidence of segment reassortment in Crimean-Congo haemorrhagic fever virus. J Gen Virol 85:3059–3070

Hinkula J, Devignot S, Åkerström S et al (2017) Immunization with DNA plasmids coding for Crimean-Congo hemorrhagic fever virus capsid and envelope proteins and/or virus-like particles induces protection and survival in challenged mice. J Virol 91:e02076–e02016. https://doi.org/10.1128/JVI.02076-16

Honig JE, Osborne JC, Nichol ST et al (2004) Crimean-Congo hemorrhagic fever virus genome L RNA segment and encoded protein. Virology 321:29–35

Hoogstraal H (1979) The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol 15:307–417

Johnson S, Henschke N, Maayan N et al (2018) Ribavirin for treating Crimean Congo haemorrhagic fever. Cochrane Database Syst Rev 6:CD012713. https://doi.org/10.1002/14651858.CD012713.pub2

Jones LD, Davies CR, Steele GM et al (1987) A novel mode of arbovirus transmission involving a nonviremic host. Science 237:775–777

Joubert JR, King JB, Rossouw DJ et al (1985) A nosocomial outbreak of Crimean0Congo haemorrhagic fever at Tygerberg Hospital. Part III. Clinical pathology and pathogenesis. S Afr Med J 68:722–728

Karabatsos N (1985) International catalogue of arboviruses (including certain other viruses of vertebrates), 3rd edn. Am Soc Trop Med Hyg, San Antonio

Karti SS, Odabasi Z, Korten V et al (2004) Crimean-Congo hemorrhagic fever in Turkey. Emerg Infect Dis 10:1379–1384

Kaya S, Yilmaz G, Ertunç B et al (2012) Parotitis associated with Crimean Congo hemorrhagic fever virus. J Clin Virol 53:159–161

Kerget F, Demirdöğen ŞO, Kerget B (2021) Case report: a rare case of Crimean-Congo hemorrhagic fever associated with epididymo-orchitis. Am J Trop Med Hyg 104:1055–1057. https://doi.org/10.4269/ajtmh.20-1143

Keshtkar-Jahromi M, Kuhn JH, Christova I et al (2011) Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antivir Res 90:85–92

Khan AS, Maupin GO, Rollin PE et al (2002) An outbreak of Crimean-Congo hemorrhagic fever in The United Arab Emirates, 1994–1995. Am J Trop Med Hyg 57(5):519–525. https://doi.org/10.4269/ajtmh.1997.57.519

Kilinc C, Gückan R, Capraz M et al (2016) Examination of the specific clinical symptoms and laboratory findings of Crimean-Congo hemorrhagic fever. J Vector Borne Dis 53:162–167

Kinsella E, Martin SG, Grolla A et al (2004) Sequence determination of the Crimean-Congo hemorrhagic fever virus L segment. Virology 321:23–28

Kondiah K, Swanepoel R, Paweska JT et al (2010) A Simple-Probe real-time PCR assay for genotyping reassorted and non-reassorted isolates of Crimean-Congo hemorrhagic fever virus in southern Africa. J Virol Methods 169:34–38

Korolev MB, Donets MA, Rubin SG et al (1976) Morphology and morphogenesis of Crimean hemorrhagic fever virus. Arch Virol 50:169–172

Kortekaas J, Vloet RP, McAuley AJ et al (2015) Crimean-Congo hemorrhagic fever virus subunit vaccines induce high levels of neutralizing antibodies but no protection in STAT1 knockout mice. Vector Borne Zoonotic Dis 15:759–764. https://doi.org/10.1089/vbz.2015.1855

Kumar JS, Parida M, Shete AM et al (2022) Development of a reverse transcription loop - mediated isothermal amplification [RT-LAMP] as a early rapid detection assay for Crimean Congo hemorrhagic fever virus. Acta Trop 231:106435. https://doi.org/10.1016/j.actatropica.2022.106435

Leblebicioglu H, Ozaras R, Irmak H et al (2016) Crimean-Congo hemorrhagic fever in Turkey: current status and future challenges. Antivir Res 126:21–34. https://doi.org/10.1016/j.antiviral.2015.12.003

Lindquist ME, Zeng X, Altamura LA et al (2018) Exploring Crimean-Congo hemorrhagic fever virus-induced hepatic injury using antibody-mediated type I interferon blockade in mice. J Virol 92:e01083–e01018. https://doi.org/10.1128/JVI.01083-18

Logan TM, Linthicun KJ, Bailey CL et al (1989) Experimental transmission of Crimean-Congo hemorrhagic fever by *Hyalomma truncatum* Koch. Am J Trop Med Hyg 40:207–212

Maltezou HC, Papa A (2010) Crimean-Congo hemorrhagic fever: risk for emergence of new endemic foci in Europe? Travel Med Infect Dis 8:139–143

Maltezou HC, Andonova L, Andraghetti R et al (2010) Crimean-Congo hemorrhagic fever in Europe: current situation calls for preparedness. Euro Surveill 15:19504–19508

Manjunathachar HV, Kumar B, Saravanan BC et al (2019) Identification and characterization of vaccine candidates against *Hyalomma anatolicum*-Vector of Crimean-Congo haemorrhagic fever virus. Transbound Emerg Dis 66:422–434. https://doi.org/10.1111/tbed.13038

Marriott AC, Nuttall PA (1992) Comparison of the S RNA segments and nucleoprotein sequences of Crimean-Congo hemorrhagic fever, Hazara, and Dugbe viruses. Virology 189:795–799

Midilli K, Gargili A, Ergönül Ö et al (2009) The first clinical case due to AP92 like strain of Crimean-Congo hemorrhagic fever virus and a field survey. BMC Infect Dis 9:90

Mishra AC, Mehta M, Mourva DT et al (2011) Crimean-Congo haemorrhagic fever in India. Lancet 378:372

Morikawa S, Saijo M, Kurane I (2007) Recent progress in molecular biology of Crimean-Congo hemorrhagic fever. Comp Immunol Microbiol Infect Dis 30:375–389

Mousavi-Jazi M, Karlberg H, Papa A et al (2012) Healthy individuals' immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. Vaccine 30:6225–6229. https://doi.org/10.1016/j.vaccine.2012.08.003

Muianga AF, Watson R, Varghese A et al (2017) First serological evidence of Crimean-Congo haemorrhagic fever in febrile patients in Mozambique. Int J Infect Dis 62:119–123. https://doi.org/10.1016/j.ijid.2017.07.024

Nabeth P, Thior M, Faye O et al (2004) Human Crimean-Congo hemorrhagic fever. Sénégal Emerg Infect Dis 10(10):1881–1882. https://doi.org/10.3201/eid1010.040586

National Institute for Communicable Diseases (2020) Crimean-Congo haemorrhagic fever. Commun Dis Communiqué [Internet] 19:3. Available from: https://www.nicd.ac.za/wp-content/uploads/2020/02/NICD_Communicable_Disease_Communique_February_2020_Issue.pdf

Negredo A, De la Calle-Prieto F, Palencia-Herrejón E et al (2017) Autochthonous Crimean-Congo hemorrhagic fever in Spain. N Engl J Med 377(2):154–161. https://doi.org/10.1056/NEJMoa1615162

Oestereich L, Rieger T, Neumann M et al (2014) Evaluation of antiviral efficacy of ribavirin, arbidol, and T-705 (favipiravir) in a mouse model for Crimean-Congo hemorrhagic fever. PLoS Negl Trop Dis 8:e2804. https://doi.org/10.1371/journal.pntd.0002804

Osman HAM, Eltom KH, Musa NO et al (2013) Development and evaluation of loop-mediated isothermal amplification assay for detection of Crimean Congo hemorrhagic fever virus in Sudan. J Virol Methods 190(1–2):4–10. https://doi.org/10.1016/j.jviromet.2013.03.004

Ozkaya E, Dincer E, Carhan A et al (2010) Molecular epidemiology of Crimean-Congo hemorrhagic fever virus in Turkey: occurrence of local topotype. Virus Res 149:64–70. https://doi.org/10.1016/j.virusres.2009.12.014

Ozkurt Z, Kiki I, Erol S et al (2006) Crimean-Congo hemorrhagic fever in Eastern Turkey: clinical features, risk factors and efficacy or ribavirin therapy. J Infect 52:207–215

Palomar AM, Portillo A, Santibanez P et al (2013) Crimean-Congo hemorrhagic fever virus in ticks from migratory birds, Morocco. Emerg Infect Dis 19:260–263

Papa A, Ma B, Kouidou S et al (2002a) Genetic characterization of the M RNA segment of Crimean Congo hemorrhagic fever virus strains, China. Emerg Inf Dis 8:50–53

Papa A, Bino S, Llagami A et al (2002b) Crimean-Congo hemorrhagic fever in Albania, 2001. Eur J Clin Microbiol Infect Dis 8:603–606

Papa A, Bozovi B, Pavlidou V et al (2002c) Genetic detection and isolation of Crimean-Congo hemorrhagic fever virus, Kosovo, Yugoslavia. Emerg Infect Dis 8:852–854

Papa A, Christova I, Papadimitriou E et al (2004) Crimean-Congo hemorrhagic fever in Bulgaria. Emerg Infect Dis 10:1465–1467

Papa A, Papadimitriou E, Bozović B et al (2005) Genetic characterization of the M RNA segment of a Balkan Crimean-Congo hemorrhagic fever virus strain. J Med Virol 75:466–469

Papa A, Bino S, Velo E et al (2006) Cytokine levels in Crimean-Congo hemorrhagic fever. J Clin Virol 36:272–276

Papa A, Drosten C, Bino S et al (2007) Viral load and Crimean-Congo hemorrhagic fever. Emerg Infect Dis 13:805–806

Papa A, Maltezou HC, Tsiodras S et al (2008) A case of Crimean-Congo haemorrhagic fever in Greece, June 2008. Euro Surveill 13:13–14

Papa A, Dalla V, Papadimitriou E et al (2010) Emergence of Crimean-Congo haemorrhagic fever virus in Greece. Clin Microbiol Infect 16(7):843–847. https://doi.org/10.1111/j.1469-0691.2009.02996.x

Papa A, Papadimitriou E, Christova I (2011) The Bulgaraian vaccine Crimean-Congo haemorrhagic fever virus strain. Scand J Infect Dis 43:225–229

Papa A, Marklewitz M, Paraskevopoulou S et al (2022) History and classification of Aigai virus (formerly Crimean-Congo haemorrhagic fever virus genotype VI). J Gen Virol 103(4). https://doi.org/10.1099/jgv.0.001734

Pavel STI, Yetiskin H, Kalkan A et al (2020) Evaluation of the cell culture based and the mouse brain derived inactivated vaccines against Crimean-Congo hemorrhagic fever virus in transiently immune-suppressed (IS) mouse model. PLoS Negl Trop Dis 14:e0008834. https://doi.org/10.1371/journal.pntd.0008834

Peyrefitte CN, Perret M, Garcia S et al (2010) Differential activation profiles of Crimean-Congo hemorrhagic fever virus- and Dugbe virus-infected antigen-presenting cells. J Gen Virol 91:189–198. https://doi.org/10.1099/vir.0.015701-0

Portillo A, Palomar AM, Santibáñez P et al (2021) Epidemiological aspects of Crimean-Congo hemorrhagic fever in Western Europe: what about the future? Microorganisms 9(3):649. https://doi.org/10.3390/microorganisms9030649

Prasad HR, Sharma A, Kothari N et al (2020) Atypical presentation of Crimean-Congo hemorrhagic fever as ascending paralysis with rhabdomyolysis. Indian J Crit Care Med 24:143–144. https://doi.org/10.5005/jp-journals-10071-23347

Pshenichnaya NY, Leblebicioglu H, Bozkurt I et al (2017) Crimean-Congo hemorrhagic fever in pregnancy: a systematic review and case series from Russia, Kazakhstan and Turkey. Int J Infect Dis 58:58–64. https://doi.org/10.1016/j.ijid.2017.02.019

Randolph SE, Rigers DJ (2007) Ecology of tick-borne disease and the role of climate. In: Whitehouse CA (ed) Ergönül Ö. Springer, Crimean-Congo haemorrhagic fever. A global perspective. Dordrecht, pp 167–1186

Rodriguez LL, Maupin GO, Ksiazek TG et al (1997) Molecular investigation of a multisource outbreak of Crimean-Congo hemorrhagic fever in The United Arab Emirates. Am J Trop Med Hyg 57:512–518

Rodriguez SE, McAuley AJ, Gargili A et al (2018) Interactions of human dermal dendritic cells and Langerhans cells treated with *Hyalomma* tick saliva with Crimean-Congo hemorrhagic fever virus. Viruses 10:381. https://doi.org/10.3390/v10070381

Rodriguez SE, Cross RW, Fenton KA et al (2019) Vesicular stomatitis virus-based vaccine protects mice against Crimean-Congo hemorrhagic fever. Sci Rep 9:7755. https://doi.org/10.1038/s41598-019-44210-6

Sahin IO, Güven AS, Kaya A et al (2016) A child with an unusual complication of Crimean-Congo hemorrhagic fever: hemorrhagic pleural effusion. J Vector Borne Dis 53:87–89

Saijo M, Tang Q, Shimayi B et al (2005a) Antigen-capture enzyme-linked immunosorbent assay for the diagnosis of Crimean-Congo hemorrhagic fever using a novel monoclonal antibody. J Med Virol 77:83–88

Saijo M, Tang Q, Shimayi B et al (2005b) Recombinant nucleoprotein-based serological diagnosis of Crimean-Congo hemorrhagic fever virus infections. J Med Virol 75:295–299

Saksida A, Duh D, Wraber B et al (2010) Interacting roles of immune mechanisms and viral load in the pathogenesis of Crimean-Congo hemorrhagic fever. Clin Vaccine Immunol 17:1086–1093

Saluzzo JF, Digoutte JP, Cornet M et al (1984) Isolation of Crimean-Congo haemorrhagic fever and Rift Valley fever viruses in Upper Volta. Lancet 1:1179

Saluzzo JF, Aubry P, McCormick J et al (1985) Haemorrhagic fever caused by Crimean Congo haemorrhagic fever virus in Mauritania. Trans R Soc Trop Med Hyg 79:268

Samudzi RR, Leman PA, Paweska JT et al (2012) Bacterial expression of Crimean-Congo hemorrhagic fever virus nucleoprotein and its evaluation as a diagnostic reagent in an indirect ELISA. J Virol Methods 179:70–76

Sanchez AJ, Vincent MJ, Nichol ST (2002) Characterization of the glycoproteins of Crimean-Congo hemorrhagic fever virus. J Virol 76:7263–7275

Sanchez AJ, Vincent MJ, Ericksen BR et al (2006) Crimean-Congo hemorrhagic fever virus glycoprotein precursor is cleaved by Furin-like and SKI-1 proteases to generate a novel 38-kilodalton glycoprotein. J Virol 80:514–525

Satrovic L, Softic A, Zuko A et al (2022) First evidence of Crimean-Congo haemorrhagic fever virus circulation in Bosnia and Herzegovina. Vet Med Sci 8(3):1271–1275. https://doi.org/10.1002/vms3.781

Schmaljohn CS, Patterson JL (1990) Bunyaviridae and their replication. Part II: replication of Bunyaviridae. In: Fields BN (ed) Virology, 2nd edn. Raven Press Ltd., New York

Scholte FEM, Spengler JR, Welch SR et al (2019) Single-dose replicon particle vaccine provides complete protection against Crimean-Congo hemorrhagic fever virus in mice. Emerg Microbes Infect 8:575–578. https://doi.org/10.1080/22221751.2019.1601030

Schwarz TF, Nitschko H, Jager G et al (1995) Crimean-Congo haemorrhagic fever in Oman. Lancet 346:1230

Şensoy G, Çaltepe Dinler G, Kalkan G et al (2011) Crimean-Congo haemorrhagic fever: peritoneal and pleural effusion. Ann Trop Paediatr 31:169–172

Seregin SV, Samakhvalov EI, Petrova ID et al (2004) Genetic characterization of the M RNA segment of Crimean-Congo hemorrhagic fever virus strains isolated in Russia and Tajikistan. Virus Genes 28:187–193

Shepherd AJ, Swanepoel R, Leman PA et al (1986) Comparison of methods for isolation and titration of Crimean-Congo hemorrhagic fever virus. J Clin Microbiol 24:654–656

Shepherd AJ, Swanepoel R, Leman PA et al (1987) Field and laboratory investigation of Crimean-Congo haemorrhagic fever virus (Nairovirus, family Bunyaviridae) infection in birds. Trans R Soc Trop Med Hyg 81:1004–1007

Shepherd AJ, Swanepoel R, Gill DE (1988) Evaluation of enzyme-linked immunosorbent assay and reversed passive hemagglutination for detection of Crimean-Congo hemorrhagic fever virus antigen. J Clin Microbiol 26:347–353

Shepherd AJ, Leman PA, Swanepoel R (1989a) Viremia and antibody response of small Africa and laboratory mammals to Crimean-Congo hemorrhagic fever virus infection. Am J Trop Med Hyg 40:541–547

Shepherd AJ, Swanepoel R, Leman PA (1989b) Antibody response in Crimean-Congo hemorrhagic fever. Rev Infect Dis 11:S801–S806

Shepherd AJ, Swanepoel R, Shepherd SP et al (1991) Viraemic transmission of Crimean-Congo haemorrhagic fever virus to ticks. Epidemiol Infect 106:373–382

Shiraki K, Daikoku T (2020) Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. Pharmacol Ther 209:107512. https://doi.org/10.1016/j.pharmthera.2020.107512

Shrivastava N, Verma A, Dash PK (2020) Identification of functional epitopes of structural proteins and in-silico designing of dual acting multiepitope anti-tick vaccine against emerging Crimean-Congo hemorrhagic fever virus. Eur J Pharm Sci 151:105396. https://doi.org/10.1016/j.ejps.2020.105396

Simpson DIH, Knoght EM, Courtois GH et al (1967) Congo virus: a hitherto undescribed virus occurring in Africa. Part 1-Human isolations-clinical notes. East Afr Med J 44:87–92

Soares-Weiser K, Thomas S, Thomson G et al (2010) Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis 10:207

Spengler JR, Patel JR, Chakrabarti AK et al (2015) RIG-I mediates an antiviral response to Crimean-Congo hemorrhagic fever virus. J Virol 89:10219–10229. https://doi.org/10.1128/JVI.01643-15

Spengler JR, Bergeron É, Rollin PE (2016) Seroepidemiological studies of Crimean-Congo hemorrhagic fever virus in domestic and wild animals. PLoS Negl Trop Dis 10(1):e0004210. https://doi.org/10.1371/journal.pntd.0004210

Spengler JR, Welch SR, Scholte FEM et al (2019) Heterologous protection against Crimean-Congo hemorrhagic fever in mice after a single dose of replicon particle vaccine. Antivir Res 170:104573. https://doi.org/10.1016/j.antiviral.2019.104573

Spengler JR, Welch SR, Scholte FEM et al (2021) Viral replicon particles protect IFNAR$^{-/-}$ mice against lethal Crimean-Congo hemorrhagic fever virus challenge three days after vaccination. Antivir Res 191:105090. https://doi.org/10.1016/j.antiviral.2021.105090

Spik K, Shurtleff A, McElroy AK et al (2006) Immunogenicity of combination DNA vaccines for Rift Valley fever virus, tick-borne encephalitis virus, Hantaan virus and Crimean-Congo hemorrhagic virus. Vaccine 24:2657–4666

Suleiman MN, Muscat-Baron JM, Harries JR et al (1980) Congo/Crimean haemorrhagic fever in Dubai. An outbreak at the Rashid Hospital. Lancet 2:939–941

Suschak JJ, Golden JW, Fitzpatrick CJ et al (2021) A CCHFV DNA vaccine protects against heterologous challenge and establishes GP38 as immunorelevant in mice. NPJ Vaccines 6:31. https://doi.org/10.1038/s41541-021-00293-9

Swanepoel R, Shepherd AJ, Leman PA et al (1987) Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. Am J Trop Med Hyg 36:120–132

Swanepoel R, Gill DE, Shepherd AJ et al (1989) The clinical pathology of Crimean-Congo hemorrhagic fever. Rev Infect Dis 11:794–800

Swanepoel R, Leman PA, Burt FJ et al (1998) Experimental infection of ostriches with Crimean-Congo haemorrhagic fever virus. Epidemiol Infect 121:427–432

Tang Q, Saijo M, Zhang Y et al (2003) A patient with Crimean-Congo hemorrhagic fever serologically diagnosed by recombinant nucleoprotein-based antibody detection systems. Clin Diagn Lab Immunol 10:489–491

Tantawi HH, Al Moslih MI, Al Janabi NY et al (1980) Crimean-Congo haemorrhagic fever virus in Iraq: isolation, identification and electron microscopy. Acta Virol 24:464–467

Tezer H, Sucakli IA, Sayli TR et al (2010) Crimean-Congo hemorrhagic fever in children. J Clin Virol 48:184–186

Tipih T, Heise M, Burt FJ (2021) Immunogenicity of a DNA-based Sindbis replicon expressing Crimean-Congo hemorrhagic fever virus nucleoprotein. Vaccines (Basel) 9:1491. https://doi.org/10.3390/vaccines9121491

Tonbak S, Aktas M, Altay K et al (2006) Crimean-Congo hemorrhagic fever virus: genetic analysis and tick survey in Turkey. J Clin Microbiol 44:4120–4124

Ture Z, Ulu Kılıç A, Celik I et al (2016) Crimean-Congo hemorrhagic fever with hyperbilirubinemia and ascites: an unusual presentation. J Med Virol 88:159–162. https://doi.org/10.1002/jmv.24295

Tuygun N, Tanir G, Caglayik DY et al (2012) Pediatric cases of Crimean-Congo hemorrhagic fever in Turkey. Pediatr Int 54:402–406. https://doi.org/10.1111/j.1442-200X.2011.03549.x

van Eeden PJ, Joubert JR, van de Wal BW et al (1985) A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part I. Clinical features. S Afr Med J 68:711–717

Wang Q, Cao R, Li L, Liu J et al (2022) In vitro and in vivo efficacy of a novel nucleoside analog H44 against Crimean-Congo hemorrhagic fever virus. Antivir Res 199:105273. https://doi.org/10.1016/j.antiviral.2022.105273

Watts DM, Ksiazek TG, Linthicum KJ et al (1989) Crimean-Congo haemorrhagic fever. In: Monath TP (ed) The arboviruses: epidemiology and ecology, vol II. CRC Press, Boca Raton

Welch SR, Ritter JM, McElroy AK et al (2019) Fluorescent Crimean-Congo hemorrhagic fever virus illuminates tissue tropism patterns and identifies early mononuclear phagocytic cell targets in Ifnar−/− mice. PLoS Pathog 15:e1008183. https://doi.org/10.1371/journal.ppat.1008183

Wilson ML, Gonzalez J-P, Cornet JP et al (1991) Transmission of Crimean-Congo haemorrhagic fever virus from experimentally infected sheep to *Hyalomma truncatum* ticks. Res Virol 142:395–404

Wölfel R, Paweska JT, Petersen N et al (2007) Virus detection and monitoring of viral load in Crimean-Congo hemorrhagic fever virus patients. Emerg Infect Dis 13:1097–1100

Wölfel R, Paweska JT, Petersen N et al (2009) Low-density macroarray for rapid detection and identification of Crimean-Congo hemorrhagic fever virus. J Clin Microbiol 47:1025–1030

World Health Organization (2021) World Health Organization Model List of Essential Medicines – 22nd List, 2021. Available from: https://apps.who.int/iris/rest/bitstreams/1374779/retrieve

Yakut N, Kepenekli E, Dogru O (2021) Crimean-Congo hemorrhagic fever with hemophagocytic lymphohistiocytosis. Rev Soc Bras Med Trop 54:e0438–e2020. https://doi.org/10.1590/0037-8682-0438-2020

Yapar M, Aydogan H, Pahsa A et al (2005) Rapid and quantitative detection of Crimean-Congo hemorrhagic fever virus by one-step real-time reverse transcriptase-PCR. Jpn J Infect Dis 58:358–362

Yashina L, Petrova I, Seregin S (2003) Genetic variability of Crimean-Congo haemorrhagic fever virus in Russia and Central Asia. J Gen Virol 84:1199–1206

Yen YC, Kong LX, Lee L et al (1985) Characteristics of Crimean-Congo hemorrhagic fever virus (Xinjiang strain) in China. Am J Trop Med Hyg 34(6):1179–1182

Zakhashvili K, Tsertsvadze N, Chikviladze T et al (2010) Crimean-Congo hemorrhagic fever in man, Republic of Georgia, 2009. Emerg Infect Dis 16:1326–1328. https://doi.org/10.3201/eid1608.100097

Zivcec M, Safronetz D, Scott DP et al (2018) Nucleocapsid protein-based vaccine provides protection in mice against lethal Crimean-Congo hemorrhagic fever virus challenge. PLoS Negl Trop Dis 12:e0006628. https://doi.org/10.1371/journal.pntd.0006628

A Review of Hendra Virus and Nipah Virus Infections in Man and Other Animals

48

Kim Halpin and Paul A. Rota

Contents

Abstract

Hendra virus (HeV) and Nipah virus (NiV) emerged in the 1990s. They have been the cause of outbreaks of respiratory and neurological disease infecting horses and pigs. Transmission from infected domestic animal species has resulted in human infections with high case fatalities. Direct transmission from the reservoir host to humans has occurred with NiV in yearly disease outbreaks in Bangladesh and India. HeV causes sporadic disease outbreaks in horses in northeast to mideastern Australia with occasional spillover from infected horses to humans. Due to their zoonotic nature, they have been ideal candidates for collaborative projects and coordinated disease outbreak investigations in the One Health space, bringing public health and animal health professionals together. This has led to practical disease outbreak investigations, disease prevention solutions including a

K. Halpin (✉)
Australian Centre for Disease Preparedness, CSIRO, Geelong, VIC, Australia
e-mail: kim.halpin@csiro.au

P. A. Rota
Division of Viral Diseases, Centres for Disease Control & Prevention, Atlanta, GA, USA
e-mail: prota@cdc.gov

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_40

horse vaccine for HeV, and NiV spillover prevention interventions in the field. As more surveillance is undertaken, their known distributions have expanded, as has the geographical range of the reservoir host species. Most bat species for which there is evidence of HeV and NiV infection belong to the group known as the Old World fruit- and nectar-feeding bats (Family *Pteropodidae,* Suborder *Yinpterochiroptera, and* Order *Chiroptera*). This chapter of HeV and NiV discusses the epidemiology, pathology, transmission, and disease symptoms in these closely related viruses which belong to the Genus *Henipavirus,* Family *Paramyxoviridae.*

Keywords

Reservoir host · Nipah virus · Hendra virus

48.1 Epidemiology of Hendra Virus in Animals

On August 1, 1994, a heavily pregnant 10-year-old thoroughbred brood mare died suddenly in a paddock in northern coastal Australia. Ironically the first of August is deemed the birthday of all thoroughbred horses in the southern hemisphere. (All thoroughbreds have the same birthday so that their ages can be standardized for comparison. In the southern hemisphere, the date is the first of August.) This date in 1994 would go down in history as the day Hendra virus (HeV) emerged. In 2021, a second variant was identified in a horse sample collected in 2015 (Annand et al. 2022). The original variant is now called HeV-g1 (genotype 1), and the new variant is HeV-g2 (genotype 2) (Wang et al. 2021).

Hendra virus was first discovered in horses, and horses remain the most infected domestic species. The reservoir host of this virus are bats of the genus *Pteropus*, family *Pteropodidae* (called *Pteropus* bats). Finding the reservoir host for this virus resulted in the first reported isolation of a zoonotic paramyxovirus from *Pteropus* bats (Halpin et al. 2000). To date, only horses have become directly infected from *Pteropus* bats. Horses act as HeV-amplifying hosts.

As of November 2022, there have been 99 horses infected, usually in events which only involved one horse, with winter having the most outbreaks, followed by spring (Fig. 1). Only two outbreaks have involved more than three horses, and the spread of the virus between the stabled horses in these outbreaks was a result of close contact and assisted mechanical transmission of the virus. Aerosol transmission is unlikely as sneezing and coughing were not features of the syndrome, and the spatial distribution of cases in the stables was not consistent with this form of spread (Baldock et al. 1996; Field et al. 2010).

Experimentally, we know that some horses can survive infection. In the outbreaks where there have been numerous horses infected, this has also been the case. In the 1994 outbreak in Hendra, 7 out of 20 horses apparently survived a lethal infection and seroconverted before being euthanized (Murray et al. 1995). In the 2008 Redlands outbreak, one horse survived for 42 days after clinical signs abated before being euthanized (Field et al. 2010).

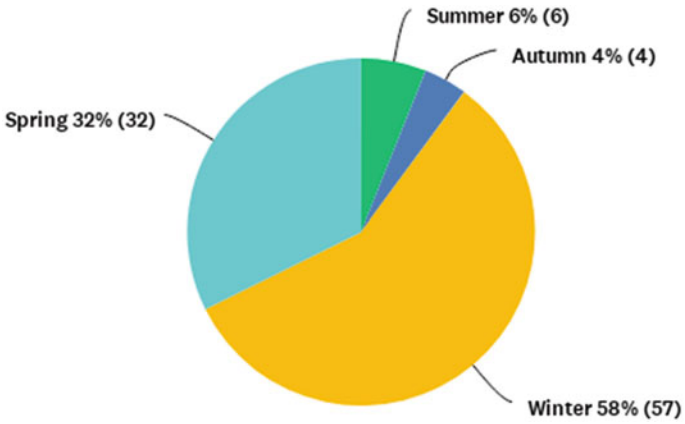

Fig. 1 Total number of HeV infected horses by season (1994–2022)

Experimentally, cats can be infected and succumb to the virus (Westbury et al. 1995; Williamson et al. 1998), but no cat has been found to be naturally infected in the field, to date. In one of the 2011 outbreaks, a dog on a property under investigation was found to have antibodies to the virus. It most likely had close contact with HeV-laden material from an infected horse but was clinically well and remained so until it was euthanized in accordance with national policy at the time (Promed 2011). In 2013, HeV RNA was detected in the EDTA-treated blood and serum, but not from the oral swab samples, from a dog which resided on the same property as an HeV-infected horse (Kirkland et al. 2015). Experimental infections in dogs have been conducted at the Australian Centre for Disease Preparedness (ACDP), to better understand the role that dogs might play in the epidemiology of the disease. In these studies, dogs could be reliably infected with HeV. Consistent with the field observation, few if any clinical signs were noted during the acute stage of infection. Viral shedding from the oral cavity occurred for a relatively short period of time, and oral secretions collected from dogs during this period were capable of transmitting infection to naïve ferrets (Middleton et al. 2017). Neutralizing antibody titers generated in these dogs were similar to that observed in the recorded canine field case of HeV infection (Promed 2011). In dogs, the key site of virus replication within the oral cavity was the tonsil, with authors concluding that it is feasible for Hendra virus to be transmitted to people from acutely infected dogs.

Infection in horses most likely occurs after close contact with *Pteropus* bat urine and birthing material which contain sufficiently high enough titers of virus to infect a horse (Halpin et al. 2000; Edson et al. 2015). Luckily for horses, these bat samples rarely contain high titers of virus. The risk of transmission to horses was found to be increased during *Pteropus* bat-reproductive periods (especially late pregnancy) and at times when the colonies were undergoing nutritional stress, such as during lactation and during food shortages (Plowright et al. 2008; Breed et al. 2011). More recent studies build on these observations and suggest that in addition to

seasonal effects, bat density and local climatic conditions interact to drive Hendra virus infection dynamics in *Pteropus* bat populations (Páez et al. 2017).

Evidence of Hendra virus infection has been found in all four mainland *Pteropus* species of bats in Australia, namely, the black flying fox (*Pteropus alecto*), the gray-headed flying fox (*P. poliocephalus*), the spectacled flying fox (*P. conspicillatus*), and the little red flying fox (*P. scapulatus*). However, spillover events to date have been restricted to regions where *P. alecto* and/or *P. conspicillatus* are found (Edson et al. 2015), noting that the geographical distribution of *P. alecto* is extending further south.

It appears that the reservoir host coexists with this virus in complete harmony. The virus spreads quite easily among *Pteropus* bats, with the HeV seroprevalence in bat colonies fluctuating over time and geographical spread. In one bat colony, seroprevalence steadily increased from 45 to 69% over a 2-year period supporting a model of endemic infection in the population (Breed et al. 2011). Absence of disease attributable to HeV infection is supported by experimental observations (Halpin et al. 2011). This is consistent with the observation that many viruses do not cause disease in their reservoir host. The long-term coexistence of viruses and their reservoir hosts has given coevolution a good chance to reach a relative equilibrium (Domingo 2010). The theory of viral coevolution with chiropteran hosts has been previously suggested, and all field observations and experimental evidence to date support this (Halpin et al. 2007).

48.2 Epidemiology of Nipah Virus in Animals

Since HeV was detected in *Pteropus* bats, they were among the first species investigated as possible reservoirs for Nipah virus (NiV) after its emergence in 1999. Neutralizing antibodies to NiV have been detected in a wide range of *Pteropus* bats, including *Pteropus hypomenalus*, and *Pteropus vampyrus* (Yob et al. 2001). In 2000, NiV was isolated from a urine sample collected underneath the roost of *Pteropus lylei* bats in Cambodia (Reynes et al. 2005). Nipah virus was eventually isolated from *Pteropus vampyrus* in Malaysia (Sohayati et al. 2011). Serologic evidence of Nipah infection was also obtained from *Rousettus leschen* and *Cynoptera sphinx* in Vietnam (Hasebe et al. 2012). Several species of Chinese bats also contained antibodies to Nipah or Nipah-like viruses (Li et al. 2008). A very thorough study of the presence of henipaviruses in Australasia indicated that NiV was present in East Timor and that non-NiV and non-HeV henipaviruses were present in Sumba, Sulawesi, and possibly Papua New Guinea (Breed et al. 2013). The authors suggested that NiV can be detected in areas where *Pteropus vampyrus* is present. In India and Bangladesh, the primary reservoir of NiV is *Pteropus medius* (Epstein et al. 2020; Yadav et al. 2019).

In Madagascar, seropositive *Pteropus rufus* and *Eidolon dupreahum* bats have been found, and 39% of *Eidolon helvum* from Ghana had NiV reactive antibodies (Hayman et al. 2008; Iehle et al. 2007). Henipavirus-like sequences were obtained from *Eidolon helvum* in Ghana (Drexler et al. 2009). The detection of antibodies to and sequences of henipaviruses in African bats suggest that the range of potential

NiV infections may be wider than previously thought, though no human cases of NiV have been reported from any region other than South Asia and Southeast Asia.

Experimentally infected *Pteropus* bats develop subclinical NiV infection with only sporadic viral excretion in urine. Some bats seroconvert, and some show evidence of infection by detection of viral antigen in tissues (Middleton et al. 2007; Halpin et al. 2011).

Regarding domestic species affected by NiV, pigs featured in the first outbreak in Malaysia (Chua et al. 2000). Pigs presumably became infected from *Pteropus* bats, and the disease spread throughout piggeries with pigs serving as an amplifying host. Most of the human infections occurred in people with direct contact to sick pigs. Serologic studies demonstrated evidence of infection among other domestic species of animals in Malaysia, including horses, dogs, and cats (Chua et al. 2000; Hooper and Williamson 2000).

In most of the outbreaks in India and Bangladesh, no intermediate domestic species has been implicated in epidemiology of the virus infection. However, in the outbreak in the Philippines, horses were infected. Epidemiologic data suggest that the most common route of virus transmission to humans was direct exposure to infected horses, contact with contaminated body fluids during slaughtering of sick horses, and/or consumption of undercooked meat from infected horses. Serological evidence of infection was also detected in four dogs; however, they were asymptomatic (Ching et al. 2015).

48.3 Epidemiology of Hendra Virus in Humans

To date, there has been no human-to-human spread of HeV; all infected people have had close contact with an infected horse. The first person to become infected and die from HeV was assisting his wife, a veterinarian, to perform an autopsy on a horse that had died suddenly in a paddock (Rogers et al. 1996). This patient recovered from a short illness but went on to die 13 months later after a relapse with encephalitis (O'Sullivan et al. 1997). In the second outbreak of Hendra virus, a horse trainer and a strapper, who had very close contact to HeV-infected horses in their racing stables, became infected (Selvey et al. 1995). The next person to become infected was a veterinarian who had performed an autopsy on a horse who had died from colic-like symptoms. At the time, colic-like symptoms had never been associated with HeV infection in horses. The veterinarian came down with a flu-like illness but recovered and for many years had neutralizing antibodies to the virus (Taylor et al. 2012). The next two people to become infected were a veterinarian who performed a nasal lavage on a horse with respiratory symptoms and the veterinary nurse who assisted with the procedure (Playford et al. 2010). The veterinarian died. The last person to become infected with Hendra virus was a veterinarian who cared for a horse which was also diagnosed with HeV infection (Field et al. 2010).

The human case fatality rate stands at 57%, with four deaths and three survivors. Interestingly to date, only male patients have died; however, with such a small sample size this should not be overinterpreted. The risk remains for people who have very close contact with bodily fluids from HeV-infected horses through performing invasive procedures, and/or by not wearing fully protective PPE.

48.4 Epidemiology of Nipah Virus in Humans

The first detected outbreak of NiV infection occurred in Malaysia with 276 human cases reported and 106 deaths (Chua et al. 2000). Nipah virus was transmitted to pigs and spread rapidly among swine herds causing primarily respiratory symptoms in pigs. Pig-to-human transmission resulted in acute febrile encephalitis mostly among adult males who worked in the pig industry. The outbreak spread to Singapore via the transport of infected live pigs from Malaysia (Chua et al. 2000; Paton et al. 1999). Culling of more than one million pigs in Malaysia was undertaken to control the outbreak (Chua et al. 2000).

Since the outbreak in Malaysia, outbreaks have been reported almost annually in Bangladesh and India from 2001 (Fig. 2) (Chadha et al. 2006; Luby and Gurley 2012; Luby et al. 2009a; Sudeep et al. 2021). The epidemiologic characteristics of the outbreaks in Bangladesh differed from the Malaysia outbreaks in several respects. Most notably, the case fatality rate in Bangladesh (2001–2010) ranged from 38% to as high as 100%, with an average mortality rate of 73%, while the mortality rate for Malaysia was approximately 38% (Luby and Gurley 2012). Infected individuals in Bangladesh were more likely to have respiratory symptoms, and there was evidence of human-to-human spread. Drinking fresh date palm sap contaminated by fruit bat saliva, urine, or excreta has been identified as the likely route of transmission from the wildlife reservoir to humans (Luby et al. 2006).

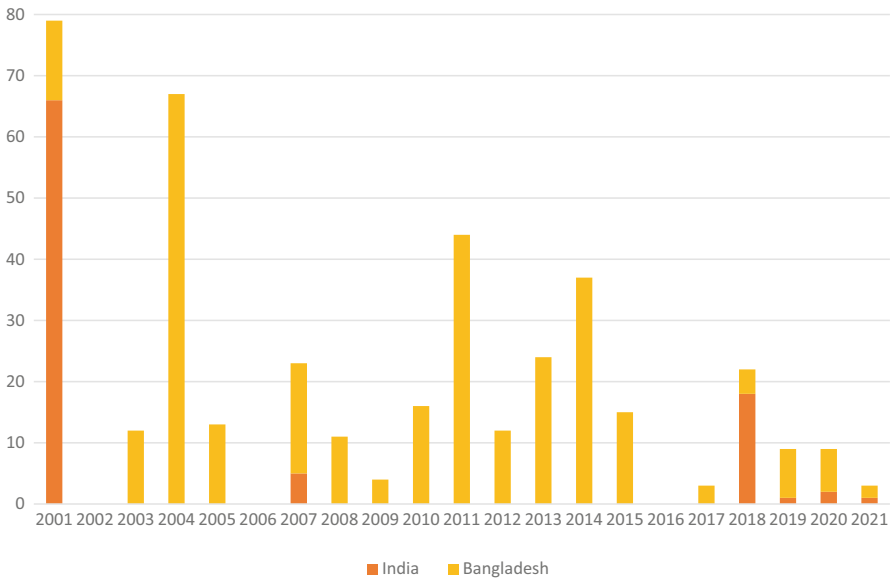

Fig. 2 Total number of deaths in NiV-infected people by year in Bangladesh and India. (Data taken from http://www.iedcr.org and http://www.searo.who.int/entity/emerging_diseases/links/nipah_virus_outbreaks_sear/en/index.html and Yadav et al. 2022)

Luby et al. (2009b) showed in their study that Nipah case-patients who had difficulty breathing were more likely than those without respiratory difficulty to transmit Nipah. Although a small minority of infected patients transmit Nipah virus, more than half of identified cases result from person-to-person transmission. In these cases, virus was spread during close contact while caring for sick individuals or preparing bodies for burial (Blum et al. 2009; Chadha et al. 2006; Luby et al. 2009b).

All confirmed Nipah outbreaks in Bangladesh have occurred in the same central and northwestern regions (Luby et al. 2009b). The first two Indian outbreaks have been in regions within 50 km of the border with Bangladesh and immediately contiguous with the affected areas in Bangladesh (Chadha et al. 2006). However, in 2018 there was a large outbreak on the western coast of India, in the Kozhikode district, Kerala state, approximately 2000 kms away from Bangladesh, where 21 people died and 2 people survived (Arunkumar et al. 2019). The source of infection for the index case is unknown but suspected to be a bat. Risk factors for infection of the other cases included proximity (i.e., touching, feeding, or nursing an NiV-infected person), enabling exposure to droplet infection (Arunkumar et al. 2019). The public health response included isolation of cases, contact tracing, and enforcement of hospital infection control practices. In the same district, a single case who survived was identified in 2019, and another case in 2021 who died (Yadav et al. 2022).

In 2014, Philippines had an outbreak of disease in horses and humans in two villages, Tinalon and Midtungok, in the municipality of Senator Ninoy Aquino, province of Sultan Kudarat, island of Mindanao. Limited sequence information from samples indicates that the virus was Nipah virus. Epidemiological data suggest that the most common route of virus transmission to humans was direct exposure to infected horses, contact with contaminated body fluids during slaughtering of sick horses, and/or consumption of undercooked meat from infected horses (Ching et al. 2015). However, for at least 5/17 cases, clinical and epidemiologic evidence suggest direct human-to-human virus transmission. No protective equipment was used by those who cared for case-patients in the home, and health care workers used gloves and a face mask but not eye protection (Ching et al. 2015).

48.5 Evidence of Animal to Human Transmission of Hendra Virus

While *Pteropus* bats are the reservoir host of the virus, humans have only become infected after close contact to infected horses. In Australia, there are many bat carers who have close contact to sick and injured *Pteropus* bats. They get bitten and scratched and come into contact with urine and fecal material, as well as placenta and birthing fluids. However, no bat carer has ever been diagnosed with HeV infection. An extensive serological survey of bat carers in Queensland was performed in the mid-1990s, and there was no serological evidence of exposure to the virus (Selvey et al. 1996). However, bat carers are at risk of becoming infected with Australian bat lyssavirus and should be vaccinated with the rabies vaccine.

In one HeV experiment, a small amount of viral RNA was detected in the nasal secretions of HeV-infected horses 2 days after exposure to the virus and at least 2 days before the onset of clinical signs, suggesting that transmission of the virus from the infected horse may be possible before it is obviously unwell (Marsh et al. 2011). However, at this early stage of infection, the amount of viral genome detected was very low and it is unlikely that this would be enough to infect another host. The findings also supported the observation in experimentally infected *Pteropus* bats that a local mucosal infection, from days 2 to approximately 6 post exposure, precedes a systemic infection (Halpin et al. 2011). Only after the systemic infection has been established does it become possible to isolate infectious virus from urine and blood.

Sequence analysis of different isolates from both horses and *Pteropus* bats reveals extreme conservation at the genome and protein levels (Marsh et al. 2010; Smith et al. 2011). In one study comparing five horse isolates from five locations (all HeV-g1) which spanned almost 2000 km, across three time points, to the original 1994 isolate, less than 1% variation at both the nucleotide and amino acid levels was shown across the 18.2-kb genome (Marsh et al. 2010). This genetic stability supports the theory of coevolution where HeV is well adapted to its host resulting in minimal pressure to change over time (Halpin et al. 2007; Smith et al. 2011).

48.6 Evidence of Animal-to-Human Transmission of Nipah Virus

In contrast to Malaysia where pigs clearly served as the amplifying host that facilitated spread of the virus from *Pteropus* bats to humans, no intermediate animal host was identified in the Bangladesh and Indian outbreaks. In the Philippines outbreak, the intermediate hosts were horses.

Several routes of transmission of NiV from *Pteropus* bats to humans have been identified by studying the nearly annual outbreaks in Bangladesh and the single outbreak in India. Consumption of contaminated date palm sap or contaminated fruit has been linked to several cases and outbreaks in Bangladesh (Rahman et al. 2012). Case-patients reported no history of physical contact with *Pteropus* bats, though community members often reported seeing bats. Infrared camera photographs have shown that *Pteropus* bats frequently visited date palm trees in those communities where sap was collected for human consumption. This provided an opportunity for intervention to prevent NiV spillover to humans. It has been shown that skirts (made from bamboo, dhoincha, jute stick, and/or polythene) covering the sap-producing areas of a tree effectively prevented bat-sap contact (Khan et al. 2012).

Genetic analysis of NiV isolates and sequences obtained from clinical samples indicated that the outbreaks in Bangladesh were the result of multiple, independent introductions of virus into the human population (Harcourt et al. 2005; Luby et al. 2009b).

The Indian flying fox (*Pteropus medius*) is the major reservoir of Nipah virus in Bangladesh and India (Yadav et al. 2019; Epstein et al. 2020). Longitudinal surveys indicate that exposure to Nipah virus is high (≈40%) in some *P. medius* populations

in Bangladesh based on serologic tests, but the prevalence of detectable Nipah virus RNA is low (<5%) at any given time (Yadav et al. 2019).

Sequences obtained from Malaysia and Cambodia are designated as genotype M, while sequences obtained from Bangladesh and India are designated genotype B. Genotypes can be assigned based on the sequence of a 729-nucleotide window in the N-terminal region of the N gene ORF. Levels of nucleotide variation among full-length ORFs between genotypes M and B ranged from 6 to 9%, and between the complete genomes nucleotide variation is approximately 8% (Lo et al. 2012). Differences in transmission patterns and mortality rates suggest that NiV-Bangladesh may be more pathogenic than NiV-Malaysia. Data has shown that NiV-B is more pathogenic in African green monkeys (Mire et al. 2016), and in ferrets NiV-B sheds more (Clayton et al. 2012).

48.7 Pathogenesis and Containment

NiV and HeV enter cells by binding to the receptor Ephrin-B2, which is expressed on neurons, smooth muscle, and endothelial cells surrounding small arteries (Bonaparte et al. 2005; Negrete et al. 2005). Ephrin-B3 serves as an alternative receptor for NiV, but not HeV (Negrete et al. 2006). After receptor binding by the attachment protein, G, the fusion protein (F) which is cleaved to create two linked polypeptides, F_1 and F_2, fuses to the host cell membrane, initiating endocytosis (Wang et al. 2001). Following fusion between the viral envelope and the host cell membrane, the viral ribonucleocapsid is released into the cytoplasm (Lamb and Parks 2007). The polymerase complex composed of the polymerase (L) and phosphoprotein (P) initiates transcription of viral mRNAs. As translation of viral mRNA occurs, viral proteins accumulate in the cell, and the polymerase switches from transcription to genome replication.

Newly made genomes are encapsidated by the nucleoprotein (N), and polymerase complexes become associated with packaged nucleocapsids. The glycoproteins are synthesized in the endoplasmic reticulum (ER), mature through the Golgi network, and are transported to the cell membrane. The processing of the fusion (F) glycoprotein occurs in the endosome (Diederich et al. 2005). The cytoplasmic tails of the F and G glycoproteins play a role in the interaction with the matrix (M) protein, which initiates virus maturation and budding (Ciancanelli and Basler 2006; Lamb and Parks 2007; Ong et al. 2009; Patch et al. 2007, 2008).

This tropism for endothelial cells results in a pathology characterized by vasculitis, thrombosis, ischemia, necrosis, and CNS parenchymal infection (Wong et al. 2002, 2009; Weingartl et al. 2009).

A postmortem study of human NiV infection determined that a systemic multiorgan vasculitis associated with infection of endothelial cells was the main pathologic feature, with infection being most pronounced in the central nervous system (CNS) (Wong et al. 2002). In the CNS vascular endothelium, immunohistochemical analysis showed intense staining of endothelial, parenchymal, and multinucleate giant cells which are characteristic of paramyxovirus infection. Evidence of

endothelial infection and vasculitis was also observed in other organs, including lung, heart, spleen, and kidney. NiV has been isolated from cerebrospinal fluid, tracheal secretions, throat swabs, nasal swabs, and urine specimens of patients (Chua et al. 2001; Goh et al. 2000; Wong et al. 2002), and detection of viral RNA by RT-PCR in urine and throat swabs samples is routinely used to confirm NiV infection (Arunkumar et al. 2019).

Both viruses are designated biosafety level (BSL) 4 agents which makes it difficult for researchers to work with these viruses. Furthermore, diagnostic tests requiring the use of live virus are restricted to laboratories that have BSL4 containment. Molecular detection of viral genome is currently the central arm of henipavirus infection diagnosis and can be done in laboratories with lower levels of containment (BSL2 and BSL3). Expanding the surveillance and laboratory capacity for rapid diagnosis of outbreaks is crucial to early detection and containment in areas at risk for NiV and HeV. Point-of-care tests are under development.

48.8 Disease Symptoms in Humans and Animals

Early cases of Hendra virus infection in horses had clinical signs of an acute respiratory disease (Murray et al. 1995). However, as more cases appeared, the spectrum of clinical signs widened to include colic-like symptoms, sudden death, and neurological manifestations. The incubation period is between 4 and 16 days (Baldock et al. 1996), after which time clinical signs such as fever, tachycardia, inappetence, depression, dyspnea, and restlessness may be observed (Marsh et al. 2011). Associated with the labored breathing, a nasal discharge which may be frothy or blood-tinged might develop. Neurological signs such as depression and ataxia are common (Rogers et al. 1996; Ball et al. 2014).

The first fatal human case of Hendra virus infection died of an acute respiratory illness (Selvey et al. 1995). The second fatal human case suffered from relapsing encephalitis (O'Sullivan et al. 1997) with the third and fourth cases succumbing to encephalitis (Field et al. 2010; Playford et al. 2010). Two of the surviving human cases suffered from a self-limited influenza-like illness at the time of HeV infection (Hanna et al. 2006). The third survivor showed development of an influenza-like illness that progressed to acute encephalitis and suffered a long and debilitating neurological illness (Playford et al. 2010).

The incubation period for NiV ranges from 6 to 14 days; after symptom onset patients deteriorated rapidly usually requiring hospitalization (Eaton et al. 2007; Hossain et al. 2008; Arunkumar et al. 2019). In humans, NiV causes acute febrile encephalitis including fever, headache, drowsiness, dizziness, myalgia, and vomiting with reduced consciousness and evidence of brainstem involvement being a poor prognostic factor. Some patients with NiV initially present with pulmonary symptoms such as cough, atypical pneumonia, and acute respiratory distress. The percentage of NiV patients presenting with respiratory disease was higher in Bangladesh (69%) than in Malaysia (25%) (Luby et al. 2009b; Tee et al. 2009). Some NiV cases experienced relapse of disease or late onset encephalitis after

initial infection, which occurred on average approximately 8 months after initial infection (range: 9 days to 22 months), and both syndromes have similar clinical manifestations (Goh et al. 2000; Tan et al. 2002; Tyler 2009). Upon postmortem examination, viral antigen was found in the brains of patients with relapse and late onset encephalitis indicating viral replication took place in these tissues. Unlike acute NiV encephalitis cases, relapse and late onset encephalitis cases did not show vasculitis in the CNS (Tan and Chua 2008; Tan et al. 2002; Tyler 2009). Several NiV-infected individuals also experienced residual neurological symptoms that ranged from mild cognitive or cerebellar disabilities to more severe cognitive impairment, with some remaining in a vegetative state (Goh et al. 2000).

In the NiV outbreak in Malaysia, a newly identified porcine respiratory and neurologic syndrome developed in some pigs infected with NiV. This syndrome was characterized by fever, barking cough, behavioral changes, uncoordinated gait, spasms, and myoclonus (Mohd Nor et al. 2000). In the 2014 outbreak in the Philippines, seven horses showed neurologic signs (head tilting, circling, and ataxia) with rapid progression of clinical signs (Ching et al. 2015).

48.9 Future Considerations

NiV and Henipavirus diseases are currently listed as one of the WHO priority diseases that pose the greatest public health risk due to their epidemic potential and the insufficient countermeasures to mitigate them (WHO 2020). There are currently no vaccines or therapeutics approved for human henipavirus infections. Numerous studies have identified potentially valuable vaccines and antiviral compounds (Gómez Román et al. 2020). A review in 2018 found there were at least 13 NiV vaccine candidates confirmed to be under development in preclinical stages (Gouglas et al. 2018). A promising therapeutic monoclonal antibody which neutralizes both HeV and NiV has undergone Phase 1 trials (Playford et al. 2020).

HeV poses a serious threat to the veterinary profession with five of the seven (71%) people infected with Hendra virus associated with this profession. Any horse which is infected with Hendra virus poses a serious threat to all who come in close contact with the animal, and this includes people, dogs, cats, ferrets, and possibly other animals. This situation prompted the development of a vaccine for horses which was released at the end of 2012 (HeV Equivac® Hendra virus vaccine). The vaccine is highly effective. In a study of 332 vaccinated horses, provided horses received at least three vaccinations (consisting of two doses 3–6 weeks apart, and a third dose 6 months later), horses had high neutralizing antibody titers and none tested negative (Halpin et al. 2021). Regarding vaccine uptake, an estimate suggested that assuming most vaccine sales are in the HeV endemic states, vaccination coverage between years 2016 and 2019 ranged from 10.1% to 13%, which is very low when compared to uptake of the equine tetanus vaccine (Halpin et al. 2021). A successful equine vaccination program has the potential to reverse the current trend of veterinarians exiting equine practice in HeV-endemic regions due to perceived personal risk and workplace liability (Mendez et al. 2012).

Two recent detections of HeV in horses have been managed with a One Health approach (Williamson et al. 2020; Taylor et al. 2022). This involved a coordinated response from animal health and human health agencies, and ecological investigations (Taylor et al. 2022). These responses serve as a blueprint for how outbreaks of zoonotic diseases, such as Hendra virus and Nipah virus, should be managed in the future.

References

Annand EJ, Horsburgh BA, Xu K, Reid PA, Poole B, de Kantzow MC, Brown N, Tweedie A, Michie M, Grewar JD, Jackson AE, Singanallur NB, Plain KM, Kim K, Tachedjian M, van der Heide B, Crameri S, Williams DT, Secombe C, Laing ED, Sterling S, Yan L, Jackson L, Jones C, Plowright RK, Peel AJ, Breed AC, Diallo I, Dhand NK, Britton PN, Broder CC, Smith I, Eden JS (2022) Novel Hendra virus variant detected by Sentinel surveillance of horses in Australia. Emerg Infect Dis 28(3):693–704

Arunkumar G, Chandni R, Mourya DT, Singh SK, Sadanandan R, Sudan P, Bhargava B, Nipah Investigators People and Health Study Group (2019) Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 219(12):1867–1878

Baldock FC, Douglas IC, Halpin K, Field H, Young PL, Black PF (1996) Epidemiological investigations into the 1994 equine morbillivirus outbreaks in Queensland, Australia. Sing Vet J 20:57–61

Ball MC, Dewberry TD, Freeman PG, Kemsley PD, Poe I (2014) Clinical review of Hendra virus infection in 11 horses in New South Wales, Australia. Aust Vet J 92(6):213–218

Blum LS, Khan R, Nahar N, Breiman RF (2009) In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. Am J Trop Med Hyg 80(1):96–102

Bonaparte MI, Dimitrov AS, Bossart KN, Crameri G, Mungall BA, Bishop KA, Choudhry V, Dimitrov DS, Wang LF, Eaton BT, Broder CC (2005) Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. Proc Natl Acad Sci U S A 102(30):10652–10657

Breed AC, Breed MF, Meers J, Field HE (2011) Evidence of endemic Hendra virus infection in flying-foxes (*Pteropus conspicillatus*)-implications for disease risk management. PLoS One 6(12):e28816

Breed AC, Meers J, Sendow I, Bossart KN, Barr JA, Smith I, Wacharapluesadee S, Wang L, Field HE (2013) The distribution of henipaviruses in Southeast Asia and Australasia: is Wallace's line a barrier to Nipah virus? PLoS One 8(4):e61316

Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE, Bellini WJ, Ksiazek TG, Mishra A (2006) Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 12(2):235–240

Ching PK, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF Jr, Bolo GC Jr, Sejvar JJ, Eagles D, Playford G, Dueger E, Kaku Y, Morikawa S, Kuroda M, Marsh GA, McCullough S, Foxwell AR (2015) Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis. 21(2):328–31

Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW (2000) Nipah virus: a recently emergent deadly paramyxovirus. Science 288(5470):1432–1435

Chua KB, Lam SK, Goh KJ, Hooi PS, Ksiazek TG, Kamarulzaman A, Olson J, Tan CT (2001) The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. J Infect 42(1):40–43

Ciancanelli MJ, Basler CF (2006) Mutation of YMYL in the Nipah virus matrix protein abrogates budding and alters subcellular localization. J Virol 80(24):12070–12078

Clayton BA, Middleton D, Bergfeld J, Haining J, Arkinstall R, Wang L, Marsh GA (2012) Transmission routes for Nipah virus from Malaysia and Bangladesh. Emerg Infect Dis 18(12): 1983–1993

Diederich S, Moll M, Klenk HD, Maisner A (2005) The Nipah virus fusion protein is cleaved within the endosomal compartment. J Biol Chem 280(33):29899–29903
Domingo E (2010) Mechanisms of viral emergence. Vet Res 41:38
Drexler JF, Corman VM, Gloza-Rausch F, Seebens A, Annan A, Ipsen A, Kruppa T, Muller MA, Kalko EK, Adu-Sarkodie Y, Oppong S, Drosten C (2009) Henipavirus RNA in African bats. PLoS ONE 4(7):e6367
Eaton BT, Mackenzie JS, Wang L-F (2007) Paramyxoviridae: Henipaviruses, vol 2. In: Knipe DM, Howley PM (eds) Fields Virology, 5th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1587–1600
Edson D, Field H, McMichael L, Vidgen M, Goldspink L, Broos A, Melville D, Kristoffersen J, de Jong C, McLaughlin A, Davis R, Kung N, Jordan D, Kirkland P, Smith C (2015) Routes of Hendra virus excretion in naturally infected flying-foxes: implications for viral transmission and Spillover risk. PLoS One 10(10):e0140670
Epstein JH, Anthony SJ, Islam A, Kilpatrick AM, Ali Khan S, Balkey MD, Ross N, Smith I, Zambrana-Torrelio C, Tao Y, Islam A, Quan PL, Olival KJ, Khan MSU, Gurley ES, Hossein MJ, Field HE, Fielder MD, Briese T, Rahman M, Broder CC, Crameri G, Wang LF, Luby SP, Lipkin WI, Daszak P (2020) Nipah virus dynamics in bats and implications for spillover to humans. Proc Natl Acad Sci U S A 117(46):29190–29201
Field H, Schaaf K, Kung N, Simon C, Waltisbuhl D, Middleton D (2010) Hendra virus outbreak with novel clinical features, Australia. Emerg Infect Dis 16:338–340
Goh KJ, Tan CT, Chew NK, Tan PS, Kamarulzaman A, Sarji SA, Wong KT, Abdullah BJ, Chua KB, Lam SK (2000) Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med 342(17):1229–1235
Gómez Román R, Wang LF, Lee B, Halpin K, de Wit E, Broder CC, Rahman M, Kristiansen P, Saville M (2020) Nipah@20: lessons learned from another virus with pandemic potential. mSphere 5(4):e00602-20. https://doi.org/10.1128/mSphere.00602-20
Gouglas D, Thanh Le T, Henderson K, Kaloudis A, Danielsen T, Hammersland NC, Robinson JM, Heaton PM, Røttingen JA (2018) Estimating the cost of vaccine development against epidemic infectious diseases: a cost minimisation study. Lancet Glob Health 6(12):e1386–e1396
Halpin K, Young PL, Field HE, Mackenzie JS (2000) Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J Gen Virol 81(Pt 8):1927–1932
Halpin K, Hyatt AD, Plowright RK, Epstein JH, Daszak P, Field HE, Wang L, Daniels PW, Henipavirus Ecology Research Group (2007) Emerging viruses: coming in on a wrinkled wing and a prayer. Clin Infect Dis 44:711–717
Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, Epstein JH, Rahman SA, Hughes T, Smith C, Field HE, Daszak P, Henipavirus Ecology Research Group (2011) Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. Am J Trop Med Hyg 85(5):946–951
Halpin K, Graham K, Durr PA (2021) Sero-monitoring of horses demonstrates the Equivac® HeV Hendra virus vaccine to be highly effective in inducing neutralising antibody titres. Vaccines (Basel) 9(7):731
Hanna JN, McBride WJ, Brookes DL, Shield J, Taylor CT, Smith IL (2006) Hendra virus infection in a veterinarian. Med J Aust 185:562–564
Harcourt BH, Lowe L, Tamin A, Liu X, Bankamp B, Bowden N, Rollin PE, Comer JA, Ksiazek TG, Hossain MJ, Gurley ES, Breiman RF, Bellini WJ, Rota PA (2005) genetic characterization of Nipah virus, Bangladesh. Emerg Infect Dis 11(10):1594–1597
Hasebe F, Thuy NT, Inoue S, Yu F, Kaku Y, Watanabe S, Akashi H, Dat DT, Mai le TQ, Morita K (2012) Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 18(3): 536–537
Hayman DT, Suu-Ire R, Breed AC, McEachern JA, Wang L, Wood JL, Cunningham AA (2008) Evidence of Henipavirus infection in West African fruit bats. PLoS One 3(7):e2739
Hooper PT, Williamson MM (2000) Hendra and Nipah virus infections. Vet Clin North Am Equine Pract 16(3):597–603
Hossain MJ, Gurley ES, Montgomery JM, Bell M, Carroll DS, Hsu VP, Formenty P, Croisier A, Bertherat E, Faiz MA, Azad AK, Islam R, Molla MA, Ksiazek TG, Rota PA, Comer JA, Rollin

PE, Luby SP, Breiman RF (2008) Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 46(7):977–984

Iehle C, Razafitrimo G, Razainirina J, Andriaholinirina N, Goodman SM, Faure C, Georges-Courbot MC, Rousset D, Reynes JM (2007) Henipavirus and Tioman virus antibodies in Pteropodid bats, Madagascar. Emerg Infect Dis 13(1):159–161

Khan SU, Gurley ES, Hossain MJ, Nahar N, Sharker MA, Luby SP (2012) A randomized controlled trial of interventions to impede date palm sap contamination by bats to prevent Nipah virus transmission in Bangladesh. PLoS One 7(8):e42689

Kirkland PD, Gabor M, Poe I, Neale K, Chaffey K, Finlaison DS, Gu X, Hick PM, Read AJ, Wright T, Middleton D (2015) Hendra Virus Infection in Dog, Australia, 2013. Emerg Infect Dis 21 (12):2182–5

Lamb RA, Parks GD (2007) Paramyxoviridae: the viruses and their replication. In: Knipe DMHP (ed) Fields virology, vol 1, 5th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1449–1496

Li Y, Wang J, Hickey AC, Zhang Y, Li Y, Wu Y, Zhang H, Yuan J, Han Z, McEachern J, Broder CC, Wang LF, Shi Z (2008) Antibodies to Nipah or Nipah-like viruses in bats, China. Emerg Infect Dis 14(12):1974–1976

Lo MK, Lowe L, Hummel KB, Sazzad HM, Gurley ES, Hossain MJ, Luby SP, Miller DM, Comer JA, Rollin PE, Bellini WJ, Rota PA (2012) Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 18(2):248–255

Luby SP, Gurley ES (2012) Epidemiology of Henipavirus disease in humans. Curr Top Microbiol Immunol 359:25–40

Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E, Comer JA, Ksiazek TG (2006) Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 12:1888–1894

Luby SP, Gurley ES, Hossain MJ (2009a) Transmission of human infections with Nipah virus. Clin Infect Dis 49:1743–1748

Luby SP, Hossain MJ, Gurley ES, Ahmed B-N, Banu S, Khan SU, Homaira N, Rota PA, Rollin PE, Comer JA, Kenah E, Ksiazek TG, Rahman M (2009b) Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 15(8):1229–1235

Marsh GA, Todd S, Foord A, Hansson E, Davies K, Wright L, Morrissy C, Halpin K, Middleton D, Field HE, Daniels P, Wang LF (2010) Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. Emerg Infect Dis 16(11):1767–1769

Marsh GA, Haining J, Hancock TJ, Robinson R, Foord AJ, Barr JA, Riddell S, Heine HG, White JR, Crameri G, Field HE, Wang LF, Middleton D (2011) Experimental infection of horses with Hendra virus/Australia/horse/2008/Redlands. Emerg Infect Dis 17(12):2232–2238

Mendez DH, Judd J, Speare R (2012) Unexpected result of Hendra virus outbreaks for veterinarians, Queensland, Australia. Emerg Infect Dis 18(1):83–85

Middleton DJ, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, Westbury HA, Halpin K, Daniels PW (2007) Experimental Nipah virus infection in pteropid bats (*Pteropus poliocephalus*). J Comp Pathol 136(4):266–272

Middleton DJ, Riddell S, Klein R, Arkinstall R, Haining J, Frazer L, Mottley C, Evans R, Johnson D, Pallister J (2017) Experimental Hendra virus infection of dogs: virus replication, shedding and potential for transmission. Aust Vet J 95(1–2):10–18

Mire CE, Satterfield BA, Geisbert JB, Agans KN, Borisevich V, Yan L, Chan YP, Cross RW, Fenton KA, Broder CC, Geisbert TW (2016) Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 6:30916

Mohd Nor MN, Gan CH, Ong BL (2000) Nipah virus infection of pigs in peninsular Malaysia. Rev Sci Tech 19:160–165

Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B, Ketterer P (1995) A morbillivirus that caused fatal disease in horses and humans. Science 268(5207):94–97

Negrete OA, Levroney EL, Aguilar HC, Bertolotti-Ciarlet A, Nazarian R, Tajyar S, Lee B (2005) EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. Nature 436 (7049):401–405

Negrete OA, Wolf MC, Aguilar HC, Enterlein S, Wang W, Muhlberger E, Su SV, Bertolotti-Ciarlet A, Flick R, Lee B (2006) Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. PLoS Pathog 2(2):e7

O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, Gould AR, Hyatt AD, Bradfield J (1997) Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet 349(9045):93–95

Ong ST, Yusoff K, Kho CL, Abdullah JO, Tan WS (2009) Mutagenesis of the nucleocapsid protein of Nipah virus involved in capsid assembly. J Gen Virol 90(Pt 2):392–397

Páez DJ, Giles J, McCallum H, Field H, Jordan D, Peel AJ, Plowright RK (2017) Conditions affecting the timing and magnitude of Hendra virus shedding across pteropodid bat populations in Australia. Epidemiol Infect 145(15):3143–3153

Patch JR, Crameri G, Wang LF, Eaton BT, Broder CC (2007) Quantitative analysis of Nipah virus proteins released as virus-like particles reveals central role for the matrix protein. Virol J 4:1

Patch JR, Han Z, McCarthy SE, Yan L, Wang LF, Harty RN, Broder CC (2008) The YPLGVG sequence of the Nipah virus matrix protein is required for budding. Virol J 5(1):137

Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, Ling AE, Chew SK, Ang B, Rollin PE, Umapathi T, Sng I, Lee CC, Lim E, Ksiazek TG (1999) Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 354(9186):1253–1256

Playford EG, McCall B, Smith G, Slinko V, Allen G, Smith I (2010) Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. Emerg Infect Dis 16:219–223

Playford EG, Munro T, Mahler SM, Elliott S, Gerometta M, Hoger KL, Jones ML, Griffin P, Lynch KD, Carroll H, El Saadi D, Gilmour ME, Hughes B, Hughes K, Huang E, de Bakker C, Klein R, Scher MG, Smith IL, Wang LF, Lambert SB, Dimitrov DS, Gray PP, Broder CC (2020) Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis 20(4):445–454

Plowright RK, Field HE, Smith C, Divljan A, Palmer C, Tabor G, Daszak P, Foley JE (2008) Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). Proc Biol Sci 275(1636):861–869

Promed (2011) Hendra virus, equine—Australia (18): (Queensland) canine [cited 2023 Jan 10]. http://www.promedmail.org. Archive no. 20110727.2257

Rahman MA, Hossain MJ, Sultana S, Homaira N, Khan SU, Rahman M, Gurley ES, Rollin PE, Lo MK, Comer JA, Lowe L, Rota PA, Ksiazek TG, Kenah E, Sharker Y, Luby SP (2012) Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis 12(1):65–72

Reynes JM, Counor D, Ong S, Faure C, Seng V, Molia S, Walston J, Georges-Courbot MC, Deubel V, Sarthou JL (2005) Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 11(7):1042–1047

Rogers RJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, Gleeson LJ, Selleck PN, Dunn KJ (1996) Investigation of a second focus of equine morbillivirus infection in coastal Queensland. Aust Vet J 74(3):243–244

Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, Lavercombe PS, Selleck P, Sheridan JW (1995) Infection of humans and horses by a newly described morbillivirus. Med J Aust 162(12):642–645

Selvey LA, Taylor R, Arklay A, Gerrard J (1996) Screening of bat carers for antibodies to equine morbillivirus. Commun Dis Intell 20:477–478

Smith I, Broos A, de Jong C, Zeddeman A, Smith C, Smith G, Moore F, Barr J, Crameri G, Marsh G, Tachedjian M, Yu M, Kung YH, Wang LF, Field H (2011) Identifying Hendra virus diversity in pteropid bats. PLoS One 6(9):e25275

Sohayati AR, Hassan L, Sharifah SH, Lazarus K, Zaini CM, Epstein JH, Shamsyul Naim N, Field HE, Arshad SS, Abdul Aziz J, Daszak P, Henipavirus Ecology Research Group (2011) Evidence for Nipah virus recrudescence and serological patterns of captive *Pteropus vampyrus*. Epidemiol Infect 139(10):1570–1579

Sudeep AB, Yadav PD, Gokhale MD et al (2021) Detection of Nipah virus in *Pteropus medius* in 2019 outbreak from Ernakulam district, Kerala, India. BMC Infect Dis 21:162. https://doi.org/10.1186/s12879-021-05865-7

Tan CT, Chua KB (2008) Nipah virus encephalitis. Curr Infect Dis Rep 10(4):315–320
Tan CT, Goh KJ, Wong KT, Sarji SA, Chua KB, Chew NK, Murugasu P, Loh YL, Chong HT, Tan KS, Thayaparan T, Kumar S, Jusoh MR (2002) Relapsed and late-onset Nipah encephalitis. Ann Neurol 51(6):703–708
Taylor C, Playford EG, McBride WJ, McMahon J, Warrilow D (2012) No evidence of prolonged Hendra virus shedding by 2 patients, Australia. Emerg Infect Dis 18(12):2025–2027
Taylor J, Thompson K, Annand EJ, Massey PD, Bennett J, Eden JS, Horsburgh BA, Hodgson E, Wood K, Kerr J, Kirkland P, Finlaison D, Peel AJ, Eby P, Durrheim DN (2022) Novel variant Hendra virus genotype 2 infection in a horse in the greater Newcastle region, New South Wales, Australia. One Health 15:100423
Tee KK, Takebe Y, Kamarulzaman A (2009) Emerging and re-emerging viruses in Malaysia, 1997–2007. Int J Infect Dis 13(3):307–318
Tyler KL (2009) Emerging viral infections of the central nervous system: part 2. Arch Neurol 66(9): 1065–1074
Wang L, Harcourt BH, Yu M, Tamin A, Rota PA, Bellini WJ, Eaton BT (2001) Molecular biology of Hendra and Nipah viruses. Microbes Infect 3(4):279–287
Wang J, Anderson DE, Halpin K, Hong X, Chen H, Walker S, Valdeter S, van der Heide B, Neave MJ, Bingham J, O'Brien D, Eagles D, Wang LF, Williams DT (2021) A new Hendra virus genotype found in Australian flying foxes. Virol J 18(1):197
Weingartl HM, Berhane Y, Czub M (2009) Animal models of henipavirus infection: a review. Vet J 181(3):211–220
Westbury HA, Hooper PT, Selleck PW, Murray PK (1995) Equine morbillivirus pneumonia: susceptibility of laboratory animals to the virus. Aust Vet J 72(7):278–279
Williamson MM, Hooper PT, Selleck PW, Gleeson LJ, Daniels PW, Westbury HA, Murray PK (1998) Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses, and cats. Aust Vet J 76:813–818
Williamson KM, Wheeler S, Kerr J, Bennett J, Freeman P, Kohlhagen J, Peel AJ, Eby P, Merritt T, Housen T, Dalton C (2020) Hendra in the Hunter Valley. One Health 10:100162
Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR (2002) Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 161 (6):2153–2167
Wong KT, Robertson T, Ong BB, Chong JW, Yaiw KC, Wang LF, Ansford AJ, Tannenberg A (2009) Human Hendra virus infection causes acute and relapsing encephalitis. Neuropathol Appl Neurobiol 35(3):296–305
World Health Organization (WHO) (2020) WHO priority diseases. World Health Organization, Geneva. https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts
Yadav PD, Shete AM, Kumar GA, Sarkale P, Sahay RR, Radhakrishnan C, Lakra R, Pardeshi P, Gupta N, Gangakhedkar RR, Rajendran VR, Sadanandan R, Mourya DT (2019) Nipah virus sequences from humans and bats during Nipah outbreak, Kerala, India, 2018. Emerg Infect Dis 25(5):1003–1006
Yadav PD, Sahay RR, Balakrishnan A, Mohandas S, Radhakrishnan C, Gokhale MD, Balasubramanian R, Abraham P, Gupta N, Sugunan AP, Khobragade R, George K, Shete A, Patil S, Thankappan UP, Dighe H, Koshy J, Vijay V, Gayathri R, Kumar PJ, Rahim A, Naveen A, Nair S, Rajendran VR, Jayasree V, Majumdar T, Jain R, Viswanathan P, Patil DY, Kumar A, Nyayanit DA, Sarkale P, Waghmare A, Baradkar S, Gawande P, Bodke P, Kalele K, Yemul J, Dhaigude S, Holepannawar M, Gopale S, Chopade G, Ray S, Waghmare P, Narayan J, Mathapati B, Kadam M, Kumar A, Suryawanshi A, Jose BP, Sivadas S, Akash NP, Vimisha TV, Keerthi KV (2022) Nipah virus outbreak in Kerala state, India amidst of COVID-19 pandemic. Front Public Health 10:818545
Yob JM, Field H, Rashdi AM, Morrissy C, van der Heide B, Rota P, bin Adzhar A, White J, Daniels P, Jamaluddin A, Ksiazek T (2001) Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 7(3):439–441

Borna Disease (Borna Disease Virus-1, BoDV-1)

49

Beware of the Bicoloured White-Toothed Shrew

Merle M. Böhmer and Markus Bauswein

Contents

Abstract

In this chapter, we describe the virologic and diagnostic aspects of Borna disease virus 1 (BoDV-1), the clinical presentation, epidemiology, transmission, and potential infection control in animals and humans. Known as an equine disease for more than 250 years, it was proven only in 2018 that BoDV-1 can cause severe encephalitis in humans and thus has zoonotic potential. BoDV-1 is an enveloped virus with negative-stranded, nonsegmented RNA belonging to the genus

M. M. Böhmer (✉)
Department of Infectious Disease Epidemiology and Surveillance, Data and Modelling Unit, Bavarian Health and Food Safety Authority, Munich, Germany
e-mail: merle.boehmer@lgl.bayern.de

M. Bauswein
Institute of Clinical Microbiology and Hygiene, Regensburg University Hospital, Regensburg, Germany
e-mail: markus.bauswein@ukr.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_52

Orthobornavirus in the family of *Bornaviridae*. The only known reservoir host for BoDV-1 is the bicolored white-toothed shrew (*Crocidura leucodon*). This species can excrete the virus, e.g., via saliva, urine, and feces without showing symptoms itself. Particularly, not only horses, sheep, and alpacas, but also a number of other mammals, including humans, are susceptible to infections with BoDV-1. They act as so-called "dead-end hosts" and do not excrete the virus. In dead-end hosts, the virus can lead to brain infections and to severe, often lethal encephalitis (Borna disease). Known endemic areas for BoDV-1 are located in parts of southern and eastern Germany, Switzerland, Austria, and Liechtenstein. BoDV-1 can be transmitted to humans via transplanted organs, but the natural transmission route in humans and also other dead-end hosts has not yet been fully clarified. Further research is needed on, among other things, the clinical spectrum of human BoDV-1 infections and the reservoir. In order to prevent BoDV-1 infections in humans, a better understanding of transmission routes is also crucial.

