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Animal-Origin Viral Zoonoses

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Livestock Diseases and Management

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Preface

In the present context of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), causing Coronavirus Disease (COVID-19) nothing is crucial than the discussions over the emergence of human viral infections having natural or intermediate animal hosts. The ongoing COVID-19 pandemic has altered us for making future preparedness plans in tackling evolving and upcoming disease which could arise in days to come. Bats, as well as several other wild animals like palm civet and pangolins, have been linked with the emergence of zoonotic viruses with the accumulation of genetic changes. With reporting of COVID-19 in a pet dog from his infected owner has further unlocked a window of dialogue on reverse zoonoses (human to animal transmission). The ongoing pandemic of SARS-CoV-2, as well as previous epidemics of coronavirus SARS-CoV of 2002 and MERS-CoV of 2012, has socially and economically affected the world.

Furthermore, to name a few more, the viral diseases/infections such as Rift Valley fever, West Nile fever, avian influenza A (H5N1), Nipah virus, Zika virus, and swine influenza A (H1N1) are frightening adversely public health globally. Therefore, the viral infections having zoonotic links became the researcher's prime choice. The restricted availability of safe and inexpensive prophylactics and therapeutics forces us to depend mainly on the control and preventive measures for limiting the transmission of emerging zoonotic viral diseases. In the current scenario, utmost need realized is for developing the capacity building for detection and differentiation of the pathogen, developing rapid, sensitive, and cost-effective door-step assays/kits, and strengthening of regional and peripheral diagnostic laboratories and clinical and surveillance of the diseases in the susceptible and in-contact animal populations.

An up-to-date resource is essential for the public and research community to apprehend the latest information and trends in the field of emerging zoonotic viruses that might help to adopt corrective actions. In the current compilation on "*Animal-origin Zoonotic Viruses*," we intend to deliver a conversant resource in this area. The collection highlights the consequence of zoonotic viral diseases to the public and livestock industry using apposite examples. This book describes the precise and up-to-date information on zoonotic animal viral diseases which have emerged in the

recent past or are re-emerging due to several complex environmental factors. Decisively, the chapters delineate current day information on the emergence and circulation of zoonotic animal viral diseases with a focus on the virus, diseases, hosts, diagnostics, prophylactics, and therapeutics. The book discusses important viruses/viral infections of public health concern in various chapters authored by national and international experts. Moreover, the book provides the essential information in the form of tables and figures, with specific references at the end for readers to obtain further details on each topic.

In total, fifteen chapters in this book are covering important zoonotic animal viruses and wild animal's role in the spread of zoonoses, including drivers of emerging viral zoonoses. The first chapter (Chap. 1) by Dr Isloor and coworkers provides an overview on the oldest and most discussed zoonotic viral disease "*Rabies*," highlighting the significance of diseases, its current worldwide status and detection ways, whereas Chap. 2 on the *Monkeypox virus* by Nikola Sklenovska provides a brief overview of virus epidemiology, immunopathobiology, and diagnostics. Likewise, comprehensive information is provided on the *Nipah virus* in Chap. 3 by Dr Saxena. Calicivirus poses severe threat globally as a cause of acute gastroenteritis in young and adults. In Chap. 4, Dr Ghosh and colleagues discussed the progress on *Animal Caliciviruses*. Influenza disease, a century-old problem, still possess a threat to the public and livestock. An overview of the *Avian Influenza virus* is given in Chap. 5 by Dr Nagarajan and associates, and Dr Saxena's team have provided an overview of the *Pandemic Influenza A virus (pH1N1)* in Chap. 6.

The burden of poxviruses is tremendous in humans and animals. Dr Amit Kumar and team in Chap. 7 have provided a comprehensive overview of the *Buffalopox virus*. In Chap. 8, *Animal Rotaviruses*, which come under the family *Reoviridae,* is discussed by Dr Vlasova's team. This chapter mainly focuses on rotaviruses affecting different animal hosts, and a few of them are also zoonotic, explaining their epidemiology, diagnosis, and control. The next chapter (Chap. 9) by Dr Venkatesan's group elaborates the *Capripoxvirus and Orf virus*, giving its current situation globally. These two viruses are well known for their economic burden in small ruminants rearing countries. In the subsequent chapter (Chap. 10), Dr Vassilis Papatsiros has overviewed *Hepatitis E viruses* which have become a big problem during these days, having relevance to animals. Dr Hemida in Chap. 11 explains about MERS-CoV that affected humans and involved camels in their transmission cycle during the outbreaks occurred initially in 2012 in Saudi Arabia and nowadays well discussed during the ongoing pandemic of SARS-CoV-2. In the next chapter (Chap. 12), Dr Das and associates provide a detailed account of the *Japanese encephalitis virus,* the economically significant encephalitis disease, where swine acts as an amplifier host.

Dr Naveen's team has dealt with *Picobirnavirus*, a small newly identified virus, affecting several animal hosts as well as human beings in Chap. 13. It is now recognized as an emerging virus problem related with coinfections and immunocompromised individuals. A detailed account on *Drivers of Emerging Viral Zoonoses* is discussed in Chap. 14 by Dr Ghatak and team. The human–wildlife interface is considered highly significant on account of the emergence of different pathogens.

The last chapter (Chap. 15) is on *Viral Zoonoses: Wildlife Perspectives* by Dr Milton and colleagues.

We believe that owing to the in-depth knowledge of crucial zoonotic animal viruses with high-quality contributions by experts, the present book will be an excellent source of information for the readers. The information compiled would be useful for veterinary professionals, clinicians, public health experts, researchers, students/scholars, animal producers, faculty, and students. Further, it would help those who have an interest in virology, viral diseases, epidemiology of viral infections, viral zoonoses, and management of viral diseases and epidemics, for countering important animal viral diseases.

We, the Editors, would like to express our gratefulness to all the contributors for their support and hard work in making this book compilation a realism. We also extend special thanks to all the peer reviewers whose competent expertise and rigorous reviewing of the manuscripts helped the authors to improve their manuscript further to reach the publication phase. The Editors are grateful to the Springer Nature Publisher for accepting the book proposal. We extend our special thanks to Dr Bhavik Sawhney, Associate Editor, Biomedicine, Springer Nature for providing all the editorial help and high cooperation while processing the manuscripts for its successful publishing.

Izatnagar, India Yashpal Singh Malik
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World Society for Virology

"Animal-Origin Viral Zoonoses" a publication from World Society for Virology.

About World Society for Virology

World Society for Virology (WSV) is a non-profit organization, 501c3-ID No. 001303257 that was established in 2017 with the mission to strengthen virology research on different viral diseases of humans, animals, plants, and others.

The WSV main objectives include but not limited to:

1. Gather the virologists worldwide in the main society that does not require a fee for its membership [a great obstacle for many virologists in many countries] and provide help to all whenever possible.
2. Build up a network of scientific collaborations among virologists worldwide.
3. Build international bridges for virology laboratories worldwide.
4. Help virologists worldwide to advance their careers and obtain awards.
5. Provide educational resources free of charge and freely available to all members.
6. Help and facilitate getting scholarship and vacancies for virologists worldwide.
7. Build up databases of virologists based on their field of specialization for remote assistance and guide in case of the existence of any disease outbreak.