Keywords

Borna disease · BoDV-1 · Encephalitis · Bicolored white-toothed shrew · *Crocidura leucodon* · Diagnostics · Pathophysiology · Transmission · Host · Reservoir · Varrigated squirrel bornavirus 1 · VSBV-1 · Disease burden · Epidemiology · Mental disorders · Dead-end host · Transplantation · Sad horse disease · Borna disease virus 1

49.1 Introduction

Borna disease was confirmed as a zoonosis only a few years ago, though the zoonotic potential of this viral disease has been discussed in scientific circles for many decades. Following transmission via organ transplantation, it was demonstrated for the first time in 2018 that Borna disease virus 1 (BoDV-1) can cause severe encephalitis in humans (Schlottau et al. 2018). The neurological symptoms and behavioral disorders typical of Borna disease, however, have been reported in horses and sheep from certain areas in Germany for more than 250 years (Durrwald and Ludwig 1997; Working Group Blood of the German Ministry of Health 2019). For many decades, the characteristic clinical picture resulting from a severe encephalitis was referred to as "heated head disease of horses." Between 1894 and 1896, an outbreak of the disease had been observed among cavalry horses in the Saxon town of Borna in eastern Germany. A few hundred horses succumbed to the disease at that time, and from then on it was called Borna disease (Durrwald and Ludwig 1997; Dürrwald et al. 2014; Working Group Blood of the German Ministry of Health 2019). The name was later also adopted in scientific nomenclature. About a decade later, in 1909, the German researchers Ernst Joest and Kurt Degen described intranuclear inclusion bodies in the ganglia of the hippocampal Ammon's horn in material from horses that had died from Borna disease (Joest and Degen 1909, 1911). Up to the present day, the detection of so-called Joest-Degen inclusion bodies is

regarded as confirmation of Borna disease in the differential diagnosis of neurological diseases in horses. These inclusion bodies were later also detected in sheep suffering from Borna disease and recently in human cases (Liesche et al. 2019). In the 1920s, Zwick and colleagues proved the viral etiology of the pathogen. Subsequently, Zwick's research team experimentally infected species such as rabbits, guinea pigs, rats, and rhesus monkeys by intracerebral inoculation, thereby giving a first indication of the virus' potential to infect a variety of warm-blooded animal species (Zwick 1927; Zwick and Seifried 1925).

In this chapter, we give an overview of the newly identified zoonosis Borna disease, methods to detect BoDV-1, pathophysiological aspects, the reservoir host (the bicolored white-toothed shrew), and possible transmission routes as well as the clinical symptoms and epidemiological situation of Borna disease in animals and humans. Additionally, we give an overview about questions that have arisen following the confirmation of Borna disease as a zoonosis. The chapter also contains a digression on another bornavirus with zoonotic potential, the varrigated squirrel bornavirus (VSBV-1).

49.2 Pathogen: Genome Organization and Viral Structure

Bornaviruses are enveloped, nonsegmented single stranded negative sense RNA viruses with the unique property among the order of *Mononegavirales* to replicate and transcribe within the host cell nucleus (Briese et al. 1994; Jordan and Lipkin 2001; Tizard et al. 2016). The virus species BoDV-1 and VSBV-1, belonging to the genus *Orthobornavirus*, are the only bornaviruses for which pathogenicity for humans has yet been demonstrated. The genome of bornaviruses has a length of 8.9 kb containing six open reading frames (ORF) that encode six viral proteins in the following order 3′-N-X-P-M-GP-L-5′ (Pyper et al. 1993).

Virus entry is mediated by the glycosylated membrane protein GP (nonglycosylated form: 56kD; glycosylated form: 84–94kD) which, after synthesis in the infected cell, is cleaved in the Golgi vesicle by the host protease furin into two subunits: an N-terminal gp56 external type I membrane protein GP1 and a C-terminal gp43 membrane-bound protein GP2 (Richt et al. 1998). Binding of GP1 to a so far unknown receptor initiates virus entry by clathrin-mediated endocytosis (Clemente and de la Torre 2009; Makino et al. 2009). Antibodies against GP are neutralizing (Furrer et al. 2001; Modrow et al. 2010). After pH-dependent membrane fusion with the early endosomal compartment, ribonucleoprotein complexes (RNP) containing the nucleocapsid protein N (40 kDa), the phosphoprotein P (23 kDa), the RNA-dependent RNA polymerase L (large protein, 190 kDa), and the RNA are released into the cytoplasm and translocate into the nucleus where viral replication and transcription take place (Clemente and de la Torre 2009; Jordan and Lipkin 2001). The matrix protein M (18 kDa) is associated with the inner membrane (Kraus et al. 2002). The accessory protein X (10 kDa) has various functions: As a counterpart of the P protein, it regulates the activity of the RNA-dependent RNA

polymerase (Poenisch et al. 2004). Further, a neuroprotective, antiapoptotic effect has been demonstrated so that the X protein likely contributes to viral persistence (Poenisch et al. 2009; Szelechowski et al. 2014). Only a small number of infectious particles are released from bornavirus-infected cells (Tizard et al. 2016). RNPs are tethered to chromosomes and are passed from cell to cell upon cell division to maintain a persistent infection (Garcia et al. 2021).

49.3 Diagnostics

The potential of BoDV-1 to cause severe human encephalitis is now accepted in the scientific community, while studies and diagnostic approaches that aimed to show a correlation between direct as well as indirect detections of BoDV-1 and human psychiatric disorders remain controversial (compare "Bornavirus infections and mental disorders – a controversial issue") (Schwemmle 2001). The assessment of diagnostic tests for BoDV-1 in humans should take this controversial aspect of bornavirus research (history) into consideration. Therefore, a diagnostic algorithm for a case definition of human BoDV-1 infections has been proposed (Eisermann et al. 2021). The fundamental pillar of this case definition is the clinical presentation of an encephalopathy or encephalitis. For a confirmed case, additionally the detection of BoDV-1-specific RNA from a neural tissue sample/cerebrospinal fluid (CSF) or the detection of a BoDV-1 protein in a tissue section by immunohistochemistry (IHC) is required. Patients presenting with an encephalopathy or encephalitis and showing a positive serological test result are defined as probable case. For the retrospective postmortem confirmation of BoDV-1 cases, direct and indirect methods have been used.

As mentioned before, in the early twentieth century Joest and Degen described nuclear inclusion bodies in ganglion cells of diseased animals and herewith provided a first specific histological indication for the disease (Joest and Degen 1909). After the identification of a virus as causative pathogen and after deciphering its sequence and genome organization in 1994 (Briese et al. 1994; Cubitt et al. 1994), diagnosing Borna disease in animal dead-end hosts nowadays relies on histological and molecular biological approaches like reverse transcription-quantitative polymerase chain reaction (qRT-PCR) from neural tissue, supplemented by detection of BoDV-1 RNA (RNA in situ hybridization) and BoDV-1 proteins (IHC) in tissue sections (Schulze et al. 2020). For humans, qRT-PCR has been shown to be positive in samples from central and in some cases also from peripheral nerve tissue (Neumann et al. 2022; Niller et al. 2020). According to a published protocol, primers and probes for qRT-PCR were designed to target two genomic regions of BoDV-1, one in the x/p region, one in the m/g region (Schlottau et al. 2018). Depending on the test evaluation algorithm, a dual target PCR assay may increase specificity or sensitivity of the test system. The short amplicon of 75 bp within the m/g gene might be particularly suitable to detect degraded RNA in tissue samples of biobanks (Niller et al. 2020). In 2010, it has been shown that elements homologous to the nucleoprotein (N) gene of bornavirus have been integrated in the genomes of several mammalian species, including humans, as so-called endogenous Borna-like N (EBLN) elements (Horie et al. 2010). Therefore, qRT-PCR primers and probes for detection of BoDV-1 should be designed for genetic regions

that are not integrated in the human genome in order to avoid false positive test results. As a second molecular biological approach, next generation sequencing (NGS) further underlines the accuracy for the detection of BoDV-1 as human pathogen. In the first published cases of a BoDV-1 encephalitis, almost the complete 8.9 kb BoDV-1 genome could be derived from tissue samples of infected patients (Korn et al. 2018; Schlottau et al. 2018). Based on NGS-obtained sequences, BoDV-1 strains can be grouped into several regional clusters. Sequences derived from shrews, infected patients, and animals in regional proximity show closest similarities, rendering the possibility of a contamination by a laboratory strain unlikely (Niller et al. 2020). In addition, BoDV-1-specific RNA and proteins were detected in tissue sections of patients (Liesche et al. 2019). Direct detection methods are accompanied by serological tests. Indirect immunofluorescence assay (iIFT) is the most abundantly used in-house serological laboratory test. Persistently BoDV-1-infected cell lines (for example, Vero cells) are heat-fixed for staining with samples and a secondary, fluorophore-conjugated antibody. Positive samples show a nuclear fluorescence pattern. As a control for unspecific staining, uninfected cells should be included (Neumann et al. 2022). Disadvantages of the test are the time-consuming evaluation and the partly subjective and examiner-dependent accuracy of test results. Additional serological tests utilizing recombinantly expressed BoDV-1 proteins (mainly nucleocapsid and phosphoprotein) in form of line assays, immunoblots, Western blots, or ELISA are recommended (Eisermann et al. 2021). Recently, an ELISA using recombinant viral N, X, and P proteins has been described (Neumann et al. 2022). These tests are not commercially available but are performed as in-house tests. Thorough validations of these in-house tests are recommended to enable an evaluation with regard to sensitivity and specificity. Whether immune reactions against expressed EBLN might lead to false positive results in these tests remains unclear (Working Group Blood of the German Ministry of Health 2019).

In contrast to a postmortem diagnosis, a timely *intravitam* diagnosis of a BoDV-1 infection is challenging for several reasons: First, patients may initially present with an encephalopathy-like or Guillan-Barré-Syndrome (GBS)-like clinical picture (Coras et al. 2019). Initial CSF may only show elevated protein, but normal cell count. As a consequence, initiating BoDV-1 diagnostic might be delayed. Second, BoDV-1 is a cell-associated virus. RNA-loads are high in neural tissue but are low or in the range of the lower limit of qRT-PCR in CSF, if detectable at all (Niller et al. 2020). A negative qRT-PCR from CSF may not completely rule out BoDV-1 infection. As brain biopsies are invasive and not the first diagnostic approach, a direct detection *intra vitam* is challenging. Third, seroconversion mainly occurs only during the later course of the disease and, depending on the serological test system used, unspecific reactions might make a serological diagnosis difficult.

49.4 Pathophysiology of Symptomatic BoDV-1 Infection

Bornaviruses do not cause a cytopathic effect in cell culture. In affected individuals, neurons, oligodendrocytes, and astrocytes are infected in a nonlytic fashion. The pathophysiology of symptomatic bornavirus infections is attributed to three main

hallmarks: T-cell-mediated immunopathology, microglial activation, and metabolic disturbances of neurons (Tizard et al. 2016).

On the one hand, evidence for the T-cell-driven immunopathogenesis of bornavirus infections comes from animal experiments: Despite persistently high viral loads in brain and CNS, rats experimentally infected with BoDV-1 do not develop neurological symptoms when the immunosuppressive drug cyclophosphamide is administered shortly after virus inoculation – in contrast to animals without this immunosuppressive treatment (Narayan et al. 1983a). In a further study with rats, the immunosuppressive drug cyclosporine prevented neurological disease in infected rats only when administered before virus inoculation (Stitz et al. 1989). In infected rabbits, treatment with cyclophosphamide and/or glucocorticoids altered clinical signs and prolonged survival (Gierend and Ludwig 1981). Further, intracerebral infection of newborn β2-microglobulin-deficient C57BL/6 and MRL mice both lacking CD8 T cells did not cause neurological symptoms (Hallensleben et al. 1998). As further evidence for the immune-driven pathogenesis, adoptive transfer of spleen or lymph cells into immunosuppressed animals induced the manifestation of clinical symptoms (Narayan et al. 1983b; Richt et al. 1989).

On the other hand, there is evidence of immunopathogenesis in human patients as well: Brain tissue sections show parenchymal and perivascular infiltrates of CD4 and CD8 lymphocytes (Liesche et al. 2019). Patients with immunosuppression due to another medical condition (e.g., after organ transplantation) tended to survive longer than patients without immunosuppression (Niller et al. 2020). One of the two so far described survivors of a symptomatic BoDV-1 infection in the literature received cyclosporin as immunosuppressive medication because of a liver transplantation (Schlottau et al. 2018). T-cell pathology is assumed to be mediated by cytotoxic CD8 T cells that require help from CD4 T cell subsets. Neuronal damage is obviously mediated by CD8 T cells that are directed against viral proteins like BoDV-1 N protein and produce both IFNγ and TNFα (Amor et al. 2014; Baruch and Schwartz 2013; Tizard et al. 2016).

In experiments with rats, the peptide ASYAQMTTY within the viral N protein has been identified as a main epitope of MHC-I-(RT1.A)-restricted cytotoxic T cell response (Planz et al. 2001). Of special note is that transgenic mice expressing the nucleoprotein in neurons are resistant to experimental infection and clinical symptoms, which is presumably attributable to untimely expression of a viral nucleocapsid component, on the one hand, and immunological tolerance, on the other hand (Rauer et al. 2004). After the discovery of endogenous bornavirus-like elements that are integrated in a broad spectrum of vertebrate genomes, but are absent in the genomes of the most frequent accidental dead-end hosts such as horse, sheep, and most bird species, one could speculate on a potentially protective function of the endogenous bornavirus-like element N (EBLN), which is transcribed and translated into a protein in different tissues (Belyi et al. 2010; Fujino et al. 2014; Horie et al. 2010; Nobach et al. 2020). While CD8 T cells are found most abundantly in the brain parenchyma, perivascular infiltrates predominantly consist of CD4 T cells (Sobbe et al. 1997).

The infiltration of T cells in the brain is preceded by high levels of chemokines, especially IP-10. In a mouse model, astrocytes have been identified as a source of IP-10 (Sauder et al. 2000). Astrocytes induce the synthesis of IP-10 following stimulation with IFNα/β and other cytokines such as IL-1β and TNFα. IP-10 serves as a chemokine to attract especially T cells to the brain. Besides T-cell recruitment, microglial activation is an independent factor for neurocytotoxicity (Tang et al. 2021; Tizard et al. 2016). In addition, metabolic disturbances contribute to symptoms of BoDV-1 infections, for example, by inhibition of glutamate reuptake by astrocytes resulting in excessive extracellular glutamate concentrations that lead to neuronal damage (Ovanesov et al. 2007). Further, it has also been shown that neurogenesis is impaired by viral proteins (Scordel et al. 2015).

49.5 Clinical Presentation in Animals

Under natural conditions, BoDV-1 mainly infects horses, sheep, alpacas, and rarely other mammals living in endemic regions (Malbon et al. 2021; Metzler et al. 1976; Richt et al. 2000; Schmidt 1912, 1952; Schulze et al. 2020; Waelchli et al. 1985). Those animals are considered so-called *dead-end hosts*, meaning a host organism, which has suboptimal conditions for a pathogen leading to no further onward transmission of the disease. As a rule, Borna disease in horses is characterized by acute, frequently severe, and lethal encephalitis. The incubation period ranges from a few weeks to several months (Richt et al. 2000). The equine disease has usually a biphasic course: It starts with nonspecific symptoms such as hyperthermia, colic, anorexia, and constipation, followed by ataxia, depression, nervousness, and lethargy (Richt et al. 1997, 2000). In addition, behavioral changes occur, such as spreading and crossing the legs, pressing the head against the wall or other objects, as well as empty chewing and "pipe smoking" (horses remain still with feed hanging out of their mouths being unable to chew and swallow it). Due to these characteristic symptoms, Borna disease is also called "sad horse disease." As the disease progresses, paralysis can occur, animals frequently fall into a coma and decease. Retinitis also frequently occurs as a disease manifestation, often resulting in blindness. In 80–100% of cases, horses succumb due to Borna disease (Richt et al. 1997; Schmidt 1912, 1952). In sheep and alpacas, the course of the disease is similar to Borna disease in horses (Jacobsen et al. 2010; Malbon et al. 2021; Metzler et al. 1976; Schulze et al. 2020; Waelchli et al. 1985). In rare cases, similar disease courses have also been in described for zoo animals that had become infected naturally with BoDV-1 in endemic regions (Schüppel et al. 1994). In addition, a large number of other mammal species (e.g., rats, bank voles, and hamsters (Anzil et al. 1973; Gosztonyi et al. 2020; Kinnunen et al. 2011)) have been experimentally infected with BoDV-1.

There is controversy among experts as to whether the so-called *feline staggering disease* in cats can be attributed to a naturally acquired BoDV-1 infection (Lundgren et al. 1997; Nowotny 1999; Nowotny and Weissenböck 1995; Wensman et al. 2014; Working Group Blood of the German Ministry of Health 2019). Final proof that the

clinical picture of staggering disease does not only occur following experimental infection in the laboratory setting is still pending (Working Group Blood of the German Ministry of Health 2019).

49.6 Clinical Presentation in Humans

The clinical picture of Borna disease in humans has so far been described only for a two-digit number of cases (Coras et al. 2019; Eisermann et al. 2021; Finck et al. 2020; Frank et al. 2022; Korn et al. 2018; Liesche et al. 2019; Meier et al. 2022; Niller et al. 2020; Pörtner et al. 2019, 2020; Schlottau et al. 2018; Tappe et al. 2021). It is assumed that the incubation period of BoDV-1 in humans ranges from a few weeks to months, similarly to other dead-end hosts (Pörtner et al. 2019). In the initial transplantation cluster, infected organ recipients started to develop symptoms around 3 months after the transplantation; however, symptom onset might have been delayed by immunosuppressive therapy (Niller et al. 2020; Schlottau et al. 2018). According to current knowledge, humans infected with BoDV-1 show a short phase with nonspecific flu-like symptoms like fatigue, headache, elevated temperatures, and reduced general performance (Niller et al. 2020; Pörtner et al. 2019, 2020). This phase is followed by the occurrence of various neurological symptoms such as gait ataxia, dysphagia, confusion, memory deficits, seizures, hemiparesis, and progressive loss of consciousness (Finck et al. 2020; Niller et al. 2020). As mentioned before, in a few cases, patients showed symptoms mimicking a GBS (Coras et al. 2019; Liesche et al. 2019). In the further course, the disease progresses rapidly after a few days to weeks with increasing inflammatory parameters and dysphagia, progressive paresis, respiratory insufficiency, somnolence, and finally deep irreversible coma and death (Eisermann et al. 2021; Finck et al. 2020; Frank et al. 2022; Korn et al. 2018; Liesche et al. 2019; Meier et al. 2022; Niller et al. 2020). Refractory fever was also described for several cases (e.g., Finck et al. 2020; Niller et al. 2020; Pörtner et al. 2019).

Neuropathologically, a nonpurulent, lymphocytic sclerosing panencephalomyelitis is typical for BoDV-1 patients (Liesche et al. 2019). BoDV-1 encephalitis is characterized by marked inflammation with diffuse parenchymal lymphohistiocytic infiltration and perivascular cuffing and strong microglial activation (Frank et al. 2022; Liesche et al. 2019). However, on individual level, the inflammatory distribution patterns vary (Liesche et al. 2019). A substantial softening of brain tissue is present only in some patients. The same goes for edema (Liesche et al. 2019). Moreover, in most cases BoDV-1 patients show a characteristic pattern in MRI in both the early and the late disease stage. Since imaging in BoDV-1 patients can mimic Creutzfeldt-Jakob disease, it should be considered in differential diagnosis (Finck et al. 2020).

With the exception of one BoDV-1-infected transplant recipient (Schlottau et al. 2018) and another patient (Frank et al. 2022), all patients with a confirmed BoDV-1 infection died after a fulminant course of disease. In the majority of cases, the diagnosis could only made *postmortem*. However, with increasing frequency, the

diagnosis is made before the patient's death, which allows room for therapeutic attempts, e.g., with antiviral drugs that have proven effective against BoDV-1 in cell culture and animal experiments (Cubitt and de la Torre 1997; Jordan et al. 1999; Mizutani et al. 1998; Tokunaga et al. 2017). Whether there are other – possibly less severe or even asymptomatic – manifestations of BoDV-1 infections in humans is subject of current research.

49.7 Reservoir

The only proven natural reservoir host for BoDV-1 is the bicolored white-toothed shrew (*Crocidura leucodon*) (Bourg et al. 2013; Dürrwald et al. 2014). It was first discovered in Switzerland that this particular shrew species acts as a reservoir for BoDV-1 (Hilbe et al. 2006; Puorger et al. 2010). Further evidence for this was subsequently obtained by numerous detections of BoDV-1 in bicolored white-toothed shrews in endemic areas in Bavaria and other German federal states (e.g., Bourg et al. 2013). In *Crocidura leucodon*, the virus replicates in a large number of tissues (e.g., nervous system, epithelial and mesenchymal tissues) without causing pathological alterations. The virus is then excreted via many different routes, e.g., urine, feces, saliva, skin, and lacrimal fluid. It is assumed that there is a self-sustaining infection cycle in bicolored white-toothed shrews, and research points to the possibility of vertical and sexual transmission within the shrew population (Dürrwald et al. 2014; Nobach et al. 2015; Puorger et al. 2010). However, it needs to be further investigated whether other closely related species of the genus *Crocidura* can serve as virus reservoirs as well. Moreover, studies on the regional distribution of *Crocidura leucodon* and the proportion of BoDV-1-infected individuals in the shrew population would also be useful to better assess the potential (region-dependent) risk of human BoDV-1 infections.

49.8 Host Range

A wide range of mammal species can be infected with BoDV-1. Under natural conditions, horses and sheep are the species most frequently affected by Borna disease (Durrwald and Ludwig 1997). However, recently also alpacas have been increasingly affected as they seem to be highly susceptible to the virus (Jacobsen et al. 2010; Malbon et al. 2021; Schulze et al. 2020). Single cases of Borna disease have also been described in zoo animals (pygmy hippopotamus and sloth (Schüppel et al. 1994)), as well as rabbits (Metzler et al. 1978), donkeys (Bilzer et al. 1995; Zimmermann et al. 1994), goats (Caplazi et al. 1999), and cattle (Caplazi et al. 1994). Additionally, domestic animals such as cats (Wensman et al. 2014) or dogs (Weissenböck et al. 1998) can contract Borna disease. Humans can be infected with BoDV-1 as well (Coras et al. 2019; Korn et al. 2018; Niller et al. 2020; Schlottau et al. 2018). However, it is assumed that infections in larger mammals and humans functioning as dead-end hosts are due to rare, accidental spillover events.

49.9 Burden of Disease in Animals

Estimating the disease burden of BoDV-1 in domestic and farm animals is rather intricate. The obligation to report BoDV-1 infections in animals was only reintroduced in Germany in April 2020, after being suspended between 2011 and 2020 (Federal Ministry of Food and Agriculture: [Notifiable animal diseases] 2021). However, based on available reporting data, it can be assumed that Borna disease in animals – similar to infection in humans – is a rare incident that mostly occurs in sporadic cases. Large outbreaks associated with high mortality (and corresponding financial losses for animal owners), such as the name-giving outbreak among cavalry horses in Borna in the nineteenth century (Durrwald and Ludwig 1997) or recently in an alpaca herd in eastern Germany (Schulze et al. 2020), may be an exception.

49.10 Burden of Disease in Humans

Currently, it is almost impossible to assess the disease burden of human BoDV-1 infections. As of February 2022, the number of identified human cases, which occurred between 1996 and 2022 in Germany (of which a vast majority is in the federal state of Bavaria), is in the mid two-digit range. Mandatory notification of BoDV-1 infection in humans was introduced in Germany not before March 2020. No human cases have been reported from other countries (such as Switzerland, Austria, and Liechtenstein) where BoDV-1 is present in animals.

With the exception of two patients (Frank et al. 2022; Schlottau et al. 2018), all cases have died as a result of severe encephalitis and had to be hospitalized and given intensive medical care beforehand due to the disease. According to current knowledge, encephalitis caused by BoDV-1 is a very rare disease. Whether and to what extent there are underdiagnoses of this disease cannot be estimated at present.

49.11 Transmission

As already mentioned above, three cases of severe encephalitis occurred in organ recipients in 2016 after transplantation of solid organs (liver, kidneys) from the same donor (Schlottau et al. 2018). The organ donor – from whom no signs of a neurological or infectious disease previous to death are known – lived in a region in Bavaria in the south of Germany that is considered an endemic area for BoDV-1. Due to the BoDV-1 infection, two of the transplanted persons (kidney recipients) died; the third person (liver recipient) survived with severe health damage (Schlottau et al. 2018). The possibility of BoDV-1 transmission from person to person via organ transplantation is thus proven, even though this is likely an extremely rare transmission route.

The precise circumstances leading to transmission of BoDV-1 from the reservoir host, the bicolored white-toothed shrew, or possibly a so far unknown intermediate host to humans in a natural setting has not yet been conclusively clarified (Pörtner

et al. 2019, 2020). Several possible transmission routes are conceivable in this regard (compare "Unresolved issues").

The exact route of infection for natural BoDV-1 infection in animal dead-end hosts, such as horses, sheep, alpacas, and other susceptible mammals is also not fully understood yet. However, it is assumed that the infection might occur intranasally (Kupke et al. 2019; Morales et al. 1988). It is conceivable that the animals mentioned above come into contact with the infectious excretions of shrews with their nose and mouth while eating grass and hay. Moreover, it is assumed that BoDV-1 could then enter the brain via the nerve endings of the olfactory epithelium and the olfactory nerve (Gosztonyi 2008; Kupke et al. 2019; Morales et al. 1988). In experimental animal tests, rats could moreover be infected with BoDV-1 intracerebrally, intraocularly, and intraperitoneally (Gosztonyi and Ludwig 1995).

49.12 Bornavirus Infections and Mental Disorders: A Controversial Issue

Since the 1980s, there has been a scientific dispute about whether BoDV-1 is transmissible to humans and if so, what range of symptoms an infection with the virus may cause. Before the first evidence of BoDV-1 as a zoonotic pathogen causing severe encephalitis was provided in 2018 (Coras et al. 2019; Korn et al. 2018; Schlottau et al. 2018), the research focus on human BoDV-1 infections was on psychiatric diseases (Dürrwald et al. 2007; Hornig et al. 2012; Lipkin et al. 2011). Psychiatric or neurological diseases such as depressive disorders, bipolar disorders, schizophrenia, anxiety disorders, primary psychosis, and chronic fatigue syndrome were considered to be associated with human BoDV-1 infection. This was supposedly proven in studies in which the seroprevalence of persons with these diseases was investigated in comparison with healthy persons (e.g., Bode et al. 2001; Mazaheri-Tehrani et al. 2014; Zaliunaite et al. 2016).

A fundamental problem of this early research on human bornavirus infections was that the diagnostic detection methods used in the respective studies were nonstandardized procedures that have – up to the present day – never been independently validated. Later, research revealed a close genetic similarity of those virus strains reported before 2018 with laboratory BoDV-1 strains (Dürrwald et al. 2007; Schwemmle 2001). These findings suggest that sample contamination in the laboratory cannot be excluded (Dürrwald et al. 2007; Rubbenstroth et al. 2019). Moreover, the results of those early seroprevalence studies were non-reproducible. Another argument that speaks against the thesis of a large-scale occurrence of BoDV-1 in humans and for (rather rare) spillover events is the regional clustering (Rubbenstroth et al. 2020). In BoDV-1 infections confirmed since 2018, the detected virus strains almost always clustered with the types occurring locally (i.e., in the region of residence of the affected person) in the population of bicolored white-toothed shrews or also with virus isolates from other dead-end hosts (succumbed horses, sheep, alpacas, etc.) (Dürrwald et al. 2006; Rubbenstroth et al. 2019). In conclusion, robust and reproducible evidence of widespread human BoDV-1 infection – also outside known endemic areas in

parts of Germany, Switzerland, Austria, and Liechtenstein – and its association with psychiatric or neurological disorders are lacking up to the present day (Rubbenstroth et al. 2020).

49.13 Conclusion

As previously mentioned, it was proven only recently that BoDV-1 has the capability to cause severe encephalitis in humans (Schlottau et al. 2018). Subsequently, a few dozen human cases of BoDV-1 infections – which have mainly occurred in the German federal state of Bavaria – have been identified and reported to the public health authorities in Germany (Eisermann et al. 2021; Frank et al. 2022; Korn et al. 2018; Liesche et al. 2019; Meier et al. 2022; Niller et al. 2020; Schlottau et al. 2018; Tappe et al. 2021). Many of those cases have been detected by the retrospective screening of preserved brain material of persons deceased from encephalitis of unknown cause (Finck et al. 2020; Liesche et al. 2019; Niller et al. 2020). The earliest known case dates back to 1996 (Coras et al. 2019). Nevertheless, quite a few issues related to human BoDV-1 infections remain unresolved until the present day. For example, the question of how BoDV-1 is transmitted to humans has not been determined yet. Is transmission possible via virus-containing dusts or aerosols, similar to the possible transmission mode of hantavirus infections? Contaminated dusts or aerosols could, for instance, be generated when shrews excrete urine or feces in sheds, garages, haylofts, or similar premises. If these are then inhaled by humans, an infection may occur (Nobach et al. 2015). Or is there possibly an intermediate host? It might be conceivable, for example, that a domestic cat catches an infected shrew leading to BoDV-1 containing blood and/or tissue residues on its mouth or paws. If there is a direct contact with a human afterward, e.g., during cuddling or petting the cat, transmission of BoDV-1 could occur via smear infection. Another possibility could be the oral transmission of the virus via food contaminated, for example, with shrew urine or feces. Further, the uptake of the virus from the environment via skin lesions seems conceivable (Pörtner et al. 2019). In any case, first results from ongoing epidemiological studies suggest that transmission from shrews to humans via a direct contact is rather unlikely (Pörtner et al. 2020). Exploring and identifying routes of transmission is, however, crucial to prevent BoDV-1 infections in humans.

Furthermore, it has not yet been possible to clarify with certainty whether there are other, possibly milder manifestations of BoDV-1 infections in humans than the known encephalitis. Therefore, it might be worthwhile to conduct seroprevalence studies in endemic regions where several cases of BoDV-1 have been reported in humans and/or in animal dead-end hosts.

The question of whether other species besides *Crocidura leucodon* can be reservoir hosts for BoDV-1 has also not yet been conclusively clarified. A recent study, for example, found no evidence that European bats serve as reservoirs for BoDV-1 (Nobach and Herden 2020). For many other species, however, this still needs to be ruled out. Therefore, this issue should be the focus of further investigation, particularly with regard to species closely related to *Crocidura leucodon*.

Finally, it should be mentioned that hitherto the regional spread of BoDV-1 in Germany, Europe, and also worldwide has not been sufficiently investigated. Every year, encephalitis of unknown cause occurs in almost every region of the world. In the interest of early detection of encephalitis caused by BoDV-1 (or other, not yet identified, bornaviruses pathogenic to humans) and the identification of endemic areas, it seems reasonable to sensitize treating physicians (especially neurologists) to the possibility of a bornavirus infection. This applies to both human and veterinary medicine.

Also Beware of Varrigated and Provost's Squirrels: Human Infections with VSBV-1

In 2015, it became known that between 2011 and 2013, three breeders of varrigated squirrels (*Sciurus variegatoides*) in eastern Germany died of a newly discovered zoonotic pathogen, the varrigated squirrel bornavirus 1 (VSBV-1) (Hoffmann et al. 2015). Extensive molecular biology and immunohistological investigations indicate that the breeders had contracted VSBV-1 from infected squirrels (Hoffmann et al. 2015). Three years later, in 2018, it was retrospectively proven that an animal caretaker, who used to work in a zoological garden in northern Germany, died of VSBV-1 in 2013 as well. This person had contracted the disease from Provost's squirrels (Tappe et al. 2018). In the meantime, VSBV-1 has also been detected in several other squirrel species (Schlottau et al. 2017a, b). Like BoDV-1, VSBV-1 belongs to the genus *Orthobornavirus* in the family *Bornaviridae* (Hoffmann et al. 2015). At present, it is not clear whether VSBV-1 is present in animals other than squirrels. Transmission routes of VSBV-1 between squirrels as well as from squirrels to humans are also currently unknown. The squirrel breeders as well as the zoo animal caretaker had very close contact with the infected squirrels; therefore, a direct transmission (e.g., by scratching or biting) is likely (Hoffmann et al. 2015; Tappe et al. 2018). The infected squirrels themselves did not show any symptoms of the disease (Hoffmann et al. 2015; Schlottau et al. 2017a, b). Similar to BoDV-1, the infected individuals developed a severe encephalitis, from which they died after a period of 2–4 months after occurrence of first symptoms (Hoffmann et al. 2015; Tappe et al. 2018, 2019).

References

Amor S, Peferoen LA, Vogel DY, Breur M, van der Valk P, Baker D et al (2014) Inflammation in neurodegenerative diseases–an update. Immunology 142(2):151–166. https://doi.org/10.1111/imm.12233

Anzil AP, Blinzinger K, Mayr A (1973) Persistent Borna virus infection in adult hamsters. Arch Gesamte Virusforsch 40(1):52–57. https://doi.org/10.1007/bf01242635

Baruch K, Schwartz M (2013) CNS-specific T cells shape brain function via the choroid plexus. Brain Behav Immun 34:11–16. https://doi.org/10.1016/j.bbi.2013.04.002

Belyi VA, Levine AJ, Skalka AM (2010) Unexpected inheritance: multiple integrations of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes. PLoS Pathog 6(7): e1001030. https://doi.org/10.1371/journal.ppat.1001030

Bilzer T, Planz O, Lipkin WI, Stitz L (1995) Presence of CD4+ and CD8+ T cells and expression of MHC class I and MHC class II antigen in horses with Borna disease virus-induced encephalitis. Brain Pathol 5(3):223–230. https://doi.org/10.1111/j.1750-3639.1995.tb00598.x

Bode L, Reckwald P, Severus WE, Stoyloff R, Ferszt R, Dietrich DE et al (2001) Borna disease virus-specific circulating immune complexes, antigenemia, and free antibodies–the key marker triplet determining infection and prevailing in severe mood disorders. Mol Psychiatry 6(4):481–491. https://doi.org/10.1038/sj.mp.4000909

Bourg M, Herzog S, Encarnação JA, Nobach D, Lange-Herbst H, Eickmann M et al (2013) Bicolored white-toothed shrews as reservoir for Borna disease virus, Bavaria, Germany. Emerg Infect Dis 19(12):2064–2066. https://doi.org/10.3201/eid1912.131076

Briese T, Schneemann A, Lewis AJ, Park YS, Kim S, Ludwig H et al (1994) Genomic organization of Borna disease virus. Proc Natl Acad Sci U S A 91(10):4362–4366. https://doi.org/10.1073/pnas.91.10.4362

Caplazi P, Waldvogel A, Stitz L, Braun U, Ehrensperger F (1994) Borna disease in naturally infected cattle. J Comp Pathol 111(1):65–72. https://doi.org/10.1016/s0021-9975(05)80112-4

Caplazi P, Melzer K, Goetzmann R, Rohner-Cotti A, Bracher V, Zlinszky K et al (1999) [Borna disease in Switzerland and in the principality of Liechtenstein]. Schweiz Arch Tierheilkd 141 (11):521–527

Clemente R, de la Torre JC (2009) Cell entry of Borna disease virus follows a clathrin-mediated endocytosis pathway that requires Rab5 and microtubules. J Virol 83(20):10406–10416. https://doi.org/10.1128/JVI.00990-09

Coras R, Korn K, Kuerten S, Huttner HB, Ensser A (2019) Severe bornavirus-encephalitis presenting as Guillain-Barre-syndrome. Acta Neuropathol 137(6):1017–1019. https://doi.org/10.1007/s00401-019-02005-z

Cubitt B, de la Torre JC (1997) Amantadine does not have antiviral activity against Borna disease virus. Arch Virol 142(10):2035–2042. https://doi.org/10.1007/s007050050220

Cubitt B, Oldstone C, de la Torre JC (1994) Sequence and genome organization of Borna disease virus. J Virol 68(3):1382–1396. https://doi.org/10.1128/JVI.68.3.1382-1396.1994

Durrwald R, Ludwig H (1997) Borna disease virus (BDV), a (zoonotic?) worldwide pathogen. A review of the history of the disease and the virus infection with comprehensive bibliography. Zentralbl Veterinarmed B 44(3):147–184. https://doi.org/10.1111/j.1439-0450.1997.tb00962.x

Dürrwald R, Kolodziejek J, Muluneh A, Herzog S, Nowotny N (2006) Epidemiological pattern of classical Borna disease and regional genetic clustering of Borna disease viruses point towards the existence of to-date unknown endemic reservoir host populations. Microbes Infect 8(3):917–929. https://doi.org/10.1016/j.micinf.2005.08.013

Dürrwald R, Kolodziejek J, Herzog S, Nowotny N (2007) Meta-analysis of putative human bornavirus sequences fails to provide evidence implicating Borna disease virus in mental illness. Rev Med Virol 17(3):181–203. https://doi.org/10.1002/rmv.530

Dürrwald R, Kolodziejek J, Weissenböck H, Nowotny N (2014) The bicolored white-toothed shrew Crocidura leucodon (HERMANN 1780) is an indigenous host of mammalian Borna disease virus. PLoS One 9(4):e93659. https://doi.org/10.1371/journal.pone.0093659

Eisermann P, Rubbenstroth D, Cadar D, Thomé-Bolduan C, Eggert P, Schlaphof A et al (2021) Active case finding of current Bornavirus infections in human encephalitis cases of unknown Etiology, Germany, 2018–2020. Emerg Infect Dis 27(5):1371–1379. https://doi.org/10.3201/eid2705.204490

Federal Ministry of Food and Agriculture: [Notifiable animal diseases] (2021). https://www.bmel.de/DE/themen/tiere/tiergesundheit/tierseuchen/meldepflichtige-tierkrankheiten.html. Accessed 20 Oct 2021

Finck T, Liesche-Starnecker F, Probst M, Bette S, Ruf V, Wendl C et al (2020) Bornavirus encephalitis shows a characteristic magnetic resonance phenotype in humans. Ann Neurol 88 (4):723–735. https://doi.org/10.1002/ana.25873

Frank C, Wickel J, Bramer D, Matschke J, Ibe R, Gazivoda C et al (2022) Human Borna disease virus 1 (BoDV-1) encephalitis cases in the north and east of Germany. Emerg Microbes Infect 11 (1):6–13. https://doi.org/10.1080/22221751.2021.2007737

Fujino K, Horie M, Honda T, Merriman DK, Tomonaga K (2014) Inhibition of Borna disease virus replication by an endogenous bornavirus-like element in the ground squirrel genome. Proc Natl Acad Sci U S A 111(36):13175–13180. https://doi.org/10.1073/pnas.1407046111

Furrer E, Bilzer T, Stitz L, Planz O (2001) Neutralizing antibodies in persistent Borna disease virus infection: prophylactic effect of gp94-specific monoclonal antibodies in preventing encephalitis. J Virol 75(2):943–951. https://doi.org/10.1128/JVI.75.2.943-951.2001

Garcia BCB, Horie M, Kojima S, Makino A, Tomonaga K (2021) BUD23-TRMT112 interacts with the L protein of Borna disease virus and mediates the chromosomal tethering of viral ribonucleoproteins. Microbiol Immunol 65(11):492–504. https://doi.org/10.1111/1348-0421.12934

Gierend M, Ludwig H (1981) Influence of immunosuppressive treatment of Borna disease in rabbits. Arch Virol 67(3):217–228. https://doi.org/10.1007/BF01318132

Gosztonyi G (2008) Natural and experimental Borna disease virus infections--neuropathology and pathogenetic considerations. APMIS Suppl 124:53–57. https://doi.org/10.1111/j.1600-0463.2008.000m8.x

Gosztonyi G, Ludwig H (1995) Borna disease–neuropathology and pathogenesis. Curr Top Microbiol Immunol 190:39–73

Gosztonyi G, Ludwig H, Bode L, Kao M, Sell M, Petrusz P et al (2020) Obesity induced by Borna disease virus in rats: key roles of hypothalamic fast-acting neurotransmitters and inflammatory infiltrates. Brain Struct Funct 225(5):1459–1482. https://doi.org/10.1007/s00429-020-02063-0

Hallensleben W, Schwemmle M, Hausmann J, Stitz L, Volk B, Pagenstecher A et al (1998) Borna disease virus-induced neurological disorder in mice: infection of neonates results in immunopathology. J Virol 72(5):4379–4386. https://doi.org/10.1128/JVI.72.5.4379-4386.1998

Hilbe M, Herrsche R, Kolodziejek J, Nowotny N, Zlinszky K, Ehrensperger F (2006) Shrews as reservoir hosts of Borna disease virus. Emerg Infect Dis 12(4):675–677. https://doi.org/10.3201/eid1204.051418

Hoffmann B, Tappe D, Höper D, Herden C, Boldt A, Mawrin C et al (2015) A variegated squirrel Bornavirus associated with fatal human encephalitis. N Engl J Med 373(2):154–162. https://doi.org/10.1056/NEJMoa1415627

Horie M, Honda T, Suzuki Y, Kobayashi Y, Daito T, Oshida T et al (2010) Endogenous non-retroviral RNA virus elements in mammalian genomes. Nature 463(7277):84–87. https://doi.org/10.1038/nature08695

Hornig M, Briese T, Licinio J, Khabbaz RF, Altshuler LL, Potkin SG et al (2012) Absence of evidence for bornavirus infection in schizophrenia, bipolar disorder and major depressive disorder. Mol Psychiatry 17(5):486–493. https://doi.org/10.1038/mp.2011.179

Jacobsen B, Algermissen D, Schaudien D, Venner M, Herzog S, Wentz E et al (2010) Borna disease in an adult alpaca stallion (Lama pacos). J Comp Pathol 143(2–3):203–208. https://doi.org/10.1016/j.jcpa.2010.01.009

Joest E, Degen K (1909) On peculiar nuclear inclusions of the ganglion cells in equine enzootic cerebrospinal meningitis. Z Infkrkh 6:348–356

Joest E, Degen K (1911) Studies on the pathological histology, pathogenesis and post-mortem diagnosis of equine Borna disease. Z Infkrkh 9:1–98

Jordan I, Lipkin WI (2001) Borna disease virus. Rev Med Virol 11(1):37–57. https://doi.org/10.1002/rmv.300

Jordan I, Briese T, Averett DR, Lipkin WI (1999) Inhibition of Borna disease virus replication by ribavirin. J Virol 73(9):7903–7906. https://doi.org/10.1128/jvi.73.9.7903-7906.1999

Kinnunen PM, Inkeroinen H, Ilander M, Kallio ER, Heikkilä HP, Koskela E et al (2011) Intracerebral Borna disease virus infection of bank voles leading to peripheral spread and reverse transcription of viral RNA. PLoS One 6(8):e23622. https://doi.org/10.1371/journal.pone.0023622

Korn K, Coras R, Bobinger T, Herzog SM, Lücking H, Stöhr R et al (2018) Fatal encephalitis associated with Borna Disease Virus 1. N Engl J Med 379(14):1375–1377. https://doi.org/10.1056/NEJMc1800724

Kraus I, Scheffczik H, Eickmann M, Kiermayer S, Stubbs MT, Garten W (2002) Crystallization and preliminary X-ray analysis of the matrix protein of Borna disease virus. Acta Crystallogr D Biol Crystallogr 58(Pt 8):1371–1373. https://doi.org/10.1107/s0907444902010132

Kupke A, Becker S, Wewetzer K, Ahlemeyer B, Eickmann M, Herden C (2019) Intranasal Borna Disease Virus (BoDV-1) infection: insights into initial steps and potential contagiosity. Int J Mol Sci 20(6). https://doi.org/10.3390/ijms20061318

Liesche F, Ruf V, Zoubaa S, Kaletka G, Rosati M, Rubbenstroth D et al (2019) The neuropathology of fatal encephalomyelitis in human Borna virus infection. Acta Neuropathol 138(4):653–665. https://doi.org/10.1007/s00401-019-02047-3

Lipkin WI, Briese T, Hornig M (2011) Borna disease virus – fact and fantasy. Virus Res 162(1–2): 162–172. https://doi.org/10.1016/j.virusres.2011.09.036

Lundgren AL, Johannisson A, Zimmermann W, Bode L, Rozell B, Muluneh A et al (1997) Neurological disease and encephalitis in cats experimentally infected with Borna disease virus. Acta Neuropathol 93(4):391–401. https://doi.org/10.1007/s004010050630

Makino A, Horimoto T, Kawaoka Y (2009) Binding properties of GP1 protein of Borna disease virus. J Vet Med Sci 71(2):243–246. https://doi.org/10.1292/jvms.71.243

Malbon AJ, Durrwald R, Kolodziejek J, Nowotny N, Kobera R, Pohle D et al (2021) New World camelids are sentinels for the presence of Borna disease virus. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14003

Mazaheri-Tehrani E, Maghsoudi N, Shams J, Soori H, Atashi H, Motamedi F et al (2014) Borna disease virus (BDV) infection in psychiatric patients and healthy controls in Iran. Virol J 11:161. https://doi.org/10.1186/1743-422x-11-161

Meier H, Bauer C, Finkenzeller W, Nentwich J, Städt M, Steininger P et al (2022) [Bornavirus encephalitis as a differential diagnosis to seronegative autoimmune encephalitis]. Nervenarzt 93:835–837. https://doi.org/10.1007/s00115-021-01259-x

Metzler A, Frei U, Danner K (1976) [Virologically confirmed outbreak of Borna's disease in a Swiss herd of sheep]. Schweiz Arch Tierheilkd 118(11):483–492

Metzler A, Ehrensperger F, Wyler R (1978) [Natural Borna virus infection in rabbits]. Zentralbl Veterinarmed B 25(2):161–164

Mizutani T, Inagaki H, Araki K, Kariwa H, Arikawa J, Takashima I (1998) Inhibition of Borna disease virus replication by ribavirin in persistently infected cells. Arch Virol 143(10):2039–2044. https://doi.org/10.1007/s007050050440

Modrow S, Falke D, Truyen U, Schätzl H (2010) Molekulare Virologie, 3rd edn. Spektrum Akademischer Verlag, Heidelberg

Morales JA, Herzog S, Kompter C, Frese K, Rott R (1988) Axonal transport of Borna disease virus along olfactory pathways in spontaneously and experimentally infected rats. Med Microbiol Immunol 177(2):51–68. https://doi.org/10.1007/bf00189527

Narayan O, Herzog S, Frese K, Scheefers H, Rott R (1983a) Pathogenesis of Borna disease in rats: immune-mediated viral ophthalmoencephalopathy causing blindness and behavioral abnormalities. J Infect Dis 148(2):305–315. https://doi.org/10.1093/infdis/148.2.305

Narayan O, Herzog S, Frese K, Scheefers H, Rott R (1983b) Behavioral disease in rats caused by immunopathological responses to persistent Borna virus in the brain. Science 220(4604):1401–1403. https://doi.org/10.1126/science.6602380

Neumann B, Angstwurm K, Linker RA, Knoll G, Eidenschink L, Rubbenstroth D et al (2022) Antibodies against viral nucleo-, phospho-, and X protein contribute to serological diagnosis of fatal Borna disease virus 1 infections. Cell Rep Med 3(1):100499. https://doi.org/10.1016/j.xcrm.2021.100499

Niller HH, Angstwurm K, Rubbenstroth D, Schlottau K, Ebinger A, Giese S et al (2020) Zoonotic spillover infections with Borna disease virus 1 leading to fatal human encephalitis, 1999–2019: an epidemiological investigation. Lancet Infect Dis 20(4):467–477. https://doi.org/10.1016/s1473-3099(19)30546-8

Nobach D, Herden C (2020) No evidence for European bats serving as reservoir for Borna disease virus 1 or other known mammalian orthobornaviruses. Virol J 17(1):11. https://doi.org/10.1186/s12985-020-1289-3

Nobach D, Bourg M, Herzog S, Lange-Herbst H, Encarnacao JA, Eickmann M et al (2015) Shedding of infectious Borna Disease Virus-1 in living bicolored white-toothed shrews. PLoS One 10(8):e0137018. https://doi.org/10.1371/journal.pone.0137018

Nobach D, Müller J, Tappe D, Herden C (2020) Update on immunopathology of bornavirus infections in humans and animals. Adv Virus Res 107:159–222. https://doi.org/10.1016/bs.aivir.2020.06.004

Nowotny N (1999) Borna disease in cats. Vet Rec 144(7):187

Nowotny N, Weissenböck H (1995) Description of feline nonsuppurative meningoencephalomyelitis ("staggering disease") and studies of its etiology. J Clin Microbiol 33(6):1668–1669. https://doi.org/10.1128/jcm.33.6.1668-1669.1995

Ovanesov MV, Vogel MW, Moran TH, Pletnikov MV (2007) Neonatal Borna disease virus infection in rats is associated with increased extracellular levels of glutamate and neurodegeneration in the striatum. J Neurovirol 13(3):185–194. https://doi.org/10.1080/13550280701258415

Planz O, Dumrese T, Hulpusch S, Schirle M, Stevanovic S, Stitz L (2001) A naturally processed rat major histocompatibility complex class I-associated viral peptide as target structure of Borna disease virus-specific CD8+ T cells. J Biol Chem 276(17):13689–13694. https://doi.org/10.1074/jbc.M009889200

Poenisch M, Unterstab G, Wolff T, Staeheli P, Schneider U (2004) The X protein of Borna disease virus regulates viral polymerase activity through interaction with the P protein. J Gen Virol 85 (Pt 7):1895–1898. https://doi.org/10.1099/vir.0.80002-0

Poenisch M, Burger N, Staeheli P, Bauer G, Schneider U (2009) Protein X of Borna disease virus inhibits apoptosis and promotes viral persistence in the central nervous systems of newborn-infected rats. J Virol 83(9):4297–4307. https://doi.org/10.1128/JVI.02321-08

Pörtner K, Frank C, Schmidt-Chanasit J, Beer M, Rubbenstroth D, Tappe D (2019) [Bornavirus infections: high lethality due to fulminant meningoencephalitides]. Deutsches Ärzteblatt 116(50):A-2350–A-2354

Pörtner K, Frank C, Wilking H, Rubbenstroth D, Tappe D, Böhmer MM. Epidemiological investigation of eight cases of lethal encephalitis caused by newly detected zoonotic Borna disease virus 1 in Germany, 2019–2020. European Scientific conference on applied infectious disease epidemiology. Online 2020. p 33

Puorger ME, Hilbe M, Müller JP, Kolodziejek J, Nowotny N, Zlinszky K et al (2010) Distribution of Borna disease virus antigen and RNA in tissues of naturally infected bicolored white-toothed shrews, *Crocidura leucodon*, supporting their role as reservoir host species. Vet Pathol 47(2):236–244. https://doi.org/10.1177/0300985809351849

Pyper JM, Richt JA, Brown L, Rott R, Narayan O, Clements JE (1993) Genomic organization of the structural proteins of Borna disease virus revealed by a cDNA clone encoding the 38-kDa protein. Virology 195(1):229–238. https://doi.org/10.1006/viro.1993.1364

Rauer M, Gotz J, Schuppli D, Staeheli P, Hausmann J (2004) Transgenic mice expressing the nucleoprotein of Borna disease virus in either neurons or astrocytes: decreased susceptibility to homotypic infection and disease. J Virol 78(7):3621–3632. https://doi.org/10.1128/jvi.78.7.3621-3632.2004

Richt JA, Stitz L, Wekerle H, Rott R (1989) Borna disease, a progressive meningoencephalomyelitis as a model for CD4+ T cell-mediated immunopathology in the brain. J Exp Med 170(3):1045–1050. https://doi.org/10.1084/jem.170.3.1045

Richt JA, Pfeuffer I, Christ M, Frese K, Bechter K, Herzog S (1997) Borna disease virus infection in animals and humans. Emerg Infect Dis 3(3):343–352. https://doi.org/10.3201/eid0303.970311

Richt JA, Furbringer T, Koch A, Pfeuffer I, Herden C, Bause-Niedrig I et al (1998) Processing of the Borna disease virus glycoprotein gp94 by the subtilisin-like endoprotease furin. J Virol 72(5):4528–4533. https://doi.org/10.1128/JVI.72.5.4528-4533.1998

Richt JA, Grabner A, Herzog S (2000) Borna disease in horses. Vet Clin North Am Equine Pract 16 (3):579–95, xi. https://doi.org/10.1016/s0749-0739(17)30097-4

Rubbenstroth D, Schlottau K, Schwemmle M, Rissland J, Beer M (2019) Human bornavirus research: back on track! PLoS Pathog 15(8):e1007873. https://doi.org/10.1371/journal.ppat.1007873

Rubbenstroth D, Niller HH, Angstwurm K, Schwemmle M, Beer M (2020) Are human Borna disease virus 1 infections zoonotic and fatal? – Authors' reply. Lancet Infect Dis 20(6):651. https://doi.org/10.1016/s1473-3099(20)30379-0

Sauder C, Hallensleben W, Pagenstecher A, Schneckenburger S, Biro L, Pertlik D et al (2000) Chemokine gene expression in astrocytes of Borna disease virus-infected rats and mice in the absence of inflammation. J Virol 74(19):9267–9280. https://doi.org/10.1128/jvi.74.19.9267-9280.2000

Schlottau K, Hoffmann B, Homeier-Bachmann T, Fast C, Ulrich RG, Beer M et al (2017a) Multiple detection of zoonotic variegated squirrel bornavirus 1 RNA in different squirrel species suggests a possible unknown origin for the virus. Arch Virol 162(9):2747–2754. https://doi.org/10.1007/s00705-017-3432-z

Schlottau K, Jenckel M, van den Brand J, Fast C, Herden C, Höper D et al (2017b) Variegated squirrel Bornavirus 1 in squirrels, Germany and the Netherlands. Emerg Infect Dis 23(3):477–481. https://doi.org/10.3201/eid2303.161061

Schlottau K, Forth L, Angstwurm K, Höper D, Zecher D, Liesche F et al (2018) Fatal encephalitic Borna disease virus 1 in solid-organ transplant recipients. N Engl J Med 379(14):1377–1379. https://doi.org/10.1056/NEJMc1803115

Schmidt J (1912) Studies on the clinical behaviour of equine Borna disease, together with information on therapeutic trials in this field. Berl Tierärztl Wochenschr 28(581–586):597–603

Schmidt J (1952) Borna disease of the horse – 55 years of research and teaching. Arch Exp Veterniarmed 6:177–187

Schulze V, Grosse R, Furstenau J, Forth LF, Ebinger A, Richter MT et al (2020) Borna disease outbreak with high mortality in an alpaca herd in a previously unreported endemic area in Germany. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13556

Schüppel KF, Kinne J, Reinacher M (1994) Bornavirus antigen detection in alpacas (*Lama pacos*) as well as in a sloth (*Cholepus didactylus*) and a pygmy hippopotamus (*Choeropsis liberiensis*). In: Hofmann RR, Ippen R (ed) Verhandlungsbericht XXXVI Internationales Symposium über Erkrankungen von Zootieren. Akademie-Verlag, Berlin, pp 189–194

Schwemmle M (2001) Borna disease virus infection in psychiatric patients: are we on the right track? Lancet Infect Dis 1(1):46–52. https://doi.org/10.1016/s1473-3099(01)00021-4

Scordel C, Huttin A, Cochet-Bernoin M, Szelechowski M, Poulet A, Richardson J et al (2015) Borna disease virus phosphoprotein impairs the developmental program controlling neurogenesis and reduces human GABAergic neurogenesis. PLoS Pathog 11(4):e1004859. https://doi.org/10.1371/journal.ppat.1004859

Sobbe M, Bilzer T, Gommel S, Noske K, Planz O, Stitz L (1997) Induction of degenerative brain lesions after adoptive transfer of brain lymphocytes from Borna disease virus-infected rats: presence of CD8+ T cells and perforin mRNA. J Virol 71(3):2400–2407. https://doi.org/10.1128/JVI.71.3.2400-2407.1997

Stitz L, Soeder D, Deschl U, Frese K, Rott R (1989) Inhibition of immune-mediated meningoencephalitis in persistently Borna disease virus-infected rats by cyclosporine A. J Immunol 143(12):4250–4256

Szelechowski M, Betourne A, Monnet Y, Ferre CA, Thouard A, Foret C et al (2014) A viral peptide that targets mitochondria protects against neuronal degeneration in models of Parkinson's disease. Nat Commun 5:5181. https://doi.org/10.1038/ncomms6181

Tang T, Guo Y, Xu X, Zhao L, Shen X, Sun L et al (2021) BoDV-1 infection induces neuroinflammation by activating the TLR4/MyD88/IRF5 signaling pathway, leading to learning and memory impairment in rats. J Med Virol 93(11):6163–6171. https://doi.org/10.1002/jmv.27212

Tappe D, Schlottau K, Cadar D, Hoffmann B, Balke L, Bewig B et al (2018) Occupation-associated fatal limbic encephalitis caused by variegated squirrel Bornavirus 1, Germany, 2013. Emerg Infect Dis 24(6):978–987. https://doi.org/10.3201/eid2406.172027

Tappe D, Schmidt-Chanasit J, Rauch J, Allartz P, Herden C (2019) Immunopathology of fatal human variegated squirrel Bornavirus 1 encephalitis, Germany, 2011–2013. Emerg Infect Dis 25(6):1058–1065. https://doi.org/10.3201/eid2506.181082

Tappe D, Portner K, Frank C, Wilking H, Ebinger A, Herden C et al (2021) Investigation of fatal human Borna disease virus 1 encephalitis outside the previously known area for human cases, Brandenburg, Germany – a case report. BMC Infect Dis 21(1):787. https://doi.org/10.1186/s12879-021-06439-3

Tizard I, Ball J, Stoica G, Payne S (2016) The pathogenesis of bornaviral diseases in mammals. Anim Health Res Rev 17(2):92–109. https://doi.org/10.1017/S1466252316000062

Tokunaga T, Yamamoto Y, Sakai M, Tomonaga K, Honda T (2017) Antiviral activity of favipiravir (T-705) against mammalian and avian bornaviruses. Antivir Res 143:237–245. https://doi.org/10.1016/j.antiviral.2017.04.018

Waelchli RO, Ehrensperger F, Metzler A, Winder C (1985) Borna disease in a sheep. Vet Rec 117 (19):499–500. https://doi.org/10.1136/vr.117.19.499

Weissenböck H, Nowotny N, Caplazi P, Kolodziejek J, Ehrensperger F (1998) Borna disease in a dog with lethal meningoencephalitis. J Clin Microbiol 36(7):2127–2130. https://doi.org/10.1128/jcm.36.7.2127-2130.1998

Wensman JJ, Jäderlund KH, Holst BS, Berg M (2014) Borna disease virus infection in cats. Vet J 201(2):142–149. https://doi.org/10.1016/j.tvjl.2013.12.012

Working Group Blood of the German Ministry of Health (2019) Bornavirus. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 62(4):519–532. https://doi.org/10.1007/s00103-019-02904-2

Zaliunaite V, Steibliene V, Bode L, Podlipskyte A, Bunevicius R, Ludwig H (2016) Primary psychosis and Borna disease virus infection in Lithuania: a case control study. BMC Psychiatry 16(1):369. https://doi.org/10.1186/s12888-016-1087-z

Zimmermann W, Durrwald R, Ludwig H (1994) Detection of Borna disease virus RNA in naturally infected animals by a nested polymerase chain reaction. J Virol Methods 46(2):133–143. https://doi.org/10.1016/0166-0934(94)90098-1

Zwick W, Seifried O, Witte J (1927) Experimental studies on the epidemic brain and spinal cord inflammation of horses (Borna disease). Z Infkrkh Haustiere 30:42–136

Zwick W, Seifried O (1925) Transmissibility of equine Borna disease to small laboratory animals (rabbits). Berl Tierärztl Wochenschr 41:129–132

Severe Acute Respiratory Syndrome Coronaviruses-2 (SARS-CoV-2)

50

Jaffar A. Al-Tawfiq and Ziad A. Memish

Contents

Abstract

The emergence of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was initially described in late 2019 in Wuhan City, China, and was reported to the World Health Organization (WHO) in December 2019

J. A. Al-Tawfiq (✉)
Infectious Disease Unit, Specialty Internal Medicine, and Quality and Patient Safety Department, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia

Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: jaltawfi@yahoo.com

Z. A. Memish
Director Research Center, King Saud Medical City, Ministry of Health, Riyadh, Saudi Arabia

Al-Faisal University, Riyadh, Saudi Arabia

Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA
e-mail: zmemish@yahoo.com

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_50

(Wang et al. 2020). The virus was identified among patients who had pneumonia and were linked to a wet-seafood market. This virus was initially known as novel coronavirus 2019 (nCoV-19) and then later was designated as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Gorbalenya et al. 2020). This chapter summarizes the current understanding of the disease, the emergence of variants, and outcome.

Keywords

SARS-CoV-2 · COVID-19 · Coronaviruses

50.1 Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was initially described in late 2019 in Wuhan City, China, and was reported to the World Health Organization (WHO) in December 2019 (Wang et al. 2020). The virus was identified among patients who had pneumonia and were linked to a wet-seafood market. This virus was initially known as novel coronavirus 2019 (nCoV-19) and later was designated as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Gorbalenya et al. 2020). This chapter summarizes the current understanding of the disease, the emergence of variants, and outcome.

50.1.1 The Virus

SARS-CoV-2 is a member of the β-type of coronaviruses. The coronaviruses are large, enveloped, positive strand RNA viruses and have four genera (alpha, beta, delta, and gamma). Human coronaviruses belong to the alpha or the beta genera (Chan et al. 2015). The initial four known coronaviruses were HCoV-229E, NL63, HCoV-OC43, and HKU1, and these caused human common cold and gastrointestinal symptoms. Two of these viruses, HCoV-229E and HCoV-OC43, were known since the 1960s. The HCoV-229E is an alphacoronavirus, and its animal host is thought to be bats and alpacas as the intermediate host (Crossley et al. 2012). The HCoV-OC43 is a betacoronavirus, with rodents as the host and cattle as the intermediate host (Corman et al. 2015). The HCoV-NL63 is an alphacoronavirus and HCoV-HKU1 is a betacoronavirus, and these emerged from bats and rodents, respectively (Tao et al. 2017; Van Der Hoek et al. 2004; Woo et al. 2005). In 2002, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) emerged and caused a limited outbreaks in many countries. The Middle East respiratory syndrome coronavirus (MERS-CoV) was initially identified in Saudi Arabia in 2012 and had caused limited outbreaks internationally. In 2019, the SARS-CoV-2 was identified initially in Wuhan, China (Lu et al. 2020; Zhu et al. 2020).

SARS-CoV-2 belongs to the coronaviruses, and the 5′-end of the genome includes the replicase gene and has two overlapping open reading frames (ORF).

Table 1 Function of coronavirus structural proteins

Structural protein	Function	Host cell receptors
Spike (S)	Facilitates viral attachment to host cell receptors Fusion between the envelope and plasma membrane Inducer of neutralizing antibodies	HLA-A2-restricted CD8 + T cells (SARS-CoV and MERS-CoV) Neutralization antibodies (SARS-CoV)
Envelope (E)	Viral envelope assembly	
Membrane (M)	Type III glycoprotein Has three parts: amino-terminal ectodomain, a triple-spanning transmembrane domain, and carboxyl-terminal inner domain	
Nucleocapsid (N) proteins	Highly basic phosphoprotein Needed in the virion Modulates viral RNA synthesis	CD4 + T cells (SARS-CoV)

Table 2 Comparison between the different coronaviruses

	Year of emergence	Infected cells	Receptors
SARS-CoV	2002	Epithelial respiratory cells, T cells	Angiotensin-converting enzyme 2 (ACE2)
MERS-CoV	2012	Alveolar epithelial cells and immune cells	DPP-4
SARS-CoV-2	2019	Epithelial respiratory cells, T cells	Angiotensin-converting enzyme 2 (ACE2)

These ORF are as follows: ORF 1a and 1b (Decaro and Lorusso 2020) and four ORFs encoding the common coronavirus structural proteins. These structural proteins are spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Decaro and Lorusso 2020) with different functions (Table 1). The receptor for the SARS-CoV-2 is the angiotensin 1-converting enzyme 2 (ACE2) (Lan et al. 2020; Shang et al. 2020) (Table 2).

50.2 Zoonotic Origin

The origin of the SARS-CoV-2 had been debated with two possibilities of being a zoonotic disease or a laboratory escape (Holmes et al. 2021). Phylogenetic analysis suggests one or more events of contacts with infected animals and/or traders with multiple spillover events (Holmes et al. 2021; Rambaut et al. 2020). One study found that SARS-CoV-2 is 96.2% identical to a bat CoV, RaTG13, and share 79.5% identity (Wrobel et al. 2020).

Zoonotic origin of previous coronaviruses had been studied and specifically for the previously emerged MERS-CoV and had been reviewed previously (Al-Tawfiq

et al. 2014). The first indication of the animal origin was related to the isolation of a bat coronavirus that resembles MERS-CoV from wild bats in the Kingdom of Saudi Arabia (Memish et al. 2013). This evidence was based on one piece of 190-nucleotide fragment of the RNA-dependent RNA polymerase (*RdRp*) from an Egyptian tomb bat (*Taphozous perforatus*) (Memish et al. 2013). The amplified sequenced was identical to that of the MERS-CoV sequence from the first index human case (Memish et al. 2013). Subsequent studies found high prevalence of MERS-CoV antibodies in dromedary camels from different regions of the globe, including the Arabian Peninsula, North Africa, and Eastern Africa (Reusken et al. 2013a, b, 2014; Hemida et al. 2013; Alexandersen et al. 2014; Nowotny and Kolodziejek 2014; Corman et al. 2014). Anti-MERS-CoV antibodies were found in camel sera collected in the 1990s (Corman et al. 2014; Hemida et al. 2014a; Alagaili et al. 2014). In addition to the detection of anti-MERS-COV antibodies, rt-PCR detected MERS-CoV from samples obtained from dromedary camels (Alagaili et al. 2014; Hemida et al. 2017; Wernery et al. 2015; Khalafalla et al. 2015; Farag et al. 2015; Stalin Raj et al. 2014; Yusof et al. 2015). Viral cultures also showed viable MERS-CoV from samples obtained from dromedary camels (Wernery et al. 2015; Stalin Raj et al. 2014; Hemida et al. 2014b; Briese et al. 2014; Haagmans et al. 2014). Sequence of the MERS-CoV spike, ORF3-4a, and nucleocapsid regions were identical from asymptomatic human contacts and their camels (Al Hammadi et al. 2015).

50.3 Clinical Features

All coronaviruses share the ability to cause mild or asymptomatic disease to severe and fatal illness. For COVID-19, a cohort of initial 41 reported cases showed a case-fatality rate of 14·6% (Huang et al. 2020a) and 11% of another 99 cases died due to multi-organ failure (Chen et al. 2020). In a meta-analysis, patients had a mean age of 51 years with 1:2:1 of male to female (Baradaran et al. 2020). The most common clinical symptoms were studied in a meta-analysis and included 281,461 patients and found that the mean age was 46.7 years and 51.8% were male (Li et al. 2021). The rate of severe disease was 22.9% and an overall case-fatality rate of 5.6%. The associated factors with higher mortality were older age, male gender, diabetes, and hypertension (Li et al. 2021). Another meta-analysis found that the most common symptoms were as follows: fever 81.2%, cough 58.5%, fatigue 38.5%, dyspnea 26.1%, and production of sputum 25.8% (Alimohamadi et al. 2020). Patients with SARS-CoV-2 infection may also have coinfection or superimposed infection. One meta-analysis included 31,953 patients with an overall pooled rate of a laboratory-confirmed bacterial infection of 15.9% (95% CI 13.6–18.2), 3.7% (95% CI 2.6–4.8) had fungal infections, and 6.6% (95% CI 5.5–7.6) had other respiratory viruses (Alhumaid et al. 2021). There had been multiple studies showing disparity in clinical outcome and presentation of patients with COVID-19 among different racial and geographic locations (Tirupathi et al. 2020). The presence of comorbidities was significantly higher in the Americas than in Asia. Most Asian patients had fever

(95% CI 0.70–0.81), most Oceanian patients had cough (95%CI 0.68–0.70), and dyspnea was common in the Americas (95%CI 0.33–0.64), and Europe (95%CI 0.29–0.64) (Tian et al. 2022). The loss of smell and taste were more common among European patients (95%CI 0.60–0.97) (Tian et al. 2022).

The case fatality rate was estimated to be 0.4% for symptomatic cases, 0.05% for those 0–49 years of age, and 1.3% for those ≥65 years (Giannouchos et al. 2020). The presence of other comorbidities, such as hypertension, obesity, and diabetes, were common among COVID-19 patients and were independent correlates of hospitalization and adverse outcomes (Guan et al. 2020a; Docherty et al. 2020). Adverse events were associated with COPD (HR 2.681), diabetes mellitus (1.59), hypertension (1.58), and malignancy (3.50). The risk of death was lower in those with one comorbidity (HR 1.79) compared to those two or more comorbidities (HR 2.59) (Guan et al. 2020b). The occurrence of comorbidities was as follows: hypertension (21%), diabetes mellitus (11%), cerebrovascular disease (2.4%), cardiovascular disease (5.8%), chronic kidney disease (3.6%), chronic liver disease (2.9%), chronic pulmonary disease (2%), malignancy (2.7%), and smoking (8.7%) (Baradaran et al. 2020). In another meta-analysis of 2401 COVID-19 patients, 66.6% of the deceased were male, with a median age of 69.9 years (Qiu et al. 2020). The deceased patients were more likely to have thrombocytopenia, higher C-reactive protein (CRP), and lactate dehydrogenase (LDH) at admission and more likely to develop acute respiratory distress syndrome (ARDS) (OR = 100) and shock (OR = 96.6) (Qiu et al. 2020). In children, multiple risk factors are also associated with severe disease and include the following: obesity (RR, 1.43), diabetes (RR, 2.26), chronic lung disease (RR, 2.62), heart disease (RR, 1.82), neurologic disease (RR, 1.18), and immunocompromised status (RR, 1.44) (Choi et al. 2022).

50.4 Diagnosis

The diagnosis of SARS-CoV-2 relies mainly on real-time PCR of respiratory tract samples. There are multiple RT-PCR based assays for the diagnosis of SARS-CoV-2. These tests detects one or more of the subunits of SARS-CoV-2 such as: the E-gene PCR (specific for bat(−related) betacoronaviruses, and thus detects both SARS-CoV-1 and SARS-CoV-2), S-gene, ORF1ab, N, or RdRp, in combination (van Kasteren et al. 2020). In a comparison of 7 RT-PCR tests for the detection of SARS-CoV-2, all tests detected all samples with the highest concentrations of SARS-CoV-2 RNA (Ct ≤ 34.5 in an in-house E-gene PCR assay). It was stated that of all 416 samples in one report, Ct value >34.5 was present in 3.6% of samples (van Kasteren et al. 2020). Since patients with mild or no symptoms or those during later stages of the infection may have a lower viral load (Zou et al. 2020), and thus the choice of the diagnostic PCR tests was limited to those from R-Biopharm AG, BGI, KH Medical, and Seegene (van Kasteren et al. 2020). The diagnostic accuracy of routinely used PCR tests are still able to detect different variants such as Delta and Omicron (World Health Organization 2021a). Omicron variant has a large number of

mutations in the S including Δ69–70 (Quarleri et al. 2021), and this deletion leads to a dropout in the TaqPath PCR test (Ferré et al. 2021).

Serologic testing of SARS-CoV-2 relies on the detection of anti-spike (S) protein, and it is not known which part of the S-protein offers the best site for antibody development (Petherick 2020). The presence of anti-SARS-CoV-2 IgM antibodies was detected in 50%–81%, and IgG antibodies were present in 81%–100% of tested patients (Zhang et al. 2020). The S1 IgG and IgA ELISAs showed lower specificity and variable sensitivity (Okba et al. 2020). In a meta-analysis, an overall sensitivity of IgG and IgM was 84.3% for enzyme-linked immunosorbent assays (ELISA), 66.0% for lateral flow immunoassays (LFIAs), and 66% for chemiluminescent immunoassays (CLIAs) (Lisboa Bastos et al. 2020). In another meta-analysis, the overall pooled sensitivity was 0.5856 for IgG, 0.4637 for IgM, and 0.6886 for IgG-IgM based on LFIA tests as compared to rRT-PCR method (Vengesai et al. 2021). It was also found that the sensitivity of commercial kits was 65% compared to 88.2% for noncommercial tests utilizing LFIAs (Lisboa Bastos et al. 2020).

The utilization of N protein as serology was evaluated in 914 sera, and the study showed that the test had a sensitivity and specificity of 76.27 and 98.78%, respectively (Deng et al. 2021). Another study showed that the positivity rate of S1 and N antigens was 64.06% of 64 COVID-19 patients (Ogata et al. 2020), and an additional study found a sensitivity and specificity of 98.4% and 79.3%, respectively, within the first 2 weeks from symptoms onset (Ahava et al. 2022). Thus, it was suggested that this type of serology for N protein could be used in the diagnosis of an acute COVID-19 disease (Le Hingrat et al. 2021; Lebedin et al. 2020).

50.5 Emergence of Variants

The emergence of multiple SARS-CoV-2 variants is of great concern globally (Temsah et al. 2021). The variants are classified as variants of interest (VOI) and variants of concern (VOC) (Centers for Disease Control and Prevention (CDC) 2021). VOC refers to variants that have increased transmissibility or virulence, and VOI refers to those with the potential to cause the disease. According to the United Sates CDC, VOC is defined as “any variant with increased transmissibility, more severe disease (as defined by increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures” (Centers for Disease Control and Prevention (CDC) 2021). On the other hand, the definition of VOI is “any variant with specific genetic associated with changes to receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity” (Centers for Disease Control and Prevention (CDC) 2021). These mutations that could appear due to mutation on the RBD and the NTD (Greaney et al. 2021). The Delta variant

was first identified in India late 2020 and then had spread worldwide and caused a new wave (Centers for Disease Control and Prevention (CDC) 2021; ECDC 2021). Delta variant, B.1.617.2, has a high rate of transmission (Yadav et al. 2021). On November 24, 2021, the WHO was notified of the emergence of the Omicron variant (World Health Organization (WHO) 2021b, c). The Omicron variant has about 50 mutations including 30 mutations in the S-protein (World Health Organization 2021; Thakur and Kanta Ratho 2021). The estimated re-infection hazard during the Omicron variant in South Africa was 2.39 (95 CI: 1.88–3.11) vs. the first wave of the epidemic (Pulliam et al. 2021). There was reduction in the neutralization of convalescent serum against the Beta and Delta variants in comparison to the initial variant (Liu et al. 2021; Wibmer et al. 2021; Planas et al. 2021).

50.6 Vaccines

Efforts to fight the SARS-CoV-2 have focused on the spike protein, which is crucial for the virus to enter the cells (Du et al. 2009). The spike protein is divided into an N-terminal S1 domain, which allows the virus to attach to the host cells through the angiotensin-converting enzyme (ACE) 2 receptor and a C-terminal S2 domain, which allows the virus to adhere to the cell membrane (Hoffmann et al. 2020; Huang et al. 2020b). The S1 domain has two specific domains known as the N-terminal domain (NTD) and a receptor-binding domain (RBD) (Lan et al. 2020). Most of the vaccines that have been developed and some monoclonal antibodies that have been used in combatting this virus have specifically focused on the S domain (Krammer 2020; Baden et al. 2021). Vaccines efficacy depends on stimulating an appropriate antibody and T cell response to S protein (Krammer 2020). The emergence of the Omicron variant had increased the debate about the need for COVID-19 vaccine booster dose, as the current dosing regimen might not provide an adequate antibody response to protect against this variant (Graham 2021).

The neutralizing antibodies against the Omicron variant were reduced at 11.4, 37.0, and 24.5 times for those who had two or three doses of the BNT162b2 vaccine (Wilhelm et al. 2021). Additionally, the neutralizing effects from heterologously vaccinated ChAdOx1 and BNT162b2 were not detected (Wilhelm et al. 2021). Receiving a booster dose of BNT162b2- vaccine was associated with a significant increase in the levels of neutralizing antibodies against Omicron variant (Wilhelm et al. 2021).

The results from the inactivated whole-virion SARS-CoV-2 (CoronaVac) vaccine did not show any detectable neutralizing antibodies against the Omicron variant and BNT162b2 vaccine showed 35.7–39.9-fold reduction in antibodies activity (Lu et al. 2021). Another study showed 29·eightfold reduction in neutralizing antibodies in patients who had homologous BNT162b2 vaccine (Dejnirattisai et al. 2021). Another study showed a 22-fold reduction in neutralizing antibodies in infected-vaccinated and vaccinated patients (Cele et al. 2021). Thus, there seems to be a need for booster doses (Burki 2021) and possibly the need to develop a universal coronavirus vaccines (He et al. 2021; Cohen 2021; Morens et al. 2021).

50.7 Therapy

The use of convalescent plasma was investigated early in the course of COVID-19. A meta-analysis included 1138 control and 1231 randomized to convalescent plasma therapy (Troxel et al. 2022). The mortality odd ratio was 0.88 at day 14 and 0.85 at day 28 (Troxel et al. 2022). Another meta-analysis showed that convalescent plasma was associated an overall discharge rate of 75.7% and a lower risk of staying in hospital with a relative risk of 0.946 (Agarwal et al. 2021).

The use of steroid for COVID-19 patients followed the publication of the RECOVERY trial and was the landmark for such improvement in the survival of patients requiring supplemental oxygen. The study used daily dexamethasone at a dose of 6 mg intravenous or oral (RECOVERY Collaborative Group 2020). The rate of death was 29.3% vs. 41.4% among those on mechanical ventilation and 23.3% vs. 26.2% among those receiving oxygen, in the dexamethasone group vs. the control group, respectively (RECOVERY Collaborative Group 2020). A meta-analysis found that all-cause mortality was lower with methylprednisolone (odds ratio [OR]: 0.16, 95% CI: 0.03–0.75] (Cheng et al. 2021).

50.8 Post-COVID-19 Syndrome

The development of persistent symptoms after COVID-19 recovery was noted in 13.3–96% of patients (Chopra et al. 2021; Davis et al. 2021; Sudre et al. 2021; Norton et al. 2021; Michielsen et al. 2003). The occurrence of such symptoms after 14 weeks was 50.9% (Moreno-Pérez et al. 2021). The exact reasons for the occurrence of post-COVID-19 symptoms is not known, and it is thought that the virus may trigger a substantial immunologic responses (Buonsenso et al. 2021; Kayaaslan et al. 2021; Menges et al. 2021).

It is also thought that disease severity, age, and comorbidity were associated with persistent symptoms (Kamal et al. 2021; Tenforde et al. 2020). The most common post-COVID-19 symptom was fatigue (Chopra et al. 2021; Davis et al. 2021; Sudre et al. 2021; Norton et al. 2021). In addition, 9.5% and 13.2 may have depression or anxiety, respectively. In a meta-analysis, the prevalence of depression, anxiety, and insomnia were 15.97%, 15.15%, and 23.87%, respectively (Cénat et al. 2021).

50.9 Conclusion

The current estimates may be variable depending on the definition used and the time frame. According to the US CDC, post-COVID conditions include: long COVID; post-acute COVID-19; long-term effects of COVID; post-acute COVID syndrome; chronic COVID; long-haul COVID; late sequelae; and post-acute sequelae of SARS-CoV-2 infection (PASC) (CDC: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/post-covid-conditions.html). According to the US CDC, "post-COVID conditions are present if recovery does not occur after the 4-week acute phase even though many patients continue to recover between 4 and 12

weeks." In conclusion, the current SARS-CoV-2 pandemic had caused significant morbidity, mortality, and healthcare disparity. Scientific studies had confirmed the efficacy and safety of vaccines and the advantage of steroid especially for patients requiring supplemental oxygen therapy. The continued emergence of SARS-CoV-2 variants is a continued challenge for ending this pandemic.

50.10 Cross-References

- ▶ Hantaviruses in a Global Perspective
- ▶ Influenza from a One Health Perspective: Infection by a Highly Versatile Virus

References

Agarwal N, Mishra S, Ayub A (2021) Convalescent plasma therapy in COVID-19 and discharge status: a systematic review. J Family Med Prim Care 10:3876. https://doi.org/10.4103/JFMPC.JFMPC_2429_20

Ahava MJ, Kurkela S, Kuivanen S, Lappalainen M, Jarva H, Jääskeläinen AJ (2022) Detection of SARS-CoV-2 nucleocapsid antigen from serum can aid in timing of COVID-19 infection. J Virol Methods 302:114469. https://doi.org/10.1016/j.jviromet.2022.114469

Al Hammadi ZM, Chu DKW, Eltahir YM, Al Hosani F, Al Mulla M, Tarnini W et al (2015) Asymptomatic MERS-CoV infection in humans possibly linked to infected dromedaries imported from Oman to United Arab Emirates, may 2015. Emerg Infect Dis 21:2197–2200. https://doi.org/10.3201/eid2112.151132

Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, de Wit E et al (2014) Middle east respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. MBio 5: e00884-14. https://doi.org/10.1128/mBio.00884-14

Alexandersen S, Kobinger GP, Soule G, Wernery U (2014) Middle east respiratory syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005. Transbound Emerg Dis 61:105–108. https://doi.org/10.1111/tbed.12212

Alhumaid S, Al Mutair A, Al Alawi Z, Alshawi AM, Alomran SA, Almuhanna MS et al (2021) Coinfections with bacteria, fungi, and respiratory viruses in patients with sars-cov-2: a systematic review and meta-analysis. Pathogens 10. https://doi.org/10.3390/pathogens10070809

Alimohamadi Y, Sepandi M, Taghdir M, Hosamirudsari H (2020) Determine the most common clinical symptoms in COVID-19 patients: a systematic review and meta-analysis. J Prev Med Hyg 61:E304–E312. https://doi.org/10.15167/2421-4248/JPMH2020.61.3.1530

Al-Tawfiq JA, Zumla A, Memish ZA (2014) Coronaviruses: severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus in travelers. Curr Opin Infect Dis 27:411–417. https://doi.org/10.1097/QCO.0000000000000089

Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R et al (2021) Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 384:403–416. https://doi.org/10.1056/nejmoa2035389

Baradaran A, Ebrahimzadeh MH, Baradaran A, Kachooei AR (2020) Prevalence of comorbidities in COVID-19 patients: a systematic review and meta-analysis. Arch Bone Jt Surg 8(1):247–255. https://doi.org/10.22038/abjs.2020.47754.2346

Briese T, Mishra N, Jain K, Zalmout IS, Jabado OJ, Karesh WB et al (2014) Middle east respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole- genome analysis and virus cultured from dromedary camels in Saudi Arabia. MBio 5. https://doi.org/10.1128/mBio.01146-14

Buonsenso D, Munblit D, De Rose C, Sinatti D, Ricchiuto A, Carfi A et al (2021) Preliminary evidence on long COVID in children. Acta Paediatr 110:2208–2211. https://doi.org/10.1111/APA.15870

Burki TK (2021) Omicron variant and booster COVID-19 vaccines. Lancet Respir Med 10:e17. https://doi.org/10.1016/S2213-2600(21)00559-2

Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H et al (2021) SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. MedRxiv Preprint 11:12. https://doi.org/10.1101/2021.12.08.21267417

Cénat JM, Blais-Rochette C, Kokou-Kpolou CK, Noorishad PG, Mukunzi JN, McIntee SE et al (2021) Prevalence of symptoms of depression, anxiety, insomnia, posttraumatic stress disorder, and psychological distress among populations affected by the COVID-19 pandemic: a systematic review and meta-analysis. Psychiatry Res 295:113599. https://doi.org/10.1016/j.psychres.2020.113599

Centers for Disease Control and Prevention (CDC). SARS-CoV-2 Variant Classifications and Definitions 2021. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fcases-updates%2Fvariant-surveillance%2Fvariant-info.html. Accessed 16 Aug 2021

Chan JFW, Lau SKP, To KKW, Cheng VCC, Woo PCY, Yuen K-Y (2015) Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev 28:465–522. https://doi.org/10.1128/CMR.00102-14

Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y et al (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395:507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

Cheng Q, Zhao G, Chen J, Jia Q, Fang Z (2021) Efficacy and safety of current treatment interventions for patients with severe COVID-19 infection: a network meta-analysis of randomized controlled trials. J Med Virol 94:1617. https://doi.org/10.1002/JMV.27512

Choi JH, Choi S-H, Yun KW (2022) Risk factors for severe COVID-19 in children: a systematic review and meta-analysis. J Korean Med Sci 37. https://doi.org/10.3346/jkms.2022.37.e35

Chopra V, Flanders SA, O'Malley M, Malani AN, Prescott HC (2021) Sixty-day outcomes among patients hospitalized with COVID-19. Ann Intern Med 174:576–578. https://doi.org/10.7326/M20-5661

Cohen J (2021) Omicron sparks a vaccine strategy debateCurrent boosters, variant-specific shots, and universal coronavirus vaccines all have fans. Science (80-) 374:1544–1545

Corman VM, Jores J, Meyer B, Younan M, Liljander A, Said MY et al (2014) Antibodies against MERS coronavirus in dromedary camels, Kenya, 1992-2013. Emerg Infect Dis 20:1319–1322. https://doi.org/10.3201/eid2008.140596

Corman VM, Baldwin HJ, Tateno AF, Zerbinati RM, Annan A, Owusu M et al (2015) Evidence for an ancestral Association of Human Coronavirus 229E with bats. J Virol 89:11858–11870. https://doi.org/10.1128/jvi.01755-15

Crossley BM, Mock RE, Callison SA, Hietala SK (2012) Identification and characterization of a novel alpaca respiratory coronavirus most closely related to the human coronavirus 229E. Viruses 4:3689–3700. https://doi.org/10.3390/v4123689

Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y et al (2021) Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine 38:101019. https://doi.org/10.1016/j.eclinm.2021.101019

Decaro N, Lorusso A (2020) Novel human coronavirus (SARS-CoV-2): a lesson from animal coronaviruses. Vet Microbiol 244:108693. https://doi.org/10.1016/j.vetmic.2020.108693

Dejnirattisai W, Shaw RH, Supasa P, Liu C, Stuart AS, Pollard AJ et al (2021) Reduced neutralisation of SARS-CoV-2 omicron B.1.1.529 variant by post-immunisation serum. Lancet. https://doi.org/10.1016/S0140-6736(21)02844-0

Deng Q, Ye G, Pan Y, Xie W, Yang G, Li Z et al (2021) High performance of SARS-Cov-2N protein antigen chemiluminescence immunoassay as frontline testing for acute phase COVID-19 diagnosis: a retrospective cohort study. Front Med 8:871. https://doi.org/10.3389/FMED.2021.676560/BIBTEX

Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L et al (2020) Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. BMJ 369. https://doi.org/10.1136/bmj.m1985

Du L, He Y, Zhou Y, Liu S, Zheng B-J, Jiang S (2009) The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol 7:226–236. https://doi.org/10.1038/nrmicro2090

ECDC. Threat Assessment Brief: emergence of SARS-CoV-2 B.1.617 variants in India and situation in the EU/EEA 2021. https://www.ecdc.europa.eu/en/publications-data/threat-assessment-emergence-sars-cov-2-b1617-variants. Accessed 16 Aug 2021

Farag EABA, Reusken CBEM, Haagmans BL, Mohran KA, Stalin Raj V, Pas SD et al (2015) High proportion of MERS-CoV shedding dromedaries at slaughterhouse with a potential epidemiological link to human cases, Qatar 2014. Infect Ecol Epidemiol 5:28305

Ferré VM, Peiffer-Smadja N, Visseaux B, Descamps D, Ghosn J, Charpentier C (2021) Omicron SARS-CoV-2 variant: what we know and what we don't. Anaesth Crit Care Pain Med 41:100998. https://doi.org/10.1016/j.accpm.2021.100998

Giannouchos TV, Sussman RA, Mier JM, Poulas K, Farsalinos K (2020) Characteristics and risk factors for COVID-19 diagnosis and adverse outcomes in Mexico: an analysis of 89,756 laboratory–confirmed COVID-19 cases. Eur Respir J:2002144. https://doi.org/10.1183/13993003.02144-2020

Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA et al (2020) The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 5:536–544. https://doi.org/10.1038/s41564-020-0695-z

Graham F (2021) Daily briefing: Omicron - what scientists know so far. Nature. https://doi.org/10.1038/D41586-021-03652-7

Greaney AJ, Starr TN, Gilchuk P, Zost SJ, Binshtein E, Loes AN et al (2021) Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. Cell Host Microb 29:44–57.e9. https://doi.org/10.1016/j.chom.2020.11.007

Guan W, Ni Z, Hu Y, Liang W, Ou C, He J et al (2020a) Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 382:1708–1720. https://doi.org/10.1056/NEJMoa2002032

Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM et al (2020b) Comorbidity and its impact on 1,590 patients with Covid-19 in China: a nationwide analysis. Eur Respir J 55:2000547. https://doi.org/10.1183/13993003.00547-2020

Haagmans BL, Al Dhahiry SHS, Reusken CBEM, Raj VS, Galiano M, Myers R et al (2014) Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis 14:140–145. https://doi.org/10.1016/S1473-3099(13)70690-X

He X, Hong W, Pan X, Lu G, Wei X (2021) SARS-CoV-2 omicron variant: characteristics and prevention. MedComm 2:838–845. https://doi.org/10.1002/MCO2.110

Hemida MG, Perera RA, Wang P, Alhammadi MA, Siu LY, Li M et al (2013) Middle east respiratory syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013. Eur Secur 18. https://doi.org/10.2807/1560-7917.ES2013.18.50.20659

Hemida MG, Perera RA, Al Jassim RA, Kayali G, Siu LY, Wang P et al (2014a) Seroepidemiology of middle east respiratory syndrome (MERS) coronavirus in Saudi Arabia (1993) and Australia (2014) and characterisation of assay specificity. Eur Secur 19:20828

Hemida MG, Chu DKW, Poon LLM, Perera RAPM, Alhammadi MA, Ng H-Y et al (2014b) MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis 20:1231–1234. https://doi.org/10.3201/eid2007.140571

Hemida MG, Alnaeem A, Chu DK, Perera RA, Chan SM, Almathen F et al (2017) Longitudinal study of Middle East Respiratory Syndrome coronavirus infection in dromedary camel herds in Saudi Arabia, 2014-2015. Emerg Microbes Infect 6:e56. https://doi.org/10.1038/emi.2017.44

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S et al (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181:271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052

Holmes EC, Goldstein SA, Rasmussen AL, Robertson DL, Crits-Christoph A, Wertheim JO et al (2021) The origins of SARS-CoV-2: a critical review. Cell 184:4848–4856. https://doi.org/10.1016/j.cell.2021.08.017

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al (2020a) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497. https://doi.org/10.1016/S0140-6736(20)30183-5

Huang Y, Yang C, Xu XF, Xu W, Liu SW (2020b) Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41:1141–1149. https://doi.org/10.1038/s41401-020-0485-4

Kamal M, Abo Omirah M, Hussein A, Saeed H (2021) Assessment and characterisation of post-COVID-19 manifestations. Int J Clin Pract 75. https://doi.org/10.1111/IJCP.13746

Kayaaslan B, Eser F, Kalem AK, Kaya G, Kaplan B, Kacar D et al (2021) Post-COVID syndrome: a single-center questionnaire study on 1007 participants recovered from COVID-19. J Med Virol 93:6566–6574. https://doi.org/10.1002/JMV.27198

Khalafalla AI, Lu X, Al-Mubarak AI, Dalab AH, Al-Busadah KA, Erdman DD (2015) MERS-CoV in upper respiratory tract and lungs of dromedary camels, Saudi Arabia, 2013-2014. Emerg Infect Dis 21:1153–1158. https://doi.org/10.3201/eid2107.150070

Krammer F (2020) SARS-CoV-2 vaccines in development. Nature 586:516–527. https://doi.org/10.1038/s41586-020-2798-3

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S et al (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581:215–220. https://doi.org/10.1038/s41586-020-2180-5

Le Hingrat Q, Visseaux B, Laouenan C, Tubiana S, Bouadma L, Yazdanpanah Y et al (2021) Detection of SARS-CoV-2 N-antigen in blood during acute COVID-19 provides a sensitive new marker and new testing alternatives. Clin Microbiol Infect 27:789.e1–789.e5. https://doi.org/10.1016/j.cmi.2020.11.025

Lebedin YS, Lyang OV, Galstyan AG, Belousov VV, Rebrikov DV (2020) Serum SARS-CoV-2 nucleocapsid antigen detection is essential for primary diagnostics of SARS-CoV-2-associated pneumonia. MedRxiv:2020.09.24.20200303. https://doi.org/10.1101/2020.09.24.20200303

Li J, Huang DQ, Zou B, Yang H, Hui WZ, Rui F et al (2021) Epidemiology of COVID-19: a systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. J Med Virol 93:1449–1458. https://doi.org/10.1002/JMV.26424

Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC et al (2020) Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370:2516. https://doi.org/10.1136/bmj.m2516

Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakhon A et al (2021) Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell 184:4220–4236.e13. https://doi.org/10.1016/j.cell.2021.06.020

Lu R, Zhao X, Li J, Niu P, Yang B, Wu H et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395:565–574. https://doi.org/10.1016/S0140-6736(20)30251-8

Lu L, Mok BW-Y, Chen L-L, Chan JM-C, Tsang OT-Y, Lam BH-S et al (2021) Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or CoronaVac vaccine recipients. Clin Infect Dis 75:e822. https://doi.org/10.1093/cid/ciab1041

Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH et al (2013) Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis 19:1819–1823. https://doi.org/10.3201/eid1911.131172

Menges D, Ballouz T, Anagnostopoulos A, Aschmann HE, Domenghino A, Fehr JS et al (2021) Burden of post-COVID-19 syndrome and implications for healthcare service planning: a population-based cohort study. PLoS One 16. https://doi.org/10.1371/JOURNAL.PONE.0254523

Michielsen HJ, De Vries J, Van Heck GL (2003) Psychometric qualities of a brief self-rated fatigue measure: the Fatigue Assessment Scale. J Psychosom Res 54:345–352. https://doi.org/10.1016/S0022-3999(02)00392-6

Moreno-Pérez O, Merino E, Leon-Ramirez JM, Andres M, Ramos JM, Arenas-Jiménez J et al (2021) Post-acute COVID-19 syndrome. Incidence and risk factors: a Mediterranean cohort study. J Infect 82:378–383. https://doi.org/10.1016/J.JINF.2021.01.004

Morens DM, Taubenberger JK, Fauci AS (2021) Universal coronavirus vaccines — an urgent need. N Engl J Med 386:297. https://doi.org/10.1056/nejmp2118468

Norton A, Olliaro P, Sigfrid L, Carson G, Paparella G, Hastie C et al (2021) Long COVID: tackling a multifaceted condition requires a multidisciplinary approach. Lancet Infect Dis 21:601–602. https://doi.org/10.1016/S1473-3099(21)00043-8

Nowotny N, Kolodziejek J (2014) Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013. Euro Surveill 19:20781. https://doi.org/10.2807/1560-7917.ES2014.19.16.20781

Ogata AF, Maley AM, Wu C, Gilboa T, Norman M, Lazarovits R et al (2020) Ultra-sensitive serial profiling of SARS-CoV-2 antigens and antibodies in plasma to understand disease progression in COVID-19 patients with severe disease. Clin Chem 66:1562–1572. https://doi.org/10.1093/clinchem/hvaa213

Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM et al (2020) Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. Emerg Infect Dis 26. https://doi.org/10.3201/eid2607.200841

Petherick A (2020) Developing antibody tests for SARS-CoV-2. Lancet 395:1101–1102. https://doi.org/10.1016/s0140-6736(20)30788-1

Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM et al (2021) Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 596:276–280. https://doi.org/10.1038/s41586-021-03777-9

Pulliam JRC, van Schalkwyk C, Govender N, von Gottberg A, Cohen C, Groome MJ et al (2021) Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. MedRxiv 2021(11):11.21266068. https://doi.org/10.1101/2021.11.11.21266068

Qiu P, Zhou Y, Wang F, Wang H, Zhang M, Pan X et al (2020) Clinical characteristics, laboratory outcome characteristics, comorbidities, and complications of related COVID-19 deceased: a systematic review and meta-analysis. Aging Clin Exp Res 32:1869–1878. https://doi.org/10.1007/s40520-020-01664-3

Quarleri J, Galvan V, Delpino MV (2021) Omicron variant of the SARS-CoV-2: a quest to define the consequences of its high mutational load. GeroScience 44:53. https://doi.org/10.1007/S11357-021-00500-4

Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C et al (2020) A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol 5:1403–1407. https://doi.org/10.1038/s41564-020-0770-5

RECOVERY Collaborative Group (2020) Dexamethasone in hospitalized patients with Covid-19 — Preliminary report. N Engl J Med. https://doi.org/10.1056/nejmoa2021436

Reusken CBEM, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B et al (2013a) Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis 13:859–866. https://doi.org/10.1016/S1473-3099(13)70164-6

Reusken CB, Ababneh M, Raj VS, Meyer B, Eljarah A, Abutarbush S et al (2013b) Middle east respiratory syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, june to September 2013. Eur Secur 18. https://doi.org/10.2807/1560-7917.ES2013.18.50.20662

Reusken CBEM, Messadi L, Feyisa A, Ularamu H, Godeke GJ, Danmarwa A et al (2014) Geographic distribution of MERS coronavirus among dromedary camels, Africa. Emerg Infect Dis 20:1370–1374. https://doi.org/10.3201/eid2008.140590

Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H et al (2020) Structural basis of receptor recognition by SARS-CoV-2. Nature 581:221–224. https://doi.org/10.1038/S41586-020-2179-Y

Stalin Raj V, Farag EABA, Reusken CBEM, Lamers MM, Pas SD, Voermans J et al (2014) Isolation of MERS coronavirus from dromedary camel, Qatar, 2014. Emerg Infect Dis 20:1339–1342. https://doi.org/10.3201/eid2008.140663

Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC et al (2021) Attributes and predictors of long COVID. Nat Med 27:626–631. https://doi.org/10.1038/S41591-021-01292-Y

Tao Y, Shi M, Chommanard C, Queen K, Zhang J, Markotter W et al (2017) Surveillance of bat coronaviruses in Kenya identifies relatives of human coronaviruses NL63 and 229E and their recombination history. J Virol 91:e01953–e01916. https://doi.org/10.1128/jvi.01953-16

Temsah MH, Barry M, Aljamaan F, Alhuzaimi AN, Al-Eyadhy A, Saddik B et al (2021) SARS-CoV-2 B.1.1.7 UK variant of concern lineage-related perceptions, COVID-19 vaccine acceptance and travel worry among healthcare workers. Front Public Health:9. https://doi.org/10.3389/fpubh.2021.686958

Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC et al (2020) Symptom duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a multistate health care systems network - United States, March-June 2020. MMWR Morb Mortal Wkly Rep 69:993–998. https://doi.org/10.15585/MMWR.MM6930E1

Thakur V, Kanta Ratho R (2021) OMICRON (B.1.1.529): a new SARS-CoV-2 variant of concern mounting worldwide fear. J Med Virol. https://doi.org/10.1002/JMV.27541

Tian Y, Wu Q, Li H, Wu Q, Xie Y, Li L et al (2022) Distinct symptoms and underlying comorbidities with latitude and longitude in COVID-19: a systematic review and meta-analysis. Can Respir J 2022:1–11. https://doi.org/10.1155/2022/6163735

Tirupathi R, Muradova V, Shekhar R, Salim SA, Al-Tawfiq JA, Palabindala V (2020) COVID-19 disparity among racial and ethnic minorities in the US: a cross sectional analysis. Travel Med Infect Dis 38:101904. https://doi.org/10.1016/j.tmaid.2020.101904

Troxel AB, Petkova E, Goldfeld K, Liu M, Tarpey T, Wu Y et al (2022) Association of convalescent plasma treatment with clinical status in patients hospitalized with COVID-19: a meta-analysis. JAMA Netw Open 5:e2147331. https://doi.org/10.1001/JAMANETWORKOPEN.2021.47331

Van Der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC et al (2004) Identification of a new human coronavirus. Nat Med 10:368–373. https://doi.org/10.1038/nm1024

van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A et al (2020) Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol 128:104412. https://doi.org/10.1016/j.jcv.2020.104412

Vengesai A, Midzi H, Kasambala M, Mutandadzi H, Mduluza-Jokonya TL, Rusakaniko S et al (2021) A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19. Syst Rev 10:1–23. https://doi.org/10.1186/s13643-021-01689-3

Wang C, Horby PW, Hayden FG, Gao GF (2020) A novel coronavirus outbreak of global health concern. Lancet 395:470–473. https://doi.org/10.1016/S0140-6736(20)30185-9

Wernery U, Corman VM, Wong EYM, Tsang AKL, Muth D, Lau SKP et al (2015) Acute Middle East respiratory syndrome coronavirus infection in livestock dromedaries, Dubai, 2014. Emerg Infect Dis 21:1019–1022. https://doi.org/10.3201/eid2106.150038

Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B et al (2021) SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med 27:622–625. https://doi.org/10.1038/S41591-021-01285-X

Wilhelm A, Widera M, Grikscheit K, Toptan T, Schenk B, Pallas C et al (2021) Reduced neutralization of SARS-CoV-2 Omicron variant by vaccine sera and monoclonal antibodies. MedRxiv 2021(12):07.21267432. https://doi.org/10.1101/2021.12.07.21267432

Woo PCY, Lau SKP, Chu C, Chan K, Tsoi H, Huang Y et al (2005) Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 79:884–895. https://doi.org/10.1128/jvi.79.2.884-895.2005

World Health Organization (WHO) (2021a) Enhancing Readiness for Omicron (B.1.1.529): Technical Brief and Priority Actions for Member States. World Heal Organ HQ:1–8. https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states. Accessed 25 Dec 2021

World Health Organization (WHO). Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of concern 2021. https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern. Accessed 10 Dec 2021b

World Health Organization (WHO). Tracking SARS-CoV-2 variants 2021. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/. Accessed 2 June 2021c

Wrobel AG, Benton DJ, Xu P, Roustan C, Martin SR, Rosenthal PB et al (2020) SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects. Nat Struct Mol Biol 27:763–767. https://doi.org/10.1038/s41594-020-0468-7

Yadav PD, Sapkal GN, Abraham P, Ella R, Deshpande G, Patil DY et al (2021) Neutralization of variant under investigation B.1.617.1 with sera of BBV152 vaccinees. Clin Infect Dis. https://doi.org/10.1093/cid/ciab411

Yusof MF, Eltahir YM, Serhan WS, Hashem FM, Elsayed EA, Marzoug BA et al (2015) Prevalence of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels in Abu Dhabi Emirate, United Arab Emirates. Virus Genes 50:509–513. https://doi.org/10.1007/s11262-015-1174-0

Zhang W, Du RH LB, Zheng XS, Yang XL, Hu B et al (2020) Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 9:386–389. https://doi.org/10.1080/22221751.2020.1729071

Zhu N, Zhang D, Wang W, Li X, Yang B, Song J et al (2020) A novel coronavirus from patients with Pneumonia in China, 2019. N Engl J Med:NEJMoa2001017. https://doi.org/10.1056/NEJMoa2001017

Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z et al (2020) SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 382:1177–1179. https://doi.org/10.1056/nejmc2001737

Part IX

Nature Is the Greatest Bioterrorist: Zoonotic Pathogens as Bioterroristic Agents

Bacterial Zoonotic Pathogens as Bioterroristic Agents

51

Stefan Hörmansdorfer

Contents

S. Hörmansdorfer (✉)
Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
e-mail: stefan.hoermansdorfer@lgl.bayern.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_42

Abstract

First reports on biological warfare date as early as the fourteenth century BC, while bioterrorism is a phenomenon of modern times. Bioterrorism means the deliberate release of viruses, bacteria, or other agents used to cause illness or death in people, animals, or plants. Infectious agents or their toxins, often known for long as the cause of classical infectious diseases may be used for bioterroristic purposes, but not all of them are equally suited as biological weapons.

Anthrax, caused by *Bacillus (B.) anthracis*, a gram-positive spore-forming rod, is a very long known animal disease with zoonotic potential. The ability to form endospores makes *B. anthracis* especially suitable for bioterroristic use as these endospores are highly resistant to environmental influences, disinfectants, heat, or radiation and can easily be aerosolized. *B. anthracis* possesses two main virulence factors, the anthrax toxin and the ability of capsule formation. Both virulence factors are plasmid-encoded. Human anthrax manifests itself as cutanaeous anthrax, alimentary anthrax, inhalational anthrax, and sometimes as injectional anthrax, especially in intravenous drug addicts.

Tularemia is a zoonosis with a broad host range. Wildlife animals are the main reservoir for humans. It is especially a disease of hares, rabbits, and other rodents. As humans are highly susceptible for tularemia, its agent, *Francisella (F.) tularensis*, which can be transmitted by arthropod vectors, by contact, by contaminated water or food, or even by aerosol, is supposed to have a bioterroristic potential, although no attempts of bioterroristic misuse have been known so far. While the most virulent subspecies *F. tularensis ssp. tularensis* is confined to North America, a less virulent subspecies, *F. tularensis ssp. holartica*, is widely distributed over the Northern hemisphere, predominantly over North America, Scandinavia, Russia, and Japan. Human tularemia is a febrile, inflammatory disease, which starts with unspecific symptoms like headache, growing pains, fever, chills, and weakness. The further course of disease depends on the agent's virulence and its route of entry.

Keywords

Bioterrorism · Anthrax · Tularemia · Bacillus anthracis · Francisella tularensis

First reports on biological warfare date as early as the fourteenth century BC, when the Hittites sent diseased rams, possibly infected with tularemia to their enemies (Barras and Greub 2014). Successful attempts of biological warfare with bubonic plague or small pox are known from medieval times up to the nineteenth century (Barras and Greub 2014; Oliveira et al. 2020). During World War I and II some countries had scientific programs for developing biological weapons, but an applicable weapon has never been delivered (Oliveira et al. 2020). During World War I, the German Reich tried to infect animals in neutral countries with anthrax or glanders, especially to hinder the delivery of horses for military use (Mogridge et al. 2010).

The Geneva Protocol for the Prohibition of the Use in War of Asphyxiating Poisonous or Other Gases and of Bacteriological Methods of Warfare was ratified in

1925 as a consequence of World War I. In 1972 the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (Biological Weapons Convention, BWC) was signed by more than 100 nations (Barras and Greub 2014; Oliveira et al. 2020).

Bioterrorism means the deliberate release of viruses, bacteria, or other agents used to cause illness or death in people, animals, or plants. Bioterrorism intends to initiate mass casualties, terror, panic, social disruption, and economic loss due to ideological, religious, or political beliefs (Jansen et al. 2014; Barras and Greub 2014; Oliveira et al. 2020; Janik et al. 2019). In the near past Anthrax spores were used in a bioterroristic raid in the USA ("Amerithrax") during autumn 2001 shortly after the attack of 9–11, when letters containing highly concentrated anthrax spores were sent by mail. Twenty-two cases were reported, 11 of them suffered from inhalational anthrax with 5 fatalities and the remaining 11 cases were cutaneous anthrax (Oliveira et al. 2020).

Infectious agents or their toxins, often known for long as the cause of classical infectious diseases may have a dual use option in using them for bioterroristic purposes. Therefore it can be quite difficult to discern a naturally occurring outbreak from the deliberate release of pathogens or their toxins, especially as for the last three decades outbreak events rose constantly in number and complexity (Barras and Greub 2014; Koch et al. 2020).

Not all naturally occurring pathogens or their toxins are equally suited as biological weapons. The CDC displays three categories of organisms or toxins according to their easiness of spread, the severity of the illness, and the public and social aspects following their release. Category A comprises biological agents and toxins, which can be easily disseminated or transmitted from person to person; result in high mortality rates and have the potential for major public health impact; might cause public panic and social disruption and require special action for public health preparedness such as *Bacillus anthracis* (anthrax), *Clostridium botulinum* toxin (botulism), *Yersinia pestis* (plague), *Variola major* virus (smallpox), *Francisella tularensis* (tularemia) or filoviruses, arenaviruses, and other hemorrhagic fever viruses (viral hemorrhagic fevers) (N N 2007; CDC 2021).

In the following, anthrax and tularemia will be presented as examples for zoonotic bacterial agents with an indwelling bioterroristic potential.

51.1 Anthrax

Anthrax is a very old disease, which is supposed to be mentioned in the Holy Bible as the fifth plague of Egypt. It has been reported since about 1500 B.C., depicted by the oldest Hebrew, Greek, and Roman authors (Böhm 1995; Koch 1885). The etiology of anthrax was proven by Robert Koch in 1876 (Selbitz 2011). Anthrax is primarily an animal disease, but with a broad host range including man. Especially in former times lots of people fell ill and often died from gastrointestinal anthrax (Koch 1885). In those days eating anthrax infected animals was a major source of human infection besides inhalational and cutaneous anthrax.

51.1.1 Etiology

Anthrax is caused by *Bacillus anthracis*, a large, facultatively anaerobic, Gram-positive rod, which is nonmotile and nonhemolytic on blood agar, although a few strains may show a weak hemolysis under the colony. The bacilli appear as single rods or in chains, especially in host tissue. *Bacillus anthracis* grows with flat, dry, rough, grayish colonies with curved and curled peripheral projections, which give them a "medusa head"-like appearance. As a non-fastidious organism, *Bacillus anthracis* grows well on a broad range of nonselective nutrient media (Picture 1).

Bacillus anthracis belongs to the *Bacillus cereus* group together with *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus weihenstephanensis*, *Bacillus cytotoxicus*, *Bacillus toyonensis*, and other species (Ehling-Schulz et al. 2019; Méndez Acevedo et al. 2020). All members share a closely related phylogeny, a highly conserved genome, and a very similar 16S RNA sequence (24). Recent genomic analyses reveal a much greater variety among the *Bacillus cereus* group, suggesting the existence of at least 57 genomospecies (Ehling-Schulz et al. 2019; Torres Manno et al. 2020). Thus, it is difficult to discern those species by mere biochemical means, so that besides phenotypical criteria like biochemical reactions, motility, penicillin susceptibility, gelatin liquefaction, or capsule formation under 10% CO_2, the detection of virulence plasmids is useful for species identification (Böhm 1995; Selbitz 2011; Quinn et al. 2011; Markey et al. 2013; Ehling-Schulz et al. 2019). Meanwhile, however, *Bacillus cereus* strains were detected, harboring anthrax-like virulence plasmids and causing an Anthrax-like disease in primates in Cameroon and Côte d'Ivoire as well as cutaneous lesions and respiratory disease resembling anthrax in metal workers (Ehling-Schulz et al. 2019; Leendertz et al. 2006; Klee et al. 2006).

Bacillus anthracis possesses the ability to form endospores. These endospores have a remarkable tenacity towards environmental factors such as heat, cold, dryness, radiation, and a great range of disinfectants. To reduce anthrax spores by 90% within a minute, temperatures up to 110 °C are necessary (Böhm 1995). The

Picture 1 *Bacillus anthracis* on sheep-blood agar (Incubation 37 °C, 18 h)

endospore formation is enhanced by oxygen and starts when *Bacillus anthracis* is set free from an infected host by bleeding or opening of the carcass. The first endospores can be detected after 4–10 h and the sporulation process is finished after 24–48 h (Mogridge et al. 2010). Those endospores remain viable in soil for decades. Under laboratory conditions a viability of up to 70 years is proven (Böhm 1995). After inhalation or ingestion the spores are phagocytosed by phagocytes and dendritic cells and transported to the lymph nodes. Some spores germinate quickly, but others can remain ungerminated for multiple days (Ehling-Schulz et al. 2019).

51.1.2 Global Distribution

Anthrax was once known all over the world, but national reduction programs in the first half of the twentieth century like the ban to bury animal carcasses and slaughter waste as well as vaccination programs have confined anthrax to some endemic regions in the Mediterranean region, Central and South America, Africa, Asia, and the Far and Middle East, known as the "anthrax belt" from Turkey to Pakistan. In North America, northern Alberta and the Northwest territories are endemic regions, while sporadic outbreaks are reported from northwest Mississippi, southeast Arkansas, and western Texas. However, sporadic cases may occur in nearly all countries (OIE 2021; WHO 2008; Ehling-Schulz et al. 2019; Pilo and Frey 2018).

51.1.3 Virulence Factors

Bacillus anthracis expresses different minor virulence factors such as phospholipases, proteases, iron uptaking proteins, siderophores, Immune Inhibitor A (InhA), and Anthrolysin O, which are encoded chromosomally or on plasmids (Mogridge et al. 2010; Ehling-Schulz et al. 2019; Schacherl and Baumann 2016). Moreover, virulent strains of *Bacillus anthracis* possess two major plasmids, pX01 and pX02. The 182 kb plasmid pX01 contains 217 genes including the three components of anthrax toxin, PA (protective antigen), EF (edema factor), and LF (lethal factor). Plasmid pX02 consists of 95 kb with 113 genes including the operon capBCDAE containing the capsule forming genes (Mogridge et al. 2010; Pilo and Frey 2018). *Bacillus anthracis* strains, which bear only one of these two virulence plasmids are non-virulent or significantly attenuated (Markey et al. 2013). Those attenuated strains may be used as live vaccines (Böhm 1995).

51.1.4 Anthrolysin O

Anthrolysin O is a cholesterol-dependent cytolysin resembling pore-forming toxins of other Gram-positive pathogens. It shows lytic effects against phagocytes by membrane destabilization and weakens the barrier function of epithelial cells (Ehling-Schulz et al. 2019).

51.1.5 Immune Inhibitor A (InhA)

InhA is a protease with low specificity thus having different effects. It damages the blood–brain barrier and contributes to hemorrhagic anthrax meningitis by cleaving zonula occludens-1 protein. InhA generally supports the spreading of *Bacillus anthracis* by degrading host tissue. InhA cleaves the vonWillebrand factor and its regulator ADAMTS13 promoting coagulopathy. InhA contributes to the activation of acute-phase response proteins in the liver supporting inflammation and by cleaving BsIA, an adhesion factor of *Bacillus anthracis* itself; it modulates adhesion and dissemination of *Bacillus anthracis* (Ehling-Schulz et al. 2019; Schacherl and Baumann 2016).

51.1.6 Capsule

The capsule consists of anionic polypeptides, i.e., linear chains of poly-γ-D-glutamic acid linked to the peptidoglycan of the bacterial cell wall. These long chains exceed a molecular weight of 400 kDa. They are protease-resistant and confer protection against the complement system and phagocytosis being poorly recognized by phagocytes due to their anionic charge. By masking immunogenic surface structures they prevent the humoral immune response. The capsule is of low immunogenicity itself. Shedding capsule poly-γ-D-glutamic acid into the bloodstream during infection leads to the production of inflammatory cytokines and contributes to the systemic inflammation at the terminal state of the infection. The capsule is expressed especially within the host. These conditions can be mimicked during culture, growing the bacteria on bicarbonate agar under enhanced CO_2 partial pressure (5–10%). This yields very moist and even slimy colonies – a phenomenon unique to *Bacillus anthracis* compared to other members of the *Bacillus cereus* group. *Bacillus anthracis* tends to lose the capsule coding plasmid pX02 easily, especially when growing in rich soil (Böhm 1995; Quinn et al. 2011; Markey et al. 2013; Mogridge et al. 2010; Ehling-Schulz et al. 2019; Pilo and Frey 2018).

51.1.7 Anthrax Toxin

The production of anthrax toxin is also enhanced by a higher CO_2 pressure (5–10%) and temperatures of about 37 °C. The highest toxin production occurs during the late logarithmic growth phase. Anthrax toxin consists of two binary components, lethal toxin and edema toxin. Both toxins are formed with lethal factor (LF, 90 kDa) or edema factor (EF, 89 kDa) as the enzymatic component and protective antigen (PA, 83 kDa), which is the receptor-binding subunit (Mogridge et al. 2010). PA recognizes two cell receptors, the capillary morphogenesis genes 2 product (CMG2 or ANTXR2) and the tumor endothelial marker 8 (TEM8 or ANTXR1) and is cleaved after binding by a furin-like protease. The resulting 63 kDa protein forms heptamers or octamers in the host cell membrane, which LF or EF binds to. These complexes are internalized by endocytosis and LF or EF is set free in the cytosol by

conformational changes of PA due to the acidic environment in the endosome (Pilo and Frey 2018).

Edema toxin is a calcium- and calmodulin-dependent adenylate cyclase. Its catalytic rate exceeds this of mammalian adenylate cyclases 100fold. Disturbing cellular signaling pathways edema toxin has numerous effects like impairing the phagocytic abilities of phagocytes and altering their cytokine production, causing necrosis in some tissues or decreasing the circulation of the lymphocyte population. High concentrations of cAMP promote water efflux from affected cells into the surrounding tissue effectively causing massive edema (Mogridge et al. 2010; Pilo and Frey 2018).

Lethal toxin is a zinc-dependent metalloprotease, which interferes in cellular signaling pathways impairing numerous cellular functions of immune cells. The inactivation of some MAPKK kinases (mitogen-activated protein kinase kinases) leads to excessive release of cytokines, apoptosis, hypoxia, and necrosis. Lethal toxin induces cell death in macrophages, dendritic cells, and neutrophils as well as in lung epithelial cells and endothelial cells (Mogridge et al. 2010; Ehling-Schulz et al. 2019; Pilo and Frey 2018).

51.1.8 Host Range

Anthrax is a disease with a broad host range. It is primarily an animal disease with ruminants, especially cattle, sheep, and goats being highly susceptible. Reports of anthrax in buffaloes, horses, camels, elephants, rein deer, and other domesticated or wild living herbivores are not seldom either.

Carnivores and humans are moderately susceptible, while pigs are of low susceptibility. Most birds are nearly unsusceptible, but rare reports on the disease in ostriches, ducks, or birds of prey exist (Böhm 1995; Selbitz 2011).

51.1.9 Epidemiology

Soil is the natural reservoir of endospores being formed during the decomposition of carcasses of animals that have died of anthrax. The endospores remain soil-bound and infectious for decades due to their high tenacity toward environmental factors. Endospores from animal carcasses having been buried can reach the surface during heavy rain and flooding of pastures after years and even decades and become a source of infection again. Herbivores catch the infection by inhalation of aerosolized endospores or their ingestion during grazing. Carnivores are infected by devouring infected animals or their carcasses. Anthrax is not a contagious disease. It can barely be transmitted from an infected animal to another except by direct contact with body fluids, especially blood, which is shed by animals near agony. Biting insects or flies are discussed as vectors and are made responsible for explosive outbreaks. Transfer to different regions is supported by human activities like trade with animal products as hides, wool, bone meal, or meat meal (Selbitz 2011; Mogridge et al. 2010; WHO 2008).

51.1.10 Infectious Dose

LD_{50} ranges from <10 spores in susceptible herbivores to 10^7 spores in less susceptible animals, when administered parenterally. The normal way of infection is inhalation or ingestion of endospores, hereby the infectious dose even in susceptible animals is in the order of tens of thousands endospores (WHO 2008).

51.1.11 Disease in Animals

The incubation period is 3 to 5 days with a range between 1 and 14 days.

Ruminants predominantly fall ill with septicemic anthrax, often suffering from sudden death within minutes or hours. The acute illness is highly febrile (fever above 40 °C) and shows signs of severe disease like apathy, dyspnea, tremor, colics, cramps, and cyanosis. Near agony there is shedding of dark, non-coagulable blood from mouth, nose, anus, and with the urine. This blood contains lots of sporulating bacilli and is highly infectious. Death occurs within about 12 h. In some cases prolonged forms occur with edema, colic, dyspnea, and indigestion (Böhm 1995; WHO 2008).

Horses normally show an acute disease with high fever (40–41 ° C), dyspnea, cyanosis, and colics. Hemorrhages, bloody diarrhea, and local, necrotizing swellings are described. Near agony bleeding from different orifices may be seen. Death occurs within 1–3 days (Böhm 1995; WHO 2008).

Septicemic anthrax with an incubation period of 2–4 days is seldom in the pig. Normally, aching, pharyngeal swellings with fever, pharyngitis, dyspnea, and edema develop. Black, necrotizing nodules may be seen in the mucosa and the skin (pharyngeal form). Icterus, vomiting, and diarrhea are signs of intestinal anthrax. Lethality is much lower in the pig compared to ruminants and horses. Chronic, localized anthrax with no clinical signs is possible and the infection is only detected at the slaughterhouse (Böhm 1995; WHO 2008).

Carnivores may fall ill with septicemic anthrax with an incubation period of 2–6 days and fever up to 40 °C. They die within hours or a few days with symptoms similar to those of ruminants. Dogs are considered much more resistant than cats. Subacute cases show diarrhea, edema predominantly in the laryngeal area or the mouth with central necrosis. Death occurs within 3–5 days. Minks are very susceptible to anthrax showing septicemic anthrax with a mortality up to 100%, depending on the level of feed contamination (Böhm 1995).

51.1.12 Human Anthrax

Humans are much less susceptible than herbivores. The infectious dose (ID_{50}) is estimated to be thousands or tens of thousands endospores (WHO 2008). Infection occurs by contact to infected animals or contaminated animal products (WHO 2008; Bauerfeind et al. 2013). Thus, human anthrax is more or less an occupational disease of farmers, veterinarians, butchers, slaughterhouse workers, or workers in the fur, leather, or wool industry, and also of transport or dock workers (Bauerfeind et al. 2013).

Endospores penetrate through small wounds or by inhalation. Ingestion by eating infected meat is of little importance nowadays, but was in former times a major route of transmission. Human anthrax manifests itself as cutanaeous anthrax, alimentary anthrax, or inhalational anthrax (Koch 1885; WHO 2008; Bauerfeind et al. 2013).

More than 95% of cases are cutaneous anthrax. The incubation period is 2–5 days with a range from ½ to 17 days. The endospores penetrate through little skin lesions or wounds and germinate. The disease starts with local reddening, followed by the formation of a papule, which becomes a vesicle within 12–24 h, containing serous or putrid liquid. After a week there is a marked edema with swollen edges while the center necrotizes and a characteristic blue-black eschar is formed. The local process is painless, but pain and fever occur when the regional lymph nodes are affected (WHO 2008; Bauerfeind et al. 2013).

Alimentary anthrax has an incubation period from 1 to 7 days. Symptoms are loss of appetite, vomiting, and nausea, later abdominal cramps, hematemesis, and bloody diarrhea with gut necrosis, ulceration, and ascites. The mesenterial lymph nodes are heavily involved. The clinical picture is that of an acute abdomen (WHO 2008; Bauerfeind et al. 2013).

Ingestion of endospores may cause oropharyngeal anthrax with sore throat, dysphagia, regionary lymphadenopathy, and a marked edema of the throat together with a pseudomembranous inflammation of the tongue and the tonsils (WHO 2008; Bauerfeind et al. 2013).

Inhalational anthrax is the most severe form of human anthrax. The ID_{50} is estimated as 8.000–50.000 endospores. The incubation period is 4–6 days. There is a preset of unspecific, influenza-like symptoms like malaise, cough, sore throat, and fever, followed by worsening dyspnea, headache, sweating, high fever, tachypnea, tachycardia, pleural effusion, cyanosis, disorientation, and, finally, coma and shock. There is massive involvement of the pleural lymphatic system leading to lung congestion and interstitial edema aggravated by toxin-mediated endothelial damage of lung capillaries (WHO 2008; Bauerfeind et al. 2013).

Since 2009, a special form of human anthrax – injectional anthrax – was seen in intravenous drug addicts. It is supposed that the injection of heroin contaminated with anthrax endospores sets a severe soft tissue infection of muscle, fasciae, and connective tissues around the injection site. The patients develop a severe local inflammation with swelling, reddening, edema, abscessation, necrotizing fasciitis, and compartment syndrome with nausea and dyspnea. The local infection may generalize to septicemic anthrax with a very poor prognosis (Bauerfeind et al. 2013; Holzmann et al. 2012; Ramsay et al. 2010). The first cases were detected in Scotland. From December 2009 to December 2010 there were 119 cases of injectional anthrax in intravenous drug users in Scotland of whom 14 people died. Another five cases with four fatalities were reported from England and two cases from Germany (N N 2011). Since June 2012 there were 14 further cases: four in Germany, two in Denmark, one in France, and seven in the UK (one in Scotland, five in England, and one in Wales). Six of these cases were fatal (ECDC 2014).

All known manifestations may lead to septicemic dissemination and anthrax meningitis with fever, somnolence, myalgia, nausea, vomiting, cramps, delirium, and toxic shock (WHO 2008; Bauerfeind et al. 2013).

51.1.13 Diagnosis

The diagnosis of anthrax comprises clinical symptoms and clinical tests together with laboratory tests. Besides the microscopic detection of Gram-positive, spore- and capsule-forming rods in chains directly from infected tissue or blood, the most important test is the isolation of the agent by culture. Culture from infected tissue should always be combined with a PCR for both virulence plasmids as an antibiotic pretreatment may effectively kill the bacteria so that culture alone may yield false negative results. Isolates may be identified and further characterized by biochemical differentiation in combination with other morphological parameters, MALDI-TOF, immunofluorescence, or other antibody techniques and molecular methods to detect anthrax-specific chromosomal sequences and plasmid-located toxin and capsule genes or reveal the clonal descent of the isolated strain. A special bacteriophage (γ-phage) may be used to identify anthrax strains (WHO 2008; Bauerfeind et al. 2013; Pilo and Frey 2018). Meanwhile, *Bacillus cereus* strains are known to harbor anthrax-like virulence plasmids and cause an anthrax-like illness (Ehling-Schulz et al. 2019; Leendertz et al. 2006; Klee et al. 2006).

51.1.14 Therapy

Bacillus anthracis is a bacterium with a wide susceptibility against antibiotics. β-lactam-antibiotics, streptomycin, tetracyclins, erythromycin, clindamycin, or ciprofloxacin are recommended for treatment (Selbitz 2011; WHO 2008; Bauerfeind et al. 2013).

51.2 Tularemia

Tularemia is a zoonosis with a broad host range. The disease was first described by McCoy in rodents in 1911 and in 1914 in humans (Pilo 2018). Wildlife animals are the main reservoir for humans. It is especially a disease of Lagomorpha (especially hares and rabbits) and rodents. As humans are highly susceptible for tularemia, its agent, *Francisella tularensis*, which can be transmitted by aerosol, is supposed to have a bioterroristic potential, although no attempts of bioterroristic misuse have been known so far.

51.2.1 Etiology

Francisella tularensis are small, coccoid, pleomorphic, Gram-negative rods. They are strictly aerobic, oxidase-negative, weakly catalase positive and non-motile. Their cell wall is rich in lipids and gives them a high tenacity in the environment allowing *Francisella tularensis* to survive in soil, mud, or water for weeks and even months (Markey et al. 2013; Bauerfeind 2011).

Picture 2 *Francisella tularensis* ssp. holarctica, isolated from a European hare

As fastidious organisms they need rich, cysteine supplemented media, e.g., cysteine-heart agar with chocolatized blood, chocolate agar with Iso-VitaleX, Thayer-Martin agar, or Martin-Lewis agar. Antibiotics should be added to suppress the contaminant flora (Picture 2). The incubation under 5% CO_2 supports the growth of *Francisella spp.* The incubation period should be at least 48 h and be extended to 10 days (Markey et al. 2013; Müller et al. 2013; Bauerfeind 2011).

Francisella (F.) is the only genus of the family *Francisellaceae*. It comprises the species *F. tularensis, F. hispaniensis, F. noatunensis, F. philomiragia*, and *F. halioticida*. The last three species are linked to water and sometimes isolated from fish. *F. tularensis* consists of four subspecies, i.e., *F. tularensis ssp. tularensis* (type A), *F. tularensis ssp. holarctica* (type B), *F. tularensis ssp. Mediasiatica*, and *F. tularensis ssp. novicida,* which share large genetic homogeneity of more than 98%. The four subspecies differ especially in geographical distribution and pathogenicity, and also in biochemical activities and host specificity (Markey et al. 2013; Larson et al. 2020; Pilo 2018). *F. tularensis ssp. tularensis* is separated in the subtypes A.I and A.II with subtype A.I being the most virulent. For *F. tularensis ssp. holarctica* 3 biovars are proposed: biovar I (erythromycin sensitive), biovar II (erythromycin resistant), and biovar japonica, which can ferment glycerol (Markey et al. 2013; Müller et al. 2013; Larson et al. 2020; Pilo 2018). *F. tularensis ssp. tularensis* is the most virulent subspecies and therefore classified into risk group 3, while the other subspecies belong to risk group 2.

51.2.2 Virulence Factors

The virulence factors of *F. tularensis* are poorly understood yet. *F. tularensis* form a capsule of polysaccharides identical to the O antigen. The capsule is responsible for serum resistance and important for the virulence of *F. tularensis*. In the host they live inside macrophages. They can manipulate the host's immune response by phase variation of their lipopolysaccharide (LPS), concerning the O antigen and the lipid A

moiety. The LPS of *F. tularensis* differs from that of other Gram-negative bacteria. Francisella-LPS is tetraacylated instead of hexaacylated, has longer fatty acids with 16–20 carbons, and a reduced or no lipid A phosphorylation. Francisella-LPS does not induce interleukin 1-release from mononuclear cells and poorly induces tumor necrosis factor. As other intracellular pathogens they modulate the phagosome biogenesis, hinder them from fusion with lysosomes, and escape to the cytoplasm (Rowe and Huntley 2015; Markey et al. 2013). *F. tularensis* as an intracellular pathogen is called a "stealth" pathogen, which manipulates the immune system, host cell entry, and intacellular pathways to limit inflammation and promotes its intracellular survival (Rowe and Huntley 2015).

51.2.3 Epidemiology and Geographical Distribution

F. tularensis is widely distributed over the Northern hemisphere, predominantly in North America, Scandinavia, Russia, and Japan (Markey et al. 2013). *F. tularensis ssp. tularensis* mainly occurs in North America with some findings of probably imported cases in Austria and Slovakia. Type A.I strains are found throughout the United States, while type A.II strains seem to be confined to the western regions of the USA. *F. tularensis spp. holarctica* – the less virulent variant – is distributed over North America, Europe, Siberia, Israel, Iran, and Asia. *F. tularensis ssp. mediasiatica* is confined to Central Asia, while *F. tularensis ssp. novicida* can be found in North America, Spain, and Australia (Markey et al. 2013; Bauerfeind et al. 2013; Larson et al. 2020).

F. tularensis has been isolated from about 250 wildlife animal species, which serve as natural reservoir for humans. The most important host animals are hares, rabbits, squirrels, voles, mice, rats, beavers, and other rodents, but also deer, foxes, bears and other carnivores, and birds as well as reptiles and amphibians or fish may be infected (Markey et al. 2013; Bauerfeind et al. 2013). Biting arthropods play a vital role in the transmission of *F. tularensis*. These include flies, mites, mosquitoes, lice, fleas, and especially ticks, of which some species may transmit the agent transovarially and are thus a natural reservoir themselves (Markey et al. 2013; Bauerfeind et al. 2013; Bauerfeind 2011). Aquatic protozoae are discussed as reservoirs, too (Bauerfeind et al. 2013; Bauerfeind 2011; Larson et al. 2020).

Tularemia is transmitted to man by direct contact with infected animals, their excretions, and organs or by bites. Further sources of infection are contaminated water or food and biting arthropods. Aerosol transmission may occur by processing agricultural products. The agent invades the host through small lesions of the skin or through the conjunctivae or the mucosae of the upper gut or respiratory tract (Markey et al. 2013; Bauerfeind et al. 2013; Bauerfeind 2011; Larson et al. 2020).

Human tularemia is an occupational disease, especially for hunters, butchers, cooks, or agricultural and forest workers. Carnivores, especially cats, may transmit the disease to humans, while dogs are relatively resistant (Markey et al. 2013; Bauerfeind et al. 2013; Bauerfeind 2011). Most human infections occur while treating and preparing hunted hares. A direct human-to-human transmission has not been described yet (Bauerfeind 2011).

51.2.4 Infectious Dose

The infectious dose for inoculation into wounds is with 10 bacteria very low, followed by an infectious dose of 10–50 bacteria for inhalation and about 10^8 bacteria for ingestion (Bauerfeind et al. 2013).

51.2.5 Disease in Animals

Animals develop an acute or chronic general disease with a wide range of symptoms from mild, regionary lymphadenopathy to acute septicemia. Further signs are depression, anorexia, fever, vomiting, diarrhea, lymphadenomegaly, ulcers, and hemorrhage (Markey et al. 2013; Bauerfeind 2011). Outbreaks with high morbidity and mortality are seen in hares. The main organs infected are lymph nodes, lung, pleura, spleen, liver, and kidneys, where miliary, whitish to yellowish necroses are formed, which resemble pseudotuberculosis lesions. In sheep, late abortions or the birth of weak lambs are described (Bauerfeind 2011).

51.2.6 Human Tularemia

The incubation period is 3–10 days with a range of 1–21 days. The disease lasts for 2–3 weeks without treatment and is followed by a long phase of reconvalescence. The case fatality rate is 4–6%, with sepsis up to 30% despite treatment. Infections with *F. tularensis ssp. holarctica* take a lighter course than those with *F. tularensis ssp. tularensis*. The disease normally leaves life-long immunity (Bauerfeind 2011).

The disease starts with unspecific symptoms like headache, growing pains, fever, chills, and weakness. The further course of disease depends on the agent's virulence and its route of entry.

The **ulceroglandular form** starts with a red papule at the site of entry, which undergoes necrosis and finally forms an ulcus. The regionary lymph nodes (often axillary or inguinal lymph nodes) are swelling and develop purulent inflammation and abscessation. If the local skin necrosis is missing, the disease is known as **glandular form**.

Invasion through the conjunctivae results in a heavy conjunctivitis with lymphadenopathy (**oculoglandular form**).

The **oropharyngeal form** manifests with stomatitis, pharyngitis, tonsillitis, and otitis with involvement of the cervical lymph nodes.

Pneumonia and pleuritis with retrosternal pain is characteristic for the **pneumonic form**, while nausea, vomiting, diarrhea, intestinal pain, and gastrointestinal hemorrhage are symptoms of the **gastrointestinal form**.

The **typhoidal or septic form** shows high, continuous or intermittent fever, chills, headache, meningitis, myalgia, apathy, diarrhea, intestinal pain, hepatosplenomegaly, renal failure, and shock with multiple organ failure (Markey et al. 2013; Bauerfeind 2011).

51.2.7 Diagnosis

Tularemia should be suspected when the above mentioned clinical symptoms meet an anamnesis of contact to wildlife animals. The diagnosis is proven by bacteriological examination or serological tests. Clinical specimens like swabs from ulcers, wounds or conjunctivae, pus, bioptates, or sputum are cultured. Importantly, *Francisella tularensis* does not grow on normal media, but needs special, cysteine supplemented media. The culture is not always successful; therefore PCR from clinical specimen should be undertaken in parallel, if tularemia is suspected. Isolates are differentiated by immunofluorescence or by PCR. Molecular methods including 16S rRNA sequencing are used to determine species and subspecies (Markey et al. 2013; Bauerfeind et al. 2013; Bauerfeind 2011).

51.2.8 Therapy

Francisella tularensis may be resistant to β-lactam antibiotics, azithromycin, and macrolides. They are susceptible to aminoglycosides, fluorquinolones, tetracyclines, and chloramphenicol. In therapy for humans, gentamicin, streptomycin, ciprofloxacin, or doxycycline is recommended (Markey et al. 2013; Bauerfeind et al. 2013; Bauerfeind 2011).

References

Barras V, Greub G (2014) History of biological warfare and bioterrorism. Clin Microbiol Infect 20: 497–502

Bauerfeind R (2011) Gattung Francisella. In: Selbitz H-J, Truyen U, Valentin-Weigand P (eds) Tiermedizinische Mikrobiologie, Infektions- und Seuchenlehre, 9th edn. Enke-Verlag, Stuttgart

Bauerfeind R, Kimmig P, Schiefer HG, Schwarz T, Slenczka W, Zahner H (2013) Zoonosen, 4th edn. Deutscher Ärzte-Verlag, Köln

Böhm R (1995) Milzbrand. In Blobel H, Schließer T (eds) Handbuch der bakteriellen Infektionen bei Tieren, vol II, part 3, 2nd edn. Gustav Fischer Verlag, Jena/Stuttgart

CDC|Bioterrorism Agents/Diseases (by category)|Emergency Preparedness & Response. Last call: 19.08.2021

ECDC (2014) Epidemiological update: anthrax cases among people who inject drugs. http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=37

Ehling-Schulz M, Koehler TM, Lereclus D (2019) The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. Microbiol Spectr 7(3). https://doi.org/10.1128/microbiolspec.GPP3-0032-2018

Holzmann T, Frangoulidis D, Simon M, Noll P, Schmoldt S, Hanczaruk M, Grass G, Pregler M, Sing A, Hörmansdorfer S, Bernard H, Grunow R, Zimmermann R, Schneider-Brachert W, Gessner A, Reischl U (2012) Fatal anthrax infection in a heroin user from southern Germany, June 2012. Euro Surveill 17(26):pii=20204. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20204

Janik E, Ceremuga M, Saluk-Bijak J, Bijak M (2019) Biological toxins as the potential tools for bioterrorism. Int J Mol Sci 20:1181. https://www.mdpi.com/1422-0067/20/5/1181

Jansen HJ, Breeveld FJ, Stijnis C, Grobusch MP (2014) Biological warfare, bioterrorism and biocrime. Clin Microbiol Infect 20:488–496

Klee SR, Özel M, Appel B, Boesch C, Ellerbrok H, Jacob D, Holland G, Leendertz FH, Pauli G, Grunow R, Nattermann H (2006) Characterization of *Bacillus anthracis*-like bacteria isolated from wild great apes from Côte d'Ivoire and Cameroon. J Bacteriol 188(15):5333–5344. https://doi.org/10.1128/JB.00303-06

Koch A (ed) (1885) Encyklopädie der Gesammten Thierheilkunde und Thierzucht. Band 1. Moritz Perles Verlag, Wien/Leipzig

Koch L, Lopes A-A, Maiguy A, Guillier S, Guillier L, Tournier J-N, Biot F (2020) Natural outbreaks and bioterrorism: how to deal with two sides of the same coin? J Glob Health. https://doi.org/10.7189/jogh.10.020317

Larson MA, Sayood K, Bartling AM, Meyer JR, Starr C, Baldwin J, Dempsey MP (2020) Differentiation of *Francisella tularensis* subspecies and subtypes. J Clin Microbiol 58: e01495–e01419. https://doi.org/10.1128/JCM.01495-19

Leendertz FH, Yumlu S, Pauli G, Boesch C, Couacy-Hymann E, Vigilant L, Junglen S, Schenk S, Ellerbrok H (2006) A new *Bacillus anthracis* found in wild chimpanzees and a gorilla from West and Central Africa. PLoS Pathog 2(1):e8. https://doi.org/10.1371/journal.ppat.0020008

Markey B, Leonard F, Archambault M, Cullinane A, Maguire D (2013) Clinical veterinary microbiology, 2nd edn. Mosby Elsevier, Edinburgh

Méndez Acevedo M, Carroll LM, Mukherjee M, Mills E, Xiaoli L, Dudley EG, Kovac J (2020) Novel effective *Bacillus cereus* group species "*Bacillus clarus*" is represented by antibiotic-producing strain ATCC 21929 isolated from soil. mSphere 5:e00882–e00820. https://doi.org/10.1128/mSphere.00882-20

Mogridge J, Shadomy S, Turnbull P (2010) *Bacillus anthracis*. In: Gyles CL, Prescott JF, Songer JG, Thoen CO (eds) Pathogenesis of bacterial infections in animals, 4th edn. Wiley-Blackwell

Müller W, Hotzel H, Otto P, Karge A, Bettin B, Bocklisch H, Braune S, Eskens U, Hörmansdorfer S, Konrad R, Nesseler A, Peters M, Runge M, Schmoock G, Schwarz B-A, Sting R, Myrtennäs K, Karlsson E, Forsman M, Tomaso H (2013) German *Francisella tularensis* isolates from European brown hares (*Lepus europaeus*) reveal genetic and phenotypic diversity. BMC Microbiol 13:61. http://www.biomedcentral.com/1471-2180/13/61

N N (2007) Biologische Gefahren I. Handbuch zum Bevölkerungsschutz, 3rd edn. Bundesamt für Bevölkerungsschutz und Katastrophenhilfe, Bonn

N N (2011) An outbreak of anthrax among drug users in Scotland, December 2009 to December 2010. Health Protection Scotland. http://www.documents.hps.scot.nhs.uk/giz/anthrax-outbreak/anthrax-outbreak-report-2011-12.pdf

OIE (2021) Anthrax – OIE – World Organisation for Animal Health. Last call: 19.08.2021.