For details, visit www.ws-virology.org

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About the Editors

Yashpal Singh Malik is presently working as "ICAR National Fellow" at the premier Veterinary Institute of the country-Indian Veterinary Research Institute (IVRI), Izatnagar, India. His major research achievements include contributions in viral disease epidemiology, virus–host interactions, microbial biodiversity, characterization, and diagnosis. He acquired advanced training in Molecular Virology from the University of Minnesota, Saint Paul, USA; Division of Virology, Ontario Research Institute, University of Ottawa, Ontario, Canada, and Wuhan Institute of Virology, Wuhan, China. He is a recipient of several prestigious national, state, and academy awards/honors including ICAR Jawaharlal Nehru Award (2001); Young Scientist Award of the Association of Microbiologists of India (2000) and Uttarakhand Council of Science and Technology (2010). He is active member of noted scientific and professional societies of international and national repute. He has been bestowed with several honors in the form of distinguished Associateships/Membership viz. Associateship of National Academy of Agricultural Sciences (2010), Membership-National Academy of Veterinary Sciences (2010); CSIR-Senior Research Fellowship (1997–2000), ICAR-Junior Research Fellowship (1995–1997); Academic Merit Scholarship in bachelor's degree (1990–1995). He is elected Fellow of the Indian Virological Society, Indian Association of Veterinary Public Health Specialists, Indian Society for Veterinary Immunology and Biotechnology, and National Academy of Biological Sciences. Dr. Malik is

member International Committee on Taxonomy of Viruses (ICTV) on Birnaviridae and Picobirnaviridae study group and managing committee member of World Society for Virology. He has supervised 3 Ph. D. and 17 M.V.Sc. students. Over the years, he has developed several technologies and diagnostic kits and also has filed two national patents. He has authored 5 books, 25 book chapters, and published 2017 scientific research and review articles in peer-reviewed national/ international journals of high impact factor. Dr. Malik has been the Editor-in-Chief of Journal of Immunology Immunopathology and also edited the special issues of several reputed journals.

Raj Kumar Singh is currently the Director-cum-Vice-Chancellor of the ICAR-Indian Veterinary Research Institute, Izatnagar. Dr. Singh is a noted scientist of high repute with specialization in veterinary microbiology, biotechnology, molecular epidemiology, diagnostics, and vaccinology. Dr. Singh has served as Head, Division of Virology, Station-in-Charge at IVRI, Mukteswar campus, Uttarakhand, and later Director, NRC on Equines and VTCC, Hisar. Dr. Singh has 10 national patents (granted-2 and filed-8), developed >8 live attenuated vaccines/vaccine candidates and >26 diagnostic tests/assays/kits. He has authored 2 books, 23 book chapters, and published over 245 scientific research papers, 52 reviews, 15 lead papers, and 24 guest editorials/compendium chapters. Dr. Singh has supervised 8 doctoral and 11 master's students. Dr. Singh received several prominent awards including prestigious ICAR Rafi Ahmed Kidwai Award and Team Research Award, DBT Tata Innovation Fellowship Award; Agriculture Research Leadership Award, FAO Fellowship for training at the University of California Davis, USA, and many others. He is serving as President ISVIB, besides having several distinguished Fellowships and life memberships of prestigious professional societies.

Kuldeep Dhama is presently working as Principal Scientist in the Division of Pathology at ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. He has research and teaching experience of more than 25 years in the field of microbiology, immunology, virology, public health, medicine, and biomedicine. He has developed several diagnostics, vaccines, and immunomodulatory modules to counter infectious diseases of animals and poultry. He has to his credit more than 600 publications, 6 books, and 65 book chapters. He has been recognized as an extremely productive researcher in the "Nature" journal publication. He is honored with 50 Best Paper Awards and other recognitions. He is NAAS (National Academy of Agricultural Sciences, India) Associate, worked as Nodal Officer—WTO, and Member—Wildlife Health Specialist Group (IUCN). He is actively serving as Editor-in-Chief, Co-EIC, Editor and Member, Editorial board of more than 20 scientific journals.

Chapter 1
Rabies

S. Isloor, R. Sharada, and S. Abdul Rahaman

Abstract Rabies is a viral disease of zoonotic importance, endemic in several countries in Asia, Africa, Western Europe and North and South America. The dog remains the most important source of infection in the countries of Asia, Africa and Latin America. Rabies is endemic in all the countries of the Indian subcontinent. This disease primarily affects the central nervous system producing abnormal behaviour and paralysis in most of the hosts it afflicts. DFA is most widely employed for post-mortem diagnosis of rabies. The development of dRIT is one of the most significant developments in the diagnosis of rabies. Further, LFA, an immunochromatography based tool, is a rapid test and highly useful for diagnosis of rabies at the field without the need for laboratory equipment. Recently, versions of RT-PCR and real-time PCRs including the LN 34 real-time PCR are becoming popular in molecular diagnosis and epidemiological studies. Further, the rabies virus neutralization tests (FAVN or RFFIT) are considered to be the gold standards to assess the anti-rabies vaccinal antibodies. As an alternative, quantitative ELISA is used. Rabies diagnosis in animals is revolutionized through recent OIE initiatives in India through twinning programme with a mandate of "Strengthening of diagnosis of rabies in animals in India". To achieve control of rabies in animals, particularly dogs, a co-ordinated multipronged approach involving various agencies is necessary. There is need to evolve the programme for vast rural India with emphasis on regular booster vaccination and seromonitoring vaccinal antibodies.

Keywords Rabies · Dogs · Diagnosis · DFA · dRIT · LFA · RFFIT · ELISA · OIE twinning · Control

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1.1 Prologue

Rabies derives its name from the Sanskrit word "Rabhas" meaning "to do violence" which describes the furious form of the disease. This neurotropic virus infects all warm-blooded animals causing a serious disease of fatality involving the central nervous system and has been documented for more than 4000 years (WHO 2018a, b). References about human deaths from bites of mad dogs exist in the Babylonian legal codes in 2300 BC. Democritus described the disease in dogs and domestic animals in 500 BC. The Iliad of Homer (eighth century) describes a character Homer, who suffered from rabies. In 1804, Zinke first demonstrated the infectious nature of saliva by inoculating it from infected animals to healthy dogs. In 1885, Louis Pasteur developed, tested and used the vaccine against rabies without knowing the nature of the virus which led to the era of prevention of diseases by vaccination. Remlinger first demonstrated the filterability of this infectious agent in 1904, and in the same year Negri demonstrated the intracytoplasmic inclusion bodies, later known as "Negri bodies" in brain tissues of rabid dogs. The rabies virus was adapted in cultures of non-neuronal cells by Kissling in 1958 which led to large scale propagation of virus in cell cultures for vaccine production (Sarma 2009).

Globally, the dog is the common source of exposure of human beings to rabies virus. Other mammals, especially wild carnivores and bats also pose a threat as they are reservoirs of the virus (OIE 2013; Birhane et al. 2017; Rupprecht et al. 2017). Wild animals such as jackals, foxes, mongoose, rats, squirrel, wolves, skunk, vampire bats act as natural reservoir hosts.