Oliveira M, Mason-Buck G, Ballard D, Branicki W, Amorim A (2020) Biowarfare, bioterrorism and biocrime: a historical overview on microbial harmful applications. Forensic Sci Int. https://doi.org/10.1016/j.forsciint.2020.110366

Pilo P (2018) Phylogenetic lineages of Francisella tularensis in animals. Front Cell Infect Microbiol 8:258. https://doi.org/10.3389/fcimb.2018.00258

Pilo P, Frey J (2018) Pathogenicity, population genetics and dissemination of *Bacillus anthracis*. Infect Genet Evol 64:115–125. https://doi.org/10.1016/j.meegid.2018.06.024

Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan PJ (2011) Veterinary microbiology and microbial disease, 2nd edn. Wiley-Blackwell

Ramsay CN, Stirling A, Smith J, Hawkins G, Brooks T, Hood J, Penrice G, Browning L M, Ahmed S, on behalf of the NHS GGC, on behalf of the Scottish National Outbreak Control Teams (2010) An outbreak of infection with *Bacillus anthracis* in injecting drug users in Scotland. Euro Surveill 15(2):pii=19465. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19465

Rowe HM, Huntley JF (2015) From the outside-in:the *Francisella tularensis* envelope and virulence. Front Cell Infect Microbiol 5:94. https://doi.org/10.3389/fcimb.2015.00094

Schacherl M, Baumann U (2016) Feeding anthrax: the crystal structure of *Bacillus anthracis* InhA protease. Structure 24. https://doi.org/10.1016/j.str.2015.12.002

Selbitz H-J (2011) Gattung Bacillus. In: Selbitz H-J, Truyen U, Valentin-Weigand P (eds) Tiermedizinische Mikrobiologie, Infektions- und Seuchenlehre, 9th edn. Enke-Verlag, Stuttgart

Torres Manno M, Repizo GD, Magni C, Dunlap CA, Espariz M (2020) The assessment of leading traits in the taxonomy of the *Bacillus cereus* group. Antonie Van Leeuwenhoek 113:2223–2242. https://doi.org/10.1007/s10482-020-01494-3

WHO (2008) Anthrax in humans and animals, 4th edn. http://whqlibdoc.who.int/publications/2008/9789241547536_eng.pdf?ua=1

Dangerous Viral Pathogens of Animal Origin: Risk and Biosecurity

52

Zoonotic Select Agents

Jean-Paul Gonzalez and Gavin Macgregor-Skinner

Contents

Abstract

Most of emerging infectious diseases affecting humans are of animal origin and transmitted under natural circumstances from either, wild or domestic vertebrate animals giving the way of zoonotic infection or epidemics. Zoonotic diseases carry a common ancient history between human and animals as a result of pathogen exchanges involving transgression of the species barrier. Nowadays, several agents have been targeted for their potential to be a major risk for human and animal populations and, have been characterized by their potential to be highly pathogenic and/or transmissible, and lacking of any means of protection. Those agents have been listed as "Select Agents" having the potential to pose a severe threat to both human and animal health, as well as to animal and plant products. Several of the most dangerous agents responsible of viral hemorrhagic

J.-P. Gonzalez (✉)
Emerging Diseases and Biosecurity, Metabiota Inc., San Francisco, CA, USA
e-mail: jpgonzalez@Metabiota.com

G. Macgregor-Skinner
Department of Public Health Sciences, College of Medicine, The Pennsylvania State University, Hershey, PA, USA
e-mail: gum13@psu.edu

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_41

fever are review in this chapter including: Ebola virus, Marburg virus, Rift valley fever virus, Kyasanur forest virus, Omsk hemorrhagic fever virus, Alkhurma hemorrhagic fever virus.

Keywords

Hemorrhagic Fever · Severe Acute Respiratory Syndrome · Rift Valley Fever · Rift Valley Fever Virus · Venezuelan Equine Encephalomyelitis Virus

52.1 Introduction

More than 75% of recently emerging infectious diseases affecting humans are of animal origin; about two third of all human pathogens have an animal source as a natural reservoir (Taylor et al. 2001). The nosologic term of "Zoonosis" has been crafted to gather all transmissible diseases harboring a potential to infect both human and animal (Palmer et al. 2001). Zoonosis (i.e. zoonotic diseases) are transmissible diseases between animals and man with an infectious (microbes and prions) or parasitic origin. In another term, a zoonotic disease represents any animal disease communicable to human and/or vice versa. Ultimately, zoonosis can be transmitted from animals to humans, directly or indirectly, sometimes by a vector or an intermediate host, or also from humans to other animals. This is considered as reverse zoonosis and called anthroponotic disease, or zooanthroponosis. Zoonoses can be of viral (Yellow Fever, HIV, hantavirus), bacterial (tularemia, leptospirosis, lyme disease), rickettsial (Q-fever), fungal (aspergillosis, histoplasmosis), parasitic (giardiasis, cryptosporidiasis), or prions (Creutzfeldt–Jakob disease) origins. Also the mechanisms of transmission are the main factors driving the risk of human infection. Infectious agents are transmissible under natural circumstances from wild or domestic vertebrate animals to humans. They can also be transmitted from animal products causing foodborne diseases, e.g., *Escherichia coli* O157:H7, Campylobacter, Calicivirus, or Salmonella.

The origin of zoonotic diseases occurred probably when humans came in close contact (scavenging or hunting) with wild animals. Indeed, several zoonoses have been known since early prehistoric times. The first hominids were in direct contact with animal groups which previously appeared on Earth some 540 million years ago (ya.). The history of mankind, starting with Australopithecus, begins about 5 million ya. and coincides with the first contact and potential of microbe exchanges between fauna and this human precursor. Also, one of the most ancient hominids, Australopithecus, was not hunter, but a pretty game (!) hunted by large and powerful carnivorous. Also sick and infected individuals were eaten by such large predators, and human epidemics turned short (Debré and Gonzalez 2013). Earlier *Homo* species from the Pleistocene era (2.6 million–11,700 ya.) utilized larger animals for subsistence (Rabinovich et al. 2008) including mammoths, long horned bisons, saber-toothed cats, giant ground sloths, among others mammals of North America,

Asia, and Europe. It is quite acceptable that these creatures were able to exchange their parasites, e.g., intestinal and blood parasites or fur ectoparasites, with humans.

Hunting remained a crucial component of hunter-gatherer societies before the domestication of livestock and the dawn of agriculture 11,000 ya. First attempts to domesticate dogs, goats, and sheep, occurred as early as 15,000–9000 ya., giving rise to domestic zoonotic parasitic disease. Ultimately, about 1000 ya., 22 species were domesticated including dog, goat, sheep, cattle, camel, pigs, and chicken. Later, during the Neolithic period, when agricultural practices appeared, domestication was well under way supporting the appearance of e.g., flea-or louse-transmitted bacterial zoonoses or pyogenic infections after contact to wild and domestic animals Domestic animals. In fact, in prehistoric times, when human populations were organized in small tribes with a limited number of 100–200 individuals, the human population was actually an accidental victim of infectious diseases, developing rapidly an herd immunity and leaving the pathogens to infect and survive in the more abundant animal populations (e.g., anthrax, rabies, tularemia, cysticercosis) (Debré and Gonzalez 2013).

Indeed, zoonotic diseases carry a common history between human and animals as a result of pathogen exchanges involving a transgression of the species barrier. Altogether, such events occur in a variety of situations involving different hosts, vectors, the pathogens natural cycle's, and the ability of a pathogen to target specific host cells or organs sharing some structural identity between taxonomically distant species (i.e.: human to non-human mammal species).

52.1.1 Zoonotic Risk

Essentially, a zoonotic risk exists and increases with the frequency of contact between infected animals and uninfected permissive human hosts, as well as with the capacity of a pathogen to infect both.

Transgressing the Species Barrier The pathogen species-jumping ability is relevant from wild as well as domestic animal species that can transmit their own microbes to human. The species barrier can easily be violated when species are sympatric and/or taxonomically closely related (e.g.: Arenavirus and different rodent species). Although some pathogens have a high infectious specificity and are usually restricted to infect one host species, some of them can pass the species barrier after a mutation or genetic re-assortment (e.g.: the SARS coronavirus from chiropteran to Palm civet, avian influenza from bird to pig) Influenza:avian and/or after an alteration of the permissive host (e.g., due to immunodeficiency). Ultimately, zoonotic diseases result from parasites, *sensu lato*, that can live apparently harmlessly in a natural host while producing disease upon entry into a different host. Some prominent examples are e.g., HIV having a non-human primate origin and influenza viruses generated from pig and bird viruses after genetic re-assortment, both subsequently evolving to be adapted to a human-to-human virus transmission.

Disease Emergence in Humans A variety of classical human viral diseases are suspected to be the consequence of such a virus jump from animal to human. The origin of such species-jumping leading to disease emergence in the human population takes place in different situations generally associated with human behavior. As mentioned above, the first pathogen exchanges between humans and animals probably occurred sequentially from hunting wild animals to animal domestication.

For example, it is hypothesized that the following diseases originated from either domestic and wild animals: smallpox from rodents more than 10,000 ya., common cold rhinovirus from cattle more than 4000 ya., influenza from pigs more than 8000 ya., measles from cattle plague 300 ya., HIV from non-human primates (NHP) less than 100 ya (Hughes et al. 2010).

Human Population at Risk While many of the zoonotic microbial agents (e.g., the bacteria causing tuberculosis or diphtheria) are resident in domestic mammals and birds, farmers, breeders and all those involved in food animal production are at risk, since the growing contact between humans and wildlife clearly increases the zoonotic risk (e.g., the example of Ebola fever) (Daszak et al. 2000). Wildlife. This can be caused either by encroachment of human activity into wilderness areas or by movement of wild animals into areas of human activity (Artsob 2004).

There are undoubtedly many zoonotic agents waiting in Nature that have the potential to be introduced into humans. Among animal reservoirs with a high and manifest risk for zoonotic transmission are the NHP because of their genetic closeness to humans (Gonzalez et al. 2013) and pigs because of the similarity of their digestive, respiratory and immune systems with the human ones (Martien et al. 2012).

Besides the "natural" risk of an emergence of a certain zoonosis that is directly linked to pathogen evolution (i.e.: change in pathogenicity) and ecology (e.g., extreme weather events, natural catastrophies, climate change) Climate change, more cryptic threats exist and are a cause of concern: the possibility of zoonotic emergence from xenotransplantation from an infected animal biological product (Allan 1996) and the deliberate release of infectious agents into human or animal populations by people (Atlas 2001).

Altogether, most of the factors involved in zoonotic emergence are of human origin, e.g., occupational (poaching, hunting, butchering), due to individual behavior (pets, eating bush meat), by man-made environmental changes (landscape fragmentation, protected area parks and recreational activities), or through social behavior (migration).

52.1.2 Biosecurity

Biosecurity is a set of preventive measures designed to reduce the risk of infection by multiple actions (quarantined pests, contain invasive alien species, master viable

genetically modified organisms [GMO], identify pathogen genetic shift, etc.) modulated by the foundations of risk in line with the assessment of biological risk. To this end, scientific research became the principal actor in a complex process aimed at understanding and mastering the emergence of pathologies (Gonzalez and Fair 2013).

Risk Assessment The biological risk can be either of natural (i.e.: the random encounter of the pathogen, the natural host and human), accidental (i.e.: unexpected "spill over" of the pathogen that infect another host including human), or deliberate origin (i.e.: an individual – criminal – or a group – terrorist – undertaking taking action to infect human or animals). Preventive measure needs a risk assessment with respect to the identified pathogen and its potential to target human and animal (or vector) populations. Several pathogens have been identified as particularly dangerous in that matter regarding their intrinsic characteristics. Ultimately, human and animal populations can consequently be identified concerning their vulnerability to the agent (i.e.: pathogenicity and occurrence in the same environment) (Table 1).

Select Agents Several classes of diseases and agents have been identified as presenting a particular high level of danger including hemorrhagic fever of viral or bacterial origin, infectious neurological syndromes, severe respiratory syndromes among others. Also, regarding the pathogenicity of infectious agents (virulence) and infectiousness (potential to spread) with respect to the risk for either the general human population or laboratory workers, they have been classified as P3–4 level of containment agents (Richmond and McKinney 1999).

For practical reasons, several agents have been targeted for their potential to be a risk for human and animal populations and characterized according their potential to be highly pathogenic or to be highly transmissible – in particular by aerosols – and the lack of any means of protection, e.g., by a vaccine. Those agents have been listed by HHS and USDA as "Select Agents" having the potential to pose a severe threat to both human and animal health, (potentially plant health), or to animal and plant products. Among these 45 Select Agents (33 viruses and 12 bacteria) 31 (69%) are zoonotic, while the remaining are known to infect only animals (Table 2) (http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20Exclusions.html).

Risk Mitigation and International Perspective Major factors have to be taken in account in order to reduce the risk of transmission between animals and humans. Besides reducing the direct contact among the two populations, tools and strategies to fight zoonoses has to be specifically developed. Select Agents have to be surveyed for their emergence, circulation and evolution. Highly pathogenic agents, as well as Select Agents, have to be diagnosed and handled by well-trained workers in certified appropriate laboratory structures (P3 and P4 laboratories, etc.) and their circulation controlled (i.e.: shipping, transferring from one laboratory to another, etc.).

Table 1 Common human and animal highly pathogenic viruses

Virus family	Virus name	Geographical origin	Hosts: Main/ secondary	Vector	Transmission and public health issues	Risk assessment	Human pathogenic	Reference
Arenavirus	Barmah forest	Australia	Possums, kangaroos and wallabies	Mosquito	HT: by bites from infected mosquitos	Z	Mild illness, ILS (pain) recovery	Marshall et al. (1982)
	Venezuelan equine encephalits	America	Horse/zebra, donkey		HT: Zoonosis fever epizootics	SA/P3/	ILS/ENC	Gardner et al. (2008)
	Chapare[a]	America	Rodent, potentially but no evidence yet/-?		HT: ?	SA/P4	H, HF: ILS + vomiting + hemorrhagic signs	Delgado et al. (2008)
Arenavirus	Junin[a]	Argentina	*Calomys musculinus* (dry lands vesper mouse or corn mouse)		HT: rodent biological fluid	SA/P4/	H, HF: neurological signs; mortality 20–30%	Maiztegui (1975)
	Lassa fever[a]	Africa	*Mastomys (Praomys) natalensis* (natal multimammate mouse)		HT: rodent biological fluid	SA/P4/	H, HF: + neurological signs; mortality 30% during epidemics	Buckley and Casals (1970)
	Lujo virus	Africa	Rodent, potentially but no evidence yet?		HT: ?	SA/P4/	H/HF: 80% fatalities	Paweska et al. (2009)

	Lymphocytic Choriomeningitis	WW	Mice with weight loss, retardation of growth and hair development, often lethal		HT: mice	Z	H: two phase, ILS and meningitis-encephalitis; E: neurological damage	Armstrong and Lillie (1934)
	Machupo[a]	Bolivia	*Calomys callosus* (large vesper mouse)		HT: rodent	SA/P4/	H/HF: slow onset with influenza like fever + Petechial; 30% mortality	Johnson et al. (1966)
	Sabia	Brazil	Rodent?		HT: aerosol	SA/P4/	H/HF one fatal case among 3 known; E: 1 death/3	Coimbra et al. (1994)
	Guanarito[a]	Brazil	*Zygodontomys brevicauda* (short-tailed cane mouse)		HT: rodent	SA/P4/	H/HF 23.1% mortality	Salas et al. (1991)
Bornavirus	Borna	WW	Horse/cattle, sheep, dog		HT: rodent?	Z	P/NS	Lipkin and Briese (2007)
Bunyavirus	Oropouche fever	Brazil	Sloth	Mosquito	HT: mosquito bite	Z	P/meningitis	Anderson et al. (1961)
Coronavirus	SARS- coronavirus (SARS-CoV)	Asia/ pandemic	*Panguma larvata*/bats? And		HT: Civets direct contact	SA/P3-4 /	H/acute RS, 10% mortality	Peiris et al. (2003)
Filovirus	Ebola[a]	Central Africa	Bats fruit eating		HT: Bats	SA/P4 /	H/HF 70% mortality	Yun (2012)

(continued)

Table 1 (continued)

Virus family	Virus name	Geographical origin	Hosts: Main/ secondary	Vector	Transmission and public health issues	Risk assessment	Human pathogenic	Reference
	Marburg[a]	Central Africa	Bats		HT: Bats	SA/P4/	H/HF 25 + % mortality	Yun (2012)
Flavivirus	Dengue fever	Asia, Africa, Americas	Monkey?	*Aedes aeypti, A. albopictus*	HT: Mosquito	P2	H/Fever to HF	Petersen and Gubler (2003)
	Eastern equine encephalitis	Americas	Horse	Ticks	HT: Mosquito	SA/P3-4/	H/ENC 25% + mortality	Zacks and Paessler (2010)
	Kyasanur forest disease	Southeast Asia	Monkey	Ticks	HT: ticks	SA/P4/	H/HF ENC 3–5% mortality	Work et al. (1959)
	Omsk hemorrhagic fever	Siberia	Rodents	Ticks	HT: tick	SA/P4/	H/ILS to HF, mortality 1–10%	Chumakov (1948)
	Tick-borne encephalitis, TBE							Barrett et al. (2008)
	TBE far eastern subtype	Europe	Game	Ticks	HT + tick bite	SA/P4/	H/ENC, mortality 1–2%	
	TBE Siberian subtype	Siberia		Ticks	HT + tick bite	SA/P4/	H/ENC, mortality 1–2%	
	West Nile virus	WW	Horse/bird	Mosquito	HT: Mosquito bite	Z	H/ENC	
	Western equine encephalitis	Americas	Horse		HT: Mosquito bite	Z	H/ENC mortality 35%	Zacks and Paessler (2010)
	Yellow fever	Africa, South America	Monkeys	Mosquito	HT: Mosquito bite	Z/P3	H/HF, ENC	Monath et al. (2008)

Hantavirus	Hantaviruses (exept KHF)	WW	Rodents		HT: rodent feces, urine	Z/P3 P4	H/HF – ReS	Lee (1989)
	Korean hemorrhagic fever, KHF[a]	Asia	Rodent		HT: rodent feces, urine; S and S:, E:	Z	H/HF	Lee et al. (1978)
	Puumala	Europe	Rodents		HT: rodent feces, urine	Z	P/ReS	Brummer-Korvenkontio et al. (1980)
Henipavirus	Hendra	Australia	Bats	Horses	HT: + bat urine, animals?	SA/P4/	H/NS – RS	Field (2009)
	Nipah	Asia (Africa?)	Bats		HT: + bat urine, human?	SA/P4/	H/RS	Halpin et al. (2000)
Herpesvirus	Cytomegalovirus (B Herpes)	WW	Primates		HT: direct contact	?	+ mild (fever)	Michaels et al. (2001)
	Herpes simian B	WW	Monkey macaque		HT: direct contact	Z	H/ENC	Gay and Holden (1933)
	Lymphocryptovirus (LCVs) gammaherpesvirus	WW	Primates (Old world and new world)		Direct contact, saliva; Epstein-Barr virus (EBV), human LCV		?	Ablashi et al. (1978)
Nairovirus	Crimean-Congo haemorrhagic fever[a]	Africa, Asia	Cattle	Ticks	HT: tick bite	SA/P4	HF	Chumakov et al. (1968)

(continued)

Table 1 (continued)

Virus family	Virus name	Geographical origin	Hosts: Main/ secondary	Vector	Transmission and public health issues	Risk assessment	Human pathogenic	Reference
Orhtomyxovirus	Influenza A virus H1N1	WW	Pigs/birds		HT: Influenza syndrome	Z	H/ILS + RS	Suarez and Schultz-Cherry (2000)
	Influenza A virus: Highly Pathogenic Avian influenza (HPAIV)[a]	WW	Ducks, shore birds, gulls (natural reservoirs of AIV[c]		Several AI strains infect human (H5N1, H7N2, H7N3, H7N, H9 H9N2, H9N and, H10N7)	USDA SA	H/RS	Chan (2002)
Paramyxovirus	Newcastle disease	WW	Avian		HT: direct contact	USDA SA	P/conjunctivitis	Nelson et al. (1952)
Phlebovirus	Rift Valley fever	Africa	Cattle	Mosquito	HT: mosquito bite	SA/P3-4 /	H/HF	
Poxvirus	Monkeypox	Central Africa	Monkeys		HT: direct contact, bite	SA/P4/	Skin lesions	Ladnyj et al. (1972)
	Orf	WW	Sheep goat		HT: direct contact (skin wound)	Z	P/MD	Geraut (2006)

Retrovirus	Human Immunodeficiency, HIV (ref. to SIV)	WW	NHP		HT (Historical: see Hahn et al.)	Z	HP/P3	Hahn et al. (2000)
	Simian Foamy (SFV)	Africa	Primates		HT: monkey bite	Z	Asymptomatic	Wolfe et al. (2004)
	Simian Immundeficiency (SIV)	Africa	Primates		HT: ?	S	Asymptomatic (?)	Switzer and Heneine (2010)
	Simian T-cell Leukemia (STLV)	Africa	Primates		HT: ?	P	Asymptomatic (?)	Mahieux and Gessain (2011)
Rhabdovirus	Rabies	WW	Canids (lethal)	Feline	HT: bite	Z	H Neurological syndrome; 100% fatal/NS	
	Vesicular stomatitis (VS)[a]	Americas	Horses, cattle, pigs		HT: aerosolization or direct exposure	USDA SA	H/IL	

WW worldwide, *HT* human transmission (+: positive, ?: unknown), *SA* national institute of allergy and infectious diseases select agent (see ref.), *SA USDA* select agent of veterinary importance (see ref. and Table 2), *P* (*3-4*) p level of security = (ref. CDC), *H* highly pathogenic, *ILS* influenza like syndrome, *P* potentially pathogenic, *S* suspected, *MD* mild disease, *NS* neurological syndrome, *ReS* renal syndrome, *RS* respiratory syndrome, *WW* world wide, *HF* hemorrhagic fever, *SA* select agent, *USDA SA* USDA select agents, *Z* recognized as zoonotic

[a]Potential biological weapon

[b]Select Agents Regulations (42 CFR Part 73, 7 CFR Part 331, 9 CFR Part 121) in the Federal Register on March 18, 2005

[c]Domestic and wild avian species (including chickens, turkeys, ducks, domestic geese, quail, pheasants, partridge, parrots, gulls, shorebirds, seabirds, emu, eagles, and others). cause disease in horses, pigs, whales, and seals; expanding to others mammalian species, i.e. cats, dogs, foxes, leopards, tigers, civets, pigs, raccoons

Table 2 Common human and animal highly pathogenic viruses

HHS select agents (zoonotic)	USDA select agents (not zoonotic)		
Virus	Bacteria/Rickettsia	Virus	Bacteria/ Rickettsia
Chapare	*Bacillus anthracis*[a]	African horse sickness	*Mycoplasma capricolum*
Crimean-Congo haemorrhagic fever	*Brucella abortus*	African swine fever	*Mycoplasma mycoides*
Eastern equine encephalitis	*Brucella melitensis*	Avian influenza	
Ebola[a]	*Brucella suis*	Classical swine fever	
Guanarito	*Burkholderia mallei*[a]	Foot-and-mouth disease[a]	
Hendra	*Burkholderia pseudomallei*[a]	Goat pox	
Junin	*Coxiella burnetii*	Lumpy skin disease	
Lassa fever	*Francisella tularensis*[a]	Newcastle disease virus	
Lujo	*Rickettsia prowazekii*	Peste des petits ruminants	
Machupo	*Yersinia pestis*[a]	Rinderpest virus[a]	
Marburg[a]		Sheep pox	
Monkeypox		Swine vesicular disease	
Nipah			
Kyasanur forest disease			
Omsk hemorrhagic fever			
Rift valley fever			
Sabia			
Tick-borne encephalitis complex			
Variola major (smallpox)[a]			
Variola minor (Alastrim)[a]			
Venezuelan equine encephalitis			

HHS (US department of) health and human Services, *USDA* US department of agriculture
[a]Tier 1 Agent

52.2 Highly Pathogenic Viral Zoonoses

52.2.1 Viral Hemorrhagic Fevers (VHF)

Viral Hemorrhagic Fevers (VHF) appear as a whole clinical entity characterized by (high) fever and bleeding that can progress to shock and death. The first severe VHF identified was the Ebola Hemorrhagic Fever (1976), although the Marburg virus

Marburg virus was isolated and characterized earlier in 1967; Marburg virus, however, appears in the medical literature as part of the nosocomial framework of VHF only in 1977 when published aside with the Ebola virus (Bowen et al. 1977). Later, several already known VHF joined the concept including: the Hemorrhagic Fevers with Renal failure (known since 1951), the Hantavirus in 1978 (Lee et al. 1978); the Lassa fever and Bolivian and Argentine HF, Yellow Fever, Rift Valley Fever, Crimean Congo Hemorrhagic fever (CCHF), and others. The group of VHF was identified as a nosologic entity associated with viruses belonging essentially to five distinct families of RNA viruses: the four Arenaviridae, Filoviridae, Bunyaviridae, and Flaviviridae. Only recently in September 2012 scientists reported the isolation of a member of the Rhabdoviridae family responsible for VHF in the Bas-Congo district of the Democratic Republic of Congo (Grard et al. 2012). Several VHFs share many important features: (1) many of them may be transmitted by arthropod-borne agents (usually mosquito vector), (2) person-to–person transmission is possible through direct contact with infected patients, their blood or other body fluids; (3) natural animal reservoirs are mainly rats and mice Mice, but also domestic livestock Livestock:domestic, monkeys or other NHP may serve as intermediate hosts. Moreover, with the increasing international travel, these mainly tropical viruses may now be imported into non-endemic countries thus posing a major global risk for human public health. Furthermore, several of these agents have been associated with nosocomial outbreaks Outbreaks:nosocomial involving health care and laboratory workers.

Due to special biosecurity concerns, we will mainly focus in the following on Filoviruses, RVFV, other flavivirus responsible of hemorrhagic fevers, Kyasanur Forest disease and Omsk HF. Alkhurma HF virus is cited in cursory detail because its limited geographic distribution.

52.2.1.1 Filoviruses (Ebola and Marburg)

Filoviruses

Ebola and Marburg viruses Marburg virus are the only members of the genus Filovirus in the *Filoviridae* family and can cause severe hemorrhagic fever in humans and NHP.

The genus *Marburgvirus* consists of a single species, *Marburg marburgvirus*, with 2 member viruses, Marburg virus (MARV) and Ravn virus (RAVV).

The genus *Ebolavirus* contains five species: *Bundibugyo ebolavirus*, *Zaire ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, and *Taï Forest ebolavirus*, whose members are Bundibugyo virus (BDBV), Ebola virus (EBOV) Ebola virus (EBOV), Reston virus (RESTV), Sudan virus (SUDV), and Taï Forest virus (TAFV), respectively (Kuhn et al. 2010). Ebola-Reston is the only known Filovirus that does not cause severe disease in humans; however, it can still be fatal in monkeys and it has been recently recovered from infected pigs in South-East Asia. A third, tentative genus ("*Cuevavirus"*) has been suggested for a novel filovirus, Lloviu virus (LLOV; species "*Lloviu cuevavirus*"), which has not yet been isolated in culture. With the exception of RESTV and possibly LLOV, all of these viruses cause severe and often fatal viral hemorrhagic fever (VHF) upon infection in humans (Negredo et al. 2011).

The Pathogen

Ebola and Marburg viruses are elongated filamentous molecules, highly variable in length, and are typically between 800–1000 nm long, and can be up to 1400 nm long due to concatamerization, with a uniform diameter of 80 nm. The viral fragment is pleomorphic, and may appear in the shape of a "6", a "U", or a circle, and it is contained within a lipid membrane. Each virion contains one molecule of single-stranded, negative-sense viral genomic RNA, complexed with the proteins *NP*, *VP35*, *VP30*, and *L* (Kiley et al. 1982; Sanchez et al. 1992; Geisbert and Jahrling 1995; Mwanatambwe et al. 2001; Pringle 2005).

Pathogenesis

Two independent studies reported that Ebola virus cell entry and replication requires the cholesterol transporter protein Niemann-Pick C1 (NPC1). The studies described that when cells from Niemann Pick Type C1 patients were exposed to Ebola virus in the laboratory, the cells survived and appeared immune to the virus, further indicating that Ebola relies on NPC1 to enter cells. The same studies described similar results with Ebola's cousin in the filovirus group, Marburg virus, showing that it too needs NPC1 to enter cells (Carette et al. 2011; Côté et al. 2011). Furthermore, NPC1 was shown to be critical to filovirus entry because it mediates infection by binding directly to the viral envelope glycoprotein (Côté et al. 2011). Miller et al. (2012) confirmed the findings that NPC1 is a critical filovirus receptor that mediates infection by binding directly to the viral envelope glycoprotein and that the second lysosomal domain of NPC1 mediates this binding. Carette et al. (2011) showed mice that were heterozygous for NPC1 were protected from lethal challenge with mouse adapted Ebola virus Ebola virus (EBOV). Together, these studies suggest NPC1 may be a potential therapeutic target for an Ebola anti-viral drug.

Clinical Signs

Ebola and Marburg virions enter the host cells through endocytosis and replication occurs in the cytoplasm. Upon infection, the virus targets the host blood coagulative and immune defense system and leads to severe immunosuppression (Harcourt et al. 1999).

Ebola virus disease is clinically indistinguishable from Marburg virus disease Marburg virus, and both are similar to many other diseases prevalent in Equatorial Africa (Grolla et al. 2005).

Early signs of infection are non-specific and flu-like, and may include sudden onset of fever, asthenia, diarrhea, headache, myalgia, arthralgia, vomiting, and abdominal pains (Bwaka et al. 1999). Less common early symptoms such as conjunctival injection, sore throat, rashes, and bleeding may also appear. Shock, cerebral oedema, coagulation disorders, and secondary bacterial infection may co-occur with onset of infection (Feldmann 2010). Hemorrhagic symptoms begin 4–5 days after onset, which includes hemorrhagic conjunctivitis, pharyngitis, bleeding gums, oral/lip ulceration, hematemesis, melena, hematuria, epistaxis, and vaginal bleeding. Hepatocellular damage, marrow depression (such as thrombocytopenia and leucopenia), serum transaminase elevation, and proteinuria may also occur.

Persons that are terminally ill typically present with obtundation, anuria, shock, tachypnea, normothermia, arthralgia, and ocular diseases. Hemorrhagic diathesis is often accompanied by hepatic damage and renal failure, central nervous system involvement, and terminal shock with multi-organ failure. Contact with the virus may also result in symptoms such as severe acute viral illness, malaise, and maculopapular rash. Pregnant women will usually abort their foetuses and experience copious bleeding. Fatality rates range between 50% and 100%, with most dying of dehydration caused by gastric problems (Casillas et al. 2003).

Diagnosis can be confirmed by virus isolation, ELISA to detect viral antigens or patient antibodies in serum or organ homogenates, RT-PCR, immunohistochemistry, and electron microscopy of tissue sections and/or biopsies (Grolla et al. 2005).

Ebola and Marburg virus are morphologically indistinguishable; laboratory studies are extremely hazardous and should be performed in a Biosafety Level 4-equivalent containment Level 4 facility. Laboratory researchers have to be properly trained in BSL-4 practices and wear proper personal protective equipment.

Ebola Virus Epidemiology

Occurrence of Ebola and Marburg virus disease has been primarily limited to countries in sub-Saharan Africa. The name, Ebola, comes from the Ebola River in the Democratic Republic of the Congo, where it was first found in 1976. Marburg virus was first discovered in 1967 and is named after the German city of Marburg.

Ebola virus disease (EVD) Ebola virus (EBOV):epidemiology was first described after almost simultaneous viral hemorrhagic fever outbreaks occurred in Zaire and Sudan in 1976 (WHO 1978a). EVD is believed to occur after an ebolavirus is transmitted to a human index case via contact with an infected animal host. Human-to-human transmission occurs via direct contact with blood or bodily fluids from an infected person (including embalming of a deceased victim) or by contact with contaminated medical equipment such as needles. In the past, explosive nosocomial transmission has occurred in underequipped African hospitals due to the reuse of needles and lack of implementation of universal precautions. Aerosol transmission has not been observed during natural EVD outbreaks, although there are reports suggesting or suspecting aerosol transmission between NHP or in humans based on epidemiological observations (Dalgard et al. 1992; Jaax et al. 1995; Johnson et al. 1995; Roels et al. 1999). The potential for widespread EVD epidemics is considered low due to the high case-fatality rate, the rapidity of demise of patients, and the remote rural areas where infections occur Ebola virus (EBOV): epidemiology.

Marburg Virus Epidemiology

In 1967, simultaneous outbreaks occurred in laboratory workers handling African green monkeys imported from Uganda in Marburg, Frankfurt (Germany), and Belgrade (Yugoslavia, now Serbia) Marburg virus:epidemiology. There were 25 reported primary laboratory-acquired cases with seven deaths. The 25 cases arose from contact and accidents with blood and tissues from infected African green monkeys and six secondary cases (medical personnel, one spouse) developed

from the primary cases (Siegert 1972). Between 1975 and 1987, isolated cases were reported in South Africa (originating from Zimbabwe), Kenya, Zimbabwe, Kenya, and the Democratic Republic of Congo (Gear 1977; Smith et al. 1982). A large long running outbreak occurred between 1998 and 2000 in the Democratic Republic of Congo, resulting in 154 cases and 128 deaths, and two different Marburg viruses, MARV and RAVV, co-circulated and caused disease (Bausch et al. 2006). The largest outbreak to date occurred in 2004 and 2005 centered in Uige, Angola where 374 cases were reported with 329 deaths (Roddy et al. 2010). Since 2007, a number of cases have been reported in Uganda, some of which have been diagnosed into other countries (i.e. USA, The Netherlands) in individuals returning from Uganda (CDC 2003; Timen et al. 2009). Marburg virus has been isolated from blood; serum; secretions, including respiratory and throat secretions; semen; urine; and various tissues and organs from human or animal hosts, or their homogenates (Fisher-Hoch 2005). Marburg virus:epidemiology.

Crossing the Species Barrier and Transmission: Ebola Virus

Between 1976 and 1998, from 30,000 mammals, birds, reptiles, amphibians, and arthropods sampled from outbreak regions, no *Ebolavirus* was detected apart from some genetic traces found in six rodents (*Mus setulosus* and *Praomys* sp.) collected from the Central African Republic (Pourrut et al. 2005). Traces of EBOV were detected in the carcasses of gorillas and chimpanzees during outbreaks in 2001 and 2003, which later became the source of human infections. However, the high lethality from infection in these species makes them unlikely as natural reservoir (Pourrut et al. 2005). Plants, arthropods, and birds have also been considered as possible reservoirs; however, bats are considered the most likely candidate. Bats were known to reside in the cotton factory in which the index cases for the 1976 and 1979 outbreaks were employed, and they have also been implicated in Marburg virus infections in 1975 and 1980 (Pourrut et al. 2005). Of 24 plant species and 19 vertebrate species experimentally inoculated with EBOV, only bats became infected (Swanepoel 1996). The absence of clinical signs in these bats is characteristic of a reservoir species. In a 2002–2003 survey of 1030 animals that included 679 bats from Gabon and the Republic of the Congo, 13 fruit bats were found to contain EBOV RNA fragments (Leroy et al. 2005). As of 2005, three types of fruit bats (*Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*) have been identified as being in contact with EBOV. They are suspected to represent the EBOV reservoir hosts (Pourrut et al. 2007).

The existence of integrated genes of filoviruses in some genomes of small rodents, insectivorous bats, shrews, tenrecs (insectivora from Madagascar), and marsupials indicates a history of infection with filoviruses in these groups as well. However, it has to be stressed that infectious Ebola virus Ebola virus (EBOV) have not yet been isolated from any nonhuman animal (Taylor et al. 2010).

Transmission between natural reservoirs and humans are rare, and outbreaks are usually traceable to a single index case where an individual has handled the carcass of a gorilla, chimpanzee, or duiker (a small antelope species) (Peterson et al. 2004). The virus then spreads person-to-person, especially within families, hospitals, and

during some mortuary rituals where contact among individuals becomes more likely (Hewlett and Amolat 2003).

The virus can be transmitted through body fluids. Transmission through oral or conjunctiva exposure is likely and has been confirmed in NHP (Jaax et al. 1995). Filoviruses are not naturally transmitted by aerosol. They are, however, highly infectious as breathable 0.8–1.2 μm droplets in laboratory conditions; because of this potential route of infection, these viruses have been classified as Category "A" biological weapons (Johnson et al. 1995; Leffel and Reed 2004) (National Institutes of Health, National Institute of Allergy and Infectious Diseases. Category A, B & C Priority Pathogens. 2013. http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/pages/cata.aspx. Accessed May 27, 2013).

Crossing the Species Barrier and Transmission: Marburg Virus

The natural reservoirs of Marburg viruses remain to be identified unequivocally. However, the isolation of both MARV and RAVV from bats and the association of several MVD outbreaks with bat-infested mines or caves strongly suggest that bats are involved in Marburg virus transmission to humans. Avoidance of contact with bats and abstaining from visits to caves is highly recommended, but may not be possible for those working in mines or people dependent on bats as a food source. Monkeys are susceptible but are incidental hosts and individuals handling infected monkeys or their fluids and cell cultures of Marburg virus have become ill (Towner et al. 2009; Timen et al. 2009; Swanepoel et al. 2007).

In 2009, the isolation of infectious MARV was reported from healthy Egyptian rousettes (*Rousettus aegyptiacus* or Egyptian fruit bat) (Towner et al. 2009). This isolation, together with the isolation of infectious RAVV, strongly suggests that Old World fruit bats are involved in the natural maintenance of marburgviruses. Further studies are necessary to establish whether Egyptian rousettes are the actual hosts of MARV and RAVV or whether they get infected via contact with another animal and therefore serve only as intermediate hosts.

The first experimental infection study of *Rousettus aegyptiacus* with MARV provided further insight into the possible involvement of these bats Bats in MARV ecology. Experimentally infected bats developed relatively low viremia lasting at least 5 days, but remained healthy and did not develop any notable gross pathology. The virus also replicated to high titers in major organs (liver and spleen), and organs that might possibly be involved in virus transmission (lung, intestine, reproductive organ, salivary gland, kidney, bladder and mammary gland). The relatively long period of viremia noted in this experiment could possibly also facilitate mechanical transmission by blood sucking arthropods or infection of susceptible vertebrate hosts by direct contact with infected blood (Paweska et al. 2012).

Biosecurity of Filoviruses

Filoviruses Biosecurity:of filoviruses (Ebola viruses and Marburg viruses) are listed as World Health Organization Risk Group 4 Pathogens, National Institute of Allergy and Infectious Diseases (NIAID) Category A Priority Pathogens, Select Agents, and Centers for Disease Control and Prevention (CDC) Category "A" Bioterrorism

Agents due to the absence of prophylaxis or treatment regimens, their high lethality (up to 90% in larger outbreaks), their high infectivity ($LD_{50} = 1$ virion in rodent models), and their stability in artificial aerosols. Research on infectious filoviruses requires Biosafety Level 4 (BSL-4) laboratories.

Filoviruses can survive up to 4–5 days on contaminated surfaces, and can survive in liquid or dried material for a number of days (Belanov et al. 1996; Bray 2003). They are susceptible to sodium hypochlorite, beta-propiolactone, 3% acetic acid (pH 2.5), phenolic disinfectants, formaldehyde and paraformaldehyde, 1% glutaraldehyde, formalin, lipid solvents, and detergents such as SDS. They are physically inactivated by heating for 30–60 min at 60 °C, boiling for 5 min, gamma irradiation (1.2×10–1.27×10 rad), and UV radiation (Elliott et al. 1982; Kurata et al. 1983; Mitchell and McCormick 1984; Mahanty et al. 1999) Biosecurity: of filoviruses.

Ebola Vaccine
Most of the Ebola virus VP proteins are capable of eliciting protective immune responses and therefore are important to consider as potential components of a vaccine to protect humans from Ebola hemorrhagic fever. An "Ebola ΔVP30" strain replication incompetent virus as been generated with a lack of the gene encoding for the VP30 protein, therefore it cannot replicate and do not form infectious progeny in wild-type cells. The genome is stable, without a single event of virus replication; experimental infection of animals did not cause disease in infected animals (Halfmann et al. 2008, 2009).

52.2.1.2 Arenavirus

Arenaviruses are negative stranded RNA viruses of the *Arenaviridae* family. They naturally and chronically infect asymptomatic rodent host-reservoirs. Each rodent species is persistently infected by a specific virus and represents a model of virus-host coevolution (Gonzalez et al. 2007). One exception is made with the Tacaribe virus that has been isolated from naturally infected chiropteran (Downs et al. 1963).

Clinical Signs Several arenaviruses can accidentally infect humans and are responsible for mild to severe zoonotic diseases. Although the arenavirus prototype species, Lymphocytic Choriomeningitis Virus of mice (LCMV) is responsible for a neurological syndrome in humans, at least seven out of the 24 arenavirus species are known to be highly pathogenic for humans and responsible of Viral Hemorrhagic Fever (VHF). Six of them are classified as Select Agents (http://www.selectagents.gov/resources/List_of_Select_Agents_and_Toxins_2012–12-4-English.pdf) including the South American Arenaviruses (Guanarito from Venezuela, Junín from Argentina, Machupo and Chapare from Bolivia and Sabía from Brazil) and the African one, Lassa Fever Virus (from Guinea, Nigeria and Sierra Leone). Also the Lujo virus, not yet a Select Agent, has been recently described in AustralAfrica and represents an emerging potential threat for the region (Paweska et al. 2009).

Although bleeding tendencies are often recorded but not always life threatening, a high mortality of 30% of infected patients can occur during epidemics. Four others

arenaviruses including Flexal (Brazil), Pichínde (Columbia), Tacaribe (Trinidad and Tobago) and White Water Arroyo (California) viruses have been found to potentially infect humans and potentially represent also highly dangerous agents (for a review, Gonzalez et al. 2007).

Epidemiology Asymptomatic infections of rodents are generally suspected to be associated with an insufficient or inappropriate host immune response (Hayes and Salvato 2012) resulting in chronic viremia and/or viruria which leads to shedding of the virus into the environment via urine or faeces.

Exceptionally, chronic infection may have a deleterious effect on their reservoir's fitness, which reduces rodent host fertility (Webb et al. 1975). NHP can be experimentally infected, but there is no evidence that these viruses are pathogenic for domestic animals (e.g.: livestock, cats, dogs), while exotic pets (hamster, mice, etc.) represent a potential source of infection.

Besides the specific association between "arenavirus species – rodent species", the geographic range of an arenavirus ecologic niche appears to be more restricted than the one of its rodent reservoir-host with a more circumscribed enzootic domain, which is often limited by natural barriers (e.g., rivers, elevations, climate, food access). This appears as one of the major characteristics of the epidemiological and dispersion patterns of arenaviruses and therefore VHFs associated with them (Salazar-Bravo et al. 2002).

Argentine HF (Junín virus) was identified in the early 1940s in Argentina and described in the 1950s in the rural area of Buenos Aires province, while the virus was characterized only in 1958. Today the virus distribution expend to 150,000 km^2 of the Pampa. The Vesper mouse (*Calomys* spp.) is the natural host and direct rodent-to-human transmission occurs via ingestion of contaminated food or water, inhalation of rodent urine infested particles or via direct contact of broken skin with rodent excrements. Currently, Argentine HF remains a major and severe enzootic disease of public importance in Argentina with an endemic risk of crossing the natural barrier of the Rio Paraná and spill over Spill over to the closest neighboring countries of Uruguay (Polop et al. 2008).

Bolivian HF (BHF) (Machupo virus) was identified after several outbreaks of BHF in 1963 in the Beni province of Bolivia. Although BHF incidence increases late during the rainy season, small outbreaks are a dominant feature of the epidemiological pattern with several years of dormancy thereafter. The natural host *Calomys callosus* invades houses during floods of the rainy season resulting in close contact and human infection (Kilgore et al. 1997).

Chapare virus was isolated once from a fatal human case of hemorrhagic fever during a unique reported outbreak of HF that occurred in 2003 in the Chapare River region close to Cochabamba in Bolivia, the original setting of Machupo virus responsible of the BHF (Delgado et al. 2008). There is no information concerning an eventual natural rodent host.

Venezuela HF emerged in 1989, with several cases that occurred in the central plains of Venezuela. A new Guanarito virus was isolated and named after the region

where the first outbreak occurred (Salas et al. 1991). The main affected populations are settlers moving into cleared forest areas to practice small agriculture. *Zygodontomys brevicauda* appears to be the principal host (i.e.: reservoir) of the virus.

Lassa fever (LF) was described in 1956 in the eponym village of Lassa. LF occurs in rural West Africa, and appears to be hyper-endemic in Sierra Leone with an antibody prevalence of 8–52%, Guinea (4–55%) and Nigeria (21%). Natural transmission of Lassa virus (LASV) occurs from its domestic, ubiquitous, prolific and common multimammate rodent virus reservoir, *Mastomys natalensis*. As for other Arenaviruses it is transmitted to humans directly through rodent urine and faeces or indirectly by contaminated food. Person-to-person transmission has been described posing a risk for healthcare workers. The virus can also be contracted by an airborne route or by direct contact with infected human blood, urine, or semen, up to 3 months after clinical recovery. LF is a prominent threat outside the endemic area with several imported cases in Germany (Gunther et al. 2000), Japan (Hirabayashi et al. 1988), the United States (Holmes et al. 1990), the United Kingdom (Kitching et al. 2009) among others. About 80% of patients experience a mild or asymptomatic infection. LF has a relatively low mortality rate up to 5%. Among the endemic countries, it is estimated that LF is responsible for about 5000 deaths a year. Pregnant women have the greatest risk of fatality. After an incubation period of 1–3 weeks an acute illness develops while the virus infects every tissue from the mucosa (e.g., intestine, lungs and urinary system) and subsequently progresses to the vascular system. Initial non-specific symptoms include fever, facial swelling, muscle fatigue, conjunctivitis and mucosal bleeding. Later on there might develop gastrointestinal tract bleeding, bloody vomiting, dysphagia, melena, accompanied with cough, dyspnea worsening to cardiovascular system dysfunctions (pericarditis, tachycardia) and hepatitis; finally hearing deficit, meningo-encephalitis and seizures occur. Death is due to multiorgan failure. With respect to this multiple organ infection and accompanying HF signs differential diagnoses include other VHFs such as Ebola or Marburg, malaria or influenza (Yun and Walker 2012; for a review (http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm; http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm; http://www.pasteur.fr/ip/easysite/pasteur/fr/presse/fiches-sur-les-maladies-infectieuses/fievre-de-lassa).

After LASV, Lujo virus is the second known to date human pathogenic arenavirus of Africa. Among the five identified cases in 2008, four died; the fifth case was treated with ribavirin early after onset of clinical disease and survived. It has been only reported from a few patients from Zambia and from a subsequent nosocomial outbreak in South Africa (Briese et al. 2009). A natural reservoir has not yet been identified.

Sabía virus was first isolated from a fatal case of Brazilian HF (BrHF) in the village of Sabía, outside of Sao Paulo, Brazil in 1990 (Lisieux et al. 1994). Two other non-fatal accidental infections were later recorded (Gandsman et al. 1997). Chapare virus infected patients were also clinically considered as BrHF cases. Both viruses

do not have an identified reservoir, however, like the other arenaviruses, they naturally appear to have only a limited geographical distribution.

Crossing the Species Barrier Transmission Virus transmission within rodent populations occurs through vertical (mother to progeny), or horizontal routes (directly through bites or indirectly by contacts with urine or feces). Arenavirus transmission from natural rodent hosts to humans occurs through contacts with infected rodent biological fluids (i.e., blood, saliva or urine), when people (through rodent bites, trapping or eating rodents) are directly exposed to the infected rodent, or indirectly, when exposed to food contaminated with rodent urine and/or by inhalation of infested rodent excreta. Also, human-to-human transmission may occur and arenaviruses can be transmitted trough aerosolized particles and sperm fluid. Moreover, transmission to humans may occur by accidental inoculation with infected body fluids and through tissue transplantation (Emonet et al. 2006; Paweska et al. 2009).

Biosecurity, Therapy and Prevention

Prevention of arenavirus infection consists of interrupting virus transmission from rodents to humans, and from humans to humans. Rodent control seems to be efficient only in certain conditions (i.e.: urban settings). Hospital based nursing barrier appears highly efficient, including personal protective measures (gloves, masks and gowns), good hygiene and appropriate sterilization of equipment. The highest-risk of infection occurs during unprotected contact with body fluids from an infected person. Linens should be handled per CDC guidelines (http://www.cdc.gov/mmwr/preview/mmwrhtml/00037085.htm). Environmental surfaces and contaminated equipment are properly disinfected by 1:10–1:100 dilution of sodium hypochlorite or other EPA-registered disinfectants. The viruses can also be inactivated by ultra-violet, gamma irradiation, temperatures of 56 °C for 20 min and, by a pH less than 5.5 or greater than 8.5.

One anti-virus drug against arenavirus infection has been identified: Ribavirin® is an anti-viral drug that interferes with RNA viral replication. It has been proved to be an efficient treatment against LASV if administered early and might in some cases also be effective against other arenaviruses including BHF, Sabía virus or Lujo virus. Also it has been shown to be effective in advanced stages of LASV infection by reducing the virus load (McCormick et al. 1986; Barry et al. 1995; Enria and Maiztegui 1994; Kilgore et al. 1997; Briese et al. 2009).

Several antiviral molecules are under development with the most promising one directed to interfere with arenavirus cell entry (Larson et al. 2008; York and Nunberg 2006; Charrel et al. 2011). Although hyperimmune serum has been effectively used in several instances, clinical experiences are limited and only circumstantial reports are available. Hyperimmune serum treatment has been used successfully for AHF patients and a plasma bank was established in Argentina (Maiztegui et al. 1979). Also, neutralizing antibodies contained in Human immune plasma appear to be

effective in patients with BHF by reducing viremia. However, LASV infection only leads to a limited neutralizing antibody reaction and hyperimmune serum treatment is not applicable.

Among all arenaviruses, only one vaccine, i.e. the live attenuated Junín virus vaccine Candid #1, has been conclusively developed and produced: its immunogenicity and efficacy in humans was proven to be greater than 84% without causing any serious adverse effects (Maiztegui et al. 1998). Other vaccines tested in animal models include: an attenuated recombinant LASV vaccine using vesicular stomatitis virus as vector that causes a protective immune response in NHP against a lethal LASV challenge (Geisbert et al. 2005); an attenuated Lassa/Mopeia construct ML-29 virus demonstrated protection against LASV challenge in guinea pigs and Rhesus macaques (Lukashevich and Patterson 2008); a yellow fever 17D vaccine expressing LASV glycoprotein precursor protected also guinea pigs against LASV challenge (Bredenbeek et al. 2006; Charrel and de Lamballerie 2010 for review).

52.2.1.3 Rift Valley Fever

Rift Valley Fever (RVF) is a viral zoonosis that primarily affects domestic livestock and also humans in Africa. RVF present a clinical spectrum from mild fever to fatal hemorrhagic syndrome Rift Valley Fever (RVF). RVF virus is spread by infected *Aedes* spp. Aedes spp. or *Culex* spp. Culex spp. mosquitoes. RVF virus is a member of the Phlebovirus genus of the Bunyaviridae family.

Clinical Signs Only a small percentage of patients develops a severe form of the disease including: ocular disease with retinal lesions (0.5–2% of patients); meningoencephalitis (<1%) with headache, loss of memory, confusion, convulsions, and coma; hemorrhagic fever (<1%) starting with severe liver impairment, jaundice, followed by hemorrhage, vomiting blood, melena, purpuric rash, nose and gums bleedings, or menorrhagia. Hemorrhagic forms have a case-fatality as high as 50%. The virus may be detected in blood for up to 10 days.

RVFV is also able to infect many animal species causing particularly severe disease in domesticated animals including cattle, sheep Sheep, camels and goats Goats. Sheep are very sensitive to infection: 90% of infected lambs die, and abortion occurs in up to 100% of infected pregnant ewes.

Epidemiology Human infections can result from direct contact with infected animal biological products, by handling of animal tissue during slaughtering or butchering, conducting veterinary procedures, or from the disposal of carcasses or fetuses. Consequently, herders, slaughterhouse workers, farmers and veterinarians are at high risk of infection. The virus can infect humans through inoculation (i.e.: wound), inhalation of aerosols, by ingesting unpasteurized or uncooked milk or from mosquito bites Bites:mosquito. To date, no human-to-human transmission of RVF has been documented. Outbreaks of RVF occur essentially in rural

environment (see WHO (http://www.who.int/mediacentre/factsheets/fs207/en/) for review).

RVF may occur as large outbreaks when heavy rains favor intense breeding of mosquito vectors. Deaths of newborn animals and abortion in pregnant sheep, goats, and cattle may happen and humans can become infected by contact with infected animal tissues or by mosquito bites. The active circulation of RVFV in Africa and the Arabian Peninsula constitutes a threat for human and animal health all over the African continent and beyond (Grobbelaar et al. 2011).

Biosecurity and Prevention Rift Valley fever belongs to the Select Agent list. It is a potential biological weapon particularly because of its high pathogenicity and its potential to be airborne transmitted (Borio et al. 2002).

Basic nursing barrier and standard infection control precautions are recommended to avoid RVFV transmission to health care workers.

A live-attenuated MP-12 RVFV strain has been developed as a vaccine; the vaccine has been shown to protect bovine and ovine dams against RVFV challenge and is safe and efficacious for use in neonatal calves and lambs (Morrill et al. 1997). Another live attenuated RVFV vaccine lacking the NSs and NSm genes cannot be transmitted by mosquitoes (Bird et al. 2011; Crabtree et al. 2012).

52.2.1.4 Kyasanur Forest Disease

The Kyasanur Forest Disease (KFD) is a tick-borne VHF endemic to and geographically limited to Karnataka State of Central-West India (Work and Trapido 1957). The KFD virus belongs to the Flaviviridae family.

In the early 1990s a new and close related highly pathogenic virus (more than 30% mortality rate), the Alkhurma virus, was isolated in Saudi Arabia and represents another threat for the local population (Charrel et al. 2001).

Clinical Signs

After an incubation period of 3–8 days, KFD starts with a sudden onset of fever, headache, severe muscle pain, cough and dehydration: later on a gastrointestinal syndrome and bleeding occurs. 10% of the patients develop low blood pressure and pancytopenia. Some patients show a biphasic form and experience after 2 weeks a second phase of fever and neurological syndrome leading to a case fatality rate (CFR) of 3–5%. Approximately 400–500 cases of KFD occur in India per year.

Epidemiology (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/kyasanur-eng.php#note10)

Although the main hosts of KFDV are rodents, shrews, bats, and monkeys may also carry the virus. Cattle, goats and sheep may become infected without playing a role in the transmission of the disease. KFDV is transmitted from the bite of an infected tick, principally *Haemaphysalis spinigera* (Work et al. 1959).

Crossing the Species Barrier
Humans can get infected from tick bites or by contact with an infected animal (often sick monkeys: *Presbytis entellus* or *Macaca radiata*). KFDV is common in young adults exposed during the dry season in the forest.

Biosecurity and Prevention
A formalin-inactivated tissue-culture vaccine has been used for vaccination campaigns since the early 1990s in the endemic area of India with an efficacy of 79.3–93.5% after respectively one or two doses (Dandawate et al. 1994).

52.2.1.5 Omsk Hemorrhagic Fever

The tick-borne arbovirus Omsk Hemorrhagic Fever Virus (OHFV) is a member of the Flaviviridae family and classified as a biosafety level 4 virus. Several tick species can transmit the virus including *Dermacentor reticulatus*, *D. marginatus* and *Ixodes persulcatus*.

Clinical Signs As for KHFD, after a one week-incubation period, a first clinical phase of infection, begins with several symptoms including fever, chills, headache, muscular pain, rash, and cervical adenopathy. After 2 weeks a neurological syndrome appears sometimes accompanied by a hemorrhagic syndrome with severe platelet loss and leucopenia. A third of patients develops pneumonia, nephritis, meningitis, or a combination of these complications. The CFR ranges from 1% to 10%, surviving patients acquire life-long immunity.

Epidemiology
The geographic distribution of the OHFV appears restricted to western Siberia (Kharitonova and Leonov 1985) in Omsk, Novosibirsk, Kurgan, and Tyumen oblasts. The main hosts of OHFV are rodents and in particular the non–native muskrat (*Ondatra zibethica*) as a natural OHFV reservoir. Muskrat was imported to Siberia from Canada in the 1920s and the virus finds a particular receptive host to replicate and spread efficiently.

The sylvatic cycle of OHFV involves rodents and in particular the non–native muskrat as a natural OHFV reservoir, but also water voles (*Arvicola terrestris*), while most animals within endemic areas can be infected and bitten by the tick vectors. OHFV survives in water and is transferred to humans via contaminated water or an infected tick.

Crossing the Species Barrier Humans become infected through tick bites or contact with blood, feces or urine of infected muskrats (and other hosts). Gamasid mites are also thought to play a minor role in transmission within the sylvatic cycle. OHFV can also spread through milk from infected goats or sheep.

Prevention
Preventing OHF consists of avoiding tick exposure; consequently persons engaged in farming, forestry, and hunting (i.e.: Siberian muskrat) are at highest risk of infection.

52.2.2 Viral Encephalitis

52.2.2.1 Eastern Equine Encephalitis

The Pathogen Eastern equine encephalitis virus (EEEV) Eastern equine encephalitis virus (EEEV) is a member of the genus Alphavirus, family Togaviridae. Other medically important alphaviruses found in the Americas include Western equine encephalitis virus (WEEV) and Venezuelan equine encephalitis virus (VEEV). EEEV has a single-stranded, positive-sense RNA genome. The virus particles are spherical and have a diameter of 60–65 nm (Snyder et al. 2009). Of the four lineages of EEEV, Group I is endemic in North America and the Caribbean and causes most human disease cases; the other three groups (IIA, IIB, and III) cause primarily equine illness in Central and South America (Zacks and Paessler 2010).

Clinical Signs The incubation period for Eastern equine encephalitis virus (EEEV) Eastern equine encephalitis virus (EEEV) disease ranges from 4 to 10 days. EEEV infection can result in one of two types of illness, systemic or encephalitic (involving swelling of the brain, referred to as EEE). The type of illness will depend on the age of the person and other host factors. It is possible that some people who become infected with EEEV may be asymptomatic.

Systemic infection has an abrupt onset and is characterized by chills, fever, malaise, arthralgia and myalgia. The illness lasts 1–2 weeks, and recovery is complete when there is no central nervous system involvement. In infants, the encephalitic form is characterized by abrupt onset; in older children and adults, encephalitis is manifested after a few days of systemic illness. Signs and symptoms in encephalitic patients are fever, headache, irritability, restlessness, drowsiness, anorexia, vomiting, diarrhea, cyanosis, convulsions, and coma.

EEE is the most severe of the arboviral encephalitis entities and has a mortality of 50–75% (Petersen and Gubler 2003). Death usually occurs 2–10 days after onset of symptoms, but can occur much later. Of those who recover, 15–50% are left with disabling and progressive mental and physical sequelae, which can range from minimal brain dysfunction to severe intellectual impairment, personality disorders, seizures, paralysis, and cranial nerve dysfunction. Many patients with severe sequelae die within a few years (Zacks and Paessler 2010).

No human vaccine against EEEV infection or specific antiviral treatment for clinical EEEV infections is available. Patients with suspected EEE should be evaluated by a healthcare provider, appropriate serologic and other diagnostic tests ordered, and supportive treatment provided.

Epidemiology EEEV is transmitted to humans through the bite of an infected mosquito. Human EEEV cases occur relatively infrequently, largely because the primary transmission cycle takes place in and around swampy areas where human populations tend to be limited. Overall, only about 4–5% of human EEEV infections result in EEE. EEEV infection is thought to confer life-long immunity against

re-infection. It does not confer significant cross-immunity against other alphaviruses (e.g., Western Equine Encephalitis Virus), and it confers no cross-immunity against flaviviruses (e.g., West Nile Virus) or bunyaviruses (e.g., La Crosse Virus).

In the United States, about six human cases of EEE are reported annually. Most cases of EEE have been reported from Florida, Georgia, Massachusetts, and New Jersey. EEEV transmission is most common in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and the Great Lakes region. Between 1964 and 2010, there were 270 confirmed cases of EEE in the US. Several states in the northeastern USA have seen increased virus activity since 2004. Between 2004 and 2006, there were 17 equine cases and at least 13 human cases of EEE reported in Massachusetts. In 2006, approximately 500,000 acres (2000 km^2) in southeastern Massachusetts were treated with mosquito adulticides to reduce the risk of humans contracting EEE. Subsequently, between 2007 and 2010, there were two confirmed human cases and six equine cases reported to CDC and USDA respectively.

In October 2007, a citizen of Livingston, West Lothian, Scotland became the first European victim of this disease. The man had visited New Hampshire during the summer of 2007 on a fishing vacation, and was diagnosed as having EEEV on 13 September 2007. He fell ill with the disease on 31 August 2007, just one day after flying home [5].

In 2012, 209 equine cases of EEE were reported from 19 US States, and 15 human cases of EEE reported from six US States. In 2012, two residents of Vermont were confirmed to have EEE, and this was the first time the illness had been reported in this state.

Crossing the Species Barrier Eastern equine encephalitis virus (EEEV) Eastern equine encephalitis virus (EEEV) is maintained in a cycle between *Culiseta melanura* mosquitoes and avian hosts in freshwater hardwood swamps. *Cs. melanura* is not considered to be an important vector of EEEV to humans, because it feeds almost exclusively on birds. Transmission to humans requires mosquito species capable of creating a "bridge" between infected birds and uninfected mammals such as some *Aedes*, *Coquillettidia*, and *Culex* species.

Wild birds are the main reservoir for transmission of EEEV. Humans, horses, and other animals (domestic fowl, feral pigs, cattle and rodents) are not significant reservoir hosts (Zacks and Paessler 2010). Amphibians and reptiles are a possible reservoir for the virus to overwinter. Mosquitoes and infected eggs are also a reservoir for the viruses (Pfeffer and Dobler 2010).

Person-to-person transmission has not been reported for EEEV viruses. Direct bird-to-human infection can occur, although humans and horses are not amplifying hosts as virus titers in their bodies are insufficient to infect mosquitoes. Eggs of mosquitoes can be infected by the female (Pfeffer and Dobler 2010).

Horses are susceptible to EEEV infection and some cases are fatal. EEEV infections in horses, however, are not a significant risk factor for human infection, because horses (like humans) are considered to be "dead-end" hosts for the virus

(i.e., the concentration of virus in their bloodstreams is usually insufficient to infect mosquitoes) (Zacks and Paessler 2010).

Biosecurity and Prevention All residents of and visitors to areas where virus activity has been identified are at risk of infection with EEEV, particularly persons who engage in outdoor work and recreational activities in these areas. Persons over age 50 and younger than age 15 are at greatest risk for severe disease (encephalitis) following infection. EEEV infection is thought to confer life-long immunity against re-infection.

EEEV is difficult to isolate from clinical samples; almost all isolates (and positive PCR results) have come from brain tissue or CSF. Laboratory acquired infections have been reported, and accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites Bites from infected laboratory arthropods or rodents are the primary hazards associated while working with these viruses.

EEEV do not persist in the environment, and are susceptible to many common disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde and formaldehyde. EEEV can be inactivated by exposure to 50% ethanol at concentration for 60 min, also by moist or dry heat, or by drying, or by UV rays (Aguilar et al. 2005).

EEEV was one of more than a dozen agents that the United States researched as potential biological weapons before the nation suspended its biological weapons program. Samples taken from people and animals with suspected EEEV infection should be handled by trained staff working in Biosafety Level 3 (BSL-3/ABSL-3) **containment** laboratories (CDC 2007).

52.2.2.2 Venezuelan Equine Encephalitis

The Pathogen Venezuelan equine encephalitis virus (VEEV) is a spherical arbovirus that belongs to the Togaviridae family and is an alphavirus (Atasheva et al. 2010). It is 70 nm in diameter and has an enveloped single stranded RNA genome (Gardner et al. 2008).

The Venezuelan equine encephalomyelitis complex contains at least six viral subtypes, I–VI. Subtype I, the Venezuelan equine encephalomyelitis virus (VEEV), is divided into five antigenic variants or serovars, AB to F. Some of the other five subtypes also have official species names; subtype II is known as Everglades virus, subtype III as Mucambo virus, and subtype IV as Pixuna virus.

VEE complex viruses are divided into epizootic (or epidemic) and enzootic (or endemic) groups. The epizootic viruses, which are amplified in equines and are responsible for most epidemics, are found in VEEV subtypes I-AB and I-C. The remaining viruses, including VEEV I-D, VEEV I-E and variants in subtypes II-VI are enzootic (sylvatic) subtypes. These viruses are generally found in limited geographic areas, where they usually occur in natural cycles between rodents and mosquitoes. The enzootic subtypes are typically non-pathogenic for

horses and are not amplified in this host; however, in 1993 an enzootic I-E variant was responsible for an outbreak of VEE among horses in Mexico (Weaver et al. 2004).

Clinical Signs In humans, VEEV usually causes mild to severe influenza-like symptoms; 4–14% of cases, however, develop neurological complications (Gardner et al. 2008). Children and young adults are more likely to develop encephalitis; however, fatalities in humans are rare reaching about 1% of all reported cases (de la Monte et al. 1985). Usually, flu-like symptoms such as headache, myalgia, fatigue, vomiting, nausea, diarrhoea, pharyngitis and fever appear abruptly, 2–5 days after exposure to the virus. The VEE virus can also cause retro-orbital and occipital headaches as well as leucopenia and tachycardia. Symptoms of encephalitis, only appearing in a minority of cases, occur 4–10 days after exposure and include somnolence, convulsions, confusion, photophobia, and coma. Fatal human cases are usually caused by encephalitis as well as brain, lung and gastrointestinal bleeding (Weaver et al. 2004). Long-term neurological damage can be caused by this virus and it can infect the foetus in pregnant women causing birth defects and stillbirths (de la Monte et al. 1985). Generally, the symptoms last between 3 and 8 days and can be biphasic, recurring 4–8 days after the initial symptoms (Sidwell et al. 1967).

Enzootic VEEV usually infects horses sub-clinically or cause mild symptoms. Epizootic subtypes may cause a generalized acute febrile disease with or without neurologic signs. Asymptomatic infections also occur.

Fatal VEE has been reported in various mammals including rabbits, goats, dogs and sheep during epizootics. Some VEE viruses also kill laboratory rodents including hamsters, guinea pigs and mice Mice; however, natural reservoir hosts for enzootic strains usually remain asymptomatic. Experimentally infected, NHP develop a nonspecific febrile illness similar to human disease.

Epidemiology Epizootic VEE viruses (VEEV I-AB and I-C) are found in South and Central America. Most VEE epidemics occur in northern and western South America, but some may spread into adjacent countries, including the US. Enzootic VEE viruses have been found in Mexico, parts of the US, and South and Central America.

The virus was first observed in horses in 1935 after outbreaks in Columbia, Venezuela and Trinidad, and was isolated in 1938. In the 1960s, over 200,000 human cases and 100,000 equine deaths were reported in Colombia and smaller epidemics occurred in Venezuela and Mexico. Between 75,000 and 100,000 infections were reported in Venezuela and Colombia in 1995. The outbreaks usually occur after a season of heavy rains, due to increases in the mosquito population (Weaver et al. 2004).

VEE can be widespread in human populations during epidemics; more than 10% of the population in an area may be affected. Between epidemics, sporadic cases of VEE are caused by enzootic viruses. Humans are highly susceptible to VEE;

approximately 90–100% of exposed individuals become infected, and nearly 100% develop clinical signs. However, most infections are mild. Less than 1% of adults develop encephalitis, with approximately 10% of these cases ending in death; the overall CFR in adults is less than 1%. Very young or elderly patients are more likely to develop severe infections. Encephalitis, with a CFR of 35%, occurs in approximately 4% of children less than 15 years of age. More severe disease, with a higher incidence of neurologic signs, might occur in both children and adults after a biological attack with aerosolized virus.

Instances of person-to-person transmission have not been reported for the VEE virus, although an infected individual can transmit the virus to mosquitoes. Generally, humans and equines become infected by mosquitoes of the *Psorophora* and *Ochlerotatus* genus. Equines can spread the virus to each other through aerosols and to mosquitoes via bites (Pfeffer and Dobler 2010).

Crossing the Species Barrier There are two types of cycles involved in the VEE virus. The enzootic cycle is maintained by rodents and mosquitoes. The epizootic cycle implicates horses, mosquitoes and humans, although there is the potential for the virus to affect many other animal species (Pfeffer and Dobler 2010). Horses are the amplifying host in the cycle and are necessary for a larger outbreak of VEE (de la Monte et al. 1985).

VEEV is typically spread by mosquitoes, although certain types of ticks and mites can spread the virus as well (Weaver et al. 2004). The *Culex (Melanoconion)* mosquito is normally responsible for the dispersal of the enzootic strain of the VEE virus (Zacks and Paessler 2010). *Ochlerotatus taeniorhynchus, Psorophora confinnis, Psorophora columbiae, Ochleratus sollicitans, Mansonia titillans* and *Anophilis aquasalis* are some of the species of mosquitoes known to carry the epizootic varieties of the VEEV (Weaver et al. 2004).

VEE epidemics typically begin in horses, with human cases developing weeks later: Unlike EEE outbreaks, which usually end with the onset of colder temperatures, VEE epidemics can last for several years. Epizootic subtypes of VEEV can cause significant morbidity and mortality in equids; the infection rate can be as high as 90%, and the morbidity rate varies from 10–40% in some areas to 50–100% in others. The CFR in horses is 38–90%. Fatal infections have also been reported in goats, rabbits, dogs and sheep during epizootics, as well as in laboratory rodents infected with some isolates.

Most enzootic VEEV subtypes do not result in serious disease or deaths in horses, but limited outbreaks of encephalitis have been reported with some variants.

Rodents are usually the natural hosts for enzootic VEEV, but birds are involved in a few cycles. The maintenance host for epizootic VEEV between outbreaks is unknown; during epidemics, these viruses are amplified mainly in equids.

Epidemic VEEV can cause serious disease in horses, mules, burros, donkeys and zebras. During epizootics, fatal cases have also been reported in domesticated rabbits, dogs, goats and sheep. Cattle, pigs, bats Bats and opossums can also be infected. Experimental infections have been reported in NHP, guinea pigs, mice

Mice and hamsters; some isolates are fatal for laboratory rodents, although they are usually asymptomatic in their normal rodent hosts.