1.2 Epidemiology

Rabies occurs in all countries except Japan, UK, Ireland, Cyprus, New Zealand, Scandinavia, Hawaii islands, Caribbean islands, Australia and Switzerland. The disease is endemic in many countries of Africa, Asia, North and South America and Western Europe. Two epidemiological cycles are established in rabies, namely the urban and the sylvatic cycle. The urban cycle is maintained in dogs and transmitted to other species through bite of a rabid dog. The dog remains the most important source of infection in the developing countries of Africa, Asia and Latin America (Zee and MacLachlan 2005). The sylvatic cycle is maintained amongst wild animals which can result in spillover infections to the domestic animals and man. Based on susceptibility, the hosts are broadly classified into four categories; most susceptible are the fox, coyote, wolf, jackal and voles. Skunk, bat, mongoose and cattle are considered increasingly susceptible. The moderately susceptible hosts are dogs, sheep, goat and horses; birds are said to be least susceptible. Foxes are important reservoirs in Western Europe, portions of Canada, Alaska and the desert south-western regions of the USA, whereas skunks and raccoons act as reservoirs in regions of North America. In Asia and Africa, mongoose is the reservoirs. Recently,

the first case report of rabies in a wolf (*Canis lupus pallipes*) from India was documented based on laboratory evidence (Isloor et al. 2014). The first confirmed case of rabies in a sloth bear (*Melursus ursinus*) from India was reported by Patel et al. (2018) from Gujarat state. Cattle and equines are considered dead-end hosts. Rats and bandicoots are naturally susceptible. Laboratory animals such as mice, rabbits and guinea pigs can be infected experimentally (Sarma 2009).

1.2.1 Global Scenario

Globally, based on the prevalence of rabies, the countries have been classified as (a) countries with enzootic canine rabies—Asia, Latin America, Africa, (b) countries where canine rabies is under control and wildlife rabies is prevalent—Western Europe, Canada, the USA, (c) rabies free countries—most islands, Australia, England, Japan (De Serres et al. 2008). In America, various species of insectivorous and vampire bats harbour the rabies virus, and 30 different variants of the virus are identified in various species of bats in North America (Nadin-Davis et al. 2001; Nadin-Davis and Loza Rubio 2006) which can spill over to other species of animals in the wild. The rabies virus is maintained in domestic dogs, vampire bats and insectivorous bats in sylvatic cycles in South America (Favi et al. 2002; Kobayashi et al. 2005). The virus is also reported in monkeys, wolves, coyotes, skunks, foxes and mongooses (Belotto et al. 2005; Everard and Everard 1988) in South and Central America. The discovery of Australian bat lyssavirus (ABLV) in 1996 made Australia lose it "rabies-free" status. ABLV was isolated from insectivorous bat and four species of flying fox bats following the death of a ten-year-old girl with clinical signs of rabies (Gould et al. 2002). The last case of dog transmitted rabies was reported in 1867, and since then Australia was considered "rabies-free" though incidences of dog rabies were occasionally reported which were attributed to importation of the dogs (Warrilow 2005). In Europe, intense vaccination campaigns were successful in controlling dog rabies in 1940s, but wild canines escalated the incidences of rabies since then. Terrestrial rabies is under control in countries such as the United Kingdom, Finland and the Netherlands through strict vaccination campaigns (Bourhy et al. 2005). There was a paucity of information regarding rabies in the African continent until late twentieth century. Rabies occurs as scattered foci and is spread by dogs in sparsely populated countries spanning the Saharan desert; occasional cases in camels have been reported (Swanepoel 2004). The rabies-related viruses, viz. Mokola, Lagos bat and Duvenhage virus have been presumed to be in circulation since hundreds of years in the African continent (Nel and Rupprecht 2007). The World Health Organization (WHO) has reported 23,700 human rabies deaths per annum in Africa (WHO 2013) which is reported mostly in poor rural communities and children and is attributed to the inadequate and costly necessary resources for rabies prevention and treatment.

1.2.2 Rabies in the Indian Subcontinent

Rabies is endemic in all the countries of the Indian subcontinent comprising of Afghanistan, Bangladesh, Bhutan, India, Nepal, Maldives, Myanmar, Pakistan and Sri Lanka. It is estimated to have claimed the lives of about 59,000 people every year (Hampson et al. 2015) with an estimate of 45% of all deaths due to rabies reported in the Indian subcontinent (Gongal and Wright 2011). Of these, about 20,000 are in India as per the WHO survey report of 2004. However, in India, the current estimates of death in human beings due to rabies is ranging from 17,000 to unconfirmed 10,000. The high prevalence of rabies in the subcontinent is attributed to a lack of awareness of post-exposure preventive measures such as washing of wounds, vaccinations and administration of immunoglobulins. Furthermore, poor supply of anti-rabies vaccine and rabies immunoglobulins (RIGs), especially in rural health-care facilities and expensive vaccines and RIGs are other contributory factors. In Bangladesh, rabies is endemic and is third in the list of countries that are endemic for rabies (Hossain et al. 2011). Annually, more than 2000 deaths in human beings are reported (Hossain et al. 2012) with most of the victims reported being children of less than 15 years of age from the poor rural population (Hossain et al. 2011, 2012). A surveillance study during 2010–2012 reported deaths due to rabies in population of several domesticated animals including cattle (2845), goats (547) and sheep (Salahuddin et al. 2016; Mondal and Yamage 2014). Interestingly, this surveillance did not document the cases of rabies in dogs. In Bhutan, only one human death was reported in 2011 (Pelzang and Tshewang 2011). In Maldives, there are no dogs and rabies is not reported in either humans or animals. However, the cats and bats are the potential threats in future. There is an active rabies awareness programme instituted by the government. In Nepal also majority of the human rabies deaths are attributed to dog bites. The reported human deaths are 37 per annum (Singh and Shrestha 2011). In Afghanistan, during 2010, 40 rabies deaths were reported. However, estimates of human rabies deaths were reported to be higher in the provinces with poor vaccination coverage (Hidaythullah 2011). In Myanmar, annually, approximately 600,000 human beings are bitten with most of them being children and annually estimated 1000 deaths. In this process, each month 2500 dogs were killed by the Yangon City Development Committee. This drew criticism from residents and animal rights activists. In India, the prevalence of rabies is high. While the exact number of rabies deaths is unknown, with estimates ranging from 10,000 to 17,000 (Hampson and Meslin 2013) it accounts for 36% of the world's deaths. Incidence of rabies is higher in Indian rural areas (1.8 per 1000) compared to urban areas (1.4 per 1000), and thousands die every year from the disease (Sudarshan et al. 2007). In India, a significant reduction in the number of human deaths due to rabies could be achieved through targeting with preventive campaigns including preventive vaccination of animals and post-exposure vaccination of humans (Suraweera et al. 2012). In India, the Association for Prevention and Control of Rabies in India (APCRI) in collaboration with World Health Organization (WHO) conducted a study to assess the burden of human rabies in 2004. In the study, it was found that the annual

incidence of human rabies was estimated to be 17,137 and an additional 20% was added to this estimate to include atypical forms. The primary animal responsible for bites was dog (96.2%), most of which were stray (Sudarshan et al. 2007). As a part of recently completed WHO-APCRI Indian multicentric rabies survey in 2017 (Sudarshan et al. 2018), laboratory-based surveillance for the status of rabies in dogs/cats was carried out in the islands of Andaman/Nicobar and Lakshadweep. The islands of Andaman are historically free from rabies but have considerable dog population. Whereas the Lakshadweep islands are not only free of rabies but also dogs. However, there is sizeable population of cats in Lakshadweep. The initial dog's brains ($n = 4$) from Andaman and cat brain samples from Lakshadweep ($n = 5$) screened were negative for rabies.