Biosecurity and Prevention VEEV can be found in the body fluids of horses, and transmission by direct contact or aerosols is theoretically possible in this species. However, natural transmission of VEEV between horses or from horses to humans has not been seen. Infected laboratory rodents can also shed this virus, and people have been infected after exposure to aerosolized debris from cages.

Vaccinations of equines with the TC-83 vaccine and protection against mosquitoes (protective clothing, insecticides) are some of the proposed ways to reduce VEE outbreaks. While the TC-83 vaccine is recommended for laboratory workers, there is no licensed vaccine available for the general population (Weaver et al. 2004).

Arboviruses may be present in blood, cerebrospinal fluid, urine and exudates. The virus may be found in nasal, eye and mouth secretions of infected animals as well as in contaminated animal bedding. The greatest risks when working with VEEV are exposure to infected aerosols, accidental subcutaneous inoculation, and contact with broken skin or contaminated animal bedding. VEEV is stable in dried blood and exudates as well as in freeze dried materials (aerosols) (Chosewood and Wilson 2009). One viral infectious particle injected subcutaneously is enough to infect an individual with VEEV (Collins and Kennedy 1983).

Like other enveloped viruses, VEEV virus is susceptible to disinfectants such as 1% sodium hypochlorite, 4% formaldehyde, 2% gluteraldehyde, 70% ethanol, 3–6% hydrogen peroxide, 2% and peracetic acid (Collins and Kennedy 1983). Microbial inactivation is possible using moist or dry heat (Block 2001). Togaviruses can be inactivated by 15 min of heat at 65 °C (Lelie et al. 1987).

During the Cold War, both the United States biological weapons program and the Soviet biological weapons program researched and weaponized VEEV. In April 2009, the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick reported that samples of VEEV were discovered missing during an inventory of a group of samples left by a departed researcher. The report stated the samples were likely among those destroyed when a freezer malfunctioned.

52.2.2.3 Tick-Borne Encephalitis

The Pathogen Tick-borne encephalitis virus (TBEV) is a single-stranded RNA virus that belongs to the genus *Flavivirus*, and was initially isolated in 1937. TBEV has three subtypes: European, Siberian, and Far Eastern, and is the most important arthropod-borne virus in Europe (Ramelow et al. 1993; Barrett et al. 2008).

The family Flaviviridae includes other tick-borne viruses affecting humans and these viruses are closely related to TBEV and Russian Spring Summer encephalitis, such as Omsk hemorrhagic fever virus in Siberia, Al Khumra virus in Saudi Arabia, and Kyasanur Forest disease virus in India. Louping ill virus (United

Kingdom) is a member of this family; it causes disease primarily in sheep and has been reported as a cause of a TBE-like illness in laboratory workers and persons at risk for contact with sick sheep (e.g.: veterinarians, butchers) (see above paragraphs 5.2.1.4 and 5.2.1.5).

Clinical Signs Tick-borne encephalitis (TBE) is a human viral infectious disease involving the central nervous system. The disease most often manifests as meningitis, encephalitis or meningoencephalitis. Although TBE is most commonly recognized as a neurologic disease, mild febrile illnesses can also occur. Long-lasting or permanent neuropsychiatric sequelae are observed in 10–20% of infected patients. Approximately two thirds of infections are asymptomatic. The median incubation period for TBE is 8 days (range, 4–28 days). The incubation period for milkborne exposure is usually shorter (3–4 days). Hemmer et al. (2005) recommended that tickborne encephalitis should be included in the differential diagnosis of meningoencephalitis in northeastern Germany, even if the patient has not been in tickborne encephalitis–endemic areas.

Among patients with central nervous system involvement, approximately 10% require intensive care and 5% need mechanical ventilation. Clinical course and long-term outcome vary by subtype of TBEV. The European subtype is associated with milder disease, a case-fatality ratio of <2%, and neurologic sequelae in up to 30% of patients. The Far Eastern subtype is often associated with a more severe disease course, including a case-fatality ratio of 20–40% and higher rates of severe neurologic sequelae. The Siberian subtype is more frequently associated with chronic or progressive disease and has a case-fatality ratio of 2–3%.

Epidemiology Tick-borne encephalitis (TBE) has become a considerable public health risk in several European countries, and on average, between 1990 and 2009, nearly 8500 cases of TBE were reported annually in Europe including Russia, although with considerable variability in incidence from year to year (Suss 2011). Many factors contribute to this increase: expanding tick populations due to climatic factors (Randolph 2009; Randolph 2010), social and behavioral changes (Kriz et al. 2004), as well as changes in land use and leisure activities (Sumilo et al. 2007). Reporting of TBE cases has improved as it is a notifiable disease in 16 European countries, including 13 European Union (EU) Member States (Austria, Czech Republic, Estonia, Finland, Germany, Greece, Hungary, Latvia, Lithuania, Poland, Slovak Republic, Slovenia, Sweden) and three non-EU Member States (Norway, Russia and Switzerland) (Donoso et al. 2008).

TBE is endemic in temperate regions of Europe and Asia (from eastern France to northern Japan and from northern Russia to Albania) and up to about 4921 ft (1500 m) in altitude. Russia has the highest number of reported TBE cases, and western Siberia has the highest incidence of TBE in the world. Other countries where the incidence is high include the Czech Republic, Estonia, Germany, Hungary, Latvia, Lithuania, Poland, Slovenia, Sweden, and Switzerland. High vaccination rates in Austria have reduced the incidence of TBE; however, unvaccinated travelers

to this country are still at risk. European countries with no reported cases are Belgium, Iceland, Ireland, Luxembourg, the Netherlands, Portugal, Spain, and the United Kingdom (Suss 2008). Asian countries known to be endemic for TBE include China, Japan, Mongolia, and South Korea (Lu et al. 2008; Walder et al. 2006).

Crossing the Species Barrier TBEV is transmitted to humans through the bite of an infected tick of the Ixodes species, primarily *I. ricinus* (European subtype) or *I. persulcatus* (Siberian and Far Eastern subtypes). The virus is maintained in discrete areas of deciduous forests. Ticks act as both vector and virus reservoir, and small rodents are the primary amplifying host. Tickborne encephalitis (TBE) can also be acquired by ingesting unpasteurized dairy products (such as milk and cheese) from infected goats, sheep or cows, and reports of this route of infections come from Slovakia, Poland, the Baltic States, and other Eastern European countries (Kerbo et al. 2005; Vaisviliene et al. 2002; Balogh et al. 2010). TBEV transmission has infrequently been reported through laboratory exposure and by slaughtering viremic animals. Direct person-to-person spread of TBEV occurs only rarely, through blood transfusion or breastfeeding (Dumpis et al. 1999).

TBE is also emerging in Europe's canine population, and the numbers of clinical cases in dogs are expected to increase (Leschnik et al. 2002; Beugnet and Marie 2009). Humans are accidental dead-end hosts for ticks and for TBEV as, humans do not transmit the disease despite showing noticeable viremia (Heinz 2008) (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18848).

Biosecurity and Prevention Reducing exposure to ticks is the best method to prevent TBE in humans. It is also recommended to avoid consuming unpasteurized dairy products (Rendi-Wagner 2004). Repellents or insecticides provide unreliable protection against tick bites, and there is no specific antiviral treatment for TBE; therapy consists of supportive care and management of complications (Ginsberg and Stafford 2005).

Being a zoonosis, TBE cannot be easily eliminated from endemic areas. However, the introduction of large-scale vaccination campaigns has proven to be highly effective in reducing the burden of disease. In Austria, where the vaccination coverage in the general population has reached approximately 90%, the number of clinical cases could be reduced to about 10%, as compared to the prevaccination era (Heinz et al. 2007). In most highly TBE-endemic countries, large-scale vaccination campaigns are not implemented (Heinz 2008). The risk of acquiring TBE in a highly endemic area in Austria was calculated at approximately 1/10,000 per person-month (Rendi-Wagner 2004). WHO (2012) recommends tick bite prevention in endemic areas during the summer months; only at-risk travellers should be offered vaccination. Travellers are considered to be at risk when hiking or camping in rural and forested areas up to altitudes of 1400 m (WHO 2012).

52.2.3 Other Severe Clinical Syndromes

52.2.3.1 Monkeypox

The Pathogen *Monkeypox* is a viral disease caused by the Monkeypox virus Monkeypox virus (MPXV), an orthopoxvirus. Human cases have been reported from nine countries in central and western Africa where the disease is endemic – Democratic Republic of Congo, People's Republic of Congo, Central African Republic, Gabon, Cameroon, Nigeria, Cote d'Ivoire, Liberia, and Sierra Leone.

The virus was first identified in the State Serum Institute in Copenhagen, Denmark, in 1958 during an investigation into a pox-like disease among monkeys. Monkeypox virus is pathogenic for both animals and humans: Human monkeypox infection was first identified in 1970 in a 9 month old child in the town of Basankusu, Equateur Province, Democratic Republic of Congo and initially NHP were suspected as the source of outbreaks (Ladnyj et al. 1972; Marrennikova et al. 1972).

Over the next year, six further human cases of monkeypox infection were reported in Liberia, Sierra Leone and Nigeria (Foster et al. 1972). From 1970 to 1979, 47 human cases of monkeypox were identified, 38 of which were from Zaire, and the majority were in close proximity to the tropical rainforest (Nalca et al. 2005). A total of 79 cases were subsequently reported over the next 12 years. In 1996–1997 a major outbreak involving 88 cases occurred; between 2001 and 2002 51 human cases were reported in the Democratic Republic of Congo (Hutin et al. 2001; Heymann et al. 2008).

During May and June 2003, the first cases of human monkey pox disease outside of the African continent were reported in an outbreak in Midwestern United States (Illinois, Indiana, Kansas, Missouri, Ohio and Wisconsin) due to direct contact with ill prairie dogs that were kept or sold as pets and which had been recently exposed to imported Monkeypox virus-infected West African rodents from Ghana (Reed et al. 2004).

There were ten confirmed cases and nine probable cases of monkeypox between September and December of 2005 reported in Unity, Sudan (now South Sudan). The particularly intriguing aspect of this outbreak is the evidence of possible human-to-human transmission. In this case, a traditional healer was linked to three of the four transmission chains in the outbreak. The healer had a confirmed case of monkeypox, and a number of the monkeypox patients were either children whom the healer had recently treated for illnesses or young adults who had gone to him for a tooth extraction procedure (removal of the incisors to signify passage into adulthood is a cultural tradition in this part of Sudan) (Nakazawa et al. 2013).

Clinical Signs Monkeypox disease is characterized by the onset of non-specific symptoms which can include fever, headache, backache, and fatigue during a prodromal period of 2–3 days (Reynolds et al. 2006). This is followed by a 2–4 week period in which a rash develops and progresses from macules, to papules,

to vesicles, and then to pustules, followed by umbilication, scabbing and desquamation (CDC 2003). The rash is usually confined to the trunk, but can spread to the palms and soles of the feet, occurring in a centrifugal distribution (Parker et al. 2007). Lesions can also develop on mucous membranes, in the mouth, on the tongue, and on the genitalia (Nalca et al. 2005). The pathogenicity of monkeypox is similar to that of smallpox except for the pronounced lymphadenopathy associated with monkeypox and generally milder symptoms (Heymann 2008). Lymphadenopathy is thus considered to be a key distinguishing feature of monkeypox (Weber and Rutala 2001). The CFR is approximately 1–10% in Africa, with higher death rates among young children (Parker et al. 2007). In children unvaccinated against smallpox, the case-fatality rate ranges from 1% to 14% (Heymann 2008). In addition, children may be more susceptible to monkeypox due to the termination of regular smallpox vaccinations following the worldwide eradication of the disease in 1980.

The incubation period varies from 6 to 16 days. The number of lesions varies from a few to several thousands, affecting oral mucous membranes (in 70% of cases), genitalia (30%), and conjunctivae (20%), as well as the cornea.

There are no drugs or vaccines available for monkeypox, although vaccination against smallpox has been proven to be 85% effective in preventing monkeypox in the past (Parker et al. 2007). Prophylactic vaccination with the smallpox vaccine may be useful within 4 days and up to 14 days after initial contact with a confirmed monkeypox case (CDC 2007).

Epidemiology Monkeypox affect all age groups; however, children under age of 16 have constituted the greatest proportion of cases (Heymann 2008).

Infections of index cases result from direct contact with blood, bodily fluids, or rashes of infected animals. In Africa, human infections have been documented through handling of infected monkeys, Gambian rats or squirrels.

Secondary transmission is human-to-human, resulting from close contact with infected respiratory tract excretions, with skin lesions of an infected person or with recently contaminated objects. Transmission via droplet respiratory particles has also been documented. Transmission can also occur by inoculation or via the placenta (congenital monkeypox). There is no evidence to date that person-to-person transmission alone can sustain monkeypox in the human population.

The differential diagnoses include usually smallpox, chickenpox, measles, bacterial skin infections, scabies, medicamentous allergies and syphilis.

Monkeypox can be definitively confirmed by a number of different tests (ELISA, antigen detection tests, PCR, virus isolation).

Crossing of the Species Barrier In Africa, monkeypox infection has been found in many animal species: rope squirrels, tree squirrels, Gambian rats, striped mice, dormice and NHP. Doubts persist on the natural history of the virus and further studies are needed to identify the exact reservoir of the monkeypox virus Monkeypox virus (MPXV) and how it is maintained in nature.

In the USA, the virus is thought to have been transmitted from African animals to a number of susceptible non-African species (like prairie dogs) with which they were co-housed.

Multiple events of human-to-human transmission have been reported, but sustained Monkeypox virus infection cycles among humans have not been documented (Damon et al. 2006; Formenty et al. 2010).

Likos et al. (2005) investigated phylogenetic relationships between Monkeypox virus isolates by examining five whole-genome sequences and confirmed the existence of two distinct groups: the first group contained isolates from the Congo Basin (Congo Basin clade), and the second group included isolates from countries in western Africa. Differences in epidemiologic and clinical features between Monkeypox virus isolates (e.g., higher morbidity and CFR caused by the Congo Basin clade) support the differentiation between these two clades.

Biosecurity and Prevention During monkeypox outbreaks, close contact with other patients is the most significant risk factor for monkeypox virus infection. In the absence of specific treatment and a vaccine, the only way to reduce infection in people is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the virus.

Public health educational messages should focus on the following risks.

- Reducing the risk of human-to-human transmission. Close physical contact with monkeypox infected people should be avoided. Gloves and protective equipment should be worn when taking care of sick people. Regular hand washing should be carried out after caring for or visiting patients.
- Reducing the risk of animal-to-human transmission. Efforts to prevent transmission in endemic regions should focus on thoroughly cooking all animal products (blood, meat) before eating. Gloves and other appropriate protective clothing should be worn while handling sick animals or their infected tissues, and during slaughtering procedures.

Restricting or banning the movement of small African mammals and monkeys may be effective in slowing the expansion of the virus outside Africa.

Captive animals should not be inoculated with smallpox. Instead, infected animals should be isolated from other animals and placed into immediate quarantine. Any animals that might have come into contact with an infected animal should be quarantined and observed for monkeypox symptoms for 30 days.

Health-care workers caring for patients with suspected or confirmed monkeypox virus infection, or handling specimens from them, should implement standard infection control precautions. Healthcare workers and those treating or exposed to patients with monkeypox or their samples should consider being immunized against smallpox. However, the smallpox vaccination should not be administered to people with comprised immune systems.

Samples taken from people and animals with suspected monkeypox virus infection should be handled by trained staff working in Biosafety Level 3 (BSL-3/ABSL-3) **containment** laboratories (CDC 2007). Orthopoxviruses are susceptible to 0.5% sodium hypochlorite, chloroxylenol-based household disinfectants, glutaraldehyde, formaldehyde, and paraformaldehyde; and are inactivated by heat (autoclaving and

incineration) (Butcher and Ulaeto 2005). Orthopoxviruses are stable at ambient temperatures when dried (CDC 2007) Orthopoxvirus(es).

52.2.3.2 Severe Acute Respiratory Syndrome

The Severe Acute Respiratory Syndrome (SARS) Coronavirus (SARS-CoV) is responsible for an acute and often fatal respiratory syndrome that was identified for the first time in the Guangdong province of South China in 2003 (Peiris et al. 2003). SARS-CoV consequently expended encompassing 37 countries and created the first emerging pandemic of the twenty-first century.

Clinical Signs SARS-CoV may cause an often-severe illness marked initially by systemic symptoms of muscle pain, headache, and fever, followed in 2–10 days by a respiratory symptoms (cough, dyspnea, and pneumonia) and a marked lymphocytopenia. Increased respiratory distress led to a CFR of 9.6% (Smith 2006).

Epidemiology SARS emerged as a unique pandemic starting as an epidemic in Guangdong Province, China in November 2002. It further expanded from person to person worldwide as a pandemic in less than 9 months and ultimately infected more than 8000 persons killing more than 700. The pandemic ended in May 2004.

The virus is supposed to have originated from its natural host, a horseshoe bat (*Rhinolophus sinicus*). Subsequently, it is thought to have been transmitted to and mutated within a secondary host, the palm civet (*Panguma larvata*) serving also as an amplification host, before it was passed into humans as a new human-pathogenic virus, the SARS-CoV (Zhong et al. 2003). SARS-CoV was found to infect also raccoon dogs (*Nyctereuteus* sp.), ferret badgers (*Melogale* spp.) and domestic cats. SARS-CoV emerged several times from the same intermediate host, the palm civet, to transgress the species barrier and infect humans. Nevertheless, SARS-CoV seems to have also emerged several times in the past in the province of Guangdong, but remained unnoticed as potential epidemic risk. The conclusion was that bats acted as a reservoir of SARS-CoV with the potential to infect other mammals including humans (Li et al. 2005).

Likewise but surprisingly, 10 years after the SARS-pandemic, a novel human coronavirus (HCoV-EMC) emerged in the Middle East in 2012 (Bermingham et al. 2012). The HCoV-EMC was identified following respiratory infections with a clinical presentation of severe acute respiratory syndrome of a Qatari man in a British hospital and, a woman who died in Saudi Arabia. The virus consequently caused 12 other confirmed cases and five deaths worldwide (Saudi Arabia, Jordan, and Britain). HCoV-EMC, that appears distant genetically from the former SARS-CoV, seems to have a zoonotic origin naturally infecting chiropteran species (Kelland 2013; Kindler et al. 2013).

Crossing of the Species Barrier SARS-CoV appears to have transgressed efficiently and successively two species barrier from bat to carnivores to humans and,

ultimately, be highly pathogenic for the later with the potential to infect human pulmonary and intestinal epithelium (Sims et al. 2008).

Interestingly, HCoV-EMC appears genetically in the same phylogenetic clade as other bat coronaviruses (Chan and Poon 2013) Coronaviruses.

In the past decade chiropterans have been confirmed as hosts or reservoirs of several emerging diseases including SARS, nipah, hendra, Ebola, Marburg and rabies viruses posing a zoonotic risk (Gonzalez et al. 2008).

Prevention Because SARS-CoV may be be transmitted by aerosol (i.e., aerosolized droplets from coughing), and due to its physical stability in the environment, the low or absent protective immunity in the human population, and the lack of effective antivirals or vaccines, infection control against SARS relied primarily on the prevention of person-to-person transmission (see for review Cheng et al. 2007).

52.3 Conclusion and Perspectives

Humans and animals did host, share and exchange their pathogens since prehistoric times.

A literature review by Olival, Bogich, Karesh et al. (pers. comm. 2013) on virus isolation from different animal hosts shows that NHP, primates and small domestic ungulates are the mammals that share the most virus species with humans; when corrected for the number of species and by the respective sampling/research methods, monkeys, rodents and bats Bats are the most important reservoirs for zoonotic agents. Moreover, if we focus on known viruses and correct for the number of species and sampling per taxonomic order, chiropterans appear to potentially harbor three and six time more different virus species than rodents and NHP, respectively. Also Rodent and Chiropteran are one of the most species richness among the vertebrate orders, they harbor a variety of viruses that can be potentially infectious for human. Moreover, apes share a so close relationship by nature with human, i.e. $>$ 90% of genomic identity, that they theoretically can easily exchange pathogens and pass such "thin" inter species barrier form NHP to Human Primates.

There is no more *terra incognita* on Earth. Humans, by migratory habits, professional or recreation occupations explored already the entire natural environments on the planet, stepping into the immense variety of its ecosystems. While the vast ocean is still open for discovery, zoonotic risk is not out of the scope. As an example, humans are more likely to interact with pinnipeds, than with any other marine mammals and a newly described influenza from seals may potentially infect humans (White 2013). Influenza B virus as well as measles can be shared by human and seals Seals. Also it is well documented that transmission occurs from human to animals like *Coxiella burneti* found infecting seals in Alaska. Moreover, *Streptococcus agalactiae*, a member of human gastrointestinal normal flora, is known to infect sea mammals as well as other marine fauna including fishes (!) among others (Delannoy et al. 2013; Duncan et al. 2012).

Understanding the fundamentals of virus emergence from an animal reservoir and its transmission to humans – but also from one animal species to another – as well as mastering the territories at risk with regard to their environments – including biological and physical environmental components (i.e. increase of the human population, climate change and exceptional weather or natural events) – are essential for controlling and preventing zoonoses and potentially emerging zoonoses.

Viruses will continue to pass the species barrier without geographical borders and acquire new abilities to survive within new hosts without losing their intrinsic pathogenic potential.

More than 60% of 335 emerging infectious diseases identified since 1940 have a zoonotic origin. Among them more than two third are from wildlife animal (Jones et al. 2008). Furthermore, specific territories or domains of emergence, within a given environment, where people, livestock and wildlife encounter each other, have been identified and characterized. An analysis of all documented events has led to develop a spatial and temporal approach for a better understanding of dynamic risk factors (so-called drivers) associated with disease emergence (Souris et al. 2010). By understanding these variable drivers of different scales (e.g. from molecular to spatial, including environmental factors) using computing assisted analysis and mathematical models we might finally be able to predict and hopefully prevent emerging zoonotic infections (Morse et al. 2012). Obviously, theoretical models will have always to be sustained by accurate survey networks coupled with multidisciplinary research. Several of these drivers have to be carefully monitored, e.g. human expansion and its propensity to invade animal territories (i.e. protected area), the emergence of new pathogens from the natural fauna, ecological and environmental conditions, human and animal behaviors, socioeconomic changes, etc.

Biodiversity plays a role in both directions, favoring the risk of exposure to new potentially pathogenic agents and protecting the host against unknown microbes. On one hand, biodiversity exists for the microorganisms as well as for all the other animals, such increasing the variety of potential human pathogens that have not yet "jumped" from animals to humans. On the other hand, the biodiversity of the human major histocompatibility complex, MCH, helps to prevent infection by new pathogens. Eventually, new pathogens may adapt to a new human host (humanization) and ultimately resist to disappearance (i.e. drug resistance) (Maillard and Gonzalez 2006).

Climate change Climate change and societal behavior favor the encounters of hosts, vectors and pathogens that never "met" before: Human and animal populations are highly reacting to climate change (e.g.: mosquitoes) and move or expand towards new territories. Human density, i.e. risk of encounter/transmission from animals to humans, and changes in behavior (pets, hunting) are the driver of emerging zoonoses.

Survey and networking, connected to research, molecular biology and/or virus discovery are the strategic key to predict and prevent the emergence of new zoonoses as well as the next pandemic zoonosis (Gonzalez et al. 2011). Moreover technological advances in molecular diagnostics, mathematical modeling, communication, and informatics enable a targeted global surveillance of emerging and previously unknown infections in both human beings and other species (Morse et al. 2012).

References

Ablashi DV, Pearson G, Rabin H, Armstrong G, Easton J, Valerio M, Cicmanec J (1978) Experimental infection of *Callithrix jacchus* marmosets with Herpesvirus ateles, Herpesvirus saimiri, and Epstein Barr virus. Biomedicine 29, 7-10 with heated, noncytopathogenic Herpesvirus saimiri. Nat New Biol 242:28–30

Aguilar PV, Paessler S, Carrara AS, Baron S, Poast J, Wang E, Moncayo AC, Anishchenko M, Watts D, Tesh RB, Weaver SC (2005) Variation in interferon sensitivity and induction among strains of eastern equine encephalitis virus. J Virol 79(17):11300–11310. https://doi.org/10.1128/JVI.79.17.11300-11310.2005

Allan JS (1996) Xenotransplantation at a crossroads: prevention versus progress. Nat Med 2:18–21

Anderson CR, Spence L, Downs WG, Aitken TH (1961) Oropouche virus: a new human disease agent from Trinidad, West Indies. Am J Trop Med Hyg 10:574–578. PMID 13683183

Anthony SJ, St Leger JA, Pugliares K, Ip HS, Chan JM, Carpenter ZW, Navarrete-Macias I, Sanchez-Leon M, Saliki JT, Pedersen J, Karesh W, Daszak P, Rabadan R, Rowles T, Lipkin WI (2012) Emergence of fatal avian influenza in New England harbor seals. MBio 3(4): e00166–e00112. https://doi.org/10.1128/mBio.00166-12

Armstrong C, Lillie RD (1934) Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St. Louis encephalitis epidemic. Pub Health Rep 49:1019–1027

Artsob H (2004) Emerging and nonemerging arboviral diseases and the veterinary/public health interface. Can Vet J 45(12):1021–1022

Atasheva S, Fish A, Fornerod M, Frolova EI (2010) Venezuelan equine Encephalitis virus capsid protein forms a tetrameric complex with CRM1 and importin alpha/beta that obstructs nuclear pore complex function. J Virol 84(9):4158–4171. https://doi.org/10.1128/JVI.02554-09

Atlas RA (2001) Bioterrorism before and after September 11. Crit Rev Microbiol 27:355–379

Balogh Z, Ferenczi E, Szeles K, Stefanoff P, Gut W, Szomor KN, Takacs M, Berencsi G (2010) Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. J Virol Methods 163(2):481–485

Barrett P, Plotkin S, Ehrlich H (2008) Tick borne encephalitis virus vaccines. In: Plotkin S, Orenstein W, Offit P (eds) Vaccines. Saunders Elsevier, Philadelphia, pp 841–856

Barry M, Russi M, Armstrong L et al (1995) Brief report: treatment of a hazard group laboratory-acquired Sabia virus infection. N Engl J Med 333:294–295

Bausch DG, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Sleurs H, Campbell P, Tshioko FK et al (2006) Marburg hemorrhagic fever associated with multiple genetic lineages of virus. N Engl J Med 355(9):909–919. https://doi.org/10.1056/NEJMoa051465

Belanov EF, Muntianov VP, Kriuk VD, Sokolov AV, Bormotov NI, P'iankov OV, Sergeev AN (1996) Survival of Marburg virus infectivity on contaminated surfaces and in aerosols. Vopr Virusol 41(1):32–34

Bermingham A, Chand MA, Brown CS, Aarons E, Tong C, Langrish C, Hoschler K, Brown K, Galiano M, Myers R, Pebody RG, Green HK, Boddington NL, Gopal R, Price N, Newsholme W, Drosten C, Fouchier RA, Zambon M (2012) Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill 17(40):20290

Beugnet F, Marie JL (2009) Emerging arthropod-borne diseases of companion animals in Europe. Vet Parasitol. https://doi.org/10.1016/j.vetpar.2009.03.028

Bird BH, Maartens LH, Campbell S, Erasmus BJ, Erickson BR, Dodd KA, Spiropoulou CF, Cannon D, Drew CP, Knust B, McElroy AK, Khristova ML, Albariño CG, Nichol ST (2011) Rift valley fever virus vaccine lacking the NSs and NSm genes is safe, nonteratogenic, and confers protection from viremia, pyrexia, and abortion following challenge in adult and pregnant sheep. J Virol 85(24):12901–12909

Block SS (ed) (2001) Disinfection, sterilization, and preservation, 5th edn. Lippincott Williams & Wilkins, Philadelphia

Borio L, Inglesby T, Peters CJ et al (2002) Hemorrhagic fever viruses as biological weapons: medical and public health management. JAMA 287(18):2391–2405

Bowen ET, Lloyd G, Harris WJ, Platt GS, Baserville A, Vella EE (1977) Viral haemorrhagic fever in southern Sudan and northern Zaire. Preliminary studies on the aetiological agent. Lancet 1(8011):571–573

Bray M (2003) Defense against filoviruses used as biological weapons. Antivir Res 57(1–2):53–60

Bredenbeek PJ, Molenkamp R, Spaan WJ, Deubel V, Marianneau P, Salvato MS, Moshkoff D, Zapata J, Tikhonov I, Patterson J, Carrion R, Ticer A, Brasky K, Lukashevich IS (2006) A recombinant yellow fever 17D vaccine expressing Lassa virus glycoproteins. Virology 345(2): 299–304

Briese T, Paweska JT, McMullan LK, Hutchison SK, Street C et al (2009) Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from Southern Africa. PLoS Pathog 5(5):e1000455. https://doi.org/10.1371/journal.ppat.1000455

Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni T, Penttinen K, Oker-Blom N, Lähdevirta J (1980) Nephropathia epidemica: detection of antigen in bank voles and serological diagnosis of human infection. J Inf Dis 141:131–134

Buckley SM, Casals J (1970) Lassa fever, a new virus disease of man from West Africa. III. Isolation and characterization of the virus. Am J Trop Med Hyg 19:680–691

Butcher W, Ulaeto D (2005) Contact inactivation of orthopoxviruses by household disinfectants. J Appl Microbiol 99(2):279–284

Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, Katwiki KR, Kibadi K, Kipasa MA, Kuvula KJ, Mapanda BB, Massamba M, Mupapa KD, Muyembe-Tamfum JJ, Ndaberey E, Peters CJ, Rollin PE, Van den Enden E, Van den Enden E (1999) Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. J Infect Dis 179(Suppl 1):S1–S7. https://doi.org/10.1086/514308

Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, Dal Cin P, Dye JM, Whelan SP, Chandran K, Brummelkamp TR (2011) Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 477(7364):340–343. https://doi.org/10.1038/nature10348

Casillas AM, Nyamathi AM, Sosa A, Wilder CL, Sands H (2003) A current review of Ebola virus: pathogenesis, clinical presentation, and diagnostic assessment. Biol Res Nurs 4(4):268–275

Centers for Disease Control and Prevention (CDC) (2003) Multistate outbreak of monkeypox-Illinois, Indiana, and Wisconsin. MMWR Morb Mortal Wkly Rep 52(23):537–540

Centers for Disease Control and Prevention (CDC) (2007) In: Richmond JY, McKinney RW (eds) Biosafety in microbiological and biomedical laboratories (BMBL), 5th edn. US GPO, Washington, DC

Centers for Disease Control and Prevention (CDC) (2008) Imported case of Marburg hemorrhagic fever-Colorado. MMWR Morb Mortal Wkly Rep 58(49):1377–1381

Chan PK (2002) Outbreak of avian influenza A (H5N1) virus infection in Hong Kong in 1997. Clin Infect Dis 34(Suppl 2):S58–S64

Chan RWY, Poon LLM (2013) The emergence of human coronavirus EMC: how scared should we be? MBio 4(2):e00191–e001913. https://doi.org/10.1128/mBio.00191-13

Charrel RN, de Lamballerie X (2010) Zoonotic aspects of arenavirus infections. Vet Microbiol 140: 213–220

Charrel RN, Zaki AM, Attoui H, Fakeeh M, Billoir F, Yousef AI, de Chesse R, De Micco P, Gould EA, de Lamballerie X (2001) Complete coding sequence of the Alkhurma virus, a tick-borne flavivirus causing severe hemorrhagic fever in humans in Saudi Arabia. Biochem Biophys Res Commun 287(2):455–461

Charrel RN, Coutard B, Baronti C, Canard B, Nougairede A, Frangeul A, Morin B, Jamal S, Schmidt CL, Hilgenfeld R, Klempa B, de Lamballerie X (2011) Arenaviruses and hantaviruses: from epidemiology and genomics to antivirals. Antivir Res 90(2):102–114. https://doi.org/10.1016/j.antiviral.2011.02.009. PMID: 21356244

Cheng VC, Lau SK, Woo PCY, Yuen KY (2007) Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev 20(4):660–694

Chosewood LC, Wilson DE (eds) (2009) Biosafety in microbiological and biomedical laboratories, 5th edn. CDC–NIH, Washington, DC

Chumakov MP (1948) [Results of a study made of Omsk hemorrhagic fever (OL) by an expedition of the Institute of Neurology] Vestnik Acad Med Nauk SSSR 2:19–26 (in Russian)

Chumakov MP, Butenko AM, Shalunova NV, Mart'yanova LI, Smirnova WE, Bashkirtsev VN, Zavodova TI, Rubin SG, Tkachenko EA, Karmy-Sheva VY, Reingold VN, Popov GV, Savinov AP (1968) New data on the virus causing Crimean hemorrhagic fever (CHF). Vopr Virusol 13: 377. (in Russian; in English, NAMRU3-T596)

Coimbra TLM, Nassar ES, Burattini MN, de Souza LTM, Ferreira IB, Iray MR, Travassos da Rosa APA, Vasconcelos PFC, Pinheiro FP, LeDuc JW, Rico-Hesse R, Gonzalez J-P, Jahrling PB, Tesh RB (1994) New arenavirus isolated in Brazil. Lancet 343(8894):391–392

Collins CH, Kennedy DA (eds) (1983) Laboratory-acquired infections, 4th edn. Butterworth-Heinermann, Oxford

Côté M, Misasi J, Ren T, Bruchez A, Lee K, Filone CM, Hensley L, Li Q, Ory D, Chandran K, Cunningham J (2011) Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. Nature 477(7364):344–348. https://doi.org/10.1038/nature10380

Courgnaud V, Van Dooren S, Liegeois F, Pourrut X, Abela B et al (2004) Simian T-cell leukemia virus (STLV) infection in wild primate populations in Cameroon evidence for dual STLV type 1 and type 3 infection in agile mangabeys (*Cercocebus agilis*). J Virol 78(9):4700–4709

Crabtree MB, Kent Crockett RJ, Bird BH, Nichol ST, Erickson BR, Biggerstaff BJ, Horiuchi K, Miller BR (2012) Infection and transmission of Rift Valley fever viruses lacking the NSs and/or NSm genes in mosquitoes: potential role for NSm in mosquito infection. PLoS Negl Trop Dis 6(5):e1639

Dalgard DW et al (1992) Combined simian hemorrhagic fever and Ebola virus infection in cynomolgus monkeys. Lab Anim Sci 42:152–157

Damon IK, Roth CE, Chowdhary V (2006) Discovery of monkeypox in Sudan. N Engl J Med 355: 962–963

Dandawate CN, Desai GB, Achar TR, Banerjee K (1994) Field evaluation of formalin inactivated Kyasanur forest disease virus tissue culture vaccine in three districts of Karnataka state. Indian J Med Res 99:152–158

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science 287:443–449

de la Monte S, Castro F, Bonilla NJ, de Gaskin UA, Hutchins GM (1985) The systemic pathology of Venezuelan equine encephalitis virus infection in humans. Am J Trop Med Hyg 34(1):194–202

Debré P, Gonzalez JP (2013) Vie et mort des épidémies. Odile Jacob, Paris, 280 p

Delannoy CM, Crumlish M, Fontaine MC, Pollock J, Foster G, Dagleish MP, Turnbull JF, Zadoks RN (2013) Human *Streptococcus agalactiae* strains in aquatic mammals and fish. BMC Microbiol 13:41. https://doi.org/10.1186/1471-2180-13-41

Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E et al (2008) Chapare virus, a newly discovered Arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. PLoS Pathog 4(4):e1000047. https://doi.org/10.1371/journal.ppat.1000047

Donoso MO, Schädler R, Niedrig M (2008) A survey on cases of tick-borne encephalitis in European countries. Euro Surveill 13(17):pii 18848

Downs WG, Anderson CR, Spence L, Aitken THG, Greenhall AH (1963) Tacaribe virus, a new agent isolated from Artibeus bats and mosquitoes in Trinidad, West Indies. Am J Trop Med Hyg 12:640–646

Dumpis U, Crook D, Oksi J (1999) Tick-borne encephalitis. Clin Infect Dis 28(4):882–890. https://doi.org/10.1086/515195

Duncan C, Savage K, Williams M, Dickerson B, Kondas AV, Fitzpatrick KA, Guerrero JL, Spraker T, Kersh GJ (2012) Multiple strains of *Coxiella burnetii* are present in the environment of St. Paul Island, Alaska. Transbound Emerg Dis. https://doi.org/10.1111/j.1865-1682.2012.01353.x

Elliott LH, McCormick JB, Johnson KM (1982) Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. J Clin Microbiol 16(4):704–708
Emonet S, Lemasson JJ, Gonzalez JP, de Lamballerie X, Charrel RN (2006) Phylogeny and evolution of old world arenaviruses. Virology 350(2):251–257
Enria DA, Maiztegui JI (1994) Antiviral treatment of Argentine hemorrhagic fever. Antivir Res 23(1):23–31
Esposito JJ, Sammons SA, Frace AM, Osborne JD, Olsen-Rasmussen M, Zhang M, Govil D, Damon IK, Kline R, Laker M, Li Y, Smith GL, Meyer H, Leduc JW, Wohlhueter RM (2006) Genome sequence diversity and clues to the evolution of variola (smallpox) virus. Science 313(5788):807–812
Feldmann H (2010) Are we any closer to combating Ebola infections? Lancet 375(9729): 1850–1852. https://doi.org/10.1016/S0140-6736(10)60597-1
Field HE (2009) Bats and emerging zoonoses: henipaviruses and SARS. Zoonoses Public Health 56(6–7):278–284. https://doi.org/10.1111/j.1863-2378.2008.01218.x
Fisher-Hoch SP (2005) Lessons from nosocomial viral haemorrhagic fever outbreaks. Br Med Bull 73–74:123–137
Formenty P, Muntasir MO, Damon IK, Chowdhary V, Opoca ML, Monimart C (2010) Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005. Emerg Infect Dis 16:1539–1545
Foster SO, Brink EW, Hutchins DL, Pifer JM, Lourie B, Moser CR, Cummings EC, Kuteyi OE, Eke RE, Titus JB, Smith EA, Hicks JW, Foege WH (1972) Human monkeypox. Bull World Health Organ 46(5):569–576
Gandsman EJ, Aaslestad HG, Ouimet TC, Rupp WD (1997) Sabia virus incident at Yale University. Am Ind Hyg Assoc J 58(1):51–53
Gardner CL, Burke CW, Tesfay MZ, Glass PJ, Klimstra WB, Ryman KD (2008) Eastern and Venezuelan equine encephalitis viruses differ in their ability to infect dendritic cells and macrophages: impact of altered cell tropism on pathogenesis. J Virol 82(21):10634–10646. https://doi.org/10.1128/JVI.01323-08
Gay FP, Holden M (1933) Isolation of herpes virus from several cases of epidemic encephalitis. Proc Soc Exp Biol Med 30:1051–1053
Gear JH (1977) Haemorrhagic fevers of Africa: an account of two recent outbreaks. J S Afr Vet Assoc 48(1):5–8
Geisbert TW, Jahrling PB (1995) Differentiation of filoviruses by electron microscopy. Virus Res 39(2–3):129–150
Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB et al (2005) Development of a new vaccine for the prevention of Lassa fever. PLoS Med 2(6):e183. https://doi.org/10.1371/journal.pmed.0020183
Géraut C (2006) Dermatoses professionnelles. Traité de pathologie professionnelle et de l'environnement, 16-533-A-10. Encyclopédie Médico-Chirurgicale. Elsevier Masson SAS
Ginsberg HS, Stafford KC (2005) Management of ticks and tick-borne diseases. In: Goodman JL, Dennis DT, Soneshine DE (eds) Tick borne diseases of humans. ASM Press, Washington, DC, pp 65–86
Gonzalez JP, Fair J (2013) From biosecurity to emerging diseases: a relentlessly changing paradigm. Trop Med Sur 1(1). https://doi.org/10.4172/tpms.1000e102
Gonzalez JP, Emonet S, de Lamballerie X, Charrel R (2007) Arenaviruses. Curr Top Microbiol Immunol 315:253–288
Gonzalez JP, Ar Gouilh M, Reynes JM, Leroy E (2008) Bat borne viral zoonoses emergence part I synthetic analyses, Chapter 6. In: Carol JCP, Kleinau E (eds) People, health and forests. Earthscan, London
Gonzalez JP, Lambert G, Legand A, Debré P (2011) Toward a transdisciplinary understanding and a global control of emerging infectious diseases. J Infect Dev Ctries 5(12):903–905
Gonzalez JP, Prugnolle F, Leroy E (2013) Men, primates, and germs: an ongoing affair. Curr Top Microbiol Immunol 365:337–353. PMID: 23239237

Grard G, Fair JN, Lee D, Slikas E, Steffen I et al (2012) A novel Rhabdovirus associated with acute hemorrhagic fever in Central Africa. PLoS Pathog 8(9):e1002924. https://doi.org/10.1371/journal.ppat.1002924

Grene D (1989) The history of the Peloponnesian War of Thucydides [trans]. University of Chicago Press, Chicago, pp 115–118

Grobbelaar AA, Weyer J, Leman PA, Kemp A, Paweska JT, Swanepoel R (2011) Molecular epidemiology of Rift valley fever virus. Emerg Infect Dis [serial on the Internet]. https://doi.org/10.3201/eid1712.111035

Grolla A, Lucht A, Dick D, Strong JE. Feldmann H (2005). Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. Bull Soc Pathol Exot 98(3):205–209

Günther S, Emmerich P, Laue T, Kühle O, Asper M, Jung A, Grewing T, ter Meulen J, Schmitz H (2000) Imported Lassa fever in Germany: molecular characterization of a new Lassa virus strain. Emerg Infect Dis 6(5):466–476. PMCID: PMC2627947

Hahn BH, Shaw GM, Sharp PM, De Cock KM (2000) AIDS as a zoonosis: scientific and public health implications. Science 287:607–614

Halfmann P, Kim JH, Ebihara H, Noda T, Neumann G, Feldmann H, Kawaoka Y (2008) Generation of biologically contained Ebola viruses. Proc Natl Acad Sci U S A 105(4):1129–1133

Halfmann P, Ebihara H, Marzi A, Hatta Y, Watanabe S, Suresh M, Neumann G, Feldmann H, Kawaoka Y (2009) Replication-deficient ebolavirus as a vaccine candidate. J Virol 83(8):3810–3815

Halpin K, Young PL, Field HE, Mackenzie JS (2000) Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J Gen Virol 81:1927–1932

Harcourt BH, Sanchez A, Offermann MK (1999) Ebola virus selectively inhibits responses to interferons, but not to interleukin-1beta, in endothelial cells. J Virol 73(4):3491–3496

Hayes M, Salvato M (2012) Arenavirus evasion of host anti-viral responses. Viruses 4(10):2182–2196

Heinz FX (2008) Tick-borne encephalitis: rounding out the picture. Euro Surveill 13(17):18844

Heinz FX, Holzmann H, Essl A, Kundi M (2007) Field effectiveness of vaccination against tick-borne encephalitis. Vaccine 25:7559–7567

Hemmer CJ, Littmann M, Löbermann M, Lafrenz M, Böttcher T, Reisinger EC (2005) Tickborne meningoencephalitis, first case after 19 years in northeastern Germany. Emerg Infect Dis [serial on the Internet]. https://doi.org/10.3201/eid1104.041110

Hewlett BS, Amolat RP (2003) Cultural contexts of Ebola in northern Uganda. Emerg Infect Dis 9(10):1242–1248

Heymann DL (2008) Control of communicable diseases manual, 19th edn. American Public Health Association, Washington, DC

Hirabayashi Y, Oka S, Goto H, Shimada K, Kurata T, Fisher-Hoch SP, McCormick JB (1988) An imported case of Lassa fever with late appearance of polyserositis. J Infect Dis 158(4):872–875

Holmes GP, McCormick JB, Trock SC, Chase RA, Lewis SM, Mason CA, Hall PA, Brammer LS, Perez-Oronoz GI, McDonnell MK et al (1990) Lassa fever in the United States. Investigation of a case and new guidelines for management. N Engl J Med 323(16):1120–1123

Hughes AL, Irausquin S, Friedman R (2010) The evolutionary biology of poxviruses. Infect Genet Evol 10(1):50–59

Hutin YJ, Williams RJ, Malfait P, Pebody R, Loparev VN, Ropp SL, Rodriguez M, Knight JC, Tshioko FK, Khan AS, Szczeniowski MV, Esposito JJ (2001) Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. Emerg Infect Dis 7(3):434–438

Jaax N et al (1995) Transmission of Ebola virus (Zaire strain) to uninfected control monkeys in a biocontainment laboratory. Lancet 346(8991):1669–1671

Johnson KM, Kuns ML, Mackenzie RB, Webb PA, Yunker CE (1966) Isolation of Machupo virus from wild rodent *Calomys callosus*. Am J Trop Med Hyg 15:103–106

Johnson E, Jaax N, White J, Jahrling P (1995) Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. Int J Exp Path 76(4):227–236

Jones KE, Patel N, Levy M et al (2008) Global trends in emerging infectious diseases. Nature 451: 990–994

Kelland K (2013) Deadly new virus well-adapted to infect humans. Medscape Medical News. http://www.medscape.com/viewarticle/779538

Kerbo N, Donchenko I, Kutsar K, Vasilenko V (2005) Tickborne encephalitis outbreak in Estonia linked to raw goat milk, May–June 2005. Euro Surveill 10(6):E050623–E050622

Kharitonova NN, Leonov YA (1985) Omsk Hemorrhagic Fever (trans.: Korzelska S). Amerind Publishing Co, New Delhi

Kiley MP, Bowen ET, Eddy GA, Isaäcson M, Johnson KM, McCormick JB, Murphy FA, Pattyn SR et al (1982) Filoviridae: a taxonomic home for Marburg and Ebola viruses? Intervirology 18(1–2):24–32

Kilgore PE, Ksiazek TG, Rollin PE, Mills JN, Villagra MR, Montenegro MJ, Costales MA, Paredes LC, Peters CJ (1997) Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. Clin Infect Dis 24(4):718–722

Kindler E, Jónsdóttir HR, Muth D, Hamming OJ et al (2013) Efficient replication of the novel human Betacoronavirus EMC on primary human epithelium highlights its zoonotic potential. MBio 4(1):e00611-12

Kitching A, Addiman S, Cathcart S, Bishop L, Krahé D, Nicholas M et al (2009) A fatal case of Lassa fever in London, January 2009. Euro Surveill 14(6):pii 19117. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19117

Kriz B, Benes C, Danielova V, Daniel M (2004) Socio-economic conditions and other anthropogenic factors influencing tick-borne encephalitis incidence in The Czech Republic. Int J Med Microbiol 293(Suppl 37):63–68

Kuhn JH, Becker S, Ebihara H et al (2010) Proposal for a revised taxonomy of the family *Filoviridae*: classification, names of taxa and viruses, and virus abbreviations. Arch Virol 155(12):2083–2103

Kurata T, Hondo R, Sato S, Oda A, Aoyama Y, McCormick JB (1983) Detection of viral antigens in formalin-fixed specimens by enzyme treatment. Ann N Y Acad Sci 420:192–207

Ladnyj ID, Ziegler P, Kima E (1972) A human infection caused by monkeypox virus in Basankusu territory, Democratic Republic of the Congo. Bull World Health Organ 46(5):593–597

Larson RA, Dai D, Hosack VT, Tan Y, Bolken TC, Hruby DE, Amberg SM (2008) Identification of a broad-spectrum arenavirus entry inhibitor. J Virol 82(21):10768–10775

Lee HW (1989) Hemorrhagic fever with renal syndrome in Korea. Rev Infect Dis 11(Suppl 4): S864–S876

Lee HW, Lee PW, Johnson KM (1978) Isolation of the etiologic agent of Korean hemorrhagic fever. J Infect Dis 137(3):298–308. PMID 24670

Leffel ER, Reed DS (2004) Marburg and Ebola viruses as aerosol threats. Biosecur Bioterror 2(3): 186–191. https://doi.org/10.1089/bsp.2004.2.186

Lelie PN, Reesink HW, Lucas CJ (1987) Inactivation of 12 viruses by heating steps applied during manufacture of a hepatitis B vaccine. J Med Virol 23(3):297–301

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Délicat A, Paweska JT et al (2005) Fruit bats as reservoirs of Ebola virus. Nature 438(7068):575–576. https://doi.org/10.1038/438575a

Leschnik MW, Kirtz GC, Thalhammer JG (2002) Tick-borne encephalitis (TBE) in dogs. Int J Med Microbiol 6(Suppl 33):66–69

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310(5748):676–679

Likos AM, Sammons SA, Olson VA, France MA, Li Y, Olsen-Rasmussen MA (2005) A tale of two clades: monkeypox viruses. J Gen Virol 86:2661–2672

Lipkin WI, Briese T (2007) Bornaviridae. In: Knipe DM, Howley PM (eds) Fields virology, 5th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1829–1851

Lisieux T, Coimbra M, Nassar ES, Burattini MN, de Souza LT, Ferreira I, Rocco IM, da Rosa AP, Vasconcelos PF, Pinheiro FP et al (1994) New arenavirus isolated in Brazil. Lancet 343(8894): 391–392

Lu Z, Broker M, Liang G (2008) Tick-borne encephalitis in mainland China. Vector Borne Zoonotic Dis 8(5):713–720
Lukashevich IS, Patterson J (2008) Safety, immunogenicity, and efficacy of the ML29 vaccine for Lassa fever in small non-human primates. Vaccine 26:5246–5254
Mahanty S, Kalwar R, Rollin PE (1999) Cytokine measurement in biological samples after physicochemical treatment for inactivation of biosafety level 4 viral agents. J Med Virol 59(3):341–345
Mahieux R, Gessain A (2011) HTLV-3/STLV-3 and HTLV-4 viruses: discovery, epidemiology, serology and molecular aspects. Viruses 3(7):1074–1090. https://doi.org/10.3390/v3071074
Maillard JC, Gonzalez JP (2006) Biodiversity and emerging diseases. Ann N Y Acad Sci 1081:1–16
Maiztegui JI (1975) Clinical and epidemiological patterns of Argentine haemorrhagic fever. Bull World Health Organ 52(657):675
Maiztegui JI, Fernández NJ, Damilano AJ (1979) Efficacy of immune plasma in treatment of Argentine Hemorrhagic Fever and association between treatment and a late neurological syndrome. Lancet 2(8154):1216–1217
Maiztegui JI, McKee KT Jr, Barrera Oro JG, Harrison LH, Gibbs PH, Feuillade MR, Enria DA, Briggiler AM, Levis SC, Ambrosio AM, Halsey NA, Peters CJ (1998) Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. J Infect Dis 177(2):277–283
Marrennikova SS, Shelukhina EM, Maltseva NN, Cimiskjan KL, Macevic GR (1972) Isolation and properties of the causal agent of a new varioloa-like disease (monkeypox) in man. Bull World Health Organ 46(5):599–611
Marshall ID, Woodroofe GM, Hirsch S (1982) Viruses recovered from mosquitoes and wildlife serum collected in the Murray Valley of south-eastern Australia, February 1974, during an epidemic of encephalitis. Aust J Exp Biol Med Sci 60:457–470
Martien AM, Groenen A, Archibald L, Uenishi H et al (2012) Analyses of pig genomes provide insight into porcine demography and evolution. Nature 491(7424):393–398
McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM (1986) Lassa fever. N Engl J Med 314(1):20–26
Michaels MG, Jenkins FJ, St George K, Nalesnik MA, Starzl TE et al (2001) Detection of infectious baboon cytomegalovirus after baboon-to-human liver xenotransplantation. J Virol 75(6): 2825–2828
Miller EH, Obernosterer G, Raaben M, Herbert AS, Deffieu MS, Krishnan A, Ndungo E, Sandesara RG, Carette JE, Kuehne AI, Ruthel G, Pfeffer SR, Dye JM, Whelan SP, Brummelkamp TR, Chandran K (2012) Ebola virus entry requires the host-programmed recognition of an intracellular receptor. EMBO J 31(8):1947–1960. https://doi.org/10.1038/emboj.2012.53
Mitchell SW, McCormick JB (1984) Physicochemical inactivation of Lassa, Ebola, and Marburg viruses and effect on clinical laboratory analyses. J Clin Microbiol 20(3):486–489
Monath T, Cetron MS, Teuwen DE (2008) Yellow fever vaccine. In: Plotkin SA, Orenstein WA, Offit PA (eds) Vaccines, 5th edn. Saunders Elsevier, Philadelphia, pp 959–1055
Morrill JC, Mebus CA, Peters CJ (1997) Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. Am J Vet Res 58(10):1104–1109
Morse SS, Mazet JA, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, Daszak P (2012) Prediction and prevention of the next pandemic zoonosis. Lancet 380(9857):1956–1965
Morvan J, Deubel V, Gounon P, Nakouné E, Barrière P, Murri S, Perpète O, Selekon B et al (1999) Identification of Ebola virus sequences present as RNA or DNA in organs of terrestrial small mammals of the Central African Republic. Microbes Infect 1(14):1193–1201. https://doi.org/10.1016/S1286-4579(99)00242-7
Mwanatambwe M, Yamada N, Arai S, Shimizu-Suganuma M, Shichinohe K, Asano G (2001) Ebola hemorrhagic fever (EHF): mechanism of transmission and pathogenicity. J Nippon Med Sch 68(5):370–375
Nakazawa Y, Emerson GL, Carroll DS, Zhao H, Li Y, Reynolds MG, Karem KL, Olson VA, Lash RR, Davidson WB, Smith SK, Levine RS, Regnery RL, Sammons SA, Frace MA, Mutasim EM, Karsani ME, Muntasir MO, Babiker AA, Opoka L, Chowdhary V, Damon IK (2013)

Phylogenetic and ecologic perspectives of a monkeypox outbreak, Southern Sudan, 2005. Emerg Infect Dis 19(2):237–245
Nalca A, Rimoin AW, Bavari S, Whitehouse CA (2005) Reemergence of monkeypox: prevalence, diagnostics, and countermeasures. Clin Infect Dis 41(12):1765–1771
Negredo A, Palacios G, Vázquez-Morón S, González F, Dopazo H et al (2011) Discovery of an Ebolavirus-like Filovirus in Europe. PLoS Pathog 7(10):e1002304. https://doi.org/10.1371/journal.ppat.1002304
Nelson CB, Pomeroy BS, Schrall K, Park WE, Lindeman RJ (1952) An outbreak of conjunctivitis due to Newcastle disease virus (NDV) occurring in poultry workers. Am J Public Health 42(6): 672–678
Palmer S, Torgerson SP, Brown DWG (2001) Biology, clinical practice, and public health control. Oxford textbook of Zoonoses. Oxford University Press, Oxford, p 994
Palmer S, Torgerson SP, Brown DWG (2011) Oxford textbook of zoonoses: biology, clinical practice, and public health control. 992 p. ISBN-10:0198570023
Parker S, Nuara A, Buller RML, Schultz DA (2007) Human monkeypox: an emerging zoonotic disease. Future Microbiol 2(1):17–34
Paweska J et al (2009) Nosocomial outbreak of novel arenavirus infection: Southern Africa. Emerg Infect Dis 15(10):1598–1602. https://doi.org/10.3201/eid1510.090211. PMC 2866397. PMID 19861052
Paweska JT, van Jansen VP, Masumu J, Leman PA, Grobbelaar AA et al (2012) Virological and serological findings in *Rousettus aegyptiacus* experimentally inoculated with vero cells-adapted hogan strain of Marburg Virus. PLoS One 7(9):e45479. https://doi.org/10.1371/journal.pone.0045479
Peiris JSM et al (2003) Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361(9366):1319–1325
Petersen LR, Gubler DJ (2003) Infection: viruses: alphaviruses. In: Warrel DA, Cox TM, Firth JD, Benz EJ (eds) Oxford text book of medicine, 4th edn. Oxford University, Oxford, pp 377–379
Peterson AT, Bauer JT, Mills JN (2004) Ecologic and geographic distribution of Filovirus disease. Emerg Infect Dis 10(1):40–47. https://doi.org/10.3201/eid1001.030125
Pfeffer M, Dobler G (2010) Emergence of zoonotic arboviruses by animal trade and migration. Parasit Vectors 3(1):35. https://doi.org/10.1186/1756-3305-3-35
Polop F, Provensal C, Scavuzzo M, Lamfri M, Caldero'n G, Polop J (2008) On the relationship between the environmental history and the epidemiological situation of Argentine hemorrhagic fever. Ecol Res 23:217–225. https://doi.org/10.1007/s11284-007-0371-2
Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Délicat A, Yaba P, Nkoghe D, Gonzalez JP et al (2005) The natural history of Ebola virus in Africa. Microbes Infect 7(7–8):1005–1014. https://doi.org/10.1016/j.micinf.2005.04.006
Pourrut X, Délicat A, Rollin PE, Ksiazek TG, Gonzalez J-P, Leroy EM (2007) Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. J Infect Dis 196(Suppl 2):S176–S183. https://doi.org/10.1086/520541
Pringle CR (2005) Order mononegavirales. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus taxonomy-eighth report of the international committee on taxonomy of viruses. Elsevier, San Diego, pp 609–614
Rabinovich R, Gaudzinski-Windheuser S, Goren-Inbar N (2008) Systematic butchering of fallow deer (Dama) at the early middle pleistocene acheulian site of Gesher Benot Ya'aqov (Israel). J Human Evol 54:134–149
Ramelow C, Süss J, Berndt D, Roggendorf M, Schreier E (1993) Detection of tick-borne encephalitis virus. RNA in ticks (*Ixodes ricinus*) by the polymerase chain reaction. J Virol Methods 45: 115–119. https://doi.org/10.1016/0166-0934(93)90145-H
Randolph SE (2009) Tick-borne disease systems emerge from the shadows: the beauty lies in molecular detail, the message in epidemiology. Parasitology 136:1403–1413
Randolph SE (2010) To what extent has climate change contributed to the recent epidemiology of tick-borne diseases? Vet Parasitol 167:92–94

Reed KD, Melski JW, Graham MB, Regnery RL, Sotir MJ, Wegner MV (2004) The initial detection of human monkeypox in the western hemisphere: association with infected prairie dogs. N Engl J Med 350(4):342–350

Rendi-Wagner P (2004) Risk and prevention of tick-borne encephalitis in travelers. J Travel Med 11(5):307–312

Reynolds MG, Yorita KL, Kuehnert MJ, Davidson WB, Huhn GD, Holman RC, Damon IK (2006) Clinical manifestations of human monkeypox influenced by route of infection. J Infect Dis 194(6):773–780

Richmond JY, McKinney RW (eds) (1999) Biosafety in microbiological and biomedical laboratories, 4th edn. ISBN:0-7881-8513–6

Roddy P, Thomas SL, Jeffs B, Nascimento Folo P, Pablo Palma P, Moco Henrique B, Villa L, Damiao Machado FP et al (2010) Factors associated with Marburg hemorrhagic fever: analysis of patient data from Uige, Angola. J Infect Dis 201(12):1909–1918. https://doi.org/10.1086/652748

Roels TH et al (1999) Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: risk factors for patients without a reported exposure. J Infect Dis 179(Suppl 1):S92–S97

Salas R, de Manzione N, Tesh RB, Rico-Hesse R, Shope RE, Betancourt A et al (1991) Venezuelan haemorrhagic fever. Lancet 338:1033–1036. https://doi.org/10.1016/0140-6736(91)91899-6

Salazar-Bravo J, Ruedas LA, Yates T (2002) Mammalian reservoirs of arenaviruses. Curr Top Microbiol Immunol 262:25–63

Sanchez A, Kiley MP, Klenk HD, Feldmann H (1992) Sequence analysis of the Marburg virus nucleoprotein gene: comparison to Ebola virus and other non-segmented negative-strand RNA viruses. J Gen Virol 73(Pt 2):347–357

Scholtissek C (1995) Molecular evolution of influenza viruses. Virus Genes 11(2–3):209–215

Sidwell RW, Gebhardt LP, Thorpe BD (1967) Epidemiological aspects of Venezuelan equine encephalitis virus infections. Bacteriol Rev 31(1):65–81

Siegert R (1972) Marburg Virus. In: Virology monograph. Springer, New York, pp 98–153

Siembieda J, Johnson CK, Boyce W et al (2008) Risk for avian influenza virus exposure at human-wildlife interface. Emerg Infect Dis 14(7):1151–1153

Sims AC, Burkett SE, Yount B, Pickles RJ (2008) SARS-CoV replication and pathogenesis in an in vitro model of the human conducting airway epithelium. Virus Res 133(1):33–44

Smith RD (2006) Responding to global infectious disease outbreaks, lessons from SARS on the role of risk perception, communication and management. Soc Sci Med 63(12):3113–3123

Smith DH, Johnson BK, Isaacson M, Swanapoel R, Johnson KM, Killey M, Bagshawe A, Siongok T et al (1982) Marburg-virus disease in Kenya. Lancet 1(8276):816–820

Smith KM, Anthony SJ, Switzer WM, Epstein JH, Seimon T et al (2012) Zoonotic viruses associated with illegally imported wildlife products. PLoS One 7(1):e29505. https://doi.org/10.1371/journal.pone.0029505

Snyder JE, Jose J, Kuhn RJ (2009) A structural and functional perspective of alphavirus replication and assembly. Future Microbiol 4(7):837–856

Souris M, Gonzalez JP, Shanmugasundaram J, Corvest V, Kittayapong P (2010) Retrospective space-time analysis of H5N1 avian influenza emergence in Thailand. Int J Health Geogr 9:3

Suarez DL, Schultz-Cherry S (2000) Immunology of avian influenza virus: a review. Dev Comp Immunol 24(2–3):269–283

Sumilo D, Asokliene L, Bormane A, Vasilenko V, Golovljova I, Randolph SE (2007) Climate change cannot explain the upsurge of tick-borne encephalitis in the Baltics. PLoS One 2: e500

Suss J (2008) Tick-borne encephalitis in Europe and beyond-the epidemiological situation as of 2007. Euro Surveill 13(26):18916

Suss J (2011) Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asian overview. Ticks Tick Borne Dis 2:2–15

Suzuki Y, Nei M (2002) Origin and evolution of influenza virus hemagglutinin genes. Mol Biol Evol 19(4):501–509

Swanepoel R (1996) Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis 2(4):321–325. https://doi.org/10.3201/eid0204.960407

Swanepoel R, Smit SB, Rollin PE, Formenty P, Leman PA, Kemp A, Burt FJ, Grobbelaar AA, Croft J, Bausch DG, Zeller H, Leirs H, Braack LE, Libande ML, Zaki S, Nichol ST, Ksiazek TG, Paweska JT, International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of, Congo (2007) Studies of reservoir hosts for Marburg virus. Emerg Infect Dis 13(12):1847–1851

Switzer WM, Heneine W (2010) Foamy virus infection of humans. In: Liu D (ed) Molecular detection of viral pathogens. CRC Press, Boca Raton, pp 131–143

Taylor LH, Latham SM, Woolhouse ME (2001) Risk factors for human disease emergence. Philos Trans R Soc Lond Ser B Biol Sci 356:983–989

Taylor DJ, Leach RW, Bruenn J (2010) Filoviruses are ancient and integrated into mammalian genomes. BMC Evol Biol 10:193. https://doi.org/10.1186/1471-2148-10-193

Timen A, Koopmans MP, Vossen AC, van Doornum GJ, Gunther S, van den Berkmortel F, Verduin KM, Dittrich S, Emmerich P, Osterhaus AD, van Dissel JT, Coutinho RA (2009) Response to imported case of Marburg hemorrhagic fever, The Netherland. Emerg Infect Dis 15(8): 1171–1175

Towner JS, Amman BR, Sealy TK, Carroll SAR, Comer JA, Kemp A, Swanepoel R, Paddock CD et al (2009) Isolation of genetically diverse Marburg viruses from Egyptian Fruit Bats (Ron AM Fouchier). PLoS Pathog 5(7). https://doi.org/10.1371/journal.ppat.1000536

U.S. Centers for Disease Control and Prevention (2012) Select agent program. http://www.selectagents.gov/resources/List_of_Select_Agents_and_Toxins_2012-12-4-English.pdf. Accessed 27 May 2013

U.S. Centers for Disease Control and Prevention. Emergency preparedness and response-bioterrorism agents/diseases. http://www.bt.cdc.gov/agent/agentlist-category.asp. Accessed 27 May 2013

Vaisviliene D, Kilciauskiene V, Zygutiene M, Asokliene L, Caplinskas S (2002) TBE in Lithuania: epidemiological aspects and laboratory diagnosis. Int J Med Microbiol 291(Suppl 33):179–181

Walder G, Lkhamsuren E, Shagdar A, Bataa J, Batmunckh T, Orth D, Heinz FX, Danichova A, Khasnatinov MA, Würzner R, Dierich MP (2006) Serological evidence for tick borne encephalitis, borreliosis, and human granulocytic anaplasmosis in Mongolia. Int J Med Microbiol 296: 69–75. https://doi.org/10.1016/j.ijmm.2006.01.031

Weaver SC, Ferro C, Barrera R, Boshell J, Navarro JC (2004) Venezuelan equine encephalitis. Annu Rev Entomol 49:141–174. https://doi.org/10.1146/annurev.ento.49.061802.123422

Webb PA, Justines G, Johnson KM (1975) Infection of wild and laboratory animals with Machupo and Latino viruses. Bull World Health Organ 52(4–6):493–499

Weber DJ, Rutala WA (2001) Risks and prevention of nosocomial transmission of rare zoonotic diseases. Clin Infect Dis 32(3):446–456

White VC (2013) A review of influenza viruses in seals and the implications for public health. US Army Med Dep J 45–50

WHO (1978a) Ebola haemorrhagic fever in Sudan 1976. Bull World Health Organ 56:247–270

WHO (1978b) Ebola haemorrhagic fever in Zaire 1976. Bull World Health Organ 56:271–293

WHO (2012) International travel and health, Chapter 6. http://www.who.int/ith/chapters/ith2012en_chap6.pdf

Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T et al (2008) Origin, spread and demography of the *Mycobacterium tuberculosis* complex. PLoS Pathog 4(9):e1000160. https://doi.org/10.1371/journal.ppat.1000160

Wolfe ND, Switzer WM, Carr JK, Bhullar VB, Shanmugam V, Tamoufe U, Prosser AT, Torimiro JN, Wright A, Mpoudi-Ngole E et al (2004) Naturally acquired simian retrovirus infections in central African hunters. Lancet 363:932–937. https://doi.org/10.1016/S0140-6736(04)15787-5

Work TH, Trapido H (1957) Kyasanur forest disease, a new virus disease in India. Indian J Med Sci 11:341

Work TH, Roderiguez FR, Bhatt PN (1959) Virological epidemiology of the 1958 epidemic of Kyasanur forest disease. Am J Public Health Nations Health 49(7):869–874
York J, Nunberg JH (2006) Role of the stable signal peptide of Junin arenavirus envelope glycoprotein in pH-dependent membrane fusion. J Virol 80(15):7775–7780
Yun NE, Walker DH (2012) Pathogenesis of Lassa fever. Viruses 4:2031–2048
Zacks MA, Paessler S (2010) Encephalitic alphaviruses. Vet Microbiol 140(3–4):281–286. https://doi.org/10.1016/j.vetmic.2009.08.023
Zhong NS, Zheng BJ, Li YM, Poon LL, Xie ZH, Chan KH et al (2003) Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet 362:1353–1358
Zinsser H (1935) Rats, lice and history. Little, Brown & Co, Boston

Part X

Controversial or Non-resolved Issues

Bovine Paratuberculosis and Human Crohn's Disease: Is There a Zoonotic Linkage?

53

Bernhard Hobmaier, Erdmute Neuendorf, and Nikolaus Ackermann

Contents

B. Hobmaier · E. Neuendorf
Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

N. Ackermann (✉)
Dept. of Public Health Microbiology, Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
e-mail: Nikolaus.Ackermann@lgl.bayern.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_43

Abstract

Mycobacterium avium ssp. *paratuberculosis* (MAP) is an acid-fast bacterium, which causes paratuberculosis, an infectious enteric disease of ruminants, also called Johne's disease (JD). Since the publication by Thomas Kennedy Dalziel in the year 1913, MAP has been discussed as probable causative agent of Morbus Crohn (syn. Crohn's disease (CD)), an inflammatory disease of the human intestinal tract. Here, we describe the history, etiology, diagnosis, clinical, and epidemiological aspects of paratuberculosis and CD to elucidate the role of MAP in the pathogenesis of CD. The theory still remains open for controversial discussion and future studies are needed to find a final conclusion. At the moment, however, there is not enough evidence to convincingly demonstrate that MAP is the etiological agent for CD.

Keywords

Mycobacterium avium subsp. *paratuberculosis* · Crohn's disease · Johne's disease · Zoonotic potential

53.1 Introduction

Morbus Crohn (syn. Crohn's disease (CD)) is an inflammatory disease that has the potential to involve any part of the human intestinal tract anywhere from the mouth to the anus. The disease is generally located at the terminal ileum and the proximal colon. A linkage between CD and *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is discussed since the publication by Thomas Kennedy Dalziel in the year 1913 (Dalziel 1989).

53.2 Johne's Disease/Paratuberculosis

Paratuberculosis, also called Johne's disease (JD) is an infectious disease of ruminants caused by MAP. Paratuberculosis can be found worldwide. Only Sweden and some states in Australia are proven to be free of this disease (Spickler 2007). Norway as well has no known cases since 2015 (Whittington et al. 2019).

Primary susceptible species are cattle, sheep, goats, deer including wild ruminant species, and other ruminants like camels (Kennedy and Benedictus 2001; Tiwari et al. 2006). The host range of JD is wide. Infections of nonruminants, like wild rabbits, foxes, stoats (a weasel species), mandrills, or macaques, and even bird species, like dows, gulls, and sparrows, are described (Beard et al. 2001; McClure

et al. 1987; Zwick et al. 2002; Waddell et al. 2016). Calves inoculated with MAP from a free-living rabbit developed typical histological lesions consistent with JD, demonstrating that wild animals other than ruminants may also contribute to the spread of the disease. But the main source of infection for calves is the exposure to feces of infected mature cattle (Beard et al. 2001).

53.3 History

Since the middle of the nineteenth century, both the clinical signs and pathological anatomy of paratuberculosis are known as chronic enteritis with marked transformation of the intestinal mucosa. Johne and Frothingham demonstrated acid-fast bacilli in altered tissues and described for the first time this disease as a singular case of tuberculosis in cattle (Johne and Frothingham 1895).

Due to the different biological properties of the infectious agent and due to the different pathological patterns, Bang proposed in the year 1906 to separate the illness from tuberculosis. Since then, bovine paratuberculosis is classified as a separate disease (Bang 1906).

In 1910/1912, Twort published the isolation and cultivation of the infectious agent for the first time. Finally, in 1933 the experimental infection of cattle with MAP succeeded. By that, the Henle-Koch postulates were fulfilled. Since then, it is proven that MAP is the monocausal agent of bovine paratuberculosis (Twort and Ingram 1912).

53.4 Etiology

53.4.1 Infectious Agent

MAP belongs to the *Mycobacteriaceae*, a family of gram-positive bacteria, which comprises more than 100 species. They proliferate intracellularly and are characterized by their acid-stability and lipid-enriched cell wall. The subspecies MAP is clearly distinct from other pathogens of the family like *Mycobacterium tuberculosis*, the infectious agent of tuberculosis, or *Mycobacterium leprae,* the infectious agent of leprosy. MAP is a mycobacterial subspecies with a slow replication rate and a generation time of over 20 hours. Its cultural growth in vitro is dependent on the addition of mycobactin, an iron chelator, that most other mycobacteria produce on their own and are therefore able to replicate outside of their host (Rowe and Grant 2006; Barclay and Ratledge 1983). MAP is a subspecies member of the *M. avium* complex (MAC), a complex of several phenotypically and serologically extremely similar mycobacteria, comprising of MAP, *Mycobacterium avium* ssp. *avium*, *Mycobacterium avium* ssp. *silvaticum*, *Mycobacterium avium* ssp. *hominissuis*, and *Mycobacterium intracellulare* (Busatto 2019). While members of the MAC typically cause disease in immunocompromised hosts, MAP has been elucidated as causative agent of JD even in immunocompetent ruminants (Behr and Kapur 2008).

53.4.2 Pathogenesis of Johne's Disease

MAP can be transmitted directly, most commonly fecal-orally or orally by colostrum, but infections by the intrauterine route or semen are also reported (Uzoigwe et al. 2007).

The vast majority of animals (ca. 85%) is infected within the first days of their life, 5% during the first year and 10% in utero. Commonly, the infection starts in the first 30 days of life; later on, an increasing age-resistance is acquired. Calves are mainly infected orally by fecally contaminated dugs or by colostrum, while older animals tend to get infected by contaminated food, water or pastures (McAloon et al. 2019; Richardson et al. 2019). Even the possibility of airborne transmission via bioaerosols and dust is being discussed (Eisenberg et al. 2011).

After oral ingestion, MAP is taken up by M cells of the Peyer's patches, mainly in the ileum, or enterocytes and transported into the submucosa (Bermudez Luiz et al. 2010; Momotani et al. 1988). Additionally, MAP weakens the tight junctions and therefore the integrity of the intestinal barrier, leading to paracellular introduction of more MAP into the submucosa (Bannantine John et al. 2013). There the organism is phagocytized by resident macrophages and persists in their phagosomes, thereby emitting interleukin-1β (IL-1 β), which leads to more macrophages being lured to the entry point and more MAP being phagocytized (Lamont et al. 2012a). By inhibiting the formation of an phagolysosome, MAP are able to persist and even proliferate in these macrophages (Lindsay Sweet et al. 2010). Furthermore, MAP decreases pro-inflammatory immune response by suppressing expression of interferon gamma (IFNγ), and tumor necrosis factor (TNF) (Khare et al. 2012).

Histologically, MAP infection can be divided into two major forms, the tuberculoid or paucibacillary form and the lepromatous or pluribacillary form (Clarke 1997).

The tuberculoid form is dominated by a cellular (Th1) immune response and therefore IFNγ-mediated activation of macrophages (Chiodini et al. 1984). The intensity of the IFNγ response is crucial for determining the further course of disease. In an infection experiment, Begg et al. (2018) demonstrated a direct correlation between IFNγ response and severity of histopathological lesions, with even single cases of apparent elimination of MAP and remission of lesions in animals with high IFNγ levels (Begg et al. 2018). Due to the histopathological features of CD, which closely resemble those found in animals with the paucibacillary form of JD, it has been suggested that the two diseases may share the same etiology (Collins et al. 2000; Grant 2003; Greenstein 2003; Moss et al. 1992).

As the infection progresses, a shift from cell-mediated Th1 immunity to a humoral Th2 response can be observed (Stabel 2000; Koets et al. 2015).

The resulting lepromatous form is characterized by the production of large amounts of antibodies, while cell-mediated immunity seems to decrease (Stabel et al. 2011). This humoral response, however, is not protective against the progression of disease and leads to a quick spread of the infectious agent and manifestation of the clinical disease (Manning and Collins 2001). The clinical disease is characterized by a profuse and intractable diarrhea that ends up in severe weight loss and death.

The incubation time is reported to range from 2 to 10 years (Chiodini et al. 1984).

The disease develops chronically and intermittently. Over a very long period, infected animals show no signs of illness. Therefore, JD usually stays undetected for

a long time. However, the spreading of bacteria starts before the onset of clinical symptoms.

Hence, mainly animals in a late subclinical stage play an important role in the propagation of paratuberculosis. Whitlock and Buergelt described that one animal with clinical symptoms represents only the tip of an iceberg. Every cow with symptoms may indicate 15–25 more affected animals in the herd (Whitlock and Buergelt 1996).

Furthermore, the intermittent spread is another cornerstone of the maintenance of MAP in the herd. While one clinically affected cow releases up to ten million infectious particles per gram feces (Whitlock et al. 2005), the excretion dose of a subclinically diseased cow ranges between 10 and 100 infectious agents per gram.

The infection dose is reported to be 10,000 agents for the infection of one calf (Gerlach 2002).

53.4.3 Time Line of Paratuberculosis in Cattle

The course of disease can be divided into four phases of different immunology, clinical signs, and pathomorphology (Whitlock and Buergelt 1996):

1. The silent early stage of infection
2. The subclinical stage
3. The clinical stage
4. The advanced clinical stage

53.4.3.1 The Silent Early Stage of Infection

In this stage, there is no detectable spread of the agent by feces. The only viable way to diagnose an infection in this stage is by histological investigation of tissue samples, as any detection of MAP-DNA or cultivation of viable MAP could also be the product of "passive shedding" after MAP had been introduced orally and are shed in the feces without infecting the animal (Begg et al. 2018).

The animal shows no signs of disease. When examining the tissue, acid-fast bacilli can be seen histologically in intestinal lymph nodes or intestinal sections and MAP can be cultivated (Whitlock and Buergelt 1996).

53.4.3.2 The Subclinical Stage

The intracellular proliferation of the pathogen in macrophages ultimately results in cell death and release of the agent into surrounding tissues and the gut lumen. As soon as the MAP cells are liberated, the humoral immune response is initiated. Acid-fast bacilli can (intermittently) be detected in the feces (Coussens 2001; Sweeney et al. 1992).

The animal still shows no signs of disease. Stages 1 and 2 correspond to the incubation time of the disease and can range between 2 and 10 years (Chiodini et al. 1984).

Pathomorphologically, a moderate hypertrophy of the mucosa and an enlargement of the mesenterial lymph nodes are accompanied by infiltration of epithelioid and Langhans giant cells and foamy macrophages containing phagocytized acid-fast

bacilli. In some cases, diagnosis via a measurable immune response – either humoral or cell mediated – can be possible (Whitlock and Buergelt 1996). Intermittent fecal shedding of MAP in this stage is possible, but due to its sporadic nature and the low prevalence of anti-MAP antibodies in the herd, many infected (and shedding) animals will likely remain undetected and therefore pose the biggest risk factor in transmission of the disease (Begg et al. 2018; McKenna et al. 2006). Additionally, those subclinical animals are responsible for a great part of the considerable economic losses due to Johne's disease in form of long-term decreased milk production and slow growth rates of subclinical infected animals on top of the more obvious losses due to culling of sick animals (Tiwari et al. 2006; Bates et al. 2018; Raizman et al. 2009).

53.4.3.3 The Clinical Stage/Advanced Clinical Stage

Initial clinical signs follow the subclinical stage. The first apparent sign is gradual weight loss. Congruent with the weight loss, the manure consistency becomes more fluid. The clinical symptoms can be seen for months with intermittent times of amelioration. Later, clinically affected animals become increasingly lethargic, weak, and emaciated. "Water-hose" or "pipe-stream" diarrhea, hypoproteinemia, and intermandibular edema (bottle jaw) characterize the advanced stage of the disease (Tiwari et al. 2006).

Morphological changes in JD include chronic inflammation involving all layers of the intestinal wall (transmural involvement), thickening of involved segments, with narrowing of the lumen, linear ulceration of the mucosa, and a submucosal edema with elevation of the surviving mucosa, producing a characteristic cobblestone appearance.

A clinical or advanced clinical stage inevitably leads to the death of the diseased animal (Eslami et al. 2019).

53.5 Therapy and Vaccination

Animals in early stages of the disease sometimes show remission and/or elimination of the infectious agent and the respective histopathological lesions (Begg et al. 2018). It is not possible to cure MAP infected, animals in a clinical state of disease. Antibiotics, although showing growth inhibition in vitro, do not lead to permanent treatment success in animals. A short-term alleviation of clinical symptoms by use of various antibiotics or antihistamines has been described in the literature but eventually all clinical cases and with the death of the diseased animal (Eslami et al. 2019). The MAP excretion, however, could not be prevented. Therefore, treating clinically ill animals not only leads to unnecessary suffering but also poses a severe threat to the rest of the herd, if strict isolation cannot be guaranteed.

In Germany, no vaccine is currently approved. However, in principle vaccinations are possible. The first vaccine was developed in 1926. Many authors describe the use of vaccines in terms of advantages and disadvantages.

The excretion of the pathogen and thus the spread of infection can be reduced by vaccination. Available vaccines, based on whole, killed, or live-attenuated bacteria, however, will not protect against infection and disease, and the use of serological methods for animal disease control in vaccinated herds is no longer suitable. Furthermore, interference with the tuberculin skin test used for the control of bovine tuberculosis is described (Bastida and Juste 2011; Juste 2012; Patton 2011; Rosseels and Huygen 2008). As this interference can lead to false-positive results in the tuberculosis skin tests, vaccination against paratuberculosis is prohibited in many countries (OIE 2021).

53.6 MAP in Milk and Dairy Products

53.6.1 Tenacity and Ability to Survive

Mycobacteria are characterized by having a thick, waxy cell wall that grants them remarkable resistance to environmental influences, disinfectants, and even antimicrobial chemotherapy (Chiaradia et al. 2017). This multilayered cell wall contains large amounts of long chained fatty acids (mycolic acids), responsible for its hydrophobic nature as well as glycopeptidolipids, which function as pathogenic factors for members of the *Mycobacterium avium* complex (Busatto 2019; Chiaradia et al. 2017). This unique tenacity leads to an eminent survivability of MAP in the environment. MAP have been described to survive long time periods in manure, on contaminated pastures, and, after being flushed away by rain, in rivers or other bodies of water (Richardson et al. 2019; Pickup et al. 2005; Pickup et al. 2006; Whittington et al. 2005). This survivability in water and resistance to established measures of drinking water decontamination leads to the possibility of MAP reaching the domestic water supply (Rhodes et al. 2014; Sousa et al. 2021).

53.6.2 MAP in Milk

One of the most discussed routes of human exposure to viable MAP is via dairy products from raw or even pasteurized milk (Todd Kuenstner et al. 2017). In principle, there are two possibilities for MAP to get into raw milk: either by direct shedding over the mammary gland of infected animals or indirectly through fecal contamination (Gerrard et al. 2018; Michael and Mullan 2019). In terms of numbers, however, the quantitative relation of fecal contamination to direct shedding is estimated to be 90–10% (Rani et al. 2019).

As growth of MAP is dependent on the presence of mycobactin as a siderophore for iron uptake, it is unlikely that MAP will multiply in milk. This, however, does not rule out milk as a possible means of transmission. In fact, MAP have been shown to change the lipid profile of their cell walls and express more proteins connected to cell invasion after being incubated in milk (Alonso-Hearn et al. 2009). Moreover,

significant changes in the MAP proteome after incubation in milk were described, including upregulation of proteins associated with stress response and immune evasion (Kleinwort et al. 2021). This can be interpreted as MAP sensing milk as a hostile environment and inducing respective counteractions (Kleinwort et al. 2021). Whether these counteractions, however, lead to an increased survivability of MAP in milk needs to be further elucidated.

Pasteurization of milk is generally considered a reliable method to protect consumers from bacterial pathogens in milk (Juffs and Deeth 2007). In the dairy industry, different heating methods are used. According to the international dairy foods association, the most important one is the high-temperature, short-time (HTST) pasteurized milk process (72–75 °C, 15–30 s), as dairy products are mainly produced on the bases of HTST milk (International Dairy Foods Association n.d.).

Despite this measure, there have been several reports of MAP in pasteurized milk products, leading to the discussion if MAP could be able to survive pasteurization (Gerrard et al. 2018; Chiodini and Hermon-Taylor 1993; Grant et al. 1999; Paolicchi et al. 2012).

To investigate the thermal resistance of MAP in milk, numerous experimental investigations were performed. After the UHT process, no surviving MAP were found in milk (Büttner et al. 2006).

In HTST-treated milk, experimentally infected with 10^2–10^3 colony-forming units (CFU)/ml of milk, viable MAP could be detected. However, there was a reduction of microbial count to five orders of magnitude. This corresponds to a reduction of 99.999%. After experimental contamination of milk with lower bacterial counts (10 CFU/ml), no viable pathogen could be detected (Büttner et al. 2006).

For interpretation of these data, it is important to consider which methodology of thermal inactivation was applied. In terms: was the experiment conducted with commercial-type pasteurizers with continuous turbulent flow for heat distribution comparable to the ones used in the dairy industry? This has been neglected in many studies and could be the cause of conflicting results (Grant et al. 1996; Meylan et al. 1996; Robertson et al. 2012). Some studies, which were conducted under industrial standards, describe an inactivation of MAP (Lynch et al. 2007; Rademaker Jan et al. 2007). Other studies, however, describe a survival of MAP under these circumstances (Grant Irene et al. 2002).

Several mechanisms for this survivability have been discussed, including clumping of MAP cells, inhomogeneous distribution, or intracellular localization in milk as well as the formation of heat resistant, spore-like forms (Gerrard et al. 2018; Lamont et al. 2012b; Grant Irene et al. 2005). Additionally, other factors like post-pasteurization contamination of the milk or malfunction of technical facilities could be reasons for the presence of viable MAP in retail milk and dairy products (Robertson et al. 2017). Independently of MAP being able to survive pasteurization or not, the repeated detection of viable MAP in pasteurized milk, even if in small numbers, leads to the possibility of human consumption of viable MAP (Michael and Mullan 2019).

53.6.3 MAP in Other Milk Products

Only limited data are available on the occurrence of MAP in raw milk cheeses: experimental production of cheese from artificially contaminated raw milk showed that MAP are also greatly reduced during the ripening process (Donaghy et al. 2004; Spahr and Schafroth 2001; Sung and Collins 2000). Besides from cheese, viable MAP have also been cultivated from calf's milk replacers and powdered infant formula (Botsaris et al. 2016; Ikonomopoulos et al. 2005; Khol et al. 2017).

53.6.4 MAP in Retail Milk

To address the occurrence of MAP in HTST-treated milk from the market field, studies in the USA (Ellingson et al. 2005), Great Britain (GB) (Grant 2003), and Ireland (O'Reilly Ciara et al. 2004) were conducted. MAP in low numbers and frequency have been detected in the USA and UK: In the UK, 67 out of 567 tested packages (11.6%) contained MAP-DNA, whereas viable MAP could only be cultivated from ten samples (1.7%). In the USA, 2.8% out of 702 examined packs were positive in MAP culture. In an Irish study, which was published in 2004, no viable MAP were cultured in any of the 357 examined milk cartons. However, MAP-DNA was detected in 35 (9.8%) of respective samples. In the Irish study, 56% of the investigated manufacturing firms treated the milk at least at 75 °C for 25 s.

53.7 Detection Methods for MAP in Food and Environment

For microbiological diagnosis of MAP, direct and indirect methods are distinguished. While the first detect the agent or parts of the agent itself, the latter are based on specific immune responses that occur after contact with the pathogen (exposure, infection, immunization).

53.7.1 Indirect Detection of MAP in Serum or Milk

Probably the most efficient way to ensure MAP-free foods is by removing MAP-shedding animals from the production chain. For the diagnosis of MAP in animals, the detection of MAP-specific antibodies by ELISA or similar methods is a popular method due to its fast turnaround time, low price, and ease of use (Slana et al. 2008). This method can be employed with either serum or milk samples. A detection of MAP-specific antibodies in cattle is an indicator for more or less recent contact to the infectious agent. It is, however, not possible to conclude active shedding of MAP from serological positivity. The most eminent problem with this detection method is that the humoral immune response against MAP is intermittent; therefore, bacterial shedding and presence of detectable antibodies do not necessarily correlate (Begg et al. 2018).

The test performance of antibody detection tests varies, depending on the used antigens. Most tests use MAP whole-cell extract, lipoarabinomannan, or MAP surface proteins (Karuppusamy et al. 2019).

In Germany, several different commercial ELISA assays are licensed for the detection of paratuberculosis-specific antibodies. The sensitivity of these methods, however, is also very limited, with none of the tests reaching a sensitivity above 60% (Friedrich Löffler Institute Germany 2021).

As mentioned above, the early stages of bovine paratuberculosis are characterized predominantly by a cell-mediated immune response. Therefore, antibody detection will most likely render those animals as false negative, which is why detection methods for cell-mediated immunity are more advised.

There are two tests for detection of a cell-mediated immunity: the gamma interferon release assay for blood samples and the skin test for delayed-type hypersensitivity. Cell-mediated immunity is typically the predominant form of immune response in early stages of infection and decreases over the course of ongoing disease development. In clinical cases, it may not be detectable at all (OIE 2021). In addition to being challenging in their interpretation due to lacking established cutoff values, these tests do struggle with specificity. MAP and other members of the *Mycobacterium avium* complex share an extraordinary similarity and are often times not distinguishable by phenotype or serological measures (Busatto 2019). Therefore, many animals may be sensitized to environmental mycobacteria or members of the *Mycobacterium avium* complex and therefore yield ambiguous results due to cross-reactivity.

According to the OIE manual both, the gamma interferon release assay and the skin test, have limited value in the field at present, and further research is needed with respect to the interpretation criteria (OIE 2021).

Because of the limited sensitivity, antibody tests are useful to determine the health status in a herd but are not an adequate tool for determining the disease in individual animals, unless typical clinical signs are observable.

53.7.2 Direct Detection of MAP

The gold standard for the diagnosis of paratuberculosis is the cultural detection of the pathogen (OIE 2021). It is considered as 100% specific, but regarding its sensitivity, it has to deal with several difficulties:

MAP is an extremely slow-growing organism and very difficult to cultivate. Not only is it dependent on the addition of an iron chelator, like mycobactin, but also on chemical decontamination of the examined material (i.e., with hexadecylpyridinium chloride), in order to prevent overgrowth of MAP cultures with nonspecific bacterial flora (OIE 2021; FLI 2020). This decontamination step markedly decreases the sensitivity of culture methods (Gao et al. 2005).

For the detection of MAP in food, however, the long incubation period is the most pivotal factor, as detection can require an incubation period of up to 18 weeks in primary culture (Whan et al. 2005; Cocito et al. 1994). At this time, examined food has either already been consumed or been taken out of stock, regardless of the culture

results. A positive result, however, is proof for the presence of living reproductive MAP in the animal or the food product.

In case of massive shedding, MAP can be detected directly in the feces by light microscopy after Ziehl-Neelsen staining, if large numbers of MAP cells with intact cell walls are present in the sample. MAP isolated from humans, however, can show a cell-wall-deficient phenotype that would not be detectable by Ziehl-Neelsen staining (Sechi et al. 2005).

Another, more sensitive, method for the direct detection of MAP is PCR (Michael and Mullan 2019). This method detects MAP by amplification of MAP-specific DNA sequences. At this time, there are six licensed real-time PCR assays for the detection of MAP in Germany (https://www.fli.de/de/institute/institut-fuer-molekulare-pathogenese-imp/referenzlabore/nrl-fuer-paratuberkulose/ (last accessed November 2021)). Probably the most popular of those target sequences is the insertion sequence 900 (IS*900*). It is highly abundant in the MAP genome and is therefore the most sensitive target for MAP-specific PCRs (Möbius et al. 2008). It has, however, been shown that IS*900* can also occur in other mycobacteria, limiting its usefulness in regard to specificity (Englund et al. 2002). Alternative target sequences to IS*900* are f57, ISMav2, and ISMap02. F57 is a single copy target, which offers far more specificity but, due to its single copy nature, a lower sensitivity than IS*900* (Möbius et al. 2008; Poupart et al. 1993). Its high sensitivity makes PCR a useful tool in the detection of infected, MAP-shedding animals. However, its sensitivity largely depends on the quality of the extracted DNA and therefore on the extraction method (Park et al. 2014). Moreover, for the detection of MAP in food, the PCR method has to cope with some additional limitations: In milk, for example, PCR is often times inhibited by high concentrations of calcium ions or fat, leading to diminished sensitivities. Additionally, PCR is not able to differentiate between viable, infectious MAP cells, and DNA originating from dead MAP cells that have been killed, i.e., during pasteurization (Slana et al. 2008).

This limitation can be circumvented by detection methods using bacteriophages. Phage-based methods for the detection of MAP all rely on the D29 bacteriophage (Grant 2021). This phage infects viable bacterial cells of several mycobacteria, is multiplied there, and causes the cell to burst, which again leads to the release of progeny phage generations. The final detection step then mostly consists on the detection of the released host DNA in combination with detection of the progeny phages via plaque assay or ELISA. A big advantage of this method is the selectivity for viable mycobacteria, ensuring that all of the detected MAP-DNA heirs from viable, infectious MAP cells. For a feasible application in complex matrices like milk or feces, however, sample preparation methods like immunomagnetic or peptide-mediated magnetic separation (PMS) are needed (Grant 2021).

Those magnetic separation methods separate MAP from accompanying nontarget bacteria and inhibitory substances and concentrate the target bacteria into a smaller volume (Husakova et al. 2017). Various types of MAP-binding molecules have been utilized in those methods, including mono- and polyclonal antibodies, MAP-binding peptides, derived from phage display, or plant lectins (Husakova et al. 2017; O'Brien et al. 2016; Stratmann et al. 2002; Hobmaier et al. 2019).

In 2019, Butot et al. published a direct comparison of the three direct detection methods in raw milk, heat-treated milk, and powdered milk In this comparison, the achieved sensitivities for each method were 94% (IS900 qPCR), 76% (f57 qPCR), 83% (culture), and 40% (PMS-phage) (Butot et al. 2019). It has to be mentioned, however, that the examined samples in this study were artificially spiked with MAP, which were previously de-clumped by filtration. Therefore, the sensitivity results are probably not transferable to the performance in naturally contaminated samples (which is likely to be markedly lower). For a systematic comparison of different methods under laboratory conditions, however, this procedure is necessary and reasonable.

In summary, at present there are various diagnostic methods suitable for the detection of animals with JD in a progressive stage. Nevertheless, in spite of intense research, they still have considerable sensitivity problems. A reliable diagnosis of the early stages of the disease is still missing. The fact that MAP is shed intermittently in feces and milk leads to postmortem screening attempts, like testing of lymph nodes in the slaughterhouse, as mesenteric lymph nodes are generally accepted to be the main locus of MAP colonization (Munster et al. 2011).

In conclusion, the time-consuming culture is still presumed to be the most significant tool to identify MAP (OIE 2021).

53.8 Crohn's Disease

CD is a chronic, relapsing inflammatory bowel disease (IBD) affecting the human gastrointestinal tract with preference for the terminal ileum and colon but possible involvement of all its other parts (Baumgart and Sandborn 2012). All age groups and both genders can be affected, with the main peak for disease onset being between the ages of 17 and 40 years (Thia et al. 2010). Patients are often febrile and suffer from painful abdominal cramps and chronic diarrhea; their stool is bloody or mucous. The Montreal classification was established to categorize the different phenotypic behavior of CD, with the majority of patients being affected by the non-stricturing non-penetrating phenotype and the remainder by the more aggressive stricturing or penetrating phenotypes, which are characterized by gut stenoses or fistulas, respectively (Satsangi et al. 2006). The etiology of the disease is unknown, but genome-wide association studies have identified an enormous amount of susceptibility loci (Franke et al. 2010; Jostins et al. 2012). Besides a genetic susceptibility to the disease, environmental factors also play a very important role in its development. A whole lot of such lifestyle factors have been found to be associated with CD, e.g., a reduction in women breastfeeding, air pollution, tobacco use, increased hygiene conditions, or the consumption of Western diet. Interestingly, CD is frequently triggered or exacerbated after an infectious gastroenteritis (Garcia Rodriguez et al. 2006). In an animal model, a virus infection was able to induce a CD-like phenotype in genetically susceptible individuals (Cadwell et al. 2010). It has been tempting to speculate from the first discovery of CD up to now that a pathogen might be the etiological agent for the development of CD.

53.8.1 Theories and Attempted Methods to Elucidate the Role of MAP in CD

As early as 1913, Thomas Kennedy Dalziel suggested that the histological characters of CD and JD are so similar as to justify the proposition that they might be the same, even though, as he also stated, the absence of the acid-fast bacillus would suggest a clear distinction (Dalziel 1989). Since then, there have been many attempts to verify the hypothesis of MAP being an etiological agent for CD, using different approaches like immunohistochemistry, attempts to cultivate the bacterium, experiments to transmit CD to animals, serological tests, molecular methods, and treatment programs of CD with antimycobacterial antibiotics.

53.8.2 Microbiological Approach

It is amazing that up to now, no final conclusion about the role of MAP in CD could be drawn. The reason for this most probably lies in the elusive behavior of MAP in the human body. Histological immunostaining of resected granuloma tissue of CD patients against MAP antigen could up to now in most cases only confirm the primary statement of Dalziel that the acid-fast bacillus is absent, even though it has been performed repeatedly by several work groups (Van Kruiningen 2011). In contrast, Jeyanathan et al. (2007) reported mycobacteria in 59% (10/17) of paraffin-embedded surgical resections of CD patients by acid-fast staining combined with in-situ hybridization for ribosomal RNA. In control patients, mycobacteria were only detected in 14% (5/35) of the samples (Jeyanathan et al. 2007). It is important to mention, however, that the methods in respective study are not able to differentiate between members of the *Mycobacterium avium* complex. Therefore, the identity of MAP cannot be completely assured (Jeyanathan et al. 2007). MAP has been described in humans to also exist in a cell-wall-deficient spheroplastic form, making acid-fast staining an insufficient measure to completely exclude the presence of MAP (Sechi et al. 2005). In the aforementioned study, results of acid-fast staining and in situ hybridization were not always concordant. A considerable amount of samples (9/52) showed positive results for in situ hybridization but were negative for acid-fast staining (Jeyanathan et al. 2007).

Cultural growth of MAP requires extremely long culture times, special selective cultural media, and experienced lab personnel (Turenne et al. 2007). Cultivation of MAP spheroplasts additionally requires specific culture media and conditions and poses a major obstacle for many researchers (Agrawal et al. 2021). This extremely difficult cultivation complicates comparison of results between different laboratories and makes determination of the real proportion of MAP-positive specimens challenging. Additionally, because of the difficult handling procedures, laboratory cross-contamination in mycobacterial laboratories is not rare, which should always be regarded when interpreting mycobacterial culture results (Van Kruiningen 2011). This might, in some cases, explain the discrepancies between different studies, where, in a few smaller studies, MAP could be detected in very small patient

collectives, while it was undetectable in major study groups. MAP was detected in breast milk samples of two patients with CD, but not in 5 controls (Naser et al. 2000a); in another study, it could be cultivated from four out of ten biopsies of children with early onset of CD but not in two ulcerative colitis or four non-IBD patients (Kirkwood et al. 2009). The group of Naser also reported the detection of MAP from blood samples of CD patients; however, there was also growth of MAP in samples from ulcerative colitis and healthy patients (Naser et al. 2004). Conversely, in a major culturing attempt on IBD samples from 191 patients, including 79 CD patients, from the USA and Denmark, not one of 3985 cultures had been positive (Collins et al. 2000).

53.8.3 Serological Approach

Alternative to culture, many studies compare antibody titers against MAP in serum of CD patients and controls. In this context, different capture antigens in the assays with expectably different specificity for MAP antibodies were used (Van Kruiningen 2011). The results gained by these antibody measurements were often inconclusive. In some cases, there were significantly higher antibody responses for CD than for control patients (Collins et al. 2000; Naser et al. 2000b); in other studies, no significant differences could be found (Bernstein et al. 2004; Cho et al. 1986; Kobayashi et al. 1988). This sometimes even occurred when using the identical test in different countries (Collins et al. 2000). As atypical mycobacteria comprise a huge group of different species, with many of them existing ubiquitously in the environment, e.g., in tap water or in the soil, and with probably also a considerable amount of still undiscovered species, it is tempting to speculate that cross-reactivity of MAP-"specific" antibodies toward antigens of different atypical mycobacteria is highly probable (Osterstock et al. 2007). Therefore, it is very difficult to interpret the meaning of positive serological test results regarding their specificity for previous or present MAP colonization, infection, or immunity.

53.8.4 Molecular Biological Approach

Comparably, an enormous amount of studies has been published with a focus on MAP-DNA detection in intestinal tissue, granulomas, or peripheral blood mononuclear cells (PBMC). In most studies, the main target gene was the IS element IS*900*, which has been postulated to be specific for MAP. Most of these studies were based on classical PCR or nested PCR on biopsies, buffy coat of blood, or PBMC (Kirkwood et al. 2009; Naser et al. 2004; Autschbach et al. 2005; Bernstein et al. 2003; Bull et al. 2003; Juste et al. 2009; Suenaga et al. 1995); one study used laser-microdissected tissue (Ryan et al. 2002), and another performed in situ labeling on paraffin-embedded tissues (Hulten et al. 2001). Also – similar to the serological analyses – there were many conflicting results between these studies, with some studies showing a significantly higher presence of IS*900* DNA in CD samples and

others showing no difference to controls. However, a meta-analysis of NAT-based techniques detected an association between MAP and CD (Abubakar et al. 2008). Given that the IS*900* element would be exclusively present in MAP and therefore indeed highly specific, then one possible explanation might be a variable sensitivity of the applied NAT in the study-specific analyzed tissues. Alternatively, as NAT are extremely sensitive methods, sample contamination could be a major issue in some of these studies. Even the water used during endoscopy for taking the biopsies could be contaminated with mycobacteria (Van Kruiningen 2011). Moreover, it becomes increasingly clear that IS*900* is indeed also present in other mycobacteria species; it could be detected by NAT in mycobacterial isolates related to *M. cookii*, *M. scrofulaceum*, and the *M. avium-intracellulare* complex (Englund et al. 2002; Van Kruiningen 2011; Cousins et al. 1999; Motiwala et al. 2004), which questions the specificity of the IS*900* PCR method and heightens the contamination risk. This may shift the focus of IS*900* PCR away from being a screening tool, to a measure for exclusion of infection.

53.8.5 Current Data Situation

A recent meta-analysis investigated data about correlation of MAP with CD, performed by culture, as well as by PCR (Patterson et al. 2021a). It is based on a collection of studies described in a review article by Naser et al. (Naser et al. 2014). Regarding cultural methods, 1795 patients were examined (820 with CD and 975 controls). MAP culture was positive for 31.41% of CD patients and 4.95% of controls, suggesting a strong correlation between MAP and CD. For PCR analysis (IS900), 3528 patients were involved (2012 CD patients and 1516 controls). MAP-DNA was detected in 28.83% of CD patients as opposed to 12.99% in controls (Patterson et al. 2021a).

The authors conclude that, despite the difficulties of detecting MAP, "...there is no denying, that Crohn's disease and *Mycobacterium avium* subspecies *paratuberculosis* are, at the very least, related to each other" (Patterson et al. 2021a).

However, when investigating the association of MAP detection with CD, it is important to keep in mind that, even if MAP is detected in a majority of CD cases, this could be either due to a causative relationship or due to CD patients being more prone to become colonized with MAP (Liverani et al. 2014).

53.8.6 Infection Experiments

According to Koch's third postulate, if an organism is causative for a disease, it must be able to cause this disease, if introduced into a susceptible host (Koch 1882; Loeffler 1884). This, however, is not applicable in some diseases. In leprosy, for example, the isolation of the bacterial agent with subsequent cultivation is not practicable. In tuberculosis, the number of infected people worldwide is estimated

to be about 2 billion. However, only 5–10% of infected people will develop a clinical disease in their lifetime (WHO 2020).

The isolation of MAP from CD patients and subsequent inoculation into a susceptible host have been attempted several times. Up to now, all inoculation experiments of animals with triturated intestinal material from CD patients were unsuccessful in induction of a JD-like infection, even though also susceptible animals, like goats or rabbits, were infected (Van Kruiningen 2011).

It is important in this case to consider that MAP, in animals, is present in its cell walled form, whereas in humans, it might be present in a cell-wall-deficient form. For infection experiments, it is therefore crucial to cultivate MAP up until the formation of a cell wall (Agrawal et al. 2021). The lack of such a readily available human isolate of a cell-wall-deficient form from infected Crohn's tissue, which can be introduced into an animal, together with the lack of a reliable method to re-isolate the cell-wall-deficient MAP from this animal, is a substantial barrier for a conclusive resolution of the question, if Koch's postulates can be seen as fulfilled (Agrawal et al. 2021).

53.8.7 Therapeutical Approach

As MAP obviously is the sole causative pathogen in cattle with JD, but very elusive and highly debated in humans, other researchers tried to demonstrate the implication of MAP or mycobacteria in CD indirectly by studying the effect of antimycobacterial antibiotic therapy on CD patients.

A recent meta-analysis of studies on this topic identified 36 clinical trial studies with a total of 3346 patients. Twelve of those 36 studies reported remission, whereas 24 "only" reported a clinical response (Patterson et al. 2021b). The authors come to the conclusion that ". . .the odds of a Crohn's Disease patient getting better is higher among those who take antibiotics. . ." (Patterson et al. 2021b).

This result, however, should not be seen as the ultimate proof for the role of MAP in CD. The application of antibiotics can influence many factors other than MAP, e.g., by their antiphlogistic effects on the immune system or maybe by elimination of other unknown causative bacteria or even sometimes by their curative effect on an existing but unrecognized gut tuberculosis. Even more problematic for this approach is the actual possibility that MAP disease might not even be curable by antibiotics, as it is the case with its hypothetical animal counterpart JD.

Another notable phenomenon in antibiotic trials is that not all antimycobacterial drugs seem to have the same extent of positive effects. So why do some therapeutics, despite being efficacious against mycobacteria, have lower effects in CD patients? A possible explanation for this may be the target of those drugs. In CD patients, a good proportion of MAP is present as cell-wall-deficient spheroplasts. Therapeutics targeting the cell wall or mycolic acid synthesis are not expected to have as much of a therapeutic effect in those cases as opposed to drugs targeting ribosomal function (Monif 2018).

In contrast to the apparent improvement of symptoms under antimycobacterial treatment stands the circumstance that no deterioration can be seen under

immnosuppressive treatment, as seen in other mycobacterial diseases like *Mycobacterium tuberculosis* (MTB) infection (Solovic et al. 2010). One difference, however, between MTB and MAP is the invasive character of MTB, whereas MAP (in CD) relies more on inducing a pro-inflammatory immune response (Agrawal et al. 2021). Furthermore, some immunomodulatory drugs, which are also used in therapy of CD, have been reported for successful antimycobacterial treatment (Chowdhry et al. 2016).

53.8.8 Epidemiological Approach

If MAP infection really were an etiological agent for CD, one would imagine that CD more often afflicts cattle farmers who are exposed to animals with JD than farmers with healthy animals. However, studies from the USA and UK could not show a higher prevalence of CD in farmers handling JD animals (Jones et al. 2006; Qual et al. 2010). A few restrictions, however, have to be added to this conclusion: First and foremost, the assumption that CD prevalence would be higher in populations with higher exposure to MAP is only applicable, if MAP is the only etiologic agent of CD. If the pathogenesis of CD, however, is a multifactorial event (e.g., a genetic disposition or other risk factors that alter the susceptibility to MAP infection), relying solely on the frequency of contact to the bacterial agent will yield lower correlation than in the case of a monocausal disease. Additionally, in the case of MAP, it is difficult to determine a suitable, nonexposed control group, as MAP is widespread in the environment and food supply and exposure to MAP can therefore not easily be ruled out (Agrawal et al. 2021).

Another point to consider is that MAP infections in susceptible species seem to be very age dependent, occurring mainly at a very young age with symptoms developing several years later in an adolescent or adult age (Windsor and Whittington 2010). Therefore, studies that correlate MAP exposure and CD prevalence should take that into account.

One epidemiological detail, which is often presented by supporters of the causation theory, is that the prevalence of CD is increasing. This is untypical for classic autoimmune diseases like rheumatoid arthritis or multiple sclerosis and suggests the involvement of an environmental factor in the pathogenesis (Molodecky et al. 2012).

53.8.9 Breastfeeding and the Hruska Postulate

The Hruska postulate describes a theory that has become more and more popular in the scientific community when discussing MAP involvement in the genesis of CD. It is based on the observation that breastfeeding seems to significantly reduce the risk of developing CD (Barclay et al. 2009; Thompson et al. 2000).

The Hruska postulate states that pathogenesis of CD is the consequence of two distinct interactions between MAP and the immune system (Monif 2018; Monif 2015):

1. Infectious challenge with MAP in absence of acquired immunity
2. Loss of immune tolerance to MAP antigens and formation of an immunological memory

The theory suggests that MAP exposure of newborn children who lack a competent acquired immune system leads to a continuous replication of MAP. This replication then has to be brought under control by innate immunity, which is followed by loss of immunological tolerance toward MAPs antigenic array, as the pro-inflammatory response is being fixated in the immunologic memory.

This conclusion is supported by similar phenomena, which can be observed with organisms like *Rubella* sp., *Herpes simplex* or *Mycobacterium tuberculosis.* Those organisms are mainly contained by cellular immunity, but if acquired immunity is absent, the reactions are markedly exaggerated in comparison to when acquired immunity is present (Monif 2018).

So how does breastfeeding and economic status play into this? As mentioned above, a pivotal element for the development of CD is exposure to MAP before acquired immunity can develop properly. Since viable MAP have repeatedly been reported to be present in retail milk, municipal water supplies, and even powdered infant formula, theory supporters suspect those products to be the main vessel for MAP exposure in neonates (Hruska and Pavlik 2014). Therefore, breastfeeding is an effective measure to postpone this exposure until competence of the acquired immune system is present. The very low incidence of CD in economically stressed population supports this theory, as breastfeeding is far more common there than in wealthier communities (Monif 2021).

53.9 Conclusions

In conclusion, the scientific community is still divided into supporters and critics for a possible role of MAP in CD. While the supporters bring forward the detection of MAP in blood, intestine, and even milk samples of human CD patients, the opponents still miss the final convincing evidence that the presence of MAP in the human body can really initiate CD. One of the main arguments of critics still stands fast, namely, that in all of recorded medical and veterinary medical history, there are no published accounts of the transmission of JD to humans (Van Kruiningen 2011). Data from a meta-analysis of CD genome-wide association scans detected considerable overlap between susceptibility loci for IBD and mycobacterial infection (Jostins et al. 2012), which could indeed mean that mycobacteria are involved in the development of IBD or that the associated changes in these susceptibility loci necessary for mycobacterial control somehow auto-induce autoimmune processes with subsequent pathogen-free granuloma formation. Therefore, the theory remains open for controversial discussion, and future studies will hopefully lead to a final conclusion. At the moment, however, there is not enough evidence to convincingly demonstrate that MAP is a possible etiological agent for CD.

53.10 Cross-References

▶ Important Zoonotic Diseases of Cattle and Their Prevention Measures
▶ Zoonotic Diseases of Swine: Food-Borne and Occupational Aspects of Infection

References

Abubakar I, Myhill D, Aliyu SH, Hunter PR (2008) Detection of *Mycobacterium avium* subspecies *paratuberculosis* from patients with Crohn's disease using nucleic acid-based techniques: a systematic review and meta-analysis. Inflamm Bowel Dis 14(3):401–410. https://doi.org/10.1002/ibd.20276

Agrawal G, Aitken J, Hamblin H, Collins M, Borody TJ (2021) Putting Crohn's on the MAP: five common questions on the contribution of *Mycobacterium avium* subspecies *paratuberculosis* to the pathophysiology of Crohn's disease. Dig Dis Sci 66(2):348–358. https://doi.org/10.1007/s10620-020-06653-0

Alonso-Hearn M, Eckstein TM, Sommer S, Bermudez LE (2009) A *Mycobacterium avium* subsp. *paratuberculosis* LuxR regulates cell envelope and virulence. Innate Immun 16(4):235–247. https://doi.org/10.1177/1753425909339811

Autschbach F, Eisold S, Hinz U, Zinser S, Linnebacher M, Giese T et al (2005) High prevalence of *Mycobacterium avium* subspecies *paratuberculosis* IS900 DNA in gut tissues from individuals with Crohn's disease. Gut 54(7):944–949. https://doi.org/10.1136/gut.2004.045526

Bang B (1906) Chronische pseudotuberkuloese Darmentzuendung beim Rinde. Berl Tieraerztl Wochenschr 42:759–763

Bannantine John P, Bermudez Luiz E, Andrews-Polymenis HL (2013) No holes barred: invasion of the intestinal mucosa by *Mycobacterium avium* subsp. *paratuberculosis*. Infect Immun 81(11):3960–3965. https://doi.org/10.1128/IAI.00575-13

Barclay R, Ratledge C (1983) Iron-binding compounds of *Mycobacterium avium*, *M. intracellulare*, *M. scrofulaceum*, and mycobactin-dependent *M. paratuberculosis* and *M. avium*. J Bacteriol 153(3):1138–1146. https://doi.org/10.1128/jb.153.3.1138-1146.1983

Barclay AR, Russell RK, Wilson ML, Harper Gilmour W, Satsangi J, Wilson DC (2009) Systematic review: the role of breastfeeding in the development of pediatric inflammatory bowel disease. J Pediatr 155(3):421–426. https://doi.org/10.1016/j.jpeds.2009.03.017

Bastida F, Juste RA (2011) Paratuberculosis control: a review with a focus on vaccination. J Immune Based Ther Vaccines 9:8. https://doi.org/10.1186/1476-8518-9-8

Bates A, O'Brien R, Liggett S, Griffin F (2018) The effect of sub-clinical infection with *Mycobacterium avium* subsp. *paratuberculosis* on milk production in a New Zealand dairy herd. BMC Vet Res 14(1):93. https://doi.org/10.1186/s12917-018-1421-4

Baumgart DC, Sandborn WJ (2012) Crohn's disease. Lancet 380(9853):1590–1605. https://doi.org/10.1016/S0140-6736(12)60026-9

Beard PM, Daniels MJ, Henderson D, Pirie A, Rudge K, Buxton D et al (2001) Paratuberculosis infection of nonruminant wildlife in Scotland. J Clin Microbiol 39(4):1517–1521. https://doi.org/10.1128/JCM.39.4.1517-1521.2001

Begg DJ, Plain KM, de Silva K, Gurung R, Gunn A, Purdie AC et al (2018) Immunopathological changes and apparent recovery from infection revealed in cattle in an experimental model of Johne's disease using a lyophilised culture of *Mycobacterium avium* subspecies *paratuberculosis*. Vet Microbiol 219:53–62. https://doi.org/10.1016/j.vetmic.2018.03.029

Behr MA, Kapur V (2008) The evidence for *Mycobacterium paratuberculosis* in Crohn's disease. Curr Opin Gastroenterol 24(1):17–21. https://doi.org/10.1097/MOG.0b013e3282f1dcc4

Bermudez Luiz E, Petrofsky M, Sommer S, Barletta Raúl G (2010) Peyer's patch-deficient mice demonstrate that *Mycobacterium avium* subsp. *paratuberculosis* translocates across the mucosal

barrier via both M cells and enterocytes but has inefficient dissemination. Infect Immun 78(8): 3570–3577. https://doi.org/10.1128/IAI.01411-09

Bernstein CN, Nayar G, Hamel A, Blanchard JF (2003) Study of animal-borne infections in the mucosas of patients with inflammatory bowel disease and population-based controls. J Clin Microbiol 41(11):4986–4990. https://doi.org/10.1128/JCM.41.11.4986-4990.2003

Bernstein CN, Blanchard JF, Rawsthorne P, Collins MT (2004) Population-based case control study of seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's disease and ulcerative colitis. J Clin Microbiol 42(3):1129–1135. https://doi.org/10.1128/JCM.42.3.1129-1135.2004

Botsaris G, Swift BMC, Slana I, Liapi M, Christodoulou M, Hatzitofi M et al (2016) Detection of viable *Mycobacterium avium* subspecies *paratuberculosis* in powdered infant formula by phage-PCR and confirmed by culture. Int J Food Microbiol 216:91–94. https://doi.org/10.1016/j.ijfoodmicro.2015.09.011

Bull TJ, McMinn EJ, Sidi-Boumedine K, Skull A, Durkin D, Neild P et al (2003) Detection and verification of *Mycobacterium avium* subsp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. J Clin Microbiol 41(7): 2915–2923. https://doi.org/10.1128/JCM.41.7.2915-2923.2003

Busatto C (2019) Júlia Silveira Vianna, Lande Vieira da Silva, Ivy Bastos Ramis, and Pedro Eduardo Almeida da Silva, *Mycobacterium avium*: an overview. Tuberculosis 114:127–134. https://doi.org/10.1016/j.tube.2018.12.004

Butot S, Ricchi M, Sevilla IA, Michot L, Molina E, Tello M et al (2019) Estimation of performance characteristics of analytical methods for *Mycobacterium avium* subsp. *paratuberculosis* detection in dairy products. Front Microbiol 10(509). https://doi.org/10.3389/fmicb.2019.00509

Büttner M, Gerbermann H, Naumann L, Neuendorf E, Rinder H, Wildner M et al (2006) Paratuberkulose beim Rind—Morbus Crohn beim Menschen: ein ursächlicher Zusammenhang. Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (ed), pp 1–114. https://www.lgl.bayern.de/tiergesundheit/tierkrankheiten/bakterielle_pilzinfektionen/paratuberkulose/doc/paratuberkulose_ag.pdf

Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE et al (2010) Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. Cell 141(7):1135–1145. https://doi.org/10.1016/j.cell.2010.05.009

Chiaradia L, Lefebvre C, Parra J, Marcoux J, Burlet-Schiltz O, Etienne G et al (2017) Dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane. Sci Rep 7(1):12807. https://doi.org/10.1038/s41598-017-12718-4

Chiodini RJ, Hermon-Taylor J (1993) The thermal resistance of *Mycobacterium paratuberculosis* in raw milk under conditions simulating pasteurization. J Vet Diagn Investig 5(4):629–631. https://doi.org/10.1177/104063879300500424

Chiodini RJ, Van Kruiningen HJ, Merkal RS (1984) Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 74(3):218–262. http://www.ncbi.nlm.nih.gov/pubmed/6375961

Cho SN, Brennan PJ, Yoshimura HH, Korelitz BI, Graham DY (1986) Mycobacterial aetiology of Crohn's disease: serologic study using common mycobacterial antigens and a species-specific glycolipid antigen from *Mycobacterium paratuberculosis*. Gut 27(11):1353–1356. https://doi.org/10.1136/gut.27.11.1353

Chowdhry S, Shukla A, D'souza P, Dhali T, Jaiswal P (2016) Treatment of severe refractory erythema nodosum leprosum with tumor necrosis factor inhibitor Etanercept. Int J Mycobacteriol 5(2):223–225. https://doi.org/10.1016/j.ijmyco.2016.02.002

Clarke CJ (1997) The pathology and pathogenesis of paratuberculosis in ruminants and other species. J Comp Pathol 116(3):217–261. https://doi.org/10.1016/S0021-9975(97)80001-1

Cocito C, Gilot P, Coene M, de Kesel M, Poupart P, Vannuffel P (1994) Paratuberculosis. Clin Microbiol Rev 7(3):328–345. https://doi.org/10.1128/CMR.7.3.328

Collins MT, Lisby G, Moser C, Chicks D, Christensen S, Reichelderfer M et al (2000) Results of multiple diagnostic tests for *Mycobacterium avium* subsp. *paratuberculosis* in patients with inflammatory bowel disease and in controls. J Clin Microbiol 38(12):4373–4381. https://doi.org/10.1128/JCM.38.12.4373-4381.2000

Cousins DV, Whittington R, Marsh I, Masters A, Evans RJ, Kluver P (1999) Mycobacteria distenct from *Mycobacterium avium* subsp. *paratuberculosis* isolated from the faeces of ruminants possess IS900-like sequences detectable IS900 polymerase chain reaction: implications for diagnosis. Mol Cell Probes 13(6):431–442. https://doi.org/10.1006/mcpr.1999.0275

Coussens PM (2001) *Mycobacterium paratuberculosis* and the bovine immune system. Anim Health Res Rev 2(2):141–161. https://doi.org/10.1079/AHRR200134

Dalziel TK (1989) Thomas Kennedy Dalziel 1861–1924. Chronic interstitial enteritis. Dis Colon Rectum 32(12):1076–1078. https://doi.org/10.1007/BF02553886

Donaghy JA, Totton NL, Rowe MT (2004) Persistence of *Mycobacterium paratuberculosis* during manufacture and ripening of cheddar cheese. Appl Environ Microbiol 70(8):4899–4905. https://doi.org/10.1128/AEM.70.8.4899-4905.2004

Eisenberg SWF, Koets AP, Nielen M, Heederik D, Mortier R, De Buck J et al (2011) Intestinal infection following aerosol challenge of calves with *Mycobacterium avium* subspecies *paratuberculosis*. Vet Res 42(1):117. https://doi.org/10.1186/1297-9716-42-117

Ellingson JL, Anderson JL, Koziczkowski JJ, Radcliff RP, Sloan SJ, Allen SE et al (2005) Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. J Food Prot 68(5):966–972. http://www.ncbi.nlm.nih.gov/pubmed/15895728

Englund S, Bolske G, Johansson KE (2002) An IS900-like sequence found in a *Mycobacterium* spp. other than *Mycobacterium avium* subsp. *paratuberculosis*. FEMS Microbiol Lett 209(2):267–271. https://doi.org/10.1111/j.1574-6968.2002.tb11142.x

Eslami M, Shafiei M, Ghasemian A, Valizadeh S, Al-Marzoqi AH, Mostafavi SKS et al (2019) *Mycobacterium avium paratuberculosis* and *Mycobacterium avium* complex and related subspecies as causative agents of zoonotic and occupational diseases. J Cell Physiol 234(8):12415–12421. https://doi.org/10.1002/jcp.28076

FLI (2020) Paratuberkulose: Amtliche Methode und Falldefinition. in Amtliche Methodensammlung und Falldefinitionen: Meldepflichtige Tierkrankheiten. Friedrich-Loeffler-Inst.: Greifswald – Insel Riems. https://www.openagrar.de/receive/openagrar_mods_00058039

Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T et al (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 42(12):1118–1125. https://doi.org/10.1038/ng.717

Friedrich Löffler Institute Germany (2021) Aus dem NRL für Paratuberkulose: Testcharakteristika der zugelassenen ELISA-Tests für den Nachweis von Antikörpern gegen *Mycobacterium avium* subsp. *paratuberculosis* beim Rind gemessen an den Referenzpanels Serum und Milch des NRL (Stand 04/2021). Available from: https://www.fli.de/fileadmin/FLI/IMP/Testcharakteristika_D_2021-04-01_bf.pdf

Gao A, Odumeru J, Raymond M, Mutharia L (2005) Development of improved method for isolation of *Mycobacterium avium* subsp. *paratuberculosis* from bulk tank milk: effect of age of milk, centrifugation, and decontamination. Can J Vet Res = Revue canadienne de recherche veterinaire 69(2):81–87. https://pubmed.ncbi.nlm.nih.gov/15971671

Garcia Rodriguez LA, Ruigomez A, Panes J (2006) Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease. Gastroenterology 130(6):1588–1594. https://doi.org/10.1053/j.gastro.2006.02.004

Gerlach GF (2002) [Paratuberculosis: the pathogen and routes of infection]. Dtsch Tierarztl Wochenschr. 109(12):504–506. http://www.ncbi.nlm.nih.gov/pubmed/12596562

Gerrard ZE, Swift BMC, Botsaris G, Davidson RS, Hutchings MR, Huxley JN et al (2018) Survival of *Mycobacterium avium* subspecies *paratuberculosis* in retail pasteurised milk. Food Microbiol 74:57–63. https://doi.org/10.1016/j.fm.2018.03.004

Grant IR (2003) *Mycobacterium paratuberculosis* and milk. Acta Vet Scand 44(3–4):261–266. http://www.ncbi.nlm.nih.gov/pubmed/15074643

Grant IR (2021) Bacteriophage-based methods for detection of viable *Mycobacterium avium* subsp. *paratuberculosis* and their potential for diagnosis of Johne's disease. Front Vet Sci 8(205). https://doi.org/10.3389/fvets.2021.632498

Grant Irene R, Hitchings Edward I, McCartney A, Ferguson F, Rowe Michael T (2002) Effect of commercial-scale high-temperature, short-time pasteurization on the viability of *Mycobacterium*

paratuberculosis in naturally infected cows' milk. Appl Environ Microbiol 68(2):602–607. https://doi.org/10.1128/AEM.68.2.602-607.2002

Grant Irene R, Williams Alan G, Rowe Michael T, Donald Muir D (2005) Efficacy of various pasteurization time-temperature conditions in combination with homogenization on inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk. Appl Environ Microbiol 71(6):2853–2861. https://doi.org/10.1128/AEM.71.6.2853-2861.2005

Grant IR, Ball HJ, Neill SD, Rowe MT (1996) Inactivation of Mycobacterium paratuberculosis in cows' milk at pasteurization temperatures. Appl Environ Microbiol 62(2):631–636. https://doi.org/10.1128/aem.62.2.631-636.1996

Grant IR, Ball HJ, Rowe MT (1999) Effect of higher pasteurization temperatures, and longer holding times at 72°C, on the inactivation of *Mycobacterium paratuberculosis* in milk. Lett Appl Microbiol 28(6):461–465. https://doi.org/10.1046/j.1365-2672.1999.00557.x

Greenstein RJ (2003) Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. Lancet Infect Dis 3(8):507–514. https://doi.org/10.1016/S1473-3099(03)00724-2

Hobmaier BF, Lutterberg K, Kleinwort KJH, Mayer R, Hirmer S, Amann B et al (2019) Characterization of plant lectins for their ability to isolate *Mycobacterium avium* subsp. *paratuberculosis* from milk. Food Microbiol 82:231–239. https://doi.org/10.1016/j.fm.2019.02.009

Hruska K, Pavlik I (2014) Crohn's disease and related inflammatory diseases: from many single hypotheses to one "superhypothesis". Vet Med 59:583–630. https://doi.org/10.17221/7822-VETMED

Hulten K, El-Zimaity HM, Karttunen TJ, Almashhrawi A, Schwartz MR, Graham DY et al (2001) Detection of *Mycobacterium avium* subspecies *paratuberculosis* in Crohn's diseased tissues by in situ hybridization. Am J Gastroenterol 96(5):1529–1535. https://doi.org/10.1111/j.1572-0241.2001.03751.x

Husakova M, Dziedzinska R, Slana I (2017) Magnetic separation methods for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in various types of matrices: a review. Biomed Res Int 2017:5869854. https://doi.org/10.1155/2017/5869854

Ikonomopoulos J, Pavlik I, Bartos M, Svastova P, Wuhib YA, Roubal P et al (2005) Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Retail Cheeses from Greece and the Czech Republic. Appl Environ Microbiol 71(12):8934–8936. https://doi.org/10.1128/AEM.71.12.8934-8936.2005

International Dairy Foods Association (n.d.) Pasteurization. Last accessed 2nd November 2021. Available from: https://www.idfa.org/pasteurization

Jeyanathan M, Boutros-Tadros O, Radhi J, Semret M, Bitton A, Behr MA (2007) Visualization of *Mycobacterium avium* in Crohn's tissue by oil-immersion microscopy. Microbes Infect 9(14):1567–1573. https://doi.org/10.1016/j.micinf.2007.09.001

Johne HA, Frothingham L (1895) Ein eigenthuemlicher Fall von Tuberculose beim Rind. Dtsch Z Tiermed Path 21:438–454

Jones PH, Farver TB, Beaman B, Cetinkaya B, Morgan KL (2006) Crohn's disease in people exposed to clinical cases of bovine paratuberculosis. Epidemiol Infect 134(1):49–56. https://doi.org/10.1017/S0950268805004681

Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY et al (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 491(7422):119–124. https://doi.org/10.1038/nature11582

Juffs HS, Deeth H (2007) cientific evaluation of pasteurisation for pathogen reduction in milk and milk products. Food Standards Australia New Zealand. ISBN: 978-0-642-34555-4. https://www.foodstandards.govt.nz/code/proposals/documents/Scientific%20Evaluation.pdf

Juste RA (2012) Slow infection control by vaccination: paratuberculosis. Vet Immunol Immunopathol 148(1–2):190–196. https://doi.org/10.1016/j.vetimm.2012.05.017

Juste RA, Elguezabal N, Pavon A, Garrido JM, Geijo M, Sevilla I et al (2009) Association between *Mycobacterium avium* subsp. *paratuberculosis* DNA in blood and cellular and humoral immune response in inflammatory bowel disease patients and controls. Int J Infect Dis 13(2):247–254. https://doi.org/10.1016/j.ijid.2008.06.034

Karuppusamy S, Kirby GM, Mutharia L, Tripathi BN (2019) An update on *Mycobacterium avium* subspecies *paratuberculosis* antigens and their role in the diagnosis of Johne's disease. World J Microbiol Biotechnol 35(8):120. https://doi.org/10.1007/s11274-019-2691-0

Kennedy DJ, Benedictus G (2001) Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species. Rev Sci Tech 20(1):151–179. https://doi.org/10.20506/rst.20.1.1274

Khare S, Lawhon SD, Drake KL, Nunes JES, Figueiredo JF, Rossetti CA et al (2012) Systems biology analysis of gene expression during in vivo *Mycobacterium avium paratuberculosis* enteric colonization reveals role for immune tolerance. PLoS One 7(8):e42127. https://doi.org/10.1371/journal.pone.0042127

Khol JL, Braun AL, Slana I, Kralik P, Wittek T (2017) Testing of milk replacers for *Mycobacterium avium* subsp. *paratuberculosis* by PCR and bacterial culture as a possible source for Johne's disease (paratuberculosis) in calves. Prev Vet Med 144:53–56. https://doi.org/10.1016/j.prevetmed.2017.05.013

Kirkwood CD, Wagner J, Boniface K, Vaughan J, Michalski WP, Catto-Smith AG et al (2009) *Mycobacterium avium* subspecies *paratuberculosis* in children with early-onset Crohn's disease. Inflamm Bowel Dis 15(11):1643–1655. https://doi.org/10.1002/ibd.20967

Kleinwort KJH, Hobmaier BF, Mayer R, Hölzel C, Degroote RL, Märtlbauer E et al (2021) Mycobacterium avium subsp. paratuberculosis proteome changes profoundly in milk. Metabolites 11(8). https://doi.org/10.3390/metabo11080549

Kobayashi K, Brown WR, Brennan PJ, Blaser MJ (1988) Serum antibodies to mycobacterial antigens in active Crohn's disease. Gastroenterology 94(6):1404–1411. https://doi.org/10.1016/0016-5085(88)90679-8

Koch R (1882) Die Ätiologie der Tuberkulose. Berliner Klinische Wochenschrift 19:221–230. https://doi.org/10.25646/5088

Koets AP, Eda S, Sreevatsan S (2015) The within host dynamics of *Mycobacterium avium* ssp. *paratuberculosis* infection in cattle: where time and place matter. Vet Res 46(1):61. https://doi.org/10.1186/s13567-015-0185-0

Lamont EA, O'Grady SM, Davis WC, Eckstein T, Sreevatsan S, Flynn JL (2012a) Infection with *Mycobacterium avium* subsp. *paratuberculosis* results in rapid interleukin-1β release and macrophage transepithelial migration. Infect Immun 80(9):3225–3235. https://doi.org/10.1128/IAI.06322-11

Lamont EA, Bannantine JP, Armién A, Ariyakumar DS, Sreevatsan S (2012b) Identification and characterization of a spore-like Morphotype in chronically starved *Mycobacterium avium* subsp. *paratuberculosis* cultures. PLoS ONE 7(1):e30648. https://doi.org/10.1371/journal.pone.0030648

Lindsay Sweet P, Singh Prachi K, Azad Abul VS, Rajaram Murugesan S, Larry S, Schorey Jeffrey S (2010) Mannose receptor-dependent delay in phagosome maturation by *Mycobacterium avium* glycopeptidolipids. Infect Immun 78(1):518–526. https://doi.org/10.1128/IAI.00257-09

Liverani E, Scaioli E, Cardamone C, Dal Monte P, Belluzzi A (2014) *Mycobacterium avium* subspecies *paratuberculosis* in the etiology of Crohn's disease, cause or epiphenomenon? World J Gastroenterol: WJG 20(36):13060. https://doi.org/10.3748/wjg.v20.i36.13060

Loeffler F (1884) Untersuchungen über die Bedeutung der Mikroorganismen für die Entstehung der Diphtherie beim Menschen, bei der Taube und beim Kalbe. In: Mitteilungen aus dem kaiserlichen Gesundheitsamte, Berlin, Germany. https://archive.org/details/39002010412584.med.yale.edu

Lynch D, Jordan KN, Kelly PM, Freyne T, Murphy PM (2007) Heat sensitivity of *Mycobacterium avium* ssp. *paratuberculosis* in milk under pilot plant pasteurization conditions†. Int J Dairy Technol 60(2):98–104. https://doi.org/10.1111/j.1471-0307.2007.00317.x

Manning EJ, Collins MT (2001) *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. Revue scientifique et technique (International Office of Epizootics) 20(1):133–150. https://doi.org/10.20506/rst.20.1.1275

McAloon CG, Roche S, Ritter C, Barkema HW, Whyte P, More SJ et al (2019) A review of paratuberculosis in dairy herds — part 1: epidemiology. Vet J 246:59–65. https://doi.org/10.1016/j.tvjl.2019.01.010

McClure HM, Chiodini RJ, Anderson DC, Swenson RB, Thayer WR, Coutu JA (1987) *Mycobacterium paratuberculosis* infection in a colony of stumptail macaques (*Macaca arctoides*). J Infect Dis 155(5):1011–1019. https://doi.org/10.1093/infdis/155.5.1011

McKenna SLB, Keefe GP, Tiwari A, VanLeeuwen J, Barkema HW (2006) Johne's disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. Can Vet J = La revue veterinaire canadienne 47(11):1089–1099. https://pubmed.ncbi.nlm.nih.gov/17147140

Meylan M, Rings DM, Shulaw WP, Kowalski JJ, Bech-Nielsen S, Hoffsis GF (1996) Survival of *Mycobacterium paratuberculosis* and preservation of immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. Am J Vet Res 57(11):1580–1585. http://www.ncbi.nlm.nih.gov/pubmed/8915433

Michael W, Mullan A (2019) Are we closer to understanding why viable cells of *Mycobacterium avium* subsp. *paratuberculosis* are still being reported in pasteurised milk? Int J Dairy Technol 72(3):332–344. https://doi.org/10.1111/1471-0307.12617

Möbius P, Hotzel H, Raßbach A, Köhler H (2008) Comparison of 13 single-round and nested PCR assays targeting IS900, ISMav2, f57 and locus 255 for detection of *Mycobacterium avium* subsp. *paratuberculosis*. Vet Microbiol 126(4):324–333. https://doi.org/10.1016/j.vetmic.2007.07.016

Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G et al (2012) Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 142(1):46–54.e42. https://doi.org/10.1053/j.gastro.2011.10.001

Momotani E, Whipple DL, Thiermann AB, Cheville NF (1988) Role of M cells and macrophages in the entrance of *mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Vet Pathol 25(2):131–137. https://doi.org/10.1177/030098588802500205

Monif GRG (2015) The Hruska postulate of Crohn's disease. Med Hypotheses 85(6):878–881. https://doi.org/10.1016/j.mehy.2015.09.019

Monif GRG (2018) The paradigms of causation for Crohn's disease. J Infect Dis Immune Ther 1:1

Monif GRG (2021) Crohn's disease: the infectious disease incorporated's perspective. Gastrointest Disord 3(3):138–141. https://doi.org/10.3390/gidisord3030015

Moss MT, Sanderson JD, Tizard ML, Hermon-Taylor J, el-Zaatari FA, Markesich DC et al (1992) Polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp *silvaticum* in long term cultures from Crohn's disease and control tissues. Gut 33 (9):1209–1213. https://doi.org/10.1136/gut.33.9.1209

Motiwala AS, Amonsin A, Strother M, Manning EJ, Kapur V, Sreevatsan S (2004) Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* isolates recovered from wild animal species. J Clin Microbiol 42(4):1703–1712. https://doi.org/10.1128/JCM.42.4.1703-1712.2004

Munster P, Volkel I, Wemheuer W, Petschenka J, Steinbrunn C, Campe A et al (2011) Detection of Mycobacterium avium ssp. paratuberculosis in ileocaecal lymph nodes collected from elderly slaughter cows using a semi-nested IS900 polymerase chain reaction. Vet Microbiol 154(1–2): 197–201. https://doi.org/10.1016/j.vetmic.2011.06.033

Naser SA, Schwartz D, Shafran I (2000a) Isolation of *Mycobacterium avium* subsp *paratuberculosis* from breast milk of Crohn's disease patients. Am J Gastroenterol 95(4):1094–1095. http://www.ncbi.nlm.nih.gov/pubmed/10763975

Naser SA, Hulten K, Shafran I, Graham DY, El-Zaatari FA (2000b) Specific seroreactivity of Crohn's disease patients against p35 and p36 antigens of *M. avium* subsp. *paratuberculosis*. Vet Microbiol 77(3–4):497–504. https://doi.org/10.1016/S0378-1135(00)00334-5

Naser SA, Ghobrial G, Romero C, Valentine JF (2004) Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's disease. Lancet 364(9439):1039–1044. https://doi.org/10.1016/S0140-6736(04)17058-X

Naser SA, Sagramsingh SR, Naser AS, Thanigachalam S (2014) *Mycobacterium avium* subspecies *paratuberculosis* causes Crohn's disease in some inflammatory bowel disease patients. World J Gastroenterol 20(23):7403–7415. https://doi.org/10.3748/wjg.v20.i23.7403

O'Brien LM, Stewart LD, Strain SAJ, Grant IR (2016) Novel monoclonal antibody and peptide binders for *Mycobacterium avium* subsp. *paratuberculosis* and their application for magnetic separation. PLoS One 11(1):e0147870. https://doi.org/10.1371/journal.pone.0147870

OIE (2021) Paratuberculosis. In: Manual of diagnostic tests and vaccines for terrestrial animals. World Organization for Animal Health: online version

O'Reilly Ciara E, O'Connor L, Anderson W, Peter Harvey R, Irene G, Donaghy J et al (2004) Surveillance of bulk raw and commercially pasteurized cows' milk from approved Irish liquid-milk pasteurization plants to determine the incidence of *Mycobacterium paratuberculosis*. Appl Environ Microbiol 70(9):5138–5144. https://doi.org/10.1128/AEM.70.9.5138-5144.2004

Osterstock JB, Fosgate GT, Norby B, Manning EJ, Collins MT, Roussel AJ (2007) Contribution of environmental mycobacteria to false-positive serum ELISA results for paratuberculosis. J Am Vet Med Assoc 230(6):896–901. https://doi.org/10.2460/javma.230.6.896

Paolicchi F, Cirone K, Morsella C, Gioffré A (2012) First isolation of *Mycobacterium avium* subsp *paratuberculosis* from commercial pasteurized milk in Argentina. Braz J Microbiol 43:1034–1037. https://doi.org/10.1590/S1517-83822012000300028

Park KT, Allen AJ, Davis WC (2014) Development of a novel DNA extraction method for identification and quantification of *Mycobacterium avium* subsp. *paratuberculosis* from tissue samples by real-time PCR. J Microbiol Methods 99:58–65. https://doi.org/10.1016/j.mimet.2014.02.003

Patterson JM, Winstead C, Milligan K (2021a) Is *Mycobacterium avium* subspecies *paratuberculosis* (MAP) associated with Crohn's Disease? medRxiv:2021.09.27.21263960. https://doi.org/10.1101/2021.09.27.21263960

Patterson JM, Winstead C, Milligan K (2021b) Effectiveness of antibiotics as a treatment option for adults with active Crohn's disease: a meta-analysis. Microb Health Dis 3(e501). https://doi.org/10.26355/mhd_20217_501

Patton EA (2011) Paratuberculosis vaccination. Vet Clin North Am Food Anim Pract 27(3):573–580, vi. https://doi.org/10.1016/j.cvfa.2011.07.004

Pickup RW, Rhodes G, Arnott S, Sidi-Boumedine K, Bull TJ, Weightman A et al (2005) *Mycobacterium avium* subsp. *paratuberculosis* in the catchment area and water of the River Taff in South Wales, United Kingdom, and its potential relationship to clustering of Crohn's disease cases in the city of Cardiff. Appl Environ Microbiol 71(4):2130–2139. https://doi.org/10.1128/AEM.71.4.2130-2139.2005

Pickup RW, Rhodes G, Bull TJ, Arnott S, Sidi-Boumedine K, Hurley M et al (2006) *Mycobacterium avium* subsp. *paratuberculosis* in lake catchments, in river water abstracted for domestic use, and in effluent from domestic sewage treatment works: diverse opportunities for environmental cycling and human exposure. Appl Environ Microbiol 72(6):4067–4077. https://doi.org/10.1128/AEM.02490-05

Poupart P, Coene M, Van Heuverswyn H, Cocito C (1993) Preparation of a specific RNA probe for detection of *Mycobacterium paratuberculosis* and diagnosis of Johne's disease. J Clin Microbiol 31(6):1601–1605. https://doi.org/10.1128/jcm.31.6.1601-1605.1993

Qual DA, Kaneene JB, Varty TJ, Miller R, Thoen CO (2010) Lack of association between the occurrence of Crohn's disease and occupational exposure to dairy and beef cattle herds infected with *Mycobacterium avium* subspecies *paratuberculosis*. J Dairy Sci 93(6):2371–2376. https://doi.org/10.3168/jds.2009-2344

Rademaker Jan LW, Vissers Marc MM, te Giffel Meike C (2007) Effective heat inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in raw milk contaminated with naturally infected feces. Appl Environ Microbiol 73(13):4185–4190. https://doi.org/10.1128/AEM.00326-07

Raizman EA, Fetrow JP, Wells SJ (2009) Loss of income from cows shedding *Mycobacterium avium* subspecies *paratuberculosis* prior to calving compared with cows not shedding the organism on two Minnesota dairy farms. J Dairy Sci 92(10):4929–4936. https://doi.org/10.3168/jds.2009-2133

Rani S, Beaver A, Schukken YH, Pradhan AK (2019) Modeling the effects of infection status and hygiene practices on *Mycobacterium avium* subspecies *paratuberculosis* contamination in bulk tank milk. Food Control 104:367–376. https://doi.org/10.1016/j.foodcont.2019.04.031

Rhodes G, Richardson H, Hermon-Taylor J, Weightman A, Higham A, Pickup R (2014) *Mycobacterium avium* subspecies *paratuberculosis*: human exposure through environmental and domestic aerosols. Pathogens (Basel, Switzerland) 3(3):577–595. https://doi.org/10.3390/pathogens3030577

Richardson H, Rhodes G, Henrys P, Sedda L, Weightman AJ, Pickup RW (2019) Presence of *Mycobacterium avium* subspecies *paratuberculosis* monitored over varying temporal and spatial scales in river catchments: persistent routes for human exposure. Microorganisms 7(5). https://doi.org/10.3390/microorganisms7050136

Robertson R, Hill B, Cerf O, Jordan K, Venter P (2012) A commentary on current perspectives on *Mycobacterium avium* subsp. *paratuberculosis*, Johne's disease and Crohn's disease: a review by Over et al. (2011). Crit Rev Microbiol 38(3):183–184. https://doi.org/10.3109/1040841X.2011.649712

Robertson RE, Cerf O, Condron RJ, Donaghy JA, Heggum C, Jordan K (2017) Review of the controversy over whether or not *Mycobacterium avium* subsp. *paratuberculosis* poses a food safety risk with pasteurised dairy products. Int Dairy J 73:10–18. https://doi.org/10.1016/j.idairyj.2017.04.009

Rosseels V, Huygen K (2008) Vaccination against paratuberculosis. Expert Rev Vaccines 7(6):817–832. https://doi.org/10.1586/14760584.7.6.817

Rowe MT, Grant IR (2006) *Mycobacterium avium* ssp. *paratuberculosis* and its potential survival tactics. Lett Appl Microbiol 42(4):305–311. https://doi.org/10.1111/j.1472-765X.2006.01873.x

Ryan P, Bennett MW, Aarons S, Lee G, Collins JK, O'Sullivan GC et al (2002) PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. Gut 51(5):665–670. https://doi.org/10.1136/gut.51.5.665

Satsangi J, Silverberg MS, Vermeire S, Colombel JF (2006) The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 55(6):749–753. https://doi.org/10.1136/gut.2005.082909

Sechi LA, Scanu AM, Molicotti P, Cannas S, Mura M, Dettori G et al (2005) Detection and isolation of Mycobacterium avium subspecies paratuberculosis from intestinal mucosal biopsies of patients with and without Crohn's disease in Sardinia. J Am Coll Gastroenterol 100(7):1529–1536. https://journals.lww.com/ajg/Fulltext/2005/07000/Detection_and_Isolation_of_Mycobacterium_avium.18.aspx

Slana I, Paolicchi F, Janstova B, Navratilova P, Pavlik I (2008) Detection methods for Mycobacterium avium subsp paratuberculosis in milk and milk products: a review. Vet Med 53(6):283. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.595.2665&rep=rep1&type=pdf

Solovic I, Sester M, Gomez-Reino JJ, Rieder HL, Ehlers S, Milburn HJ et al (2010) The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. Eur Respir J 36(5):1185. https://doi.org/10.1183/09031936.00028510

Sousa T, Costa M, Sarmento P, Manso MC, Abreu C, Bull TJ et al (2021) DNA-based detection of Mycobacterium avium subsp. paratuberculosis in domestic and municipal water from Porto (Portugal), an area of high IBD prevalence. AIMS Microbiology 7(2):163–174. https://doi.org/10.3934/microbiol.2021011

Spahr U, Schafroth K (2001) Fate of Mycobacterium avium subsp. paratuberculosis in Swiss hard and semihard cheese manufactured from raw milk. Appl Environ Microbiol 67(9):4199–4205. https://doi.org/10.1128/AEM.67.9.4199-4205.2001

Spickler AR (2007) Paratuberculosis. (Last updated 2017) Center for Food Security & Public Health, Iowa State Unversity. Available from: http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php

Stabel JR (2000) Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet Microbiol 77(3):465–473. https://doi.org/10.1016/S0378-1135(00)00331-X

Stabel JR, Waters WR, Bannantine JP, Lyashchenko K (2011) Mediation of host immune responses after immunization of neonatal calves with a heat-killed *Mycobacterium avium* subsp. *paratuberculosis* vaccine. Clin Vaccine Immunol 18(12):2079–2089. https://doi.org/10.1128/CVI.05421-11

Stratmann J, Strommenger B, Stevenson K, Gerlach Gerald F (2002) Development of a peptide-mediated capture PCR for detection of *Mycobacterium avium* subsp. *paratuberculosis* in Milk. J Clin Microbiol 40(11):4244–4250. https://doi.org/10.1128/JCM.40.11.4244-4250.2002

Suenaga K, Yokoyama Y, Okazaki K, Yamamoto Y (1995) Mycobacteria in the intestine of Japanese patients with inflammatory bowel disease. Am J Gastroenterol 90(1):76–80. http://www.ncbi.nlm.nih.gov/pubmed/7801954

Sung N, Collins MT (2000) Effect of three factors in cheese production (pH, salt, and heat) on *Mycobacterium avium* subsp. *paratuberculosis* viability. Appl Environ Microbiol 66(4):1334–1339. https://doi.org/10.1128/AEM.66.4.1334-1339.2000

Sweeney RW, Whitlock RH, Rosenberger AE (1992) *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. J Clin Microbiol 30 (1):166–171. https://doi.org/10.1128/jcm.30.1.166-171.1992

Thia KT, Sandborn WJ, Harmsen WS, Zinsmeister AR, Loftus EV Jr (2010) Risk factors associated with progression to intestinal complications of Crohn's disease in a population-based cohort. Gastroenterology 139(4):1147–1155. https://doi.org/10.1053/j.gastro.2010.06.070

Thompson NP, Montgomery SM, Wadsworth MEJ, Pounder RE, Wakefield AJ (2000) Early determinants of inflammatory bowel disease: use of two national longitudinal birth cohorts. Eur J Gastroenterol Hepatol 12(1). https://doi.org/10.1097/00042737-200012010-00006

Tiwari A, VanLeeuwen JA, McKenna SL, Keefe GP, Barkema HW (2006) Johne's disease in Canada Part I: clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. Can Vet J 47(9):874–882. http://www.ncbi.nlm.nih.gov/pubmed/17017652

Todd Kuenstner J, Naser S, Chamberlin W, Borody T, Graham DY, McNees A et al (2017) The Consensus from the *Mycobacterium avium* ssp. *paratuberculosis* (MAP) Conference 2017. Front Public Health 5:208. https://doi.org/10.3389/fpubh.2017.00208

Turenne CY, Wallace R Jr, Behr MA (2007) *Mycobacterium avium* in the postgenomic era. Clin Microbiol Rev 20(2):205–229. https://doi.org/10.1128/CMR.00036-06

Twort FW, Ingram GLY (1912) A method for isolating and cultivations the *Mycobacterium enteritidis chronicae pseudotuberculosae bovis*, Johne, and some experiments on the preparation of a diagnostic vaccine for pseudo-tuberculosis enteritis of bovines. Proc Soc London 99:1323–1329

Uzoigwe JC, Khaitsa ML, Gibbs PS (2007) Epidemiological evidence for *Mycobacterium avium* subspecies *paratuberculosis* as a cause of Crohn's disease. Epidemiol Infect 135(7):1057–1068. https://doi.org/10.1017/S0950268807008448

Van Kruiningen HJ (2011) Where are the weapons of mass destruction – the *Mycobacterium paratuberculosis* in Crohn's disease? J Crohns Colitis 5(6):638–644. https://doi.org/10.1016/j.crohns.2011.08.004

Waddell L, Rajić A, Stärk K, McEwen SA (2016) *Mycobacterium avium* ssp. *paratuberculosis* detection in animals, food, water and other sources or vehicles of human exposure: a scoping review of the existing evidence. Prev Vet Med 132:32–48. https://doi.org/10.1016/j.prevetmed.2016.08.003

Whan L, Ball HJ, Grant IR, Rowe MT (2005) Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in untreated water in Northern Ireland. Appl Environ Microbiol 71(11): 7107–7112. https://doi.org/10.1128/AEM.71.11.7107-7112.2005

Whitlock RH, Buergelt C (1996) Preclinical and clinical manifestations of paratuberculosis (including pathology). Vet Clin North Am Food Anim Pract 12(2):345–356. https://doi.org/10.1016/S0749-0720(15)30410-2

Whitlock RH, Sweeney RW, Fyock T, Smith J (2005) MAP super-shedders: another factor in the control of Johne's disease. In: Manning EJB, Nielsen SS (eds) Proceedings of the 8th International Colloquium on Paratuberculosis. International Association for Paratuberculosis, Madison, WI

Whittington RJ, Marsh IB, Reddacliff LA (2005) Survival of *Mycobacterium avium* subsp. *paratuberculosis* in dam water and sediment. Appl Environ Microbiol 71(9):5304–5308. https://doi.org/10.1128/AEM.71.9.5304-5308.2005

Whittington R, Donat K, Weber MF, Kelton D, Nielsen SS, Eisenberg S et al (2019) Control of paratuberculosis: who, why and how. A review of 48 countries. BMC Vet Res 15(1):198. https://doi.org/10.1186/s12917-019-1943-4

WHO (2020) Global tuberculosis report 2020. World Health Organization, Geneva. ISBN: 978-92-4-001313-1. https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf

Windsor PA, Whittington RJ (2010) Evidence for age susceptibility of cattle to Johne's disease. Vet J 184(1):37–44. https://doi.org/10.1016/j.tvjl.2009.01.007

Zwick LS, Walsh TF, Barbiers R, Collins MT, Kinsel MJ, Murnane RD (2002) Paratuberculosis in a mandrill (*Papio sphinx*). J Vet Diagn Invest 14(4):326–328. https://doi.org/10.1177/2F104063870201400409

Clostridia: Botulinum-Neurotoxin (BoNT)-Producing Clostridia, *Clostridium perfringens*, and *Clostridioides difficile*

54

Ute Messelhäusser

Contents

Abstract

Clostridium spp., anaerobic spore-forming bacteria, are not commonly counted among the classical zoonotic agents. They are regularly found in the environment, e.g., in soil, dust, or sludge, and also in the intestine of healthy humans and animals without causing any symptoms. Botulism is a typical intoxication, normally caused by the ingestion of contaminated food or animal feeding stuff or in rare cases by the bacterial contamination of deep wounds. Based on the classical foodborne transmission route, the European Union classified botulism as a zoonotic intoxication. During the last few years, some scientists thought to have

A notorious and underestimated class of (potentially) zoonotic agents?