1.3 Classification

Rabies is a disease caused by the rabies virus of the genus *lyssavirus* of the family *Rhabdoviridae*, order *Mononegavirales*. The lyssa viral species are divided into two phylogroups based on their genetic distance and serological cross-reactivity. The rabies virus belongs to the phylogroup1 (ICTV 2017; Rupprecht et al. 2002). The other lyssaviruses produce disease like rabies and bats are an important reservoir host of several of these viruses (Zee and MacLachlan 2005). Monoclonal antibody analysis has demonstrated considerable antigenic variation among virus isolates from outbreaks associated with different wildlife vectors or from different geographical areas (Debbie and Trimarchi 1992; Sarma 2009).

Highly virulent strains of rabies virus isolated from naturally occurring cases are referred to as "Street viruses". The attenuated laboratory strains are referred to as "Fixed viruses". These are street viruses adapted to the laboratory by passaging them in unnatural hosts like rabbits or in cell culture. These strains differ from each other in their biological properties in laboratory animals, for example, virulence, incubation period and distribution and nature of lesions in target tissues. The street viruses have a long incubation period, an affinity for salivary glands and produce intracytoplasmic inclusion bodies but do not produce any cytopathic effect in cell cultures. The fixed strains have short and defined incubation period of 5–7 days and are more neurotropic for rabbits but have no affinity for the salivary glands; they multiply faster and are stable viruses. They are comparatively less pathogenic and do not produce inclusion bodies, thus are used for vaccine production. These can infect only by the CNS route. Further, these are not seen in the saliva and other peripheral secretions. For example Flury strain, Street Alabama Dufferin (SAD), Vnukovo, Kelev. The antigenic variation between the strains can be distinguished by their reaction with monoclonal antibodies.

1.4 Virion Properties

Rabies virus is a bullet-shaped (80 nm × 180 nm), an enveloped, negative-sense—single-stranded RNA (11–15 kb) virus. The nucleocapsid is helically coiled and cylindrical in shape. The genome of rabies virus codes for five different viral proteins, namely nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA dependent RNA polymerase (L) in the order 3′–5′. The RNA exists as ribonucleoprotein (RNP) with the N protein tightly encasing the RNA. The N protein is present in abundance and is an important structural component of the viral ribonucleoprotein core which is required for propagation of virus. Furthermore, N protein is the primary target for diagnosis of rabies. The phosphoprotein is a cofactor component of the viral polymerase. The M protein facilitates virion budding by linking the nucleocapsid to the lipid envelope that contains the G glycoprotein. The glycoprotein is the major surface protein and is peplomers or spikes seen on the surface of the virion. These form approximately 400 trimeric spikes or peplomers of 6–7 nm in length which are closely aligned on the viral envelope. The G protein is highly antigenic and has the epitopes for vaccine-induced neutralizing antibodies and rabies immunoglobulins (RIGs). The G spike protein controls major aspects of host cell infection such as receptor binding, antigenicity and host adaptation. Moreover, it is involved in the trans-synaptic spread within the central nervous system. The nucleoprotein includes epitopes involved in cell-mediated immunity. The polymerase aids in transcription and replication of the virus (ICTV 2017).

The lipid component of virions is derived from the cell membranes of the host. The virus contains approximately 67% proteins, 26% lipid, 4% RNA and 3% carbohydrate and has a buoyant density of 1.19–1.20 g/cm^3 in caesium chloride and 1.17–1.19 g/cm in sucrose gradients. The sedimentation value ranges between 500 and 1000S (Sarma 2009; Chandra et al. 2015). It is heat-labile and inactivated by heating at 56 °C for 30 min. The virus is ether sensitive and readily inactivated by exposure to sunlight or UV radiation, formalin (1%), cresol (3%), beta-propiolactone (0.1%), mercuric chloride (0.1%), aqueous solutions of household bleach, quaternary ammonium compounds and hospital disinfectants. The virus persists in the infected brain tissue for up to 10 days at room temperature and several weeks at 4 °C but is relatively susceptible to disinfection. It can be preserved indefinitely at −70 °C and by freeze-drying. The virus is susceptible to pH below 7.0 and above 10 (Debbie and Trimarchi 1992; Zee and MacLachlan 2005; Chandra et al. 2015). Laboratory adapted strains haemagglutinates goose erythrocytes at 0–4 °C and pH 6.8 (Sarma 2009).

1.5 Viral Replication

The rabies virus infects all warm-blooded animals and replicates in them. Thus the virus can be propagated in chick embryo or duck embryo or various cell culture systems like baby hamster kidney cells (BHK21), human diploid cells (WI-26), mouse neuroblastoma cells (MNA) (Zee and MacLachlan 2005). The glycoproteins are essential in the entry of rabies virus into the susceptible cells through receptor mediation. The virus is taken in by pinocytosis via the clathrin-coated pits in vesicles of cytoplasm. The fusion of viral membrane with the endosomal membrane is dependent on pH after it is endocytosed by a process known as viropexis. This results in release of the ribonucleoproteins (RNPs) by uncoating (Roche and Gaudin 2004; Gaudin 2000). The RNA is synthesized by viral origin polymerases L and P through stuttering transcription. In the replication mode, complete length ribonucleoproteins are generated. Further, the structural components of the viral envelope, the matrix protein and glycoproteins are necessary for assembly and release of matured virus particles (Rose and Whitt 2001). The virions mature as they are released by the budding through the plasma membrane. The rabies virus buds through the intracytoplasmic membranes of the infected neurons or plasma membranes of the salivary glands and epithelial cells. The rabies virus replicates slowly and usually does not induce cytopathic effect (CPE) as it does not shut down the synthesis of host cell protein and nucleic acid synthesis. However, it results in formation of prominent inclusion bodies that are of diagnostic importance. The M protein is an important factor in budding of virus and a regulatory protein (Finke et al. 2003; Finke and Conzelmann 2003).

1.6 Clinical Features

The clinical signs of rabies are similar in most species but vary between individuals. The incubation period is prolonged, varying from 2 weeks to 6 months and even longer in some exceptional circumstances (WHO 2018a, b). The CNS disturbance manifests as behavioural changes like nervousness, irritability, hyperexcitability, ataxia, altered phonation. The affected animal prefers to be in isolation. A change in temperament of the animal is a common feature noticed in rabies infections wherein a docile animal becomes vicious or aggressive, and an aggressive animal turns docile. The clinical signs of the disease with minor exceptions for different species can split into three phases that could be overlapping at times. A prodromal phase exists which lasts for 1–3 days before the overt clinical disease which is normally overlooked. In this phase the animal shows only vague clinical signs which intensify rapidly after the onset of paralysis. The affected animal succumbs to death within 10 days of onset of clinical signs. Excitative phase referred to as the furious form of the disease follows the prodromal phase and is the most commonly encountered form. It is also referred to as the “Mad-Dog Syndrome”. The animal exhibits

restlessness with nervous signs and could be aggressive. It turns aggressive on slightest provocation (of sound or noise) and uses its teeth, claws, horns or hooves and thus bites at anything that gains its attention. The affected animal will exhibit an alert posture with dilated pupils and an anxiety expression. It loses the sense of fear, swallows foreign objects and shows hyperaesthesia. Carnivores tend to roam aimlessly attacking any moving objects or personnel on its way. As the disease progresses, furious symptoms reduce and paralytic signs sets in, incoordination and seizures are seen and the animal finally succumbs to death due to progressive paralysis within 2–14 days after the onset of clinical signs. Paralytic phase also termed as the dumb form manifests as hydrophobia and profuse salivation or inability to swallow due to paralysis of the pharyngeal muscles. Dropping of lower jaw is characteristic. The animal remains dumb and rarely bites, thus posing a risk of infection. The paralysis progresses rapidly resulting in coma and death within few hours.

1.6.1 Dogs

The incubation period in dogs vary from 10 days to 2 months, and the clinical signs attributable to the CNS are paramount in dogs. There may be hyperexcitability or lethargy, pharyngeal paralysis and thus frothing of saliva, posterior paresis or paralysis, sudden coma and death. Behavioural changes are common during the early phases of the disease when the dog behaves abnormally, hides in dark corners, shows unusual agitations, becomes restless. Fever, dilatation of the pupils and photophobia are sometimes present. The furious form follows the prodromal phase and the affected dogs may bite without any provocation. It may bite itself and inflict serious injuries. Some dogs exhibit only a paralytic stage with the characteristic dropped jaw and incoordination. Progressive paralysis begins with the muscles of the head and neck region. The tone of bark changes due to partial paralysis of vocal cords. Convulsions are seen in the terminal phase followed by incoordination and posterior paresis. Once the clinical signs set in, the disease progresses rapidly to the death of the animal due to respiratory failure generally within 3–8 days. It is during this clinical period and up to 5 days before recognition of clinical signs that the virus may be present in the saliva. This mandates the 14 days confinement and observation of a dog that has bitten a person or other animals (Zee and MacLachlan 2005). The excretion of virus in the saliva of infected dogs is intermittent and is variable (Hemachudha et al. 2013).

1.6.2 Cats

The clinical signs in cats are of a furious type and are similar to that in dogs, but the affected cats have a greater tendency to hide in secluded places and are more vicious

than dogs. The cat might strike in air with its forepaws. After 2–4 days of the excitation phase, the paralysis of posterior third of the body follows.

1.6.3 Cattle, Buffalo, Sheep and Goat

The incubation period may vary from 2 weeks to many months. In cattle, the prodromal signs may manifest as an animal being off feed and water and a drop in milk production which is of little diagnostic value. Lactation ceases abruptly, grinding of teeth, salivation or pharyngeal paralysis is often misdiagnosed as a choke. Cattle become aggressive with rabies infrequently. In furious form of the disease, the cattle loses its placid expression and becomes alert and restless. The eyes and ears of affected animal follow the sound and movement and thus butts moving objects, attacks man and animals nearby. Salivation, choking, absence of rumination, rectal straining and paralysis of hindquarters are noticed. There may be sexual excitement, and the animal starts bellowing abnormally due to vocal paralysis and intermittently until it succumbs to death within 12–24 h. Once the clinical signs are evident, the disease progresses rapidly to the death of the animal generally within 5–7 days. During this period and up to 5 days before recognition of clinical signs, the virus may be excreted in the saliva.

1.6.4 Horses and Mules

In horses, the signs are similar to tetanus. Initially, there is a weakness or lameness. The infected horses and mules appear distressed and agitated. They start rolling, which is normally confused with colic. The animal may bite or strike viciously and becomes unmanageable causing self-inflicted wounds. Tremors and spasms are noted in specific muscles. Difficulty in swallowing, progressive paralysis, stiffening of the hindquarters, ataxia and eventual death within 2–4 days.

1.6.5 Pigs

The symptoms are characterized by excitement, irritation, rooting up the ground or rubbing at the surface, aggressiveness, biting of hard objects, other animals and man followed by paralysis and death in 2–4 days.

1.6.6 Humans

Human infections are most commonly due to rabid dog bites. The early signs include headache, extreme thirst, vomiting and anorexia. Later, the painful spasms of the pharyngeal muscles when drinking (hydrophobia) is experienced. This is followed by excitement to sensory stimuli which progresses to generalized paralysis. Death is the inevitable outcome once clinical signs develop (Zee and MacLachlan 2005). The rabies virus can be detected especially in saliva, lacrimal secretions, urine and tissues of nervine origin, thus posing a risk when exposed to these secretions and excretions. Transmission among humans has been reported as a result of infected tissue or organ transplantation (Rupprecht et al. 2016; WHO 2018a, b). A single case of perinatal transmission has been reported (Aguèmon et al. 2016; Rupprecht et al. 2016).

1.6.7 Monkeys

In monkeys, clinical signs are similar to that exhibited in humans with hydrophobia, paralysis, anxiety. However, non-human primates do not play a major role in the transmission of rabies.

Herbivores do not transmit the disease. Exposure of the virus to mucous membranes and conjunctiva can result in infection, but infection through respiratory route is very rare. Inhalation of aerosols containing the virus in bat-infested caves can result in rabies. Transmission from bats is of increasing concern in the canine rabies-free areas since transmission can occur without any history of bite. The disease is also spread by frozen meat, urine and milk in bats. Intrauterine infections are reported in man, cattle, skunk, mice (Aguèmon et al. 2016; Rupprecht et al. 2016). Livestock is vulnerable victims of rabid carnivores and mongoose. In foxes, virus excretion is higher in urine and the nosing behaviour maybe a non-bite transmission mechanism in sylvatic rabies (Sarma 2009).

1.7 Pathogenesis

The rabies virus has an affinity to the nervous system (NS) (Wunner 1987) and reaching the CNS is critical for the virus to establish the infection. Various mechanisms are used by the virus to strategically evade the immune system of the host, but the detailed mechanism of evading the host and its pathogenesis during the early stages is not well understood. The glycoprotein and phosphoprotein have a major role in axonal transport. The virus cannot penetrate intact skin, and thus the disease transmission is most commonly by a bite. The susceptibility of the virus is less by oral route than the intramuscular route. Thus ingestion is not a common mode of infection. In the affected animals, the incubation period depends on various factors

like the location of bite (its proximity to the CNS), depth of the wound, load and virulence of the virus, susceptibility of the species of animal which is bitten, the stress condition and immune status of the animal all these play role in pathogenesis and course of the disease. The actual events that occur in the incubation period are not certain, but at the site where the viruses enter the host, the movement of the virus may get delayed. Hyaluronidase present in the saliva of biting animals particularly wild carnivores increases the permeability of the virus in tissues and eases the entry of virus. It is well proved that the nervous system is capable of sensing the attack of rabies virus and that it can also mount an immune response at the earliest.