U. Messelhäusser (✉)
Bavarian Health and Food Safety Authority (LGL), Oberschleißheim, Germany
e-mail: ute.messelhaeusser@lgl.bayern.de

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_44

indications for a zoonotic transmission of an infective form of botulism, so-called chronic botulism. However, there is no resilient evidence for this theory. The transmission routes for *Clostridium (C.) perfringens* resemble those of Botulinum-neurotoxin (BoNT)-producing clostridia. Normally the ingestion of food highly contaminated with spores of *C. perfringens* causes a self-limiting watery diarrhea, whereas the contamination of deep wounds can lead to a clostridial myositis/myonecrosis. Even if *C. perfringens* normally do not rank among the classical zoonotic agents, at least the toxicoinfection via food fulfills the criteria of a zoonosis. An infection with *Clostridioides difficile* can result in heavy diarrhea in humans and is supposed to infect also animals, but the importance of a transmission via food or the classical way of zoonotic infection, the direct transmission between human and animal, is still unclear. However, scientific data suggest that the zoonotic potential of the organism might be higher than thought until now. Therefore, the contact with food or animals, carrying *Clostridioides difficile*, could be one source of human colonization known also from other antibiotic-resistant bacteria.

Keywords

BoNT · *Clostridium perfringens* · *Clostridiodes difficile*

54.1 Botulinum-Neurotoxin (BoNT)-Producing Clostridia

54.1.1 Botulinum-Neurotoxin (BoNT)-Producing Clostridia: The Organisms and the Toxins

Botulinum-neurotoxin (BoNT)-producing clostridia are a strictly anaerobic, spore-forming, rod-shaped, gram-positive bacteria mainly occurring in the soil of both terrestrial and aquatic environments. Spores of the organisms can also be found in the intestine of healthy humans and animals. The term "BoNT-producing clostridia" comprises four genetically and physiologically different groups of bacteria, which are connected through their ability to produce Botulinum-neurotoxin (BoNT). Therefore, for some years the scientific world does not speak of "*Clostridium botulinum*" any longer, but of "Botulinum-neurotoxin-producing clostridia." BoNT exists in seven different confirmed antigenic types (BoNT/A to BoNT/G), of which BoNT/E and BoNT/F are known to be toxic for humans, BoNT/C and BoNT/D mainly for different animals, and BoNT/A and BoNT/B for both animals and humans. The toxicity of BoNT/G is largely unknown so far. In 2013 a possible eighth antigenic type (BoNT/H) was described, but the terminology for this type is not yet consistent in scientific literature. In different reports the novel BoNT is referred to as "BoNT/H," "BoNT/FA," or BoNT/HA" (Peck et al. 2017). In addition at least the seven confirmed antigenic types are subdivided into a different amount of subtypes, e.g., BoNT/A in the subtypes BoNT/A1 to A8 and BoNT/B in the subtypes BoNT/B1 to B8 (Peck et al. 2017). This huge amount of genetic and

subsequently immunological variations of the BoNT complicates the development and use of in vitro diagnostics like ELISA-based methods. Therefore, the mouse bioassay is still the "gold standard" detecting BoNT in human samples especially serum.

BoNT/A, B, E, and F are simply constructed polypeptides, consisting of a 100 KDa "heavy" chain and a 50 kDa "light "chain linked through a disulfide bond. The light chain is a zinc-containing endopeptidase, which blocks the release of acetylcholine. This is the reason of the atonic paralysis, the classical symptom of BoNT intoxication. BoNT is one of the most lethal biological toxins; the lethal dose for a healthy adult person is as low as 0.1–1 μg.

The formation of neurotoxin occurs under strict anaerobic conditions after germination of the spores in the last growth phase and the toxin is released after lysis of the bacterium. Growth and toxin production can take place in nearly every food matrix or tissue except those with a low pH < 4.6 or low $a_w < 0.94$ (EFSA 2005). The temperature for optimal growth and toxin formation differs between the groups of BoNT-producing clostridia, and proteolytic strains are normally not able to grow and produce toxin below 10 °C. In contrast non-proteolytic strains, also called "psychotrophic" strains, can grow at temperatures as low as 3 °C (Graham et al. 1997). BoNT belongs to the group of heat-labile toxins; normal cooking temperatures can destroy (pre)formed toxin, but not the spores. Spores are safely eliminated using a temperature-time combination of 3 min at 121 °C for proteolytic strains or of 10 min at 90 °C for non-proteolytic strains (ACMSF 1992).

54.1.2 The Four "Classical" Forms of Botulism

54.1.2.1 Foodborne Botulism

Foodborne botulism is the most common form of botulism in animals and humans. Illness is caused by ingestion of food or animal feeding stuff like silage containing (pre)formed toxin. In both animals and humans the classical symptoms of botulism are atonic paralysis of extremities and at an advanced state respiratory paralysis. In humans intoxication manifests itself after 12–48 h first with the classical symptoms of a foodborne illness like nausea, vomiting, and acute abdominal pain followed by double vision, dysphagia, and atonic paralysis of extremities. Without early treatment (antitoxin therapy and artificial respiration, sometimes over months), botulism mostly leads to death. In cattle and horses the first symptoms are an atonic paralysis of the mastication muscles with prolapse of the tongue also followed by atonic paralysis of the extremities. Death occurs as a result of respiratory paralysis. Poultry and waterfowl show after an incubation time of a few hours to 3 days paralysis of the wings and weakness and death within 10 days; waterfowl drown with clear sensorium. Foodborne botulism can also be acquired by minks and ferrets. Dogs, cats, and pigs are much less susceptible to the toxin; foodborne botulism is rarely described in these animals.

Sources of human foodborne botulism are insufficiently heated, mostly homemade and canned foods, such as meat, sausages, and vegetables, or cured meat

products with low salt concentrations. In animals foodborne botulism mainly occurs in cases of deficient ensiling or of contamination of hay or silage with cadavers. Via cadavers the spores and the toxin can also reach the environment. Waterfowl is mostly poisoned in the summer months by ingestion of toxin-containing slurry.

54.1.2.2 Wound Botulism

In rare cases spores of BoNT-producing clostridia can contaminate deep wounds, e.g., through drug abuse or iatrogenic contamination. The spores can germinate under anaerobic conditions, grow, and produce the toxin directly in the tissue. Four to 14 days after the entry of the spores the affected person shows the classical symptoms of botulism as described above.

54.1.2.3 Infant Botulism (Infant Intestinal Toxemia Botulism)

Infant botulism is a toxicoinfection in children under 6 months and in rare cases also of older children up to 12 months of age. Spores of BoNT-producing clostridia are ingested with contaminated food, e.g., honey or infant formula, or from the environment. Due to a less developed and diversified intestinal microflora, the spores can colonize and germinate in the large intestine of the infant. The produced toxin leads to the classical botulism symptoms, which are preceded at first by constipation, weakness, and poor feeding. Later a typical atonic paralysis occurs. Fortunately, today the toxicoinfection leads only in very rare cases to the death of the patient. Normally, treated patients recover fully from infant botulism (Arnon 1995).

54.1.2.4 Infective Botulism (Adult and Toddler Intestinal Toxemia Botulism)

Approximately a dozen cases of intestinal toxemia botulism have been described in toddlers and adults so far. In most cases there are predisposing factors, which negatively affect the intestinal microflora and allow spores of BoNT-producing clostridia to colonize an adult intestine. Possible factors can be a longstanding treatment with broad-spectrum antibiotics and illnesses, which affect the intestinal anatomy and physiology, e.g., inflammatory bowel diseases, vagotomy, or considerable decreased intestinal motility (Arnon 1995). The patients develop the classical symptoms of botulism described above. Importantly, intestinal toxemia botulism without the typical atonic paralysis has not been described in the scientific literature so far.

In the veterinary literature a similar disease is reported, the equine grass sickness. The disease results in a partial or complete paralysis of the gastrointestinal tract and normally ends with the death of the horse (Hunter et al. 1999). However, a link between equine grass sickness and BoNT has not been scientifically verified until now. In recent times the scientific community assumed that equine grass sickness could be more likely a pasture mycotoxicosis than an infective botulism (McGorum et al. 2021).

54.1.2.5 "Chronic or Visceral Botulism"

Since the mid-1990s some veterinary research groups postulate – besides the classical forms of botulism – the existence of a chronic disease entity in cattle called "chronic" or "visceral botulism" (Böhnel et al. 2001). This disease is said to originate from the ingestion of spores of BoNT-producing clostridia from the feed or the environment. The path of infection and intoxication is compared by these research groups to the adult intestinal toxemia botulism; however, the chronic form of botulism would not lead to an acute and life-threatening intoxication, but to a chronic intake of minimal dose rates of BoNT. This chronic contamination is hypothesized to cause unspecific symptoms in the individual cow, e.g., apathy, indigestion, edemas, chronic lameness, and sudden unexpected death (Böhnel et al. 2001). On farms affected by this so-called "chronic botulism," the groups claim that the milk yield and the fertility rate decrease significantly (Krüger et al. 2014). Medical symptoms simultaneously and coincidentally found among a few farmers on affected farms such as weakness of the extremities and dysfunction of vegetative nerve system, e.g., pupillary motoric, were claimed by one medical research group to be linked to a chronic intake of BoNT (Dressler and Saberi 2009; Rodloff and Krüger 2012). The story of a new, emerging zoonotic disease, the "chronic" or "visceral botulism," which affects farmers, veterinarians, and cattle and can be transmitted, alternating between humans and animals, was born. Until today, more than 20 years later, scientifically sound evidence for the existence of a chronic form of botulism and also for the transmission of spores or vegetative cells of BoNT-producing clostridia between humans and animals in terms of a zoonosis is still missing. The discussion found an (preliminary) end with the results of two extensive scientific studies on dairy farms in two different regions in Germany neither detecting BoNT in fecal samples nor showing differences of shedding of spores of BoNT-producing clostridia between case and control animals (Seyboldt et al. 2015; Fohler et al. 2016; Dietsche et al. 2017).

54.1.2.6 Occurrence of Botulism Cases

In summary, botulism can be classified as a classical foodborne zoonosis transmitted via food from healthy animals, shedding spores of BoNT-producing clostridia (Rasetti-Escargueil et al. 2019). In contrast to other zoonotic illnesses, the transmission of the spores occurs only very rarely and in cases of subsequent toxin production in the contaminated food to a disease in humans. In Europe the confirmed case rate of human botulism constantly ranges between 0.02 and 0.03 cases per 100,000 population. The most frequent reported form of botulism in Europe is foodborne botulism; infant botulism and wound botulism are only seen in single cases (ECDC 2013). This main foodborne transmission route in Europe is one of the reasons why the European Union classified botulism as a bacterial zoonotic intoxication which has to be monitored in the different member states depending on the epidemiological situation according to Annex I B no. 1 of Directive 2003/99/EC (EU 2003).

In the USA the CDC reported for the year 2018 231 confirmed human botulism cases, of which 104 (70%) cases were due to infant botulism (CDC 2021).

54.2 *Clostridium perfringens*

54.2.1 *Clostridium perfringens*: Much More than a Bacterial Contamination of Food

Clostridium (C.) perfringens was first described by Welch and Nutall in 1892 and assigned to the genus *Bacillus*. After a change in nomenclature the bacterium was named after its discoverer "*Clostridium welchii*," until it get its present name "*Clostridium perfringens*." The name "*perfringens*" (Latin for "disrupt") is derived from one of the clinical pictures caused by *C. perfringens* so-called "gas gangrene." Concerning the morphology *C. perfringens* is similar to other clostridia, but in contrast to BoNT-producing clostridia, the organism is less susceptible against oxygen and can therefore survive and grow under not strictly anaerobic conditions.

According to the ability of producing four "major toxins" and a number of "minor toxins," the species *C. perfringens* is subdivided into different toxinotypes. In 2018 the old scheme of five toxinotypes A to E was revised and extended to seven toxinotypes A to G with six major toxins (Rood et al. 2018). The scheme comprises toxinotypes causing illness exclusively in different animals (B, E, and G), exclusively in humans (F), and in both humans and animals (A, C, D) (EFSA 2005). Relevant for humans is the new toxinotype F carrying the *cpa* gene (encoding the alpha toxin) and the *cpe* gene (encoding the enterotoxin) but also the toxinotypes A, C, and D. Strains of toxinotype F are responsible for foodborne toxicoinfections and antibiotic-associated diarrhea, while the toxinotypes C and D are associated mainly with necrotic enteritis. Toxinotype A carrying the *cpa* gene (encoding the alpha toxin) is responsible for the classical illness first associated with *C. perfringens* so-called "gas gangrene" (myonecrosis).

In veterinary diagnostics, it is distinguished between two different disease complexes caused by *C. perfringens*:

- Necrotizing enteritis in young animals (e.g., lamb dysentery [type B] or necrotizing enteritis in suckling piglets [type C])
- Enterotoxemia, e.g., also in older animals, after a sudden change in feed, overfeeding, and too little raw fiber ("protein disease")

In veterinary medicine, the term "enterotoxemia" refers to peracute diseases in which toxin formation takes place in the intestine, is absorbed, and enters the bloodstream. Furthermore, *C. perfringens*, along with other animal pathogens *Clostridium* spp., plays an important role in gas edema diseases and can cause necrotizing mastitis in ruminants. The symptoms and the development of the different diseases in animals are very similar in comparison to human diseases caused by *C. perfringens* described below (EFSA 2005).

54.2.1.1 Foodborne Toxicoinfections

The *C. perfringens* enterotoxin (CpE) is produced during sporulation mainly in the ileum but also in other parts of the intestine. The enterotoxin binds on the epithelial cells in the intestine, destroys the intestinal villi, and results in a desquamation of the

cell membranes. As a consequence, the adsorption of electrolytes and water from the intestine is blocked and this results in the predominant AAD symptom of a *C. perfringens* toxicoinfection, a watery diarrhea. Besides *C. perfringens* type F also strains of *C. perfringens* type C and D can carry the *cpe* gene that is responsible for the production of CpE.

C. perfringens can grow under completely anaerobic conditions, e.g., in oxygen-free canned food but also with a rest amount of oxygen at the bottom of big pots of stew and soups (preferable with meat), and cause the so-called "toxicoinfections." In contrast to an intoxication, the bacterial pathogens produce in this case the toxin not directly in the food but after sporulation in the human intestine. *C. perfringens* toxicoinfections occur mainly in (mass) catering facilities, in canteens, or on buffets, where food is kept warm over a longer period under inadequate temperature conditions. Mostly self-limiting symptoms of a foodborne *C. perfringens* toxicoinfection, especially watery diarrhea, arise after 8–24 h and last not more than 24–48 h.

54.2.1.2 Sporadic and Antibiotic-Associated Diarrhea

Besides foodborne toxicoinfection, *C. perfringens* type F can also cause sporadic and antibiotic-associated diarrhea (AAD) similar to *Clostridioides difficile*. A worldwide systematic review showed that in AAD cases the prevalence of *C. perfringens* was 14.9% behind *Clostridioides difficile* (19.6%) and *Klebsiella oxytoca* (27.0%) (Motamedi et al. 2021). A necessary precondition for developing an antibiotic-associated diarrhea caused by *C. perfringens* type F is an intensive treatment with broad-spectrum antibiotics. This can lead to the destruction of the normal intestinal flora and an uncontrolled growth and toxin productions of *C. perfringens* type F in the intestine.

54.2.1.3 Enteritis Necroticans

Between 1946 and 1948 a new epidemic disease was observed in different cities in the northern part of Germany. Because of progress and symptoms, the disease was called "enteritis necroticans." Researchers described the disease in the way that healthy adults developed, few hours after consumption of canned meat, game or fish abdominal pain and diarrhea. In some cases symptoms were slight and self-limiting, but in other cases the patients developed bloody diarrhea with serious abdominal pain and died of dehydration and cardiovascular failure or of intestinal obstruction (Hobbs et al. 1953; Kreft et al. 2000). In fecal samples of patients and at autopsies, huge amounts of *C. perfringens* type C with the ability to produce beta toxin were detected. Additionally the spores of the isolates were characterized by a very high heat resistance. Therefore, the spores survived the heat treatment of the preservation process. Meanwhile enteritis necroticans is very rare in Europe, but in crisis areas, it can occur again at any time because one of the predisposing factors responsible for the occurrence of this disease is malnutrition. Malnutrition can lead i.a. to an inadequate production of trypsin, which enables the effect of the beta toxin formed in the intestine.

54.2.1.4 Gas Gangrene

The classical illness first associated with *C. perfringens* was not diarrhea but myonecrosis so-called "gas gangrene." The cause of gas gangrene is

C. perfringens spores from the environment getting into deep wounds, where the spores germinate and multiply in this anaerobic environment. According to the current state of science, the symptoms underlying such an infection process can essentially be assigned to two pathogenicity factors formed by *C. perfringens* type A, alpha and theta toxin. The alpha toxin (phospholipase C), a lecithinase, splits the lecithin present in the membrane into phosphorylcholine and diacylglycerol. Theta toxin (perfringolysin O) is a cytolysin that leads to complete hemolysis of red blood cells (Harris et al. 1991). Like most anaerobes, *C. perfringens* is one of the gas-forming microorganisms. When the wound is palpated, gas formation can sometimes be perceived as a characteristic "crackling." Without treatment the patient may develop septic shock and dies within hours due to multiorgan failure (Di Fazio et al. 2022).

54.2.2 *Clostridium perfringens*: Only an Environmental Contamination or a Zoonotic Agent?

C. perfringens exists, like other clostridia, in a form of spores ubiquitous in the environment and can be found in higher amounts in soil and dust. Therefore, it can be assumed that *C. perfringens* spores are part of the normal flora in soil. Furthermore *C. perfringens* is found regularly in feces of healthy humans and animals in higher concentrations (around 10^4–10^6 cfu/g feces). Considering *C. perfringens* as a normal part of the intestinal flora, human fecal samples are not routinely tested for *C. perfringens* in case of diarrhea or other intestinal illnesses. So *C. perfringens* is not counted to the obligate pathogenic organisms, but depending on the subtype or strain-specific ability to produce different major or minor toxins, it has the potential to cause the entire spectrum from mild self-limiting to severe life-threatening diseases. As a normal inhabitant of the human or animal intestine *C. perfringens* can be transmitted directly or indirectly (via food) between humans and animals causing under special circumstances also illnesses. Therefore, *C. perfringens* meet, like the other (potentially) pathogenic clostridia, all criteria of a (bacterial) zoonotic agent as defined by the European Union in Directive 2003/99/EC (EU 2003).

54.3 *Clostridioides difficile*

54.3.1 *Clostridioides difficile*: The Causative Agent of Nosocomial Pseudomembranous Colitis in Humans

Clostridioides (C.) difficile is also a gram-positive, rod-sharped, strictly anaerobic bacterium, which was first described as "*Clostridium difficile*" by Hall and O'Toole in 1935 as part of the microbial intestinal flora of infants (Hall and O'Toole 1935). The species was assigned to the new genus "*Clostridioides*," which means "similar to clostridia," and renamed in August 2016 based on 16S rRNA gene sequence

analysis (Lawson et al. 2016). *C. difficile* is counted to the group of facultative pathogenic bacteria, because it can be detected also in the intestine of healthy persons without any characteristic symptoms. Predominantly the organism causes nosocomial disease linked to antibiotic treatment; however, there are also community-acquired *C. difficile* infections without any correlation to hospitalization. The pathogenicity of *C. difficile* is based on the ability to produce different toxins, an enterotoxin A, a cytotoxin B, and in some strains (ribotype 027) also a binary toxin, which is linked to more severe symptoms (Schroeder 2005).

Usually a *C. difficile* infection (CDI) develops after an intensive therapy with broad-spectrum antibiotics, which destroy the normal microbial intestinal flora. In such a situation *C. difficile* spores can colonize the intestine, germinate, and overgrow the remaining normal microbial flora. The severity of the symptoms depends on the immune status of the patient and can vary from mild diarrhea to a pseudomembranous colitis with a case fatality rate of 6–30%. For a long period it was assumed that most patients who develop severe symptoms like a pseudomembranous colitis are infected in the hospital and that only a small part carries the causative organism already before hospitalization (Schroeder 2005). However, recent studies showed that by using, e.g., whole-genome sequencing, according to genetic correlation only a substantially low number of isolates suggested a hospital-acquired transmission. In a high number of cases alternative environmental reservoirs besides the hospital have to be taken into account (Eyre et al. 2013; Lim et al. 2021). Therefore, it can be assumed that *C. difficile* is a zoonotic agent, which also can be transmitted via animals, food, or the environment to humans. However, these new findings are not in contradiction with the fact that hygienic deficiencies in hospitals and healthcare facilities can also cause transmission of *C. difficile* between patients and lead to outbreaks with a major number of deaths, especially if hypervirulent strains of increasing importance such as ribotype 027 are involved.

54.3.2 *C. difficile* in Animals, Food, and the Environment

Like different potentially pathogenic clostridia, e.g., BoNT-producing clostridia and *C. perfringens*, *C. difficile* is ubiquitous in the environment and can be found more or less frequently in soil, dust, and water but also in compost and biogas plants (Fröschle et al. 2015; Le Maréchal et al. 2020; Rodriguez Diaz et al. 2018). The exposure of *C. difficile* in the environment arises most probably from animal (and human) feces, because the mammalian gastrointestinal tract is known as the primary reservoir of *C. difficile*. Similar to *C. perfringens* the *C. difficile* strains can colonize the mammalian gastrointestinal tract without causing any symptoms (Knight and Riley 2019).

The prevalence of *C. difficile* in farm animals differs depending on the age of the animals investigated, but also on the geographic region/country and relating thereto the different veterinary and agricultural practices (Knight and Riley 2019). In general the prevalence of *C. difficile* decreases with the age of the animals. In calves, for example, detection rates up to 56.4% are reported, whereas the detection rates in

cows vary between 0.6% and 12.9% (Knight and Riley 2019). In piglets and pigs the prevalence seems to be in general higher, rising up to 100% (Lim et al. 2019). The relevance of *C. difficile* as a pathogenic agent in animals in general is not completely clarified until now. It is known that *C. difficile* can be isolated from feces of foals, piglets, dogs, or cats with clinical signs of diarrhea (Schneeberg et al. 2013; Songer 2010), but also from healthy animals (Rodriguez-Palacios et al. 2013). It seems that *C. difficile* cause clinical signs of disease mostly in young animals; in older ones a colonization without any clinical signs (like a commensal) is reported. This is also known from other zoonotic agents and from potentially pathogenic clostridia like *C. perfringens*.

Via farm animals *C. difficile* is also entering the food chain and can be detected in different processed and non-processed food of animal origin (de Boer et al. 2011). But also in food of non-animal origin *C. difficile* is found regularly with remarkable differences in the detection rates in Europe and North America (3–8%) on one side and Australia (20–56%) on the other side (Knight and Riley 2019).

54.3.3 *C. difficile*: A Prime Example for the One Health Approach

Summarizing the current state of scientific knowledge, the zoonotic potential of *C. difficile* is unquestionable and there is no point of discussion any longer. Normally an indirect zoonotic transmission between animals and humans via food or the environment can be assumed, but also a direct zoonotic transmission between pigs and pig farmer is reported (Knetsch et al. 2014, 2018). Against the background of an increasing number of community-acquired *C. difficile* infections in healthy people without the classical risk factors for CDI (e.g., old age, prolonged hospital stay, a recent surgical procedure) and the ubiquitous occurrence of *C. difficile*, a One Health approach for long-term management of this zoonosis should be taken into account (Lim et al., 2019). Based on the ubiquitous nature of this spore-performing organism, it seems to be impossible to prevent humans getting in contact with *C. difficile*. However, for normal immunocompetent persons this contact can lead to an asymptomatic colonization, but usually not to a symptomatic infection. Therefore, it is important to investigate more in depth the predisposing factors for community-acquired CDI, especially the question what has to be happened to change the status of *C. difficile* in the mammalian gastrointestinal tract from "commensal" (colonization) to "pathogen" (infection)? (Lim et al., 2019). Active sentinel surveillance in the State of New York over a period of 6 months showed, for example, that 76% of patients with a community-acquired CDI had used antimicrobial drugs within 12 weeks before infection. Strong evidence that certain kinds of food or animal exposure were involved was not found (Dumyati et al. 2012). But the use of antimicrobial substances cannot be the only major risk factor for a community-acquired CDI. Therefore, in future it is necessary to identify further predisposing factors to develop prevention strategies especially for severe and life-threatening progressions of disease not only in intensive care or healthcare units with critically ill persons but also in the community.

References

Advisory Committee on the Microbiological Safety of Food (ACMSF) (1992) Report on vacuum packaging and associated processes. HMSO, London

Arnon S (1995) Chapter 19: botulism as an intestinal toxemia. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL (eds) Infection of the gastrointestinal tract. Raven Press, New York, pp 257–271

Böhnel H, Schwagerick B, Gessler F (2001) Visceral botulism--a new form of bovine *Clostridium botulinum* toxication. J Vet Med A Physiol Pathol Clin Med 48(6):373–383

Centers for Disease Control and Prevention (CDC) (2021) Botulism annual summary, 2018. U.S. Department of Health and Human Services, CDC, Atlanta, p 2021

de Boer E, Zwartkruis-Nahuis A, Heuvelink AE, Harmanus C, Kuijper EJ (2011) Prevalence of *Clostridium difficile* in retailed meat in the Netherlands. Int J Food Microbiol 144(3):561–564

Di Fazio N, Baldari B, Del Fante Z, De Simone S, Aromatario M, Cipolloni L (2022) Gas gangrene, not a common infection: case report of post-traumatic fulminant death. Clin Ter 173(1):1–5. https://doi.org/10.7417/CT.2022.2382

Dietsche J, Metzner M, Messelhäußer U, Mansfeld R, Sauter-Louis C, Hörmansdorfer S, Hoedemaker M, Dorner M, Knubben-Schweizer G (2017) Significance of potentially toxinogenic *Clostridium* spp with regard to herd health problems in Bavarian dairy herds. Berl Munch Tierarztl Wochenschr 130. https://doi.org/10.2376/0005-9366-16078

Dressler D, Saberi FA (2009) Botulinum Toxin: vom Medikament zum Toxin . . . . Fortschr Neurol Psychiat 77(Suppl 1):549–554

Dumyati G, Stevens V, Hannett GE, Thompson AD, Long C, Maccannell D, Limbago B (2012) Community-associated *Clostridium difficile* infections, Monroe County, New York, USA. Emerg Infect Dis 18(3):392–400. https://doi.org/10.3201/eid1803.102023

European Centre for Disease Prevention and Control (ECDC) (2013) Disease data from ECDC Surveillance Atlas – botulism. https://www.ecdc.europa.eu/en/botulism/surveillance/atlas. Last accessed 06 Mar 2022

European Food Safety Authority (EFSA) (2005) Opinion of the scientific panel on biological hazards on a request from the Commission related to *Clostridium* spp in foodstuffs. EFSA J 199:1–65

European Union (2003) Directive 2003/99/EC of the European Parliament and of the Council on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EE

Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CLC, Golubchik T, Batty EM, Finney JM, Wyllie DH, Didelot X, Piazza P, Bowden R, Dingle KE, Harding RM, Crook DW, Wilcox MH, Peto TEA, Walker AS (2013) Diverse sources of *C. difficile* infection identified on whole-genome sequencing. N Engl J Med 369(13):1195–1205. https://doi.org/10.1056/NEJMoa1216064

Fohler S, Discher S, Jordan E, Seyboldt C, Klein G, Neubauer H, Hoedemaker M, Scheu T, Campe A, Charlotte Jensen K, Abdulmawjood A (2016) Detection of *Clostridium botulinum* neurotoxin genes (A-F) in dairy farms from Northern Germany using PCR: a case-control study. Anaerobe 97-104:97. https://doi.org/10.1016/j.anaerobe.2016.03.008

Fröschle B, Messelhäusser U, Höller C, Lebuhn M (2015) Fate of *Clostridium botulinum* and incidence of pathogenic clostridia in biogas processes. J Appl Microbiol 119(4):936–947. https://doi.org/10.1111/jam.12909

Graham AF, Mason DR, Maxwell FJ, Peck MW (1997) Effect of pH and NaCl on growth from spores of non-proteolytic *Clostridium botulinum* at chill temperature. Lett Appl Microbiol 24: 95–100

Hall IC, O'Toole E (1935) Intestinal flora in newborn infants with a description of a new pathogenic anaerobe *Bacillus difficilis*. Am J Dis Child 49:390

Harris RW, Sims PJ, Tweten RK (1991) Evidence that *Clostridium perfringens* theta-toxin induces colloid-osmotic lysis of erythrocytes. Infect Immun 59(7):2499–2501. https://doi.org/10.1128/iai.59.7.2499-2501

Hobbs BC, Smith ME, Oakley CL, Warrack GH, Cruickshanks JC (1953) *Clostridium welchii* food poisoning. J Hyg (Lond) 51(1):75–101. https://doi.org/10.1017/s0022172400015515

Hunter LC, Miller JK, Poxton IR (1999) The association of *Clostridium botulinum* type C with equine grass sickness: a toxicoinfection? Equine Vet J 31(6):492–499

Knetsch CW, Connor TR, Mutreja A, van Dorp SM, Sanders IM, Browne HP, Harris D, Lipman L, Keessen EC, Corver J, Kuijper EJ, Lawley TD (2014) Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011. Eur Secur 19:20954. https://doi.org/10.2807/1560-7917.ES2014.19.45.20954

Knetsch CW, Kumar N, Forster SC, Connor TR, Browne HP, Harmanus C, Sanders IM, Harris SR, Turner L, Morris T, Perry M, Miyajima F, Roberts P, Pirmohamed M, Songer JG, Weese JS, Indra A, Corver J, Rupnik M, Wren BW, Riley TV, Kuijper EJ, Lawley TD (2018) Zoonotic transfer of *Clostridium difficile* harboring antimicrobial resistance between farm animals and humans. J Clin Microbiol 56(3):e01384–e01317. https://doi.org/10.1128/JCM.01384-17

Knight DR, Riley TV (2019) Genomic delineation of zoonotic origins of *Clostridium difficile*. Front Public Health 7:164. https://doi.org/10.3389/fpubh.2019.00164

Kreft B, Dalhoff K, Sack K (2000) Necrotizing enterocolitis: a historical and current review. Med Klin (Munich) 95(8):435–441. https://doi.org/10.1007/s000630050003

Krüger M, Neuhaus J, Herrenthey AG, Gökce MM, Schrödl W, Shehata AA (2014) Chronic botulism in a Saxony dairy farm: sources, predisposing factors, development of the disease and treatment possibilities. Anaerobe:220–225. https://doi.org/10.1016/j.anaerobe.2014.06.010

Lawson PA, Citron DM, Tyrrell KL, Finegold SM (2016) Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prévot 1938. Anaerobe 40:95–9. https://doi.org/10.1016/j.anaerobe.2016.06.008.

Le Maréchal C, Gateau C, Poezevara T, Couturier J, Rouxel S, Syed Zaidi R, Houard E, Pourcher AM, Denis M, Barbut F (2020) Characterization of *Clostridioides difficile* strains isolated from manure and digestate in five agricultural biogas plants. Anaerobe 62:102180. https://doi.org/10.1016/j.anaerobe.2020.102180

Lim SC, Knight DR, Riley TV (2019) *Clostridium difficile* and one health. Clin Microbiol Infect 26 (7):857–863. https://doi.org/10.1016/j.cmi.2019.10.023

Lim SC, Collins DA, Imwattana K, Knight DR, Perumalsamy S, Hain-Saunders NMR, Putsathit P, Speers D, Riley TV (2021) Whole-genome sequencing links *Clostridium (Clostridioides) difficile* in a single hospital to diverse environmental sources in the community. J Appl Microbiol 11:1156. https://doi.org/10.1111/jam.15408

McGorum BC, Chen Z, Glendinning L, Gweon HS, Hunt L, Ivens A, Keen JA, Pirie RS, Taylor J, Wilkinson T, McLachlan G (2021) Equine grass sickness (a multiple systems neuropathy) is associated with alterations in the gastrointestinal mycobiome. Anim Microbiome 3(1):70. https://doi.org/10.1186/s42523-021-00131-2

Motamedi H, Fathollahi M, Abiri R, Kadivarian S, Rostamian M, Alvandi A (2021) A worldwide systematic review and meta-analysis of bacteria related to antibiotic-associated diarrhea in hospitalized patients. PLoS One 16(12):e0260667. https://doi.org/10.1371/journal.pone.0260667. eCollection 2021

Peck MW, Smith TJ, Anniballi F, Austin JW, Bano L, Bradshaw M, Cuervo P, Cheng LW, Derman Y, Dorner BG, Fisher A, Hill KK, Kalb SR, Korkeala H, Lindström M, Lista F, Lúquez C, Mazuet C, Pirazzini M, Popoff MR, Rossetto O, Rummel A, Sesardic D, Singh BR, Stringer SC (2017) Historical perspectives and guidelines for botulinum neurotoxin subtype nomenclature. Toxins (Basel) 9(1):38. https://doi.org/10.3390/toxins9010038

Rasetti-Escargueil C, Lemichez E, Popoff MR (2019) Public health risk associated with botulism as foodborne zoonoses. Toxins (Basel) 12(1):17. https://doi.org/10.3390/toxins12010017

Rodloff AC, Krüger M (2012) Chronic *Clostridium botulinum* infections in farmers. Anaerobe 18 (2):226–228

Rodriguez Diaz C, Seyboldt C, Rupnik M (2018) Non-human *C. difficile* reservoirs and sources: animals, food, experiment. Adv Exp Med Biol 1050:227–243. https://doi.org/10.1007/978-3-319-72799-8_13

Rodriguez-Palacios A, Borgmann S, Kline TR, Lejeune JT (2013) *Clostridium difficile* in foods and animals: history and measures to reduce exposure. Anim Health Res Rev 16:1–19

Rood JI, Adams V, Lacey J, Lyras D, McClane BA, Melville SB, Moore RJ, Popoff MR, Sarker MR, Songer JG, Uzal FA, Van Immerseel F (2018) Expansion of the *Clostridium perfringens* toxin-based typing scheme. Anaerobe 53:5–10. https://doi.org/10.1016/j.anaerobe.2018.04.011

Schneeberg A, Neubauer H, Schmoock G, Grossmann E, Seyboldt C (2013) Presence of *Clostridium difficile* PCR ribotype clusters related to 033, 078 and 045 in diarrhoeic calves in Germany. J Med Microbiol 62(Pt 8):1190–1198. https://doi.org/10.1099/jmm.0.056473-0

Schroeder MS (2005) *Clostridium difficile*--associated diarrhea. Am Fam Physician 71(5):921–928

Seyboldt C, Discher S, Jordan E, Neubauer H, Jensen KC, Campe A, Kreienbrock L, Scheu T, Wichern A, Gundling F, DoDuc P, Fohler S, Abdulmawjood A, Klein G, Hoedemaker M (2015) Occurrence of *Clostridium botulinum* neurotoxin in chronic disease of dairy cows. Vet Microbiol 177(3–4):398–402. https://doi.org/10.1016/j.vetmic.2015.03.012

Songer JG (2010) Clostridia as agents of zoonotic disease. Vet Microbiol 140(3–4):399–404

Part XI

Economic and Ecological Aspects of Zoonoses

Burden of Zoonoses

55

Making Sense and Acting on the Socio-economic Impact of Zoonoses in Our Food Systems

Sara Babo Martins, Peregrine Rothman-Ostrow, Grace Patterson, Barbara Häsler, and Jonathan Rushton

Contents

Abstract

Livestock food systems are changing rapidly with increasingly complex, dynamic, and globally connected production, harvesting, distribution, and consumption patterns. These changes carry new challenges from zoonotic diseases,

S. Babo Martins
Faculty of Medicine, Institute of Global Health, University of Geneva, Geneva, Switzerland
e-mail: Sara.BaboMartins@unige.ch

P. Rothman-Ostrow · J. Rushton (✉)
Department of Livestock and One Health, Institute of Infection Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK
e-mail: Peregrine.Rothman-Ostrow@liverpool.ac.uk; jrushton@liverpool.ac.uk

G. Patterson
Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

B. Häsler
Veterinary Epidemiology, Economics and Public Health Group, Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, UK
e-mail: bhaesler@rvc.ac.uk

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_45

which require an understanding of the socio-economic impact to develop, prioritize, and advocate for proportionate mitigation actions.

A full assessment of the socio-economic impacts of zoonotic diseases from a societal perspective can be challenging. Recognizing pathways for burden and loss and selecting appropriate tools, frameworks and data, form the foundation for that assessment. The value-add of this investment in understanding zoonotic impacts is the ability to recognize where the balance lies between costs of mitigation actions and avoidance of losses due to ill-health and poor animal welfare. This balance may equate to a wider economic efficiency view, which includes investments in research, education, and coordination that allows people and livestock keepers improved access to zoonotic disease mitigation options.

This chapter presents the case for better use of socio-economic impact assessments to improve our understanding of the burden and subsequent control of zoonoses.

Keywords

Zoonoses · Economic assessment · Burden of disease · Food systems · Impact

55.1 Introduction

The initial successes in the control of infectious diseases in animals were the control and eradication of rinderpest and contagious bovine pleuropneumonia in Europe in the late 1800s (Fisher 1998). The distribution of livestock diseases began to change more rapidly in the 1960s and 1970s as European and North American countries and Japan began to make serious inroads into the control of a range of both transboundary and endemic animal diseases. This was achieved through significant investments in human skills, building on previous investments in veterinary organizations, education, and infrastructure from the mid-nineteenth century onwards. With the establishment of the World Organisation for Animal Health (WOAH, formerly OIE) in 1924, an important foundation was created for standard setting in animal health. Moreover, WOAH helped to build capacity for national veterinary services with their Performance of Veterinary Services (PVS) Pathway.

In human health, attempts to manage diseases affecting societies appear to have been in place in Europe as early as the 1500s and addressed the problems caused by the plague (Harrison 2004). The period of enlightenment expanded the knowledge of causal agents of disease (Hays 2009), and there were breakthroughs in the control of diseases such as smallpox with the use of vaccines, ultimately leading to the official eradication of the disease in 1979 (Harrison 2004). In terms of overall service delivery, much work has gone into the management of human health, with most countries recognizing the need for coordinated health services. This culminated in the recognition of the need for the International Health Regulations in 2005. These initiatives have been prompted largely by disease shocks and the core contagious diseases that spread between humans.

While both animal and human health sectors have strengthened their capacity to tackle major diseases, the use of collaborative approaches across sectors in the prevention and control of zoonoses using a One Health perspective has also been expanding. At the heart of long-standing policies and research approaches for endemic zoonotic and foodborne diseases, the recognition of One Health has been accelerated by its central place in global health efforts to tackle antimicrobial resistance (AMR) and by recent crises linked to emerging pathogens, most notably avian influenza virus, Middle East respiratory syndrome–related coronavirus (MERS), and the COVID-19 pandemic. Also driving the recognition of One Health is a better understanding of the burden of zoonoses and other health issues arising at the interface of the health of animals, humans, and the ecosystems they share. A systems thinking approach, such as that expounded by the One Health concept, allows for the recognition and capture of disease impacts across sectors and fosters an umbrella under which multiple species' health and welfare can be monitored in the context of disease, control programs, and overall influence on food systems.

The heightened awareness of problems with salmonella in the 1980s, followed by the emergence of bovine spongiform encephalopathy (BSE), drew attention towards the insidious nature of zoonoses in the food system. A rise in challenges surrounding the management of highly pathogenic avian influenza (HPAI) in poultry systems, the re-emergence of the pandemic form of H1N1 from pigs, and a range of coronaviruses associated with wild and domesticated animals further brought zoonoses into stark focus. Concurrent to these major zoonotic disease threats, changing patterns in foodborne diseases have driven a change in food security and investment, with campylobacter being a pathogen of greatest significance. These disease shifts reflect the increasing complexity of our food systems and have led to investments in rigorous and organized programs that employ epidemiology and economics research to assist in decision-making. Similar to the disease and response processes of the major diseases in animals and humans, major foodborne diseases have created a need to invest and further investigate the impact of zoonoses in global food systems.

The economic impact of zoonoses in food systems is dependent on epidemiological parameters such as incidence, mortality, and morbidity effects in animals, the effects on human health and welfare, and the efforts to respond to the disease across relevant species groupings. Further impacts include food security, nutritional availability, and environmental management (Häsler et al. 2017; Rushton et al. 2018), while knock-on impacts due to food scares, trade restrictions, labor shortages, and value chain bottlenecks can drive significant negative effects on food security both in the short and long term (Chapot et al. 2021). We review how zoonoses impact food system sectors in the context of an increasingly complex value chain, address the economic concepts behind the balance between losses due to direct costs of disease and expenditures in reaction to disease presence, and identify possible economic tools and frameworks to assess the burden of zoonoses and the value of interventions to tackle them.

55.2 Context

The increasing complexity of livestock production systems and their associated value chains is underpinned by changes in political and institutional environments. From the late 1940s to the 1970s, state action was accepted as pivotal in economic and agricultural development. However, during the 1980s, there was a change in thinking that positioned the market as the primary way to organize economic activity, supported by a minimal role for the state. Different elements of these changes in public policy have had varying impacts on zoonoses management and public health in general. The 1970s saw the emergence of a perceived problem of government and its role in society as a major organizer and contributor to society. The 1980s saw the rise in deregulation of economic activity and the privatization of state-owned companies and services. This change also applied to veterinary services in many countries and was particularly contentious in Africa (Leonard 2000, 2004). Pressure on public budgets and an increasing role for the private sector saw the privatization of many former governmental veterinary services and activities. The public health systems suffered less, but zoonoses control often fell between weakened veterinary systems and relatively powerful human health institutions and agencies with a focus on diseases and health problems that largely affect people.

In addition to the changes in the public funding of health infrastructure, global food systems have evolved in response to growth and change in demand and dietary preferences, creating more difficulties in the management of pathogens.

55.2.1 Changing Context of the Food Systems That Feed Us

Over a period of around 200 years, the world has moved from relatively simple livestock value chains to increasingly complex ones. The pace of this change has accelerated in the livestock sector with the increasing use of intensive systems where animals are housed and fed defined diets and no longer allowed to scavenge or graze. In the case of poultry, this has been particularly dramatic. Scavenge-based systems for poultry production were common and used mainly local resources, with the household consumption of products and infrequent sale to local markets (see Fig. 1). The presence of these systems in a local environment allowed people to observe the

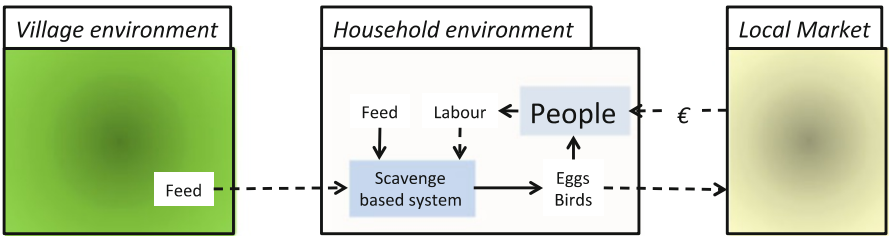

Fig. 1 Scavenge-based poultry system

health of the animals, and it is also probable that they witnessed the slaughter of the animals. The information on production and processing allowed people to make individual judgments of how best to prepare and eat their food while also limiting the distance of the value chain.

In the complex food value chains that are now dominant in many parts of the world, primary production has complex relationships with consumers through processing and marketing companies. The links in the chain are maintained by middlemen, transport companies, and finance groups. Where the value chains become integrated, that is, owned and controlled by one company, the middlemen disappear. In addition, consumer demands have become more sophisticated, with demand rising for processed food and an expectation to purchase foods with zero risk of food-borne diseases (Rushton 2009a). For the intensive poultry systems that are increasingly dominant in the provision of meat across the world, the system is global. Day-old birds and feed are produced in different parts of the world, and the fattening of birds and their slaughter take place some distance from the families that ultimately consume the meat (see Fig. 2). These systems do not allow an individual to gain much direct insight into, or information on, the origin of the meat eaten. Instead, the consumer is given that information through labeling and/or systems of trust on the quality and food safety of the product, for example, through having trusted relationships with a supplier.

The changes in the livestock food systems have been gradual, though there have been jumps associated with major technological changes. These have come at different points in the food system. For example, the ability to freeze meat allowed the slaughter and transport of carcasses from distant places to points of consumption. These changes have also been stimulated by the social and economic shifts in society, the growth of human populations, and the greater proportion of people found in urban, rather than rural areas (Wahba Tadros et al. 2021). The urban-based populations require food to be produced and processed for them, driving food systems globally to become more technologically and economically efficient to provide relatively cheap food in comparison to other goods in society. Overall, this has driven a reduction in the need for labor in primary

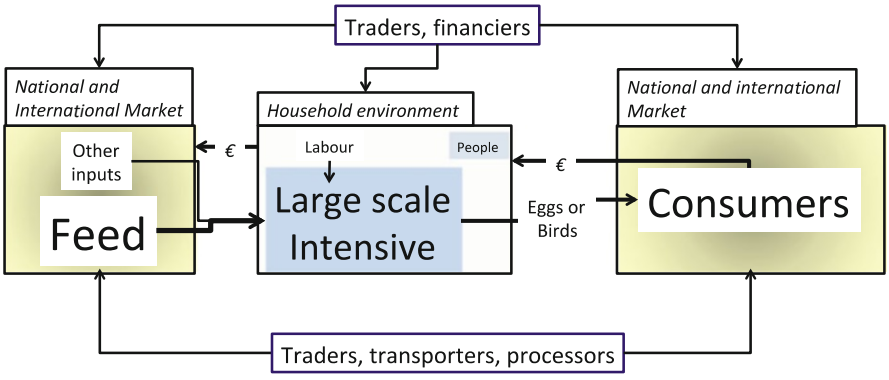

Fig. 2 A schematic diagram of the complex intensive poultry food systems (Rushton 2009a)

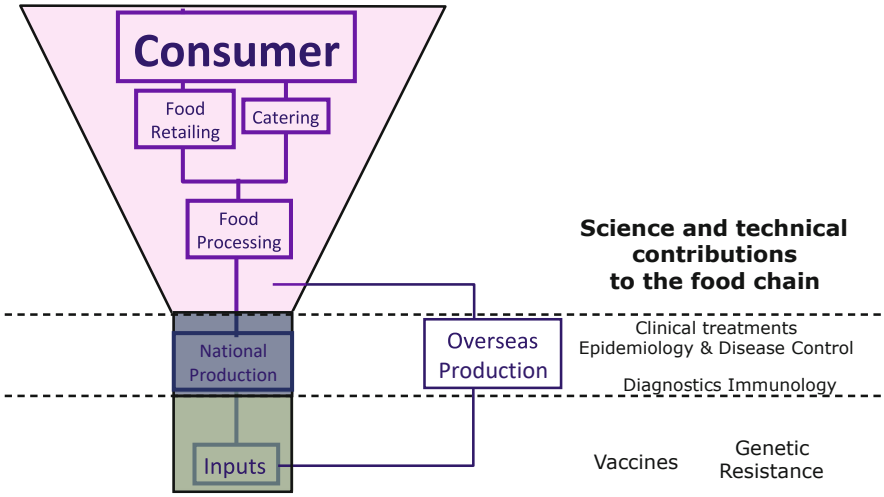

Fig. 3 A schematic diagram of the food systems (Rushton et al. 2012)

production. In the case of livestock, this has meant that animals are managed in larger herds or flocks with a greater animal to human ratio in stewardship. These changes have engendered a shift in the economic activity of the food system, which is now predominantly found in the processing, catering, and retailing areas where food preparation that would have traditionally been done in the home is now carried out by third parties. The shape of the food system in many countries worldwide in terms of numbers of people is therefore like a pyramid with a large number of consumers being supplied with animal source proteins produced by fewer and fewer farmers who are selling to complex food processing, retailing, and catering companies (see Fig. 3). There are still countries where very many farmers supply food to the system (e.g., dairy producers in India), but the trend overall, in particularly in higher income economies, is one of fewer farmers supplying to an increasingly large consumer base.

In the UK, out of the estimated 4.3 million people employed in the agri-food sector in 2018, it is estimated that only 11% were engaged directly in farming. While processing and manufacturing employees accounted for about the same number of employees as farming, most people in the food system were employed in retailing and hospitality. The UK agri-food sector contributed £121 billion or 9.4% to the National Gross Value Added (GVA) in 2018 (Hasnain et al. 2020). This food system relies on imports from other countries and feeds, daily, more than 67 million people. In short, never have so many been fed by so few. While primary production still employs a majority of people in many African and several Asian and Latin American nations (World Bank 2021), similar patterns of consolidation limit the economic impact and international reach of the majority of small-scale producers.

The length, breadth, and density of current food system value chains, and the additional complexity brought on by the resulting increase in number of people and links in the supply chain, suggests that the presence of infectious diseases, including

zoonoses, will have wider and more far-reaching impacts on a greater number and variety of stakeholders, sectors, and people. This alone brings new and complex challenges to disease control. Aside from controlling disease within food production systems, lengthy international value chains are also at risk of disruption from external major crises, including disease outbreaks. A recent example of this is given by the early response to the COVID-19 pandemic-imposed travel restrictions limiting the movement of people and goods between countries (Patterson et al. 2020). These restrictions limited travel for migrant field workers, international shipment of foods and inputs such as livestock feeds, and consumer access to goods. In places reliant on international trade for agricultural inputs, production was slowed or halted. For example, the day-old-chicks, feed, feed additives, and vaccines required as imported inputs in the commercial Ethiopian poultry sector were inaccessible during the early months of the COVID-19 pandemic, causing massive disruption across poultry production systems (Ethiopia-Netherlands Trade for Agricultural Growth, 2020). A telephone survey of commercial poultry producers and stakeholders in Ethiopia found that, in the first few months of the the pandemic in 2020 production rates fell by 92% for feed, 68% for day-old chicks, 100% for pullets, and 87% for broilers totalling losses of ETB 226,485,788 (approximately USD $ 5,186,072). production of feed, day old chicks, pullets, and broilers each fell more than 60%, resulting in significant economic losses for the surveyed producers. Globally, chicken meat systems were disrupted, because of their complex interconnection and dependencies and the ripple effects in the system stemming from disruptions in the hospitality sector, movement restrictions, closure of markets, and negative behaviors such as panic buying or avoidance of products due to food scares (Chapot et al. 2021).

Reduction in the overall number of primary producers and increases in food processing, catering, and retail sectors have also led to an emphasis on quantity over quality to feed a growing global population and keep the price of food low. Fewer international producers are taking up larger portions of the global market share in grains and livestock (Howard 2017). Several transnational corporations wield strong industry influence yet have minimal societal name recognition (Swinburn et al. 2019). Reduction in genetic diversity of livestock breeds and crops has reduced the resilience of plants and livestock to disease (Bennett et al. 2018; Khoury et al. 2014), and in some cases, modern, high yield crop strains have lost the high micronutrient content found in wild progenitor strains (Velu et al. 2019). Researchers are now working to retain and rebuild that lost diversity to maintain average productivity while improving resilience to threats wrought by climate change (drought, flood, and shifting patterns of disease) (Wolfe and Ceccarelli 2020). Modern food systems are often increasingly inequitable on an international scale. Global food demands from countries with greater economic influence can drive economic and environmental externalities related to unsustainable modes of food production that fall disproportionately on communities with less power in the global market (Gonzalez 2010).

The background of food system change is a necessary component of a socio-economic assessment of zoonotic diseases. The food system dictates the costs of production and the price of food, while the public and private rules that govern the food system impact how zoonoses are managed.

55.2.2 Classification of Zoonoses

Zoonoses have been defined as diseases and infections that are naturally transmissible between vertebrate animals and humans (WHO 2021a). This group of diseases is generally classified according to the zoonotic agent itself and the route of disease transmission from animals to humans (Karesh et al. 2012; Lloyd-Smith et al. 2009). This classification offers a means to group zoonoses into diseases that are mainly transmitted to humans through food consumption, such as salmonellosis or campylobacteriosis; those that are mainly transmitted to humans through means other than food, for example, vectors, direct contact, or proximity to infected animals, such as avian influenza, and zoonoses with multiple routes of transmission, such as brucellosis (transmitted through food or direct contact with infected animals) and Rift Valley fever (conferred either through direct contact with an infected animal or the bite of an infected mosquito) (EFSA 2013).

While foodborne zoonoses are particularly important in terms of direct economic ramifications for the food sector, indirect costs of zoonoses transmitted by means other than food can also produce a significant impact on the food industry (McLeod et al. 2006; Rassy and Smith 2012). The designation of a disease as a zoonosis comes with wide-reaching social and political ramifications when considering ownership and leadership of control and management strategies, and while a One Health perspective on disease management has gained considerable traction in recent years, economic ramifications are still often sector-specific. Further, the designation of a disease as a zoonosis is not without ambiguity. A disease such as rabies, which is transmitted to humans through the bite of an infected dog in 99% of global cases, is a clear example of a zoonosis (WHO 2021b). Ambiguity around the appropriateness of the designation of a disease as a zoonosis arises however when human-to-human spread becomes the dominant route of transmission; these diseases (including COVID-19) have been labeled as "infectious disease of probable animal origin" (Haider et al. 2020). Independent of the designation, these pathogens can result in a significant direct and indirect economic impact on animal-source food value chains (Rassy and Smith 2012) – as seen in the case of (H1N1)pdm09 virus.

Zooanthroponosis, or "reverse zoonosis," defined as the human transmission of a disease to animals, must also be regarded as a threat to food systems and the wider economy (Messenger et al. 2014). In the case of COVID-19, its ability to act as a reverse zoonosis has resulted in devasting impacts on select animal populations, most notably, the mass culling of mink in 2020 following human-to-mink transmission of SARS-CoV-2. For instance, porcine cysticercosis (*Taenia solium*), though rightfully designated a zoonotic disease given that humans can become infected through eating undercooked pork, should also be regarded as a zooanthroponotic pathogen when considering that humans are the only definitive host (García et al. 2003), while pigs serve as an intermediate. While it could be argued that increased hygiene and sanitation in the human sector might aid in the reduction of transmission, the pork industry is often held largely responsible for disease prevention from a meat inspection standpoint and frequently shoulders heavy economic losses when

cysts are discovered during the butchering process (Carabin and Traoré 2014). Zooanthroponotic disease transmission can also have negative impacts on conservation initiatives with recent reports of neurocysticercosis (*T. solium*) detected in a wild Bengal tiger and suspected human to elephant spillover of *Mycobacterium tuberculosis* (Miller et al. 2019).

55.3 Understanding the Burden of Zoonoses

The impact of zoonotic diseases results in losses at the human-animal-environment interface and within each of those sectors, individually. Furthermore, the pattern of loss across each sector is widely influenced, both directly and indirectly, by the impacts in other sectors (Grace et al. 2012; Keusch et al. 2009). This is illustrated by the decades-long international battle to control and eradicate brucellosis (see Box 1), a disease with significant direct and indirect costs across livestock and public health.

55.3.1 Pathways for Burden

55.3.1.1 Public Health

The burden of diseases on public health can be seen through a health-related viewpoint, primarily by the assessment of changes in epidemiological parameters, such as prevalence or incidence of disease in the population or mortality rates. Additionally, in human health, the impact of both mortality and morbidity rates can be combined using metrics such as the disability-adjusted life years (DALYs) or quality-adjusted life years (QALYs). From a socio-economic perspective, impact also includes the value of the resources that are utilized as a result of disease, including health sector costs (e.g., visit a physician, laboratory or treatment expenses, or control and surveillance and disease control programs) and the value of decreased or lost productivity by the patient, as well as intangible costs (e.g., psychological costs due to pain or suffering).

The indirect effects mentioned above, stemming from disruptions to food and nutrition security, carry important weight as well, but are more difficult to measure due to their more distal and multistep effects on public health. Consequently, the direct effects on human health can be seen as a minimum burden caused by foodborne disease. In countries where there are no substitution possibilities for foods potentially contaminated with pathogens, consumers may put themselves at risk for foodborne disease when consuming the food due to the lack of alternatives, or they may increase the risk of malnutrition by excluding nutritious foods from their diets. Foodborne disease leading to diarrhea reduces appetite and/or the absorption of nutrients in the body. Malnutrition impairs the immune response and predisposes affected people to infection thereby creating a vicious cycle of malnutrition and infectious disease (Ibrahim et al. 2017).

Box 1 Economic Impact of Zoonoses – Brucellosis

Brucellosis is a highly transmissible disease of livestock, wildlife, and humans causing significant economic losses in livestock due to reduced milk and meat production caused by poor fertility. A 2015 study in India estimated that the livestock industry suffers a median loss of USD $ 3.4 billion in revenue due to brucellosis (Singh et al. 2015). Humans are typically infected via direct contact with infected animals and their products, such as during husbandry, at slaughterhouses, or by drinking unpasteurized milk. In humans, brucellosis infection can cause a recurring undulating fever and joint pain, leading to significant loss of work. In India, annual median losses due to human brucellosis were estimated at USD $10.46 million/year (Singh et al. 2018). Brucellosis also significantly threatens food security by reducing milk output and fertility rates. In 2002, the FAO estimated that eradication of brucellosis in Sub-Saharan Africa (SSA) would have resulted in generation of approximately 371,000 additional tons of meat and 616,000 additional tons of milk in the region (Mangen et al. 2002). Many species of *Brucella* infect livestock, the most important being *B. abortus*, which mainly infects cattle, and *B. melitensis*, which mainly impacts small ruminants (Corbel 2006). *B. melitensis* causes more human cases and more severe disease than *B. abortus*. While vaccines for use in animals exist for both *B. abortus* and *B. melitensis*, they have different safety and effectiveness profiles that contribute to the historical difficulty in controlling *B. melitensis* as compared to *B. abortus* (Corbel 1997). *B. abortus* is more prevalent among livestock, but has proven easier to control than *B. melitensis,* with more countries reaching bovine brucellosis-free status than ovine/caprine brucellosis-free status (Corbel 2006).

Concerted efforts to eradicate or minimize brucellosis began in the early 1900s, but these actions have largely only been accessible to countries with the structural organization and money necessary to sustain vaccination and surveillance for decades. Vaccination, test-and-slaughter, and hybrid campaigns have each been employed with varying levels of success, but this success was contingent on strong, high-level implementation over many years (Zhang et al. 2018). In the US, efforts to eradicate bovine brucellosis cost USD $3.5 billion over 63 years (Dadar et al. 2021), and pockets of brucellosis persist among wildlife in the Greater Yellowstone Ecosystem. Government mistrust, insufficient capital or infrastructure, and social and religious concerns, such as reluctance to cull animals or pasteurize milk, can hamper or totally inhibit control efforts. Nevertheless, if these barriers can be overcome, mass vaccination may be more cost-effective than reactive measures even for developing countries (Zhang et al. 2018). In Mongolia, mass vaccination of small ruminants and cattle against brucellosis was predicted to generate a net positive impact of USD $ 18.3 million (Roth et al. 2003).

(continued)

Box 1 (continued)

Significant trade barriers exist between brucellosis free and brucellosis endemic areas, designated according to OIE guidelines in compliance with the Sanitary and Phytosanitary Agreement of the World Trade Organization (Franc et al. 2018). Other organizations such as the European Union apply additional rules surrounding international surveillance and trade. Consistent risk assessment and monitoring is required to maintain brucellosis free status. In 2010, Great Britain was EU certified as "Officially Brucellosis Free" (OBF), while the Republic of Ireland was not. A risk assessment for import of breeding cattle from Ireland to GB identified a high risk of importing brucellosis. Indeed, in 2003 36 infected heifers were imported into GB from the Republic of Ireland, 10 years after the last reported case of bovine brucellosis in the UK (Jones et al. 2004). For countries with high incidence of brucellosis, animals to be exported must be isolated and tested for disease prior to movement (OIE 2018), making export more costly for producers (Peck and Bruce 2017). Replacement of depopulation policies with quarantine policies cost producers in the USD $ 94,000 for a 400-head herd. However, little work has been done to quantify the overall economic impact of lost trade due to brucellosis status of herds or countries. Investment in veterinary capacity-building can help some nations attain improved brucellosis status, and therefore improved trade prospects (Rossiter and Hammadi 2009). Relaxing restrictions on international trade of low-risk processed meats could also improve equitability of market access for producers in areas endemic for brucellosis.

The true extent of the economic and health impacts of brucellosis on livestock and humans is unknown. Underreporting and poor surveillance, lack of capacity, and mistrust of government intervention all contribute to the persistence of the disease and our inability to quantify it in low and middle-income countries in particular (Franc et al. 2018).

55.3.1.2 Animal Health and Production

The presence of zoonoses in food-producing animals may be associated with losses due to morbidity and mortality, which can lead to a reduction in expected output (e.g., decreased milk yield, lowered body weight) and an increase in expenditures in treatment costs (Bennett and IJpelaar 2005).

Morbidity and mortality of animals due to zoonotic diseases also carry other losses related to the wider social, cultural, and economic value of animals and their health and welfare. Animals can be a source of income and employment, provide draught power and fertilizer, serve as a means of transport, particularly in low-income settings, and serve as guardians of livestock and households and companions to people (Meslin 2006; Torgerson 2013). They also act as a form of insurance and social status. Losses of domestic animals can therefore represent an important socio-economic impact in affected communities. For low-income families

or those with limited wealth, an unexpected loss in earnings may inhibit their ability to seek healthcare when needed (Eckhardt et al. 2018). Among rural households in Kenya that held livestock, the number of cattle owned increased the likelihood of healthcare-seeking when family members fell ill (Thumbi et al. 2015). Zoonoses may also lead to the erosion of the health benefits due to animal interaction, animal ownership, and the human-animal or human-nature bond. More broadly, social value impact that can also be associated with zoonoses, including effects on health equity, may also be significant.

To counteract these losses in the animal health sector, and the potential impact on public health, another economic cost accrues from efforts to prevent, control, or eradicate a disease. In high-income countries, these impacts can be enormous, dwarfing the production losses due to disease. Examples illustrating this are provided by control and prevention measures carried out to tackle some zoonotic events, such as brucellosis (see Box 1), BSE (see Box 2), HPAI (see Box 3), or bovine TB, where control and surveillance activities were estimated to cost GBP £74–99 million/year in the UK (Torgerson and Torgerson 2008).

55.3.1.3 Ecosystem Health

Impacts on the ecosystem can either lead to production losses (e.g., when pollinators such as bees are affected by disease in turn causing harvest losses) or the reduction of ecosystem services to people. For example, if an area cannot be accessed anymore, because of a risk of zoonotic disease transmission, a value loss occurs to people in that they cannot use the area as they usually would.

Land-use change, habitat fragmentation, deforestation, and alteration of biodiverse natural ecosystems for agriculture and infrastructure expansion have long been associated with risks to human health (Myers et al. 2013). While such landscape alterations may offer an opportunity for improved nutrition and livelihoods, there is a growing need to account for the value of natural capital stocks and ecosystem services that are provided by intact, biodiverse environments and consider how disruption and fragmentation through unsustainable practices may impact these wider services.

Box 2 Economic Impact of Zoonoses – *BSE*

The impact of the BSE crisis has been the subject of numerous assessments in several of the countries affected (reviewed by World Bank 2010). The disease has led to important direct and indirect losses. Before the link between BSE in cattle to Creutzfeldt–Jakob disease (CJD) in humans was established, the disease losses were mainly linked to the loss in value of infected carcasses and to the costs of establishing control measures, namely the disposal of specified risk material (Atkinson 1999). The establishment of the link between the two diseases meant the additional emergence of important indirect costs linked to market impacts, including the contraction in domestic demand of beef products, loss of export markets and a fall of beef cattle prices (Atkinson

(continued)

Box 2 (continued)
1999). Beef consumption and domestic prices of cattle, beef and beef products were reported to have substantial drop-offs in many countries (Probst et al. 2013; Serra 2011).

To respond to the crisis, a series of preventive and control measures have been implemented in the countries affected. In the UK, the estimation of the costs associated with control and regulation compliance in the years of 1996/1997 indicated additional costs of around GBP £25–50 million, to which added costs associated with slaughtering and culling of GBP £220 million (Atkinson 1999). In Germany, the total costs associated with prevention, control and surveillance of BSE were estimated to range between EUR €1.8 and 2.0 billion, with approximately 54% of the costs being incurred by the extension of the feed ban for animal protein to all farmed livestock and 21% to active surveillance (Probst et al. 2013). An analysis of the cost-effectiveness of these measures in the Netherlands, indicated a cost of EUR €4.3–17.7 million, from 2002 to 2005, per life year saved (Benedictus et al. 2009).