The strains of rabies virus which cause acute infections escape the host innate immune response at least partially. The various mechanisms adopted by the virus for evading the immune surveillance explain why rabies remains one of the few infections with a mortality rate of almost 100%. (Jackson 2016). The virus enters the host through the bite wound or scratch (very rarely through mucous membranes). Following exposure, the virus is deposited and persists in the local muscle tissue for hours or days (3–9 weeks). The initial replication of virus occurs in the cytoplasm of the muscle cells or epithelial cells in the lower layers of epidermis near the bite. Since the replication is minimum at the site of bite, no detectable immune response is seen during the incubation period. Viremic phase does not exist since the virus does not move through the blood or lymphatics. On entry into the host, binding occurs at the postsynaptic muscle membrane to the nicotinic acetylcholine receptor (nAchR) which enriches the virus at the neuromuscular junction or synaptic cleft thus enabling an efficient infection of the motor neurons. Some studies indicate that infection of muscle cells might be aided by the nAchRs, suggesting initial virus replication in muscle cells. Further, the virus enters neurons through neural cell adhesion molecule (NCAM) and p75 neurotrophic receptor and is transported to the cell body through the axon in vesicles. Though two mechanisms are proposed for this movement of virus in vesicles as (1) whole virion or (2) only the virus capsid, the evidence favours intact virion transport in vesicles through the axons (Lentz et al. 1982; Dodding and Michael 2011). Once the virus enters the axon, antibodies cannot inhibit its transport, and the virus moves to the CNS at a rate of 12–100 mm per day by fast axonal transport (Kucera et al. 1985; Tsiang et al. 1991). The viral infection induces production of inflammatory cytokines and chemokines which in turn attracts activated lymphocytes leading to their migration through blood–brain barrier. Viral strains causing encephalitis tend to maintain the physical integrity of the neuronal network and the neurons to facilitate invasiveness right from the entry site to the site of exit producing a non-cytopathogenic kind of infection. The pathogenic strains are capable of inducing peripheral immunosuppression inhibiting an immune response and thus favouring its survival and invasion of the entire nervous system. The movement of the virus in the neurons involves the interaction of amino acid residues in the phosphoprotein at position 143 and 147 with the cytoplasmic dynein light chain (LC8) (Jacob et al. 2000; Raux et al. 2000; Poisson et al. 2001). The LC8, a component of myosin V and dynein is of 10 kDa. In the axons, this LC8 is associated with the actin-based vesicle transport and the microtubule directed organelle

transport. In the CNS, the rabies virus uses the axonal microtubules for its retrograde movement.

Furthermore, phosphoprotein (P) was identified to be responsible for inhibition of type I interferons. Experiments in mammalian cells have revealed that the P protein prevents the expression of interferon stimulating gene by interaction with STAT1 (Vidy et al. 2005). The virions ascend in the axons of nerve cells causing neuronal infection. Further replication or amplification of virus occurs in the dorsal root ganglion before its ascent to the brain from the spinal cord. The virions bud from the infected cell causing infection to the neighbouring cells. This centripetal passive movement causes ascending neuronal dysfunction and also forms the pathognomonic Negri bodies. The virions reach the CNS via the spinal cord and multiply extensively in the limbic system of the brain causing release of cortical control of behaviour resulting in fury and behavioural signs being exhibited. After proliferation in the brain, the virus disseminates within the brain. Multiplication of the viruses in the neocortex results in paralytic or dumb form. This is when the animal becomes anorexic, stays in the dark with profuse salivation. The virus can also persist in the brain of infected skunks, rats, raccoons, bats and foxes for many months without exhibiting any overt clinical signs. The virus further travels centrifugally through peripheral nerves to the salivary glands, retina, cornea, tonsils and nasal mucous membranes. The virus replicates rapidly in the salivary glands, and thus infected saliva is the major source of infection which precedes the clinical signs in some animals. The virus is excreted in all secretions and excretions.

The expression of neuronal dysfunction accounts for the clinical signs of rabies that causes the animal to attack (Debbie and Trimarchi 1992; Zee and MacLachlan 2005). The virus may be excreted through the milk of rabid animals, but its role in causing the disease has not been documented (WHO 2018a, b). If the cerebrum or cerebellum is not infected, it results in an abortive type of infections where no clinical signs are seen.

The ability of the rabies virus to induce very mild pathological changes in the CNS which indicates dysfunction and not the death of neurons is important in disease production though rabies is a neurological disease (Jackson 2002; Lafon 2011). The successful invasion of the CNS is attributed to the two important complementary characteristics of the virus (1) ability to escape from the host immune response and (2) ability to protect the infected neurons from apoptosis or premature destruction since the neurotropic viruses cause cell death by either apoptosis or necrosis (Griffin and Hardwick 1999; Allsopp and Fazakerley 2000; Fazakerley and Allsopp 2001). The glycoprotein (G) spikes present on the surface of viruses and its ability to bind to the receptors on cells determine the neuropathogenicity of the virus. The G proteins are also responsible for the induction of apoptosis. Thus the ability to avoid apoptosis improves the pathogenic potential of the virus and also correlates with the degree of attenuation of virus (Morimoto et al. 1999). The rate of replication of the virus and its glycoprotein expression levels correlates inversely with the pathogenesis, whereas the kinetics of virus uptake and its spread directly correlates to the pathogenesis.

The infected animal will have no antibodies when they first show the signs of illness. This virus does not induce the immune system and does not produce cytopathic effects. However, a detectable level of antibodies is seen in serum and cerebrospinal fluid (CSF) after 8–10 days of onset of clinical signs. Neutralizing antibodies are detected only during the terminal stages when the animal is about to succumb to death. Experimental studies have revealed that T lymphocytes from immunized animals are cytotoxic for rabies-infected cells and these rabies-infected cells are lysed by antibodies in the presence of complement (Sarma 2009). The T cell response resulting in antibody production is a crucial factor for clearance of virus from the nervous system and thus survival. The CD8+ T cells play a dual role by functioning together with the antibodies in controlling the infection by clearing viruses from the nervous system and also induce neuronal apoptosis. These cells thus initiate an immunopathological reaction associated with clinical paralysis (Jackson 2016).

1.8 Pathology

In general, gross pathological lesions are not visible in rabid dogs except fresh bite wounds and signs of self-mutilation. Microscopic changes are limited to CNS. The histopathology of brain tissue revealed moderate neuronal damage with encephalomyelitis and perivascular cuffing of lymphocytes, mononuclear infiltration and polymorphonuclear cells. Cytoplasmic eosinophilia, cytoplasmic vacuolation, pyknosis and karyorrhexis are more commonly seen in freshly fixed and adequately treated brain tissue. Acidophilic round or oval inclusions in the cytoplasm of infected neurons are seen with a clear halo around it which are referred to as Negri bodies. These are found in the pyramidal cells of Ammon's horns, Purkinje cells of the cerebellum and brain stem. A large number of small inclusions can also be demonstrated in the smears of brain tissue by immunofluorescence or immunoenzyme methods. Experimental inoculation of mice with fixed rabies virus may also show numerous virus particles without the formation of Negri body (Chandra et al. 2015).

1.9 Diagnosis

Accurate and rapid diagnosis is critical for initiating post-exposure prophylaxis and public health control strategies. Various methods are used for the diagnosis of the disease. However, proper collection and submission of post-mortem specimens with special reference to brain tissues from animals suspected for rabies constitute the basis for confirmatory diagnosis of rabies (Isloor et al. 2017).

1.9.1 Preliminary Safety

All individuals and laboratory personnel involved in the handling of rabies suspected cases should undergo pre-exposure immunization and regular boosters as required. These personnel are at risk of rabies infection through various means. Hence, personal protective equipment (PPE) must be used at all levels starting from necropsy procedure.

1.9.2 Agent Identification

As rabies virus tends to get rapidly inactivated, the specimens collected should be sent on ice to the laboratory by the fastest means available. Various techniques are employed to diagnose rabies and are particularly employed on brain tissue, but other organs such as salivary glands. For laboratory diagnosis, both cerebellum and brain stem are recommended to be collected since the virus will be present in abundance in these and aid laboratory diagnosis. These parts of the brain can be obtained after removing the entire brain through the skull open method during necropsy.

1.9.3 Collection of Samples

In a rabies-infected animal, the brain, spinal cord, saliva, salivary glands may contain the virus, and fresh, non-fixed tissue is acceptable for diagnosis of the disease. The brain tissue is the choice of specimen for rabies diagnosis, and thus the animal suspected for rabies should be euthanized in a manner such that the brain is not damaged. Animal heads are accepted for diagnosis; care should be taken so that the neck should be severed at the midpoint between the base of the skull and shoulders. Only veterinarians or animal control officers who have been vaccinated and perfectly trained should remove the animal heads. The post-mortem should be done in a ventilated area using protective gear. After opening the skull, appropriate samples like the brain stem and cerebellum are collected. This is a laborious task and hazardous too in field conditions or even when the prosector is not well trained. An alternate method of brain sample collection without the need to break open the skull has been developed and is referred to as the occipital foramen route of brain sampling.

Brain Sample Collection Through Occipital Foramen Route
The brain sample is collected through the occipital foramen by introducing a drinking straw of 5 mm or a disposable plastic pipette of about 2 mL or by using the artificial insemination sheath which is about 10 cm long into the foramen in the direction of the eye. Samples of brain stem and cerebellum can be collected from the juice straw or artificial insemination sheath (Fig. 1.1). This approach was reported to

Fig. 1.1 Collection of the brainstem from foramen magnum approach in dog

be user-friendly, rapid and risk-free for accurate diagnosis of rabies (Ghouse et al. 2018). This encourages collection and submission of more number of brain samples from the field for the laboratory confirmation.

1.9.4 Transportation of Samples to the Laboratory

The specimens collected shall be transported to the laboratory at the earliest either by post or by courier or by air as suitable. The specimens suspected for rabies should be shipped on ice in a leak-proof container to the laboratory so that it does not pose a threat of contamination. Any undue delay can wither away the cooling effect of ice especially in tropical climates enhancing putrefaction of sample making it unsuitable for diagnosis. If it is not possible to send the samples in a refrigerated condition, other preservation techniques may also be used. The preservative used shall be based on the tests to be employed for diagnosis. However, in most of the situations, either the brain sample may be packed as such without any preservatives or shipped in glycerol saline in refrigeration.

1.9.4.1 Transportation of Specimens without Preservatives

This is the most commonly used method of sending samples to a diagnostic laboratory. The suspected brain samples are first placed in a sealed, rigid container and then labelled. As soon as the head is separated from the body of the animal, it is placed in a small plastic bag. Before packaging, the specimen has to be cooled in a refrigerator. If only the cerebellum and brain stem are transported, these should be first placed in a small plastic container and then placed in a small plastic bag. The

entire head when collected should be first wrapped in absorbing paper and then placed in resistant plastic bag. The primary package is then placed in a secondary container which is also tightly sealed and further put in an insulated container preferably made of expanded polystyrene. Absorbing materials to prevent leakage and cooling materials are placed in this tertiary container and finally sealed with an adhesive tape. The information relevant to the sample is placed in an envelope and attached on the outer surface of the box. The box should be labelled clearly as "BEWARE! BIOLOGICAL SPECIMEN FOR RABIES DIAGNOSIS. INFECTIOUS HAZARD!"

1.9.4.2 Transport Using Preservative Solutions

Preservative solutions are used if transit time to the laboratory is long or if transportation on refrigerants is not possible. The laboratory technique that is used for diagnosis determines the preservative to be used.

- The use of formalin solution is safe since it inactivates the rabies virus, but the sample becomes unsuitable for isolation/inoculation tests. These specimens are suitable for FAT and histology.
- Specimens can also be transported in glycerine solution which does not inactivate the virus rapidly but is capable of inhibiting the growth of contaminants temporarily.

1.9.4.3 Preservative Solutions for Diagnosis

For transit over short distances, the specimens for diagnosis of rabies are sent on ice in wide-mouth leak-proof containers. If the transit time is longer, samples are placed in preservative solutions as described below

(a) One half of the brain in either 10% formal saline or Zenker's fluid and the other half in 50% glycerol saline.
(b) Salivary glands in 50% glycerol saline.
(c) CSF, saliva and urine are transported in tissue culture medium with 2% saline.
(d) Specimens for cytological tests or histopathological diagnosis are transported in 10% neutral buffer formalin or in Bouin's solution.

In packaging, the materials capable of causing injury shall be avoided.

1.9.4.4 Labelling of the Specimens

The specimen container should be properly labelled using permanent markers before dispatching it to the laboratory. The label should inform about the date of collection,

species, type and number of samples being sent along with the preservative used. The parcel should also be appropriately labelled.

1.9.4.5 Sample Submission Form

The sample submission form accompanying the specimens sent should be complete providing all the necessary information like detailed history of the case, species and breed of an animal infected, vaccination history, clinical signs observed, probable contact with other animals/humans, the mode and date of death, etc. This ensures proper identification of the animal and proper reporting of results.