Exports were also strongly impacted by trade restrictions put in place. For the UK, the export market of beef and trade in live calves, worth GBP £670 million in 1995, was lost with the trade ban imposed in 1996 (Atkinson 1999). These losses associated to a loss in output from beef and associated products, were balanced across the economy through a shift in consumption to substitute products (Wigle et al. 2007). In Europe, for example, poultry, pork, vegetables and milk products benefited from the BSE crisis (Benedictus et al. 2009). In May 2003 the Canadian government reported the detection of a single case of bovine spongiform encephalopathy in a national cattle population of nearly 13.5 million animals. This led to 40 countries banning the import of a large range of live animals and livestock products from Canada. Mitura and Di Piétro (2004) estimated that the impact of the international livestock trade ban was significant for Canada. In 2003, Canadian farm cash receipts from cattle and calves were estimated at CAD $5.2 billion, a sharp drop of CAD $2.5 billion (33%) from 2002. At farm-level it was estimated that on average a family farm with an unincorporated beef unit would have lost CAD $20,000. The more wide-ranging impact of the trade ban was the movement of cattle from Mexico to the USA to fill the demand for store cattle that would have come from Canada. While this has created a positive impact for cattle producers in Mexico, it has meant that beef prices in Mexico have risen affecting Mexican consumers, and that the USA is potentially importing animals from areas with low tuberculosis status (Ayala and Velasco 2005). Later in 2003 the USA also declared the discovery of a single animal with BSE (out of an estimated cattle population of 96 millionc) which led to 53 countries banning the imports of American beef. Coffey et al. (2005) estimated that the losses associated with this trade ban were between USD $3.2 and 4.7 billion. These

(continued)

Box 2 (continued)
authors also estimated that BSE has had considerable costs in terms of increased needs for surveillance at a farm and slaughterhouse level. On an international level the use of BSE cases in Canada and USA as a trade barrier can have a negative impact on disease reporting. Livestock exporting countries, whose economies have far less ability to absorb rapid changes in export demand for livestock products, are unlikely to report minor levels of animal disease where there is a risk of exaggerated and rapid trade bans.

Estimations of losses on the public health sector are not as readily available in the literature as for the animal health sector and food chain. An estimate from the UK points to running costs associated to staff time and expert committees of GBP £2.5 million, in 1988–1996. The cost of patient care for CJD patients were considered too uncertain to be quantified (Phillips 2000).

Zoonotic diseases have a correlative relationship with biodiverse ecosystems. For instance, human-dominated landscapes and unsustainable land management practices are associated with higher zoonotic pathogen diversity and prevalence, while conversely, healthy biodiverse ecosystems and sustainable farming practices are associated with increased human health and decreased pathogen diversity and host prevalence (Gibb et al. 2020). Heightened mixing between domestic animals, humans, and wildlife, particularly at the borders of urban settlements or industrial production settings, can lead to increased transmission of zoonotic disease from domesticated animals to wildlife and negatively impact native ecosystem structures, further threatening endangered wildlife species (Mathews 2009; Reaser et al. 2021). This impacts species conservation initiatives both directly and indirectly through knock-on effects in the wider food and biodiverse functional chain.

"Natural capital" places value on naturally occurring resources such as soil, water, air, geological formations, and biodiversity and positions them as assets to health and the wider economy. These resources are the stocks from which benefits flow to human health, welfare, and the world economy (i.e., ecosystem services). In 2011, these services were given an estimated global value of between USD $125 and $145 trillion, annually (Costanza et al. 2014). Measuring the economic value of naturally occurring systems that facilitate human health and welfare through water filtration, nutrient cycling, erosion control, pollination, and climate regulation is a relatively new practice and does not yet adequately consider the substantial impact that global natural ecosystems have on human health and disease prevention. However, a recent model estimated that the cost of implementation over 10 years of a suite of preventive measures to prevent the next major zoonotic spillover event and pandemic would total merely 2% of the estimated USD $5 trillion global cost of the COVID-19 pandemic. Such actions, including reducing deforestation, routine disease surveillance of domestic and wild animals, and halting the illegal wildlife trade, would also have ancillary environmental benefits in reducing CO_2 emissions (Dobson et al. 2020). While many studies focus on ecosystem services for human health, it should not be forgotten that the same services sustain animals and enable

livestock production around the globe. With livestock being the predominant biomass on the planet (followed by humans), it is important to promote ecosystem health for the benefit of people, animals, plants, and the planet as a whole. Striving for a socio-ecological equilibrium that facilitates the health of multiple populations in the long term sits at the core of modern One Health agendas.

Understanding the value of the relationship between health and the environment is vital to assessing the full impact of zoonotic diseases on society, and when we consider biodiversity of pivotal impact in the prevention of zoonotic disease emergence and potential pandemics, this value serves to contextualize intact ecosystems within the context of the global economy.

55.3.1.4 Trade and Travel

The presence and risks of zoonoses can have substantial impacts on consumption patterns and/or trade and travel restrictions imposed as a consequence of zoonotic disease outbreaks can be extensive and last beyond the duration of an outbreak, depending on the risk perception of trade partners and tourists.

Box 3 Economic Impact of Zoonoses – *Highly Pathogenic Avian Influenza in Chile*

In 2002, Chile reported for the first time an outbreak of HPAI. This was also the first isolation of avian influenza virus in South America (Lupiani and Reddy 2009). The poultry industry in the country produced, at that time, 400,000 tons/year of fresh poultry meat, with exports, mainly to Mexico and the European Union, following an increasing trend (USD $69 million in 2001, $44 million in 2002 and $72 million in 2003) (Orozco 2005). Following the outbreak, the access to export markets was closed (Orozco 2005).

As a response, the national authorities have put in place a series of mitigation measures, including stamping out of affected farms, setting up of surveillance, pre-diagnosis quarantine, depopulation, movement control, and increased biosecurity and to regain access to the export markets as soon as possible, a zooning strategy was adopted (Max et al. 2007; Orozco 2005). The culling of the two infected farms to stamp out the disease resulted in the destruction of 560,000 breeding chicken and turkeys (Rojas 2009). Within 7 months, Chile was declared free from HPAI (Max et al. 2007).

The initial financial impact of the disease was calculated by Verdugo et al., (2006, cited Rojas 2009) to be USD $31.7 million, with costs largely borne by the private sector. An economic impact assessment of HPAI in Chile has been estimated that over the whole economy losses reached USD $250 million (Wright 2004).

The example of the severe acute respiratory syndrome (SARS) epidemic demonstrated the economic impacts of travel restrictions affecting tourism and its contribution to reductions in Gross domestic product (GDP) growth in some countries, through reductions on service exports, particularly tourism-related exports (Keusch et al. 2009; Xiaoqin Fan 2003). For China, Taiwan, Hong Kong, and Singapore, this

impact has been estimated to be USD $13 million, or 0.5–1.1% of the GDP (Keusch et al. 2009). The 1994 plague outbreak in India also led to economic losses due to internal and external travel restrictions (Keusch et al. 2009). The H1N1 emergence in Mexico resulted in a reduction of almost a million overseas visitors and losses of around USD $2.8 billion for the country's economy (Rassy and Smith 2012). More recently, and as mentioned earlier, the ongoing COVID-19 pandemic-imposed travel restrictions limiting the movement of people and goods between countries have also impacted the trade of animal and animal products.

Losses due to changes in consumption patterns and trade disruption can also be highly visible. In the European Union, and following the BSE food scare, more than half of the surveyed consumers were fearful of BSE and unsure as to whether beef was safe for consumption (Bánáti 2011) (see Box 2). This has led to important losses, particularly via trade bans that in 2003 cost CAD $2.5 billion (Mitura and Di Piétro 2004) and cost the USD $3.2–4.7 billion (Coffey et al. 2005). While BSE has led to a significant drop in consumption and a market shock, other scares have been relatively short-lived with consumption returning to previous levels, but disrupting the food system which caused some businesses to go bankrupt and led to the loss of employment and/or restructuring of the industry.

Product recalls have become an important component of the food system as value chains have lengthened. Of the recent major food recall incidents, many have been related to *E. coli* including beef in the USA in 1997 (McKenzie and Thomsen 2001) and salmonella (Roos n.d.). In Europe, the costs of a recall are strongly related to the traceability systems in place. If traceability is poor, then large amounts of products have to be recalled that are likely to be unaffected by the problem. However, the development and implementation of improved traceability systems are onerous and costly. New technologies are emerging, such as blockchain, that may improve traceability by improving transparency and data privacy, reducing manual errors, and increasing the efficiency and speed of identifying contaminated products (Feng et al. 2020). Increasingly advanced traceability systems are a positive step towards food safety and consumer awareness and autonomy. However, the use of advanced traceability technology requires a high degree of collaboration and trust as well as consensus on and implementation of international standards and regulations. As with all cutting-edge technologies, uneven adoption across countries and economies due to lack of resources or infrastructure could limit access of small-scale producers to international markets. Traceability is yet another issue that must be considered in terms of impact not just on food safety, but across domains of equity in a globalized food system.

55.3.2 Assessing the Burden of Zoonoses and the Value of Mitigation Actions

Bringing together the burden of zoonotic diseases across the domains of health of people, animals, and the environment is a large task. Its assessment requires an adequate representation of the complexity of loss across species, sectors, and society.

This in turn requires knowledge of disease levels, morbidity, and mortality of the disease in people and their animals and an assessment of the impacts of the pathogen and its mitigation and transmission patterns in the environment. As such, while some estimates have incorporated aspects of animal and human costs (Bennett and IJpelaar 2005; Budke et al. 2006; Charypkhan et al. 2019; Choudhury et al. 2013), metrics and tools available to measure economic impacts tend to be specific to each sector and difficult to translate across fields, with the different components of impact often considered individually rather than in an integrated way (Grace et al. 2012).

In the field of public health, DALY is one of the most commonly used health gap summary measures and became the key metric for quantifying burden of disease, being used to measure the global burden of disease estimates regularly produced by the World Health Organization (WHO 2021c) and The Global Burden of Disease program (GBD). The DALY captures the loss of life due to reduced life expectancy and reduced quality of life while alive due to impediments in what people can do when affected by a health issue (Murray and Lopez 1996). This framework is relatively simple in its concept, yet complex in its application requiring data and information on disease levels, morbidity, and mortality. The DALY avoids the need to place an economic value on early death and only estimates the disutility to the individual of being ill, not capturing the medical direct and indirect costs of illness to the individual or society. However, the DALY can be used to compare the costs of averting a DALY through a mitigation action.

In health economics, cost burden is commonly assessed using cost-of-illness studies, which cover both the direct and indirect costs of disease and place a monetary value on these costs. There are two aspects in this assessment (Rushton et al. 1999; Rushton 2009b): (i) the expenditure on health care interventions, and mitigation actions such as surveillance, control, and prevention measures – in human health economics referred to as direct costs; and (ii) the loss in health that leads to increased mortality and reduced ability to work in the case of people and generate goods and services in the case of animals – in human health economics referred to as indirect costs.

When assessing morbidity and mortality in animals due to zoonotic disease, units of physical losses (e.g., number of animals that died, number of animals suffering from milk loss, rate of milk loss) can be combined with data on production (e.g., milk yield) and/or market prices (e.g., market value of the animal, milk price). Illustrations of how such losses can be calculated can, for example, be found in Mcdermott et al. (2013) and Herrera et al. (2008). Generally, morbidity and mortality in animals are measurable in monetary units. Frequently used tools include cost-analysis, incorporating the losses mentioned above (Bennett and IJpelaar 2005), and decision tree analysis, which might model different scenarios of production (Carabin et al. 2005; Choudhury et al. 2013). An overview of various techniques suitable to assess the economics of animal disease can be found in Rushton et al. (1999).

There have been attempts to mirror burden estimates for animal diseases, but significant important conceptual difficulties arise (reviewed in Torgerson et al. 2018). In livestock health as the majority of these animals are maintained for economic purposes, the direct and indirect costs can be valued at market prices.

Even where the goods and services these animals generate do not have markets, it is possible to impute a value by estimating what it would cost to generate such goods and services in alternative ways, for example, the use of a tractor rather than oxen for ploughing or the use of artificial fertilizer rather than manure. In addition, it is possible to use contingency or willingness-to-pay valuations. Despite these differences in human and animal health burden estimations, there is still a need to look at the Global Burden of Animal Diseases (GBADs), yet the approach is different from human health. There needs to be an understanding of the gap in production – the difference between current levels of output and input use with the health status as it stands, versus a situation where the animals would have no disease, adequate nutrition and water, and no risks of injury, accidents, or predation. This gap has been termed an Animal Health Loss Envelope (Rushton et al. 2021; Torgerson and Shaw 2021) and is the basis for the estimation of the burden of diseases in livestock.

Willingness-to-pay and the contingent valuation approach have also been used to attribute value to ecosystem services and in animal health to attribute a value to food safety or animal welfare. The concept of "willingness to pay" (WTP) is based on the assumption that the maximum amount an individual is willing to pay for a commodity reflects the value it has for this person. Miller and Unnevehr (2001), for example, conducted a household survey to investigate consumers' WTP for enhanced pork meat safety. They found that roughly 80% of the consumers were willing to pay at least USD $0.10 more per pound of certified safer pork. Another study used a hypothetical market scenario in the UK to investigate people's WTP to support legislation to phase out the use of battery cages in egg production in the EU by 2005 (Bennett 1998). The survey showed a mean WTP of £0.43 increase in price per dozen eggs (with a market price of around £1.40 per dozen), purporting to indicate the value respondents attributed to improved animal welfare. The main criticism of the WTP approach is that it does not give reliable valuations, since the choices are often hypothetical and people tend to overestimate their willingness to pay. Another drawback is that nonusers of a good or service might find it difficult to attribute a value to it because their knowledge of it is very limited. WTP methodologies have had inconsistent success in the valuation of ecosystem services such as water filtration or conservation of a species (Dasgupta 2021). In these instances, people were found at times to vary their answers when asked a question differently or standardize their perceived value of preserving a species regardless of the total number of animals at risk.

As mentioned before, various outbreaks of zoonotic disease in the past have shown that food safety scares can alarm consumers to the extent that they reject consuming certain products. Such a reduction in demand caused by a loss in consumer confidence can lead to a reduction in market prices. Similarly, zoonotic disease outbreaks can lead to movement or export bans, which impact the quantity of products placed on the market and consequently affects prices. The value of these changes in supply and demand can be assessed using surplus models to measure producer, processor, and consumers surplus changes, as illustrated for avian influenza outbreaks in South East Asia and the United States, respectively (Hall et al.

2006; Paarlberg et al. 2007), and BSE (Weerahewa et al. 2007). Furthermore, there is a wide range of different methods available to assess food and nutrition security indicators; a review has been published by Pangaribowo et al. (2013).

When attempting to more holistically assess the effects of disease in different sectors, it can be challenging to capture and effectively pinpoint burden across public health, animal health, and the environment. Recently, Morel et al. (2020) have carefully explained how to incorporate all the data needed for a comprehensive socio-economic assessment of AMR in terms of the cost burden and have placed this under the One Health approach. New methods have also been developed in terms of metrics. The recently developed zDALY metric is an adjusted indicator to estimate the burden of zoonotic diseases that enables DALYs associated with human ill health to be combined with a component measuring losses in animals (Torgerson et al. 2018). This component, termed the animal loss equivalent (ALE), allows for the conversion of losses due to animal disease to an equivalent metric using a local gross national income per capita deflator. The examples of the use of this metric show the importance of measuring both the burden of disease in humans and animals and the actual impact of the losses felt by the population (Torgerson et al. 2018). Other authors suggested a method to monetize the human health benefits derived from zoonotic disease reduction using the annualized value of statistical life to form a "combined" monetary burden (Herrera-Araujo et al. 2020).

Determining the absolute levels of direct and indirect costs of this zoonotic disease burden is necessary yet not sufficient in defining proportionate levels of mitigation actions. The reason is that for every increase in direct costs, there should be an impact on indirect costs as the expenditure on mitigation is aimed at avoiding the losses of health and productivity. A priori, the effectiveness of mitigation actions will be diminished as more money is spent and will reach a point where the incremental levels of indirect costs avoidance will reduce. From an economic perspective, incremental expenditure on mitigation actions should cease when the last dollar spent returns a dollar of avoided health loss.

To assess disease mitigation strategies from an economic perspective, commonly used tools are cost-benefit analyses and cost-effectiveness analysis. Cost-benefit analyses compare the total discounted benefits of a project in monetary units with its total discounted costs and recommend the implementation of the project if the benefits exceed the costs. It includes the definition of the useful life of the project, the estimate in physical units of benefits (losses avoided) and costs (mitigation resources used), translation of the physical units into economic values, the conversion of future values into present values by discounting, and finally the calculation of the net benefit (net present value = total discounted costs − total discounted benefits). Benefit-cost ratios as choice criteria can be misleading when multiple options are compared, and some authors recommend using the net present value instead of the benefit-cost ratio (Howe et al. 2013; McInerney 1991; Tisdell 1995). In any assessment of this nature, it is therefore important to review all measures of project worth, namely, the net present value, the benefit-cost ratio, and internal rate of return and timing permitting an examination of the estimation of the benefit and cost streams.

A similar basic principle is seen in cost-effectiveness analysis, which is commonly used to assess human health interventions but has been less applied to animal health decision-making problems (Babo Martins and Rushton 2014). Cost-effectiveness analysis aims to assess the effect of a program in nonmonetary units in relation to its cost. In human health economics, the effect often refers to the avoidance of illness or death, but the outcome of any objective can – in theory – be measured in various technical terms, for example, reduction of CO_2 emissions or detection of cases of disease. However, the value of the effect in question must reflect a (nonmonetary or monetary) value so that it can be interpreted for decisions on resource allocation. A special case to cost-effectiveness analysis is the least-cost analysis where two or more programs or projects achieve the same effect. The economic assessment then aims to identify the cheaper option.

Importantly, all these fundamental concepts explained above only provide an estimation of the economic efficiency (optimal balance, acceptable combination, and least-cost option) of technically feasible ways of dealing with zoonotic disease. Some authors have proposed frameworks that take into account wider issues, including risk management options and the understanding of the factors impacting those options (Grace et al. 2012; Narrod et al. 2012). It is the case of the framework proposed by Narrod et al. (2012), consisting of a modified risk analysis framework to enhance the reduction of zoonotic disease burden, including the analysis outputs of animal and human disease transmission models and economic impact models.

55.4 Conclusions

Economics provides several tools and frameworks that can be used in the measurement of the impacts of disease and to aid in informing resource allocation for zoonotic disease mitigation. However, the measurement of the impact of zoonotic diseases presents several challenges. The impacts of zoonoses are felt in multiple sectors, in multiple links of increasingly complex value chains, and the interface between sectors and jurisdictions. These multiple dimensions are generally complex to capture as a whole, with many studies focusing on impact assessments per sector and failing to capture the entire realm of effects.

In addition to the need for addressing impacts in a wider context, aspects such as unused human, financial and capital capacity in the food system, reduced confidence in the marketplace, particularly for export markets, and important lags created in terms of confidence and investment – often taking years to recover capacities, skills, and markets – should be incorporated in future, more refined, impact assessments of zoonoses.

Data availability and quality to populate assessments represent a further challenge. Underreporting of cases of zoonotic disease, particularly in low-income settings are, for example, believed to be contributing to the underestimation of the burden of these diseases in the human and animal populations, therefore influencing disease mitigation decisions and contributing to the perpetuation of their impact. However, the use of big data and participatory approaches including citizen science

could improve access to reporting and data availability in the future. The changing context of our food systems should also be considered, including how we as a society move towards a sustainable food system, ensuring that, using a systems approach and stakeholder engagement, gains in one sector or region do not saddle those in other sectors or regions with unmitigated negative consequences.

References

Atkinson N (1999) The Impact ofBSE on the U.K. Economy. Paper presented to the 1st Symposium on Animal and Human TSEs, Buenos Aires

Ayala G, Velasco RJ (2005) Informe de Evaluacion Nacional Subprograma Salud Animal. Available at https://www.agricultura.gob.mx/sites/default/files/sagarpa/document/2018/11/14/1533/14112018-2004-nal-sa.pdf

Babo Martins S, Rushton J (2014) Cost-effectiveness analysis – adding value to animal health, welfare and production assessment. Rev Sci Tech 33(3):681–689

Bánáti D (2011) Consumer response to food scandals and scares. Trends Food Sci Technol 22: 56–60. https://doi.org/10.1016/j.tifs.2010.12.007

Benedictus A, Hogeveen H, Berends BR (2009) The price of the precautionary principle: cost-effectiveness of BSE intervention strategies in The Netherlands. Prev Vet Med 89:212–222. https://doi.org/10.1016/j.prevetmed.2009.03.001

Bennett R (1998) Measuring public support for animal welfare legislation: a case study of cage egg production. Anim Welf 7:1–10

Bennett R, IJpelaar J (2005) Updated estimates of the costs associated with thirty four endemic livestock diseases in Great Britain: a note. J Agric Econ 56:135–144. https://doi.org/10.1111/j.1477-9552.2005.tb00126.x

Bennett CE, Thomas R, Williams M, Zalasiewicz J, Edgeworth M, Miller H et al (2018) The broiler chicken as a signal of a human reconfigured biosphere. R Soc Open Sci 5. https://doi.org/10.1098/RSOS.180325

Budke CM, Deplazes P, Torgerson PR (2006) Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis 12:296. https://doi.org/10.3201/EID1202.050499

Carabin H, Traoré AA (2014) *Taenia solium* taeniasis and cysticercosis control and elimination through community-based interventions. Curr Trop Med Rep 1:181–193. https://doi.org/10.1007/S40475-014-0029-4

Carabin H, Budke CM, Cowan LD, Willingham AL, Torgerson PR (2005) Methods for assessing the burden of parasitic zoonoses: echinococcosis and cysticercosis. Trends Parasitol 21: 327–333. https://doi.org/10.1016/j.pt.2005.05.009

Chapot L, Whatford L, Compston P, Tak M, Cuevas S, Garza M, Bennani H, Bin Aslam H, Hennessey M, Limon G et al (2021) A global media analysis of the impact of the COVID-19 pandemic on chicken meat food systems: key vulnerabilities and opportunities for building resilience. Sustainability 13:9435. https://doi.org/10.3390/su13169435

Charypkhan D, Sultanov AA, Ivanov NP, Baramova SA, Taitubayev MK, Torgerson PR (2019) Economic and health burden of brucellosis in Kazakhstan. Zoonoses Public Health 66:487–494. https://doi.org/10.1111/ZPH.12582

Choudhury AAK, Conlan JV, Racloz VN, Reid SA, Blacksell SD, Fenwick SG et al (2013) The economic impact of pig-associated parasitic zoonosis in northern Lao PDR. EcoHealth. https://doi.org/10.1007/s10393-013-0821-y

Coffey B, Mintert J, Fox JA, Schroeder TC, Valentin L (2005) The economic impact of BSE on the U.S. beef industry: product value losses, regulatory costs, and consumer reactions. Available at https://krex.k-state.edu/handle/2097/12460

Corbel MJ (1997) Brucellosis: an overview. Emerg Infect Dis 3:213. https://doi.org/10.3201/EID0302.970219

Corbel MJ (2006) Brucellosis in humans and animals. Food and Agriculture Organization of the United Nations/World Health Organization/World Organization for Animal Health, Rome

Costanza R, de Groot R, Sutton P, van der Ploeg S, Anderson SJ, Kubiszewski I et al (2014) Changes in the global value of ecosystem services. Glob Environ Change 26:152–158. https://doi.org/10.1016/J.GLOENVCHA.2014.04.002

Dadar M, Tiwari R, Sharun K, Dhama K (2021) Importance of brucellosis control programs of livestock on the improvement of one health. Vet Q 41:137–151. https://doi.org/10.1080/01652176.2021.1894501

Dasgupta P (2021) The economics of biodiversity: the Dasgupta review. HM Treasury, London

Dobson AP, Pimm SL, Hannah L, Kaufman L, Ahumada JA, Ando AW et al (2020) Ecology and economics for pandemic prevention: investments to prevent tropical deforestation and to limit wildlife trade will protect against future zoonosis outbreaks. Science 369:379–381. https://doi.org/10.1126/SCIENCE.ABC3189

Eckhardt M, Santillán D, Faresjö T, Forsberg BC, Falk M (2018) Universal health coverage in rural Ecuador: a cross-sectional study of perceived emergencies. West J Emerg Med 19:889. https://doi.org/10.5811/WESTJEM.2018.6.38410

EFSA (2013) Zoonotic diseases. http://www.efsa.europa.eu/en/topics/topic/zoonoticdiseases.htm

Ethiopia-Netherlands Trade for Agricultural Growth (2020) COVID-19 effects on the Ethiopian poultry sector. Available at https://benefitethiopia.files.wordpress.com/2020/06/coronaeffect_poultrysector_062020-1.pdf

Feng H, Wang X, Duan Y, Zhang J, Zhang X (2020) Applying blockchain technology to improve agri-food traceability: a review of development methods, benefits and challenges. J Clean Prod 260:121031. https://doi.org/10.1016/J.JCLEPRO.2020.121031

Fisher JR (1998) Cattle plagues past and present: the mystery of mad cow disease. J Contemp Hist 33:215–228

Franc KA, Krecek RC, Häsler BN, Arenas-Gamboa AM (2018) Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC Public Health 18:1–9. https://doi.org/10.1186/S12889-017-5016-Y

García HH, Gonzalez AE, Evans CA, Gilman RH (2003) *Taenia solium* cysticercosis. Lancet 362:547–556. https://doi.org/10.1016/S0140-6736(03)14117-7

Gibb R, Redding DW, Chin KQ, Donnelly CA, Blackburn TM, Newbold T et al (2020) Zoonotic host diversity increases in human-dominated ecosystems. Nature 584(7821):398–402. https://doi.org/10.1038/s41586-020-2562-8

Gonzalez CG (2010) The global food crisis: law, policy, and the elusive quest for justice. Yale Hum Rights Dev Law J 13:462–479

Grace D, Gilbert J, Randolph T, Kang'ethe E. (2012) The multiple burdens of zoonotic disease and an Ecohealth approach to their assessment. Trop Anim Health Prod 44(Suppl 1):S67–S73. https://doi.org/10.1007/s11250-012-0209-y

Haider N, Rothman-Ostrow P, Osman AY, Arruda LB, Macfarlane-Berry L, Elton L et al (2020) COVID-19 – zoonosis or emerging infectious disease? Front Public Health 8:763. https://doi.org/10.3389/FPUBH.2020.596944

Hall DC, Benigno C, Kalpravidh W (2006) The impact of avian influenza on small and medium scale poultry producers in South East Asia (preliminary findings). Sel. Pap. Prep. Present. Am. Agric. Econ. Assoc. Annu. Meet. Long Beach, California, 23–26 July 2006, pp 1–27

Harrison M (2004) Disease and the modern world 1500 to the present day. Polity Press, Cambridge, UK

Häsler BN, Dominguez-Salas P, Fornace K, Garza M, Grace D, Rushton J (2017) Where food safety meets nutrition outcomes in livestock and fish value chains: a conceptual approach. Food Sec 9:1001–1017. https://doi.org/10.1007/s12571-017-0710-2

Hasnain S, Ingram J, Zurek M (2020) Mapping the UK Food System – a report for the UKRI Transforming UK Food Systems Programme. Available at https://www.eci.ox.ac.uk/research/food/downloads/Mapping-the-UK-food-system-digital.pdf

Hays J (2009) The burdens of disease. Epidemics and human response in Western history. Rutgers University Press, New Brunswick

Herrera E, Palomares G, Díaz-Aparicio E (2008) Milk production increase in a dairy farm under a six-year Brucellosis control program. Ann N Y Acad Sci 1149:296–299. https://doi.org/10.1196/annals.1428.011

Herrera-Araujo D, Mikecz O, Pica-Ciamarra U (2020) Placing a monetary value on the human health component of zoonotic diseases: a methodological note with an application to cysticercosis in Africa. Prev Vet Med 175:104862. https://doi.org/10.1016/J.PREVETMED.2019.104862

Howard PH (2017) Corporate concentration in global meat processing: the role of government subsidies. Available at https://philhowardnet.files.wordpress.com/2017/06/corporate-concentration-in-global-meat-processing1.pdf

Howe KS, Häsler B, Stärk KDC (2013) Economic principles for resource allocation decisions at national level to mitigate the effects of disease in farm animal populations. Epidemiol Infect 141:91–101. https://doi.org/10.1017/S095026881200060X

Ibrahim MK, Zambruni M, Melby CL, Melby PC (2017) Impact of childhood malnutrition on host defense and infection. Clin Microbiol Rev 30:919–971. https://doi.org/10.1128/CMR.00119-16

Jones RD, Kelly L, England T, MacMillan A, Wooldridge M (2004) A quantitative risk assessment for the importation of brucellosis-infected breeding cattle into Great Britain from selected European countries. Prev Vet Med 63:51–61. https://doi.org/10.1016/J.PREVETMED.2004.01.008

Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M et al (2012) Ecology of zoonoses: natural and unnatural histories. Lancet 380:1936–1945. https://doi.org/10.1016/S0140-6736(12)61678-X

Keusch GT, Pappaioanou M, González MC, Scott KA, Tsai P (2009) Sustaining global surveillance and response to emerging zoonotic diseases. National Academies Press, Washington, DC. https://doi.org/10.17226/12625

Khoury CK, Bjorkman AD, Dempewolf H, Ramirez-Villegas J, Guarino L, Jarvis A et al (2014) Increasing homogeneity in global food supplies and the implications for food security. Proc Natl Acad Sci U S A 111:4001–4006. https://doi.org/10.1073/PNAS.1313490111

Leonard DK (2000) The new institutional economics and the restructuring of animal health Services in Africa. In: Leonard DK (ed) Africa's changing markets for health and veterinary services. The new institutional issues. Macmillan Press Ltd, London, pp 1–40

Leonard DK (2004) Tools from the new institutional economics for reforming the delivery of veterinary services. Rev Sci Tech 23:47–57. Discussion 391–401

Lloyd-Smith JO, George D, Pepin KM, Pitzer VE, Pulliam JRC, Dobson AP et al (2009) Epidemic dynamics at the human-animal interface. Science 326:1362–1367. https://doi.org/10.1126/science.1177345

Lupiani B, Reddy SM (2009) The history of avian influenza. Comp Immunol Microbiol Infect Dis 32:311–323. https://doi.org/10.1016/j.cimid.2008.01.004

Mangen MJ, Otte J, Pfeiffer D, Chilonda P (2002) Bovine brucellosis in sub-Saharan Africa: estimation of sero-prevalence and impact on meat and milk offtake potential. FAO, Rome

Mathews F (2009) Zoonoses in wildlife integrating ecology into management. Adv Parasitol 68:185–209. https://doi.org/10.1016/S0065-308X(08)00608-8

Max V, Herrera J, Moreira R, Rojas H (2007) Avian influenza in Chile: a successful experience. Avian Dis 51:363–365

McDermott J, Grace D, Zinsstag J (2013) Economics of brucellosis impact and control in low-income countries. Rev Sci Tech 32:249–261

McInerney J (1991) Cost-benefit analysis of livestock disease: a simplified look at its economic foundations. In: Proc 6th Int Symp Vet Epidemiol Econ, pp 149–153

McKenzie AM, Thomsen MR (2001) The effect of *E. coli* O157:H7 on beef prices. J Agric Resour Econ 26:431–444. https://doi.org/10.2307/40987119

McLeod A, Morgan N, Prakash A, Hinrichs J (2006) Economic and social impacts of avian influenza

Meslin FX (2006) Impact of zoonoses on human health. Vet Ital 42:369–379

Messenger AM, Barnes AN, Gray GC (2014) Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. PLoS One 9:e89055. https://doi.org/10.1371/JOURNAL.PONE.0089055

Miller GY, Unnevehr LJ (2001) Characteristics of consumers demanding and their willingness to pay for certified safer pork. J Agribus 2:101–119
Miller MA, Buss P, Roos EO, Hausler G, Dippenaar A, Mitchell E et al (2019) Fatal tuberculosis in a free-ranging African elephant and one health implications of human pathogens in wildlife. Front Vet Sci 6:18. https://doi.org/10.3389/FVETS.2019.00018
Mitura V, Di Piétro L (2004) Canada's beef cattle sector and the impact of BSE on farm family income 2000–2003. Available at https://ageconsearch.umn.edu/record/28038/files/wp040069.pdf
Morel CM, Alm RA, Årdal C, Bandera A, Bruno GM, Carrara E et al (2020) A one health framework to estimate the cost of antimicrobial resistance. Antimicrob Resist Infect Control 9(1):187. https://doi.org/10.1186/S13756-020-00822-6
Murray CJL, Lopez AD (1996) Summary – the Global Burden of Disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. The Harvard School of Public Health on behalf of the World Health Organization and The World Bank distributed by Harvard University Press
Myers SS, Gaffikin L, Golden CD, Ostfeld RS, Redford KH, Ricketts TH et al (2013) Human health impacts of ecosystem alteration. Proc Natl Acad Sci U S A 110:18753–18760. https://doi.org/10.1073/PNAS.1218656110
Narrod C, Zinsstag J, Tiongco M (2012) A one health framework for estimating the economic costs of zoonotic diseases on society. EcoHealth 9:150–162. https://doi.org/10.1007/s10393-012-0747-9
OIE (2018) Chapter 8.4: Infection with *Brucella abortus*, *B. melitensis* and *B. suis*. In: Terrestrial animal health code. Available at https://www.woah.org/fileadmin/Home/eng/Health_standards/tahc/2018/en_chapitre_bovine_brucellosis.htm
Orozco C (2005) The SPS agreement and crisis management: the Chile-EU avian influenza experience. Compil. 45 case Stud. which Doc. disparate Exp. among Econ. addressing challenges Particip. WTO. Available at https://www.wto.org/english/res_e/booksp_e/casestudies_e/case10_e.htm
Paarlberg PL, Seitzinger AH, Lee JG (2007) Economic impacts of regionalization of a highly pathogenic avian influenza outbreak in the United States. J Agric Appl Econ 2:325–333
Pangaribowo E, Gerber N, Torero M (2013) Food and nutrition security indicators: a review. FOODSECURE working paper 04. Available at: https://www.wecr.wur.nl/WECRGeneral/FoodSecurePublications/05_Pangaribowo%20Gerber%20Torero_FNS%20Indicators.pdf
Patterson GT, Thomas LF, Coyne LA, Rushton J (2020) Moving health to the heart of agri-food policies; mitigating risk from our food systems. Glob Food Sec 26:100424. https://doi.org/10.1016/J.GFS.2020.100424
Peck D, Bruce M (2017) The economic efficiency and equity of government policies on brucellosis: comparative insights from Albania and the United States of America. Rev Sci Tech 36:291–302
Phillips L (chair) (2000) The BSE inquiry report, vol 10. Her Majesty's Stationery Office, London
Probst C, Gethmann JM, Heuser R, Niemann H, Conraths FJ (2013) Direct costs of bovine spongiform encephalopathy control measures in Germany. Zoonoses Public Health. https://doi.org/10.1111/zph.12032
Rassy D, Smith RD (2012) The economic impact of H1N1 on Mexico's tourist and pork sectors. Health Econ. https://doi.org/10.1002/hec.2862
Reaser JK, Witt A, Tabor GM, Hudson PJ, Plowright RK (2021) Ecological countermeasures for preventing zoonotic disease outbreaks: when ecological restoration is a human health imperative. Restor Ecol 29. https://doi.org/10.1111/REC.13357
Rojas H (2009) Animal diseases management in a new livestock trade environment: the case of Chile. In: Rushton J (ed) The economics of animal health and production. CABI, Wallingford, pp 274–279
Roos R (n.d.) USDA estimates *E. coli*, *Salmonella* costs at $3.1 billion. CIDRAP. https://www.cidrap.umn.edu/news-perspective/2010/05/usda-estimates-e-coli-salmonella-costs-31-billion. Accessed 21 Sept 2021
Rossiter PB, Hammadi NA (2009) Living with transboundary animal diseases (TADs). Trop Anim Health Prod 41:999–1004
Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G, Cosivi O et al (2003) Human health benefits from livestock vaccination for brucellosis: case study. Bull World Health Organ 81:867–876
Rushton J (2009a) The economics of animal health and production. CABI, Wallingford

Rushton J (2009b) Economic analysis tools. In: Rushton J (ed) The economics of animal health and production. CABI, Wallingford, pp 65–106
Rushton J, Thornton PK, Otte MJ (1999) Methods of economic impact assessment. Rev Sci Tech 18:315–342
Rushton J, Häsler B, De Haan N, Rushton R (2012) Economic benefits or drivers of a "One Health" approach: why should anyone invest? Onderstepoort J Vet Res 79:461. https://doi.org/10.4102/ojvr.v79i2.461
Rushton J, Bruce M, Bellet C, Torgerson P, Shaw A, Marsh T et al (2018) Initiation of global burden of animal diseases programme. Lancet 392:538–540. https://doi.org/10.1016/S0140-6736(18)31472-7
Rushton J, Huntington B, Gilbert W, Herrero M, Torgerson PR, Shaw APM et al (2021) Roll-out of the Global Burden of Animal Diseases programme. Lancet 397:1045–1046. https://doi.org/10.1016/S0140-6736(21)00189-6
Serra T (2011) Food scare crises and price volatility: the case of the BSE in Spain. Food Policy 36:179–185. https://doi.org/10.1016/j.foodpol.2010.11.006
Singh BB, Dhand NK, Gill JPS (2015) Economic losses occurring due to brucellosis in Indian livestock populations. Prev Vet Med 119:211–215. https://doi.org/10.1016/J.PREVETMED.2015.03.013
Singh BB, Kostoulas P, Gill JPS, Dhand NK (2018) Cost-benefit analysis of intervention policies for prevention and control of brucellosis in India. PLoS Negl Trop Dis 12:e0006488. https://doi.org/10.1371/JOURNAL.PNTD.0006488
Swinburn BA, Kraak VI, Allender S, Atkins VJ, Baker PI, Bogard JR et al (2019) The global syndemic of obesity, undernutrition, and climate change: the Lancet Commission report. Lancet 393:791–846. https://doi.org/10.1016/S0140-6736(18)32822-8
Thumbi SM, Njenga MK, Marsh TL, Noh S, Otiang E, Munyua P et al (2015) Linking human health and livestock health: a "One-Health" platform for integrated analysis of human health, livestock health, and economic welfare in livestock dependent communities. PLoS One 10. https://doi.org/10.1371/journal.pone.0120761
Tisdell C (1995) Assessing the approach to cost-benefit analysis of controlling livestock diseases of McInerney and others. University of Queensland, Brisbane
Torgerson PR (2013) One world health: socioeconomic burden and parasitic disease control priorities. Vet Parasitol 195:223–232. https://doi.org/10.1016/j.vetpar.2013.04.004
Torgerson PR, Shaw APM (2021) A simple metric to capture losses. Available at https://bulletin.woah.org/?panorama=03-2-2021-1_ahle
Torgerson P, Torgerson D (2008) Does risk to humans justify high cost of fighting bovine TB? Nature 455:1029. https://doi.org/10.1038/4551029a
Torgerson PR, Rüegg S, Devleesschauwer B, Abela-Ridder B, Havelaar AH, Shaw APM et al (2018) zDALY: an adjusted indicator to estimate the burden of zoonotic diseases. One Health 5:40–45. https://doi.org/10.1016/j.onehlt.2017.11.003
Velu G, Crespo Herrera L, Guzman C, Huerta J, Payne T, Singh RP (2019) Assessing genetic diversity to breed competitive biofortified wheat with enhanced grain Zn and Fe concentrations. Front Plant Sci 9:1971. https://doi.org/10.3389/FPLS.2018.01971
Wahba Tadros SN, Wellenstein A, Das MB, Palmarini N, D'Aoust OS, Singh G, Restrepo Cadavid P, Goga S, Terraza HC, Lakovits C, Baeumler AEN, Gapihan AT (2021) Demographic Trends and Urbanization (English). Washington, D.C. : World Bank Group. http://documents.worldbank.org/curated/en/260581617988607640/Demographic-Trends-and-Urbanization
Weerahewa J, Meilke KD, LeRoy D (2007) An economic assessment of the BSE crisis in Canada: impacts of border closure and BSE recovery programs, pp 1–35. Available at https://www.uoguelph.ca/catprn/PDF/Working%20Paper%202007-1a.pdf
WHO (2021a) Zoonoses. https://www.who.int/news-room/fact-sheets/detail/zoonoses. Accessed 28 Sept 2021
WHO (2021b) Rabies. https://www.who.int/news-room/fact-sheets/detail/rabies. Accessed 19 Sept 2021
WHO (2021c) Global Health Observatory. https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates. Accessed 28 Sept 2021

Wigle R, Weerahewa J, Bredahl M, Samarajeewa S (2007) Impacts of BSE on world trade in cattle and beef: implications for the Canadian economy. Can J Agric Econ 55:535–549. https://doi.org/10.1111/j.1744-7976.2007.00108.x

Wolfe MS, Ceccarelli S (2020) The increased use of diversity in cereal cropping requires more descriptive precision. J Sci Food Agric 100:4119–4123. https://doi.org/10.1002/JSFA.9906

World Bank (2010) People, pathogens and our planet. Volume 1: towards a One Health approach for controlling zoonotic diseases, report no. 50833-GLB. World Bank, Washington, DC

World Bank (2021) Employment in agriculture (% of total employment) (modeled ILO estimate). https://data.worldbank.org/indicator/SL.AGR.EMPL.ZS?end=2019&most_recent_value_desc=true&start=2019&view=map. Accessed 2 June 2021

Wright C (2004) The avian influenza outbreak in Chile. Poult Int 2004:34–39

Xiaoqin Fan E (2003) SARS: economic impacts and implications. Available at https://www.adb.org/publications/sars-economic-impacts-and-implications

Zhang N, Huang D, Wu W, Liu J, Liang F, Zhou B et al (2018) Animal brucellosis control or eradication programs worldwide: a systematic review of experiences and lessons learned. Prev Vet Med 160:105–115. https://doi.org/10.1016/J.PREVETMED.2018.10.002

Zoonoses and Poverty: The Multiple Burdens of Zoonoses in Low- and Middle-Income Countries

56

Delia Grace and Elizabeth Cook

Contents

Abstract

Poor people have greater exposure to zoonoses through livestock keeping; living in agricultural communities; greater exposure to peri-domestic and wild animals; and less access to clean water and sanitation. Although their consumption of animal source products is low, the quality of these products is poor.

In addition to human health burdens, zoonoses reduce livestock productivity and are important barriers to trade in livestock products, as well as causing more difficulty to quantify harms such as spillover to wildlife populations. These

D. Grace (✉)
Natural Resources Institute, Chatham Maritime, UK

International Livestock Research Institute, Nairobi, Kenya
e-mail: d.grace@cgiar.org

E. Cook
International Livestock Research Institute, Nairobi, Kenya
e-mail: e.cook@cgiar.org

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9_46

additional impacts also contribute to poverty in developing countries. However, the relation between poverty and zoonoses is complicated.

Assessing the impacts of zoonoses helps prioritize management. Among the most important zoonoses in developing countries are leptospirosis, cysticercosis, brucellosis, tuberculosis, and rabies and zoonoses causing foodborne disease. The COVID-19 pandemic also showed how lack of resilience leads to greater vulnerability of poor people to emerging zoonoses of high economic impact.

Investment and innovation are urgently needed to tackle zoonoses in developing countries where they currently impose massive burdens on human, animal, and ecosystem health.

Keywords

Zoonoses · Low- and middle-income countries · Poverty

56.1 Introduction

This chapter focuses on links between poverty and zoonoses in low- and middle-income countries (LMICs) and highlights recent developments in our understanding of these links. We first provide a typology of zoonoses according to their epidemiology and next present evidence that the burden of endemic zoonoses falls mainly on the poor, because of greater exposure risk. We describe the endemic zoonoses of greatest impact on poor people. We then discuss how emerging zoonoses can have more harmful impacts on the poor even if health and economic impacts are less, using evidence from the COVID pandemic. We, last, summarize major advances in approaches to understanding and managing the zoonoses of poverty in the last decade.

56.2 Poverty and Endemic Zoonoses

56.2.1 Zoonoses

"The poor you will always have with you" says the Christian Bible, and while wealth disparities are inevitable, are poverty and disease inevitability linked? Over decades and centuries, there has been a positive trend of global reduction in infectious diseases, especially those associated with environmental conditions and food systems as fewer people were directly exposed to the conditions favoring the transmission of disease. However, at the start of the twenty-first century, many people, particularly those in poor, remote, and rural communities, continue to be vulnerable to the avoidable diseases they contract as a result of direct and indirect contact with animals.

Zoonoses are diseases transmissible between animals (livestock and wildlife) and humans. Around 60% of all human diseases and around 75% of emerging infectious diseases are zoonotic (Woolhouse et al. 2005). The first section of the chapter provides an overview of zoonoses and poverty, and the second section provides more details on zoonoses, which have been identified as priorities in poor countries.

Zoonoses threaten human health and well-being in different ways.

- Endemic zoonoses are continually present to a greater or lesser degree in certain animal and human populations. Examples are cysticercosis, brucellosis, bovine tuberculosis, leptospirosis, and foodborne zoonoses. They have mainly been eradicated from wealthy countries but remain common in poor populations. Most at risk are people in LMICs in frequent contact with animals, and perhaps as a result, endemic zoonoses have been neglected by the international donor, standard setting, and research communities.
- Outbreak or epidemic zoonoses typically occur intermittently. Examples are anthrax, rabies, Rift Valley fever, and leishmaniasis. Endemic zoonoses may also occur as outbreaks in naïve populations or when triggered by events such as climate changes, flooding, waning immunity, or concomitant hunger or disease. The overall health impact of epidemic zoonoses is much less than endemic zoonoses, but because they can "shock" systems they reduce resilience. Most are absent from or controlled in HICs, and the burden largely falls on poor rural communities in LMICs. Outbreaks may result in slaughter bans or movement restrictions that have impacts on the wider community including people who are not in contact with livestock. Donors and decision-makers are concerned over epidemic zoonoses because of the risks they pose to trade and economies.
- Emerging zoonoses are those that newly appear in a population or have existed previously but are now rapidly increasing in incidence or geographical range. They are relatively rare, around 300 events in the last 70 years (Grace et al. 2012b). Most are of minimal impact, but some such as COVID-19 have changed the way civilizations operate. Especially if transmission shifts from animal-to-human to human-to-human, people in HICs and LMICs are both at risk. Donors and decision-makers are often concerned about emerging diseases, because of their potentially large impacts on global human health and economies.

Zoonoses do not just threaten human health; they also reduce livestock productivity and impose costs on domestic and international trade (McDermott et al. 2013). Some zoonoses impose a large morbidity and mortality burden in the livestock host (e.g., brucellosis). In other cases, disease impacts in animals may be few (e.g., salmonellosis in chicken), but there may be high costs for the food sector from inspection and intermittent food recalls. Other costs of zoonoses may be more difficult to quantify, for example, the cost of lost biodiversity when animal diseases spill over to wildlife (as when rabies in Ethiopia threatens survival of the Ethiopian wolf, the rarest canid in the world). The environmental impacts are often overlooked, but in the past deforestation and insecticides such as DDT have been used to reduce tsetse fly abundance and hence human African trypanosomiasis with lasting impacts on the ecosystem.

56.2.2 Poverty Context

Poverty can be defined as a pronounced deprivation in well-being. No single indicator exists to measure all dimensions of poverty simultaneously; however,

internationally comparable metrics, such as the "extreme poverty" threshold (less than $2.15 per person per day at 2017 purchasing power parity), are useful for spatial and temporal comparisons. Since 1990, the number of people in extreme poverty was declining, a trend temporarily interrupted by COVID-19 (World Bank 2022). However, the geography of poverty is shifting. While in 1990, 36% of the world lived in extreme poverty and 80% of these were in Asia, by 2030 7% of the world will be in extreme poverty but 85% of these will be in sub-Saharan Africa (Kharas and Dooley 2022).

Livestock keepers, especially pastoralists, are overrepresented among the poor and the poorest of the poor (Grace et al. 2017). The reasons for this are complex: Many livestock-keepers live in rural or remote areas, most are underserved, and many keep livestock because they are poor, rather than to escape poverty (Perry and Grace 2009).

56.2.3 Poverty and Zoonoses

Globally, the greatest burden of infectious disease is borne by poor countries. Since 1990, when systematic efforts first started to estimate the global health burden of disease, estimates have become more granular and precise (Murray 2022). Burden of disease is calculated using the disability-adjusted life year (DALY) which combines years of life lost due to premature mortality and years of life lost due to time lived in states of less than full health.

However, global burden of disease (GBD) estimates have some challenges in assessing zoonoses. First, zoonoses (especially in poor countries) are widely underreported, and underreporting is relatively greater for zoonoses than for non-zoonotic diseases of comparable prevalence (Schelling et al. 2007). Second, no burden estimates exist for several priority zoonoses (di Bari et al. 2022). Third, the GBD is organized around diseases and not pathogens, so assumptions need to be made about the proportion of a disease that is due to zoonotic pathogens, for example, tuberculosis is one category in the GBD, but the proportion that is due to zoonotic diseases is not estimated.

Even with these assumptions, it is clear that poor countries bear the greatest burdens of zoonotic disease (Grace et al. 2012a). The burden of endemic zoonoses falls mainly on the poor and causes at least ten million DALYs annually (di Bari et al. 2022). The great majority of the global foodborne disease burden (33 million DALYs) falls on LMICs, and most of the responsible pathogens are zoonotic (Havelaar et al. 2015). These estimates do not include the burden due to HIV-AIDS (48 million DALYs) (Wu et al. 2021) or COVID-19 (42 million DALYs) which are emerging zoonoses (Fan et al. 2021). Moreover, zoonoses make a substantial contribution (25%) to the estimated 420 million DALYs lost from all infectious diseases (GBD 2020).

We next discuss why people in poor countries may be more vulnerable to zoonotic infections, and why the most poor may be the most vulnerable. There are several reasons why poor people might bear a greater burden of zoonoses. First, they may be exposed to higher levels of pathogens; second, they may be more vulnerable to disease; and third, they may be less able to prevent or treat zoonoses. Finally, even

if they are equally or less vulnerable to disease, the impacts of disease may be relatively greater because of the lower resilience of the poor. This is especially the case for high-impact emerging zoonoses such as COVID-19.

56.2.4 Poverty and Exposure to Zoonoses

This section focuses on the first and most obvious reason poor people are more at risk, which is because they have much greater exposure to pathogens harbored by animals responsible for endemic and epidemic zoonoses.

- Poor people are much more likely to keep or be in contact with animals than rich people. Poorer households are more likely to keep small ruminants and richer households to keep large ruminants. Poultry keeping tends to be evenly distributed across wealth groups. However, poorer people are more likely to keep livestock in the house or close at hand and biosecurity and hygiene practices may be lower.
- Within poor countries, the poorest of the poor often keep less livestock. However, the less poor may keep more livestock than the relatively better off. A 12-country study supports this, finding that on average, around 68% of rural households in the bottom 40% as regards expenditure kept some farm animal compared to 65–58% of those in the top 40%; in urban areas, 22–26% of the poor kept livestock, and 8–12% of the well-off (Pica-Ciamarra et al. 2011). Consumption of bushmeat is also much more common in developing countries.
- Because of poor sanitation and waste disposal, there is greater contact with peridomestic animals such as bats and rodents in poor communities. There are also large numbers of community-owned dogs and cats, which are often semi-scavenging though they may return to one household at night. Poor people are also more exposed to vectors, both through work in agriculture and because they are unable to afford bed nets and other prevention measures.
- Foodborne transmission is a common route for exposure to zoonoses. Overall, livestock consumption is much lower in poor countries than in rich countries. The better off in poor countries have higher consumption of meat, milk, fish, and eggs which are indirect routes for zoonoses transmission and so would be more at risk from infection through this route than poor people. Studies in developing countries typically find that food contains high levels of pathogens; often the majority of samples will exceed international standards (Grace 2015). Waterborne transmission is important from some zoonoses, notably *Cryptosporidium parvum, Escherichia coli* O157, *Salmonella*, *Campylobacter,* and *Taenia solium*). Because poverty is strongly linked to lack of access to clean water, the poor are more at risk by transmission through this pathway.

56.2.5 Poverty and Greater Vulnerability to Zoonoses

People in poor countries may be more biologically vulnerable to endemic and epidemic zoonoses. Compared to rich countries, the proportion of infants, pregnant

or lactating women, and immune-suppressed people is higher. Moreover, high levels of malnutrition and high exposure to toxins (especially mycotoxins) increase vulnerability to infection.

In addition, private and public health services are often under-resourced and underperforming. Common problems include lack of diagnostic facilities; lack of trained personnel; lack of appropriate drugs; high prevalence of fake drugs; and expenses associated with obtaining treatment.

Unlike endemic and epidemic zoonoses which are transmitted by contact with domestic livestock, emerging zoonoses are most serious when they evolve to be transmitted between humans. This was how the Spanish flu, which killed more than 40 million people, is believed to have emerged, and was also the origin of HIV-AIDS, and more recently COVID. Once a disease has become pandemic, the victim profile changes and is more dependent on transmission modalities, virulence properties, immunological status, and treatment and prevention options. For example, the Spanish flu caused greater mortality among younger people, perhaps because older people had some immunity from a previous flu pandemic (Russian flu in 1889–1890) (Gagnon et al. 2013). Because of its transmission through bodily fluids, certain populations are more at risk of HIV-AIDS: In 2021, at-risk groups (sex workers and their clients, men who have sex with men, and people who inject drugs) and their sexual partners accounted for 70% of HIV infections globally (UNAIDs 2023). In the case of COVID-19, LMICs seem to have caused less mortality in LMICs, possibly because of younger age population, fewer comorbidities, cross-immunity from other coronaviruses, and climate conditions (Okonji et al. 2021; Pedersen et al. 2022).

However, while the direct health impacts of emerging zoonoses may be no worse, or even less in LMICs, the indirect livelihood and economic effects may be more severe. Movement restrictions, travel bans, and curfews may prevent opportunities to earn income, purchase food, or access health services for marginalized people.

Although poor people are at greater risk from zoonoses as a result of their greater contact with animals or their lack of resources and hence greater vulnerability, animals and animal source food also bring many benefits. Livestock support livelihoods through generating income, provide nutrient-rich food for household consumption, are assets that can guarantee risks or be monetized to pay for routine or unexpected expenses, may provide manure for soil fertility, fuel, and building materials, may provide power (ploughing, transport), and often have cultural value and provide psychosocial benefits. Livestock can even directly reduce risk of human illness. Zooprophylaxis, the diversion of disease carrying insects from humans to animals, may reduce transmission of diseases such as malaria (Asale et al. 2017).

56.3 Zoonoses of Poverty

56.3.1 Most Important Zoonoses in Poor Countries

In recent years, several prioritization exercises have been developed for zoonoses and animal health, although most did not focus on poor countries. These were summarized by Grace et al. (2012b), finding, in declining order of importance:

- First place: Salmonellosis
- Joint second place: Leptospirosis = rabies
- Joint fourth place: Campylobacteriosis = tuberculosis = West Nile virus infection = toxoplasmosis
- Joint eighth place: Listeriosis = anthrax = echinococcosis = *E. coli* infection = BSE = botulism
- Joint 14th place: Cryptosporidiosis = Japanese encephalitis = Q fever = Rift Valley fever = tetanus

The same study identified 56 priority zoonoses from five different credible sources. These 56 zoonoses were then ranked by five criteria: human mortality; human morbidity; livestock sector impacts; amenability to intervention; and risk of emergence. This produced a short-listing of 12 zoonoses, which were responsible for an estimated 2.2 million human deaths and 2.4 billion cases of illness each year. Total 9 of the 13 top-ranked zoonoses were considered to have high impact on livestock, all have a wildlife interface, and all were amenable to agriculture-based interventions (Table 1).

Another study was carried out for the World Organisation for Animal Health (WOAH) in the WOAH African region on priority diseases (Grace et al. 2015). Questionnaires were sent to the Veterinary Authorities of the 54 OIE Member Countries in Africa. In all, 34 countries responded. As regards zoonoses in general, rabies was most commonly the first priority (44%) followed by anthrax (15%), and then zoonotic influenza, Rift Valley fever, and bovine tuberculosis tied (9% each).

Table 1 Top zoonoses from a literature-based study

Disease	Wildlife interface	Deaths human annual	Affected humans annual
Gastrointestinal (zoonotic)	Important	1,500,000	2,333,000,000
Leptospirosis	Very important	123,000	1,700,000
Tuberculosis (zoonotic)	Some importance	100,000	554,500
Rabies	Important	70,000	70,000
Cysticercosis	Some importance	50,000	50,000,000
Leishmaniasis	Important	47,000	2,000,000
Brucellosis	Some importance	25,000	500,000
Echinococcosis	Important	18,000	300,000
Toxoplasmosis	Important	10,000	2,000,000
Q fever	Important	3000	3,500,000
Trypanosomosis (zoonotic)	Important	2500	15,000
Anthrax	Some importance	1250	11,000

Grace et al. (2012b)

The remaining countries selected brucellosis (n = 2), leptospirosis, or salmonellosis (n = 1). When asked more specifically about the priority emerging zoonoses, avian influenza was selected by 44% of respondents, followed by Rift Valley fever (25%) and Ebola (11%). A few other diseases were selected (anthrax, brucellosis, bovine tuberculosis, monkey pox, and trypanosomosis). Among priority foodborne diseases, two-thirds reported salmonellosis, 11% bovine tuberculosis, and 7% colibacillosis, anthrax, or cysticercosis; the remaining country reported brucellosis.

The last decade has seen dozens of zoonoses prioritization exercises conducted at a national level, summarized in Table 2. Most (n = 22) used the methodology developed by the US Centres for Disease Control and Prevention (CDC), and the One Health Zoonotic Disease Prioritization (OHZDP) Tool, while eight used their own methods.

While these prioritizations were done at different times, and covered different countries, for different purposes, the agreement is striking. The most important zoonoses are the classical zoonoses (rabies, anthrax, brucellosis, leptospirosis, and tuberculosis), foodborne (dominated by salmonellosis, cysticercosis, and campylobacteriosis), viral hemorrhagic fevers (especially Ebola and Rift Valley fever), and emerging (avian influenzas). All of these studies were conducted before the COVID pandemic starting around 2020, but COVID would undoubtedly be near the top of a current list of zoonoses. This shows the need to consider "Disease X" in prioritization and preparedness, that is, a disease currently not

Table 2 The top diseases as ranked by prioritization exercises in 30 countries

	Asian (n = 13)	African (n = 17)	All (n = 30)
Zoonotic influenza/HPAI	12	16	28
Rabies	13	14	27
Anthrax	8	12	20
VHF[a] (any of Ebola, Marburg, Lassa, yellow fever, Crimean-Congo hemorrhagic fever [CCHF], and RVF)	4	14	18
Brucellosis	8	7	15
Zoonotic tuberculosis	2	8	10
Foodborne bacteria	3	4	7
Trypanosomiasis	NA	5	5
Leptospirosis	5	0	5
Q fever	1	0	1
Plague	1	0	1
Streptococcus suis	1	0	1
Echinococcus	1	0	1
Trichinella	1	0	1
Nipah virus	1	NA	1
Dengue	0	1	1
Mpox	NA	1	1

[a]VHF covered different viruses for different countries; here the count is for any mention of any VHF
NA: disease is currently geographically limited to one region (Source: authors)

known but with the potential of being at least as important as current priorities (Van Kerkhove et al. 2021). This results in a short list of ten top zoonoses which will be considered in more depth in the next section. That a small number of zoonoses are considered responsible for the great majority of the problem is compatible with the Pareto principle which states roughly 80% of consequences come from 20% of causes. This phenomenon is commonly observed in disease rankings (Grace et al. 2012b).

56.3.2 Top 11 Zoonoses in Poor Countries

In the last section of the chapter, we consider some of the priority zoonoses in greater depth.

56.3.2.1 Classical Zoonoses

Rabies: Rabies is one of the most feared of all zoonoses. It is a disease of poor and vulnerable communities where deaths are often unreported: More than 95% of human deaths occur in Africa and Asia. Dogs are the most important reservoir, but many domestic and wild animals can be affected. Where rabies is common, it can be of minor importance as a cause of mortality in livestock.

Anthrax: Anthrax is endemic in sub-Saharan Africa and many parts of Asia. It is highly lethal to ruminants, but in humans the most typical manifestation are skin lesions (malignant pustule). However, if spores are inhaled or ingested, death may result. Anthrax is not common in most developing countries, but some areas are prone to outbreaks where localized losses can be high.

Brucellosis: The most important species of *Brucella* are zoonotic: *B. abortus*, responsible for bovine brucellosis; *B. melitensis*, the main etiologic agent of ovine and caprine brucellosis and an increasing cause of cattle brucellosis; and *B. suis,* causing pig brucellosis. The main risks for people are occupational (contact with livestock) and consumption of dairy products. In some areas, brucellosis may be maintained in reservoir wild animal hosts (African buffaloes and North American bison); in other cases, disease spills over to wildlife and, if eliminated in cattle, brucellosis will die out in wildlife. Brucellosis is an important disease of livestock in developing countries because of the impacts on animal fertility and reproduction as well as the public health impacts.

Leptospirosis: Leptospirosis is an infectious disease caused by pathogenic organisms belonging to the genus *Leptospira*. There are many serovars (>250), but typically only around 10–20 are found in a given region. Serovars can be grouped into 25 serogroups. Infected animals often become carriers. Wild animals are affected and can be important reservoir hosts. Although the impact of leptospirosis on animal production has not been quantified, it may be an important cause of disease, particularly abortions, in animals in developing countries. Evidence is especially strong for swine production in Asia. There are occupational risks to dairy farmers, milkers, and abattoir workers in these settings.

Tuberculosis: Worldwide and historically, most human tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (which probably gave rise to cattle TB). *M. bovis* is responsible for cattle tuberculosis. It affects a wide range of animals and is responsible for zoonotic TB in humans. In West Africa, *M. africanum* causes up to half of human tuberculosis. Atypical mycobacteria are found in the soil and environment and can infect both people and animals. Tuberculosis is an important cause of reduced productivity in livestock but is believed to make only a small contribution to the global TB burden (Müller et al. 2013). However, it can be a more serious problem in some high-risk communities (especially cattle-keepers in Africa).

56.3.2.2 Foodborne Zoonoses

Nontyphoidal salmonellosis (NTS): NTS is one of the most frequently isolated foodborne pathogens. Livestock (cattle, swine, and poultry) are the main reservoirs of NTS. Infections are characterized by nonbloody diarrhea, vomiting, nausea, headache, abdominal cramps, and myalgias (hence often called "gastric flu"). Complications can occur such as pancreatitis. Antibiotic-resistant strains have emerged and are often of increased virulence.

Campylobacteriosis: *Campylobacter* species are widely distributed in most warm-blooded animals. Infections are generally mild but can be fatal among very young children, elderly, and immunosuppressed individuals. Although diarrhea is the most common symptom, more severe symptoms such as bacteremia or flaccid paralysis can occur. Subclinical infection has been associated with stunting in children (too short for their age).

Cysticercosis: Cysticercosis is a systemic parasitic infestation caused by the pork tapeworm (*Taenia solium*). Humans are at risk not from consumption of pork with cysts but from consumption of tapeworm eggs shed by themselves or another human carrier. The disease persists in poor, pig-keeping communities where pigs have access to human feces. Cysticercosis is believed to be the most common cause of adult-onset epilepsy in poor, pig-keeping communities. The disease is not an important cause of illness in livestock but reduces the value of meat produced.

56.3.2.3 Viral Hemorrhagic Fevers

Ebola virus disease (EVD). Formerly known as Ebola hemorrhagic fever, EVD is a severe, often fatal illness. It is transmitted to people from wild animals (the natural reservoir is thought to be fruit bats) and spreads in the human population through direct contact with the blood, secretions, organs or other bodily fluids of infected people, and with contaminated surfaces and materials. Antibodies have also been detected in livestock, but the implication is not known. Previously mainly confined to African rainforests, more recently its range has increased and intense transmission has taken place in urban areas.

Rift Valley fever (RVF): This is a viral disease most commonly seen in domesticated animals in sub-Saharan Africa, such as cattle, buffalo, sheep, goats, and camels. People can get RVF through contact with blood, body fluids, or tissues of infected animals, or through bites from infected mosquitoes. Spread from person to person has not been documented. Most symptoms in people are mild, but around 8–10% may develop hemorrhage, eye disease, or encephalitis.

56.3.2.4 Emerging Zoonoses

Zoonotic influenzas: Zoonotic influenza refers to disease caused by animal influenza viruses that cross the animal–human divide to infect people. Avian, swine, and other zoonotic influenza virus infections in humans may cause disease ranging from mild upper respiratory tract infection to death. A major concern is that zoonotic influenzas may mutate to novel influenza with the ability to cause sustained human-to-human transmission. Three worldwide (pandemic) outbreaks of influenza virus with avian origins occurred in the twentieth century: in 1918, 1957, and 1968.

Coronavirus infection: Coronaviruses (CoV) are a family of RNA viruses that typically cause mild respiratory disease in humans. In the twenty-first century, there have been three major emergences capable of causing severe disease in humans. Severe acute respiratory syndrome (SARS) was detected in China in 2003, Middle East respiratory syndrome (MERS) in Saudi Arabia in 2011, and COVID also in China in 2019. The reservoir host is likely bats, but spillover is associated with intermediate hosts: civet cats in the case of SARS and camels in the case of MERS.

56.4 Conclusion

Livestock-keepers in developing countries are not all alike, and there are important differences between isolated and neglected pastoralists in a declining and increasingly climate insecure economy, and the rapidly growing urban centers with their accompanying intensifying livestock systems. The most critical systems where improved understanding on the prevalence, impact, and dynamics of zoonoses are needed include the following: rapidly intensifying agricultural systems; urban systems; and systems where substantial land-use change is occurring especially irrigation in arid areas, and farming on forest margins.

As the importance of zoonoses in poor countries is increasingly recognized, so is the need to generate a stronger evidence-base on problems and solutions to support decision-making. Information is needed on the impacts of zoonoses, the multiple costs and benefits of control, and the sustainability, feasibility, and acceptability of zoonoses management.

History in the developed world shows that the burden of zoonotic diseases can be dramatically reduced. High zoonotic disease prevalence is both a cause and consequence of poverty, and investment and innovation are urgently needed to tackle zoonoses in developing countries where they currently impose massive burdens on human, animal, and ecosystem health.

56.5 Cross-References

▶ Brucellosis
▶ *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control
▶ Cryptosporidium and Cryptosporidiosis: Trickle or Treat?

- ▶ Crimean-Congo Hemorrhagic Fever Virus: An Emerging and Re-emerging Pathogen of Public Health Concern
- ▶ Cystic and Alveolar Echinococcosis: Fraternal Twins Both in Search of Optimal Treatment
- ▶ Cysticercosis
- ▶ Elimination of Rabies: A Missed Opportunity
- ▶ Enterohemorrhagic *E. coli* (EHEC): Environmental-Vehicle-Human Interface
- ▶ Human African Trypanosomiasis: The Smoldering Scourge of Africa
- ▶ Influenza from a One Health Perspective: Infection by a Highly Versatile Virus
- ▶ Q Fever (*Coxiella burnetii*)
- ▶ The Zoonotic Agent Salmonella
- ▶ Toxoplasmosis: A Widespread Zoonosis Diversely Affecting Humans and Animals

References

Asale A, Duchateau L, Devleesschauwer B, Huisman G, Yewhalaw D (2017) Zooprophylaxis as a control strategy for malaria caused by the vector *Anopheles arabiensis* (Diptera: Culicidae): a systematic review. Infect Dis Poverty 6(1):160

di Bari C, Venkateswaran N, Pigott D, Flastl C, Devleesschauwer B (2022) The global burden of neglected zoonotic diseases: current state of evidence. Eur J Public Health 32(Suppl 3): ckac129.757

Fan CY, Fann JC, Yang MC, Lin TY, Chen HH, Liu JT, Yang KC (2021) Estimating global burden of COVID-19 with disability-adjusted life years and value of statistical life metrics. J Formosan Med Assoc = Taiwan yi zhi 120(Suppl 1):S106–S117

Gagnon A, Miller MS, Hallman SA, Bourbeau R, Herring DA, Earn DJ, Madrenas J (2013) Age-specific mortality during the 1918 influenza pandemic: unravelling the mystery of high young adult mortality. PLoS One 8(8):e69586

GBD 2019 Diseases and Injuries Collaborators (2020) Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396(10258):1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

Grace D (2015) Food safety in low- and middle-income countries. Int J Environ Res Public Health 12(9):10490–10507

Grace D, Gilbert J, Randolph T, Kang'ethe E (2012a) The multiple burdens of zoonotic disease and an ecohealth approach to their assessment. Trop Anim Health Prod 44(S1):67–73

Grace D, Mutua F, Ochungo P, Kruska R, Jones K, Brierley L, Lapar L, Said M, Herrero M, Phuc PM, Thao NB, Akuku I, Ogutu F (2012b) Mapping of poverty and likely zoonoses hotspots. Zoonoses Project 4. Report to the UK Department for International Development. ILRI, Nairobi

Grace D, Songe M, Knight-Jones T (2015) Impact of neglected diseases on animal productivity and public health in Africa. Paper written for 21st conference of the World Organisation for Animal Health (OIE) regional commission for Africa, Rabat, Morocco, 16–20 February 2015. ILRI, Nairobi

Grace D, Lindahl J, Wanyoike F, Bett B, Randolph T, Rich KM (2017) Poor livestock keepers: ecosystem-poverty-health interactions. Philos Trans R Soc Lond Ser B Biol Sci 372(1725): 20160166

Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ et al (2015) World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med 12(12):e1001923

Kharas H, Dooley M (2022) The evolution of global poverty, 1990–2030, Brookings Global working paper 166. Brookings Institution, Washington, DC

McDermott J, Grace D, Zinsstag J (2013) Economics of brucellosis impact and control in low-income countries. Sci Tech Rev 32(1):249–261

Müller B, Dürr S, Alonso S, Hattendorf J, Laisse CJM, Parsons SDC, van Helden PD, Zinsstag J (2013) Zoonotic mycobacterium bovis–induced tuberculosis in humans. Emerg Infect Dis 19 (6):899–908

Murray CJL (2022) The Global Burden of disease study at 30 years. Nat Med 28:2019–2026

Okonji EF, Okonji OC, Mukumbang FC, Van Wyk B (2021) Understanding varying COVID-19 mortality rates reported in Africa compared to Europe, Americas and Asia. Trop Med Int Health: TM & IH 26(7):716–719

Pedersen J, Koumakpayi IH, Babuadze G et al (2022) Cross-reactive immunity against SARS-CoV-2 N protein in Central and West Africa precedes the COVID-19 pandemic. Sci Rep 12:12962

Perry B, Grace D (2009) The impacts of livestock diseases and their control on growth and development processes that are pro-poor. Philos Trans R Soc B 2009(364):2643–2655

Pica-Ciamarra U, Tasciotti L, Otte J, Zezza A (2011) Livestock assets, livestock income and rural households. FAO, Rome

Schelling E, Grace D, Willingham AL, Randolph TF (2007) Which research approaches for pro-poor control of zoonoses? Food Nutr Bull 2 Suppl:S345–S356

UNAIDS (2023). https://www.unaids.org/en/resources/fact-sheet

Van Kerkhove MD, Ryan MJ, Ghebreyesus TA (2021) Preparing for "Disease X". Science 374 (6566):377

Woolhouse MEJ et al (2005) Emerging pathogens: the epidemiology and evolution of species jumps. TRENDS Ecol Evol 20(5):238–244

Wu J, Tianwen L, Han H, Wang S, Lyu J (2021) Global, regional and national disability-adjusted life years due to HIV from 1990 to 2019: findings from the Global Burden of Disease Study 2019. Trop Med Int Health

Index

A. Sing (ed.), *Zoonoses: Infections Affecting Humans and Animals*,
https://doi.org/10.1007/978-3-031-27164-9