1.9.4.6 Some Materials Suggested for Use and Proper Handling of the Specimen

1. Primary Container
   This contains the clinical specimen and hence should be one which can be tightly sealed. Thus zip lock bags or heavy-duty garbage bags of appropriate size can be used. If the specimen has a sharp protrusion such as a shattered bone, this can be wrapped in several layers of newspaper.
2. Secondary Container
   The primary container is placed in a secondary container which should be packed with an absorbent material like cotton or absorbent pads or paper towels to prevent leakage of the sample. This can preferably be a plastic or metal container with a lid or even another suitable zip lock bag. The sample submission form should also be enclosed in a plastic bag within the secondary container or taped outside this container.
3. Shipping Container
   The secondary container is then placed in the shipping container which is a thick-walled container with or without an exterior fibreboard liner. Frozen cool packs and cushioning materials are put in these containers to prevent damage to the specimen during transport. This container should be labelled as "BEWARE! BIOLOGICAL SPECIMEN FOR RABIES DIAGNOSIS. INFECTIOUS HAZARD!" In conclusion, the specimens collected for rabies diagnosis are infectious, and thus appropriate precautions should be taken during handling and transportation to ensure the safety of the personnel and the environment involved like the sample collector/submitter, transportation carriers, laboratory staff and the public at large. The samples should be transported on ice to prevent their decomposition. Frozen cold packs are preferred over wet ice which may leak through the container resulting in rejection of the specimen. Triple packaging of the sample is preferred. If more than one specimen is transported, each specimen should be packaged in a separate primary container properly labelled and then placed in a secondary container. The tertiary container should be cleaned on its outside surface with a disinfectant to reduce the risk of exposure to anyone handling

the package. Proper packaging helps in proper maintenance of the samples to aid accurate diagnosis of rabies.

1.9.5 Diagnosis of Rabies in Animals

The laboratory techniques for the diagnosis of rabies were initiated as early as 1800 BC. Adelchi Negri in 1903 discovered the Negri bodies and their diagnostic significance was demonstrated by his wife Lina Negri-Luzzani in 1913. This paved the way for the development of a plethora of laboratory techniques for confirmation of rabies which is detailed in the WHO publication "Laboratory Techniques in Rabies" (Meslin et al. 1996) and also in the "OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals" (OIE 2012).

1.9.5.1 Direct Microscopy: Sellar's Staining

Seller's staining technique is a simple and rapid test. It is a histological test employed on brain impressions to demonstrate the characteristic cell lesions, viz. "Negri bodies". These are aggregates of viral particles seen as intracytoplasmic inclusion bodies varying from 3 to 30 μm size in infected neuronal cells. The Negri bodies are circular or oval bodies with basophilic granules in an eosinophilic matrix. This method has very low sensitivity on unfixed tissue smears (WHO 2013; Meslin et al. 1996; OIE 2012).

1.9.5.2 Direct Fluorescent Antibody Assay (DFA)

Direct fluorescent antibody assay is recommended by the World Health Organization (WHO) and World Organization for Animal Health (OIE) and is the most widely employed test for post-mortem confirmatory diagnosis of rabies. This gold standard test was developed by Goldwasser and Kissling in 1958. Here, the "Nucleoprotein antigen" (N) of the rabies virus present in fresh brain impressions of rabies suspected animals is demonstrated (Fig. 1.2). The brain impression of non-rabid animals does not possess such rabies viral inclusions (Fig. 1.3). Further, the specificity and sensitivity of the DFA is almost 99% in a standard laboratory (Isloor et al. 2017).

The DFA is sensitive and specific. The sensitivity of this test depends on the quality of the specimen (on how comprehensively the brain is sampled and also on the degree of autolysis), type of lyssavirus and on the proficiency of the diagnostic staff. Impressions are taken from a composite sample of brain tissue which should include the brain stem. It is air-dried and then placed in 100% high-grade cold acetone for 1 h for fixing the impression. The impression is removed from the acetone, air-dried and then stained by adding a drop of the specific conjugate.

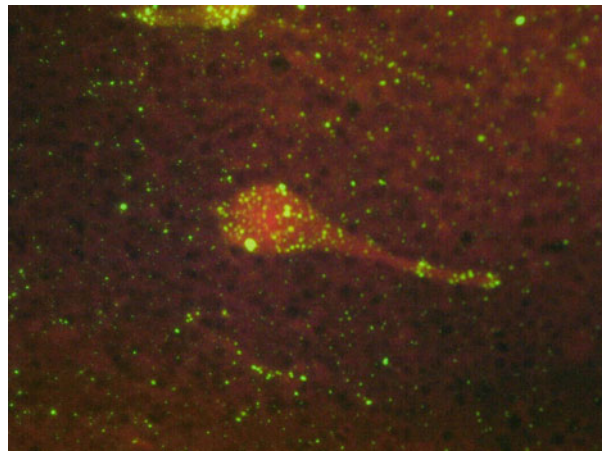

Fig. 1.2 Rabid animal brain impression (200×) stained with the rabies virus anti-nucleocapsid IgG-FITC conjugate (Rabies DFA III, Light Diagnostics, Cat # 6500) with counterstain (Evan's blue)

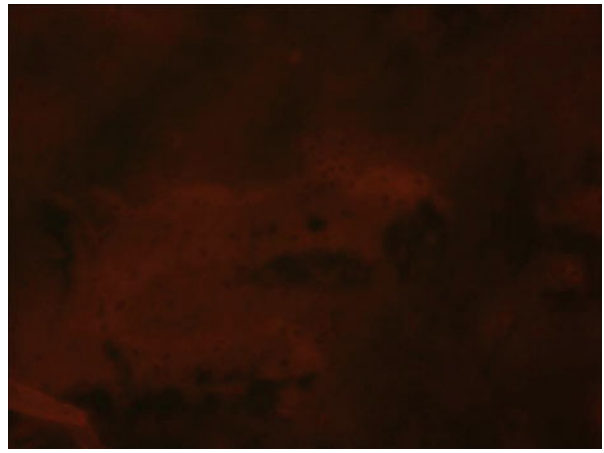

Fig. 1.3 Non-rabid animal brain impression (200×) stained with the rabies virus anti-nucleocapsid IgG-FITC conjugate (Rabies DFA III, Light Diagnostics, Cat # 6500) with counterstain

Further, the impression is incubated at 37 °C for 1 h. Anti-rabies fluorescent conjugates are commercially available as either polyclonal or monoclonal antibodies (MAbs) that is specific to the entire virus or the N protein of rabies virus, conjugated to the fluorescing dye, fluorescein isothiocyanate (FITC). The DFA slides should be examined in a fluorescent microscope having a filter appropriate for the wavelength of the fluorescent conjugate used. The most commonly used fluorescent dye is FITC, which is excited at 490 nm and re-emits at 510 nm. The presence of nucleocapsid protein aggregates is identified by specific apple green coloured fluorescence of bound conjugate.

This test is reliable when brain tissue used is a fresh one. The bacterial contamination of partially decomposed brains results in nonspecific fluorescence which is difficult to differentiate from specific fluorescence due to N antigen, making it unsuitable for this test. Specimens preserved in 50% glycerol saline needs washing with normal saline, whereas for formalin preserved specimens, treatment with

proteolytic enzymes is necessary before employing DFA. Furthermore, limited usage of DFA in developing countries is attributed to expensive fluorescence microscope, its maintenance and need for skilled personnel for interpretation of the test (Isloor et al. 2017).

1.9.5.3 Virus Isolation

Virus isolation is carried out, especially for epidemiological purposes to characterize the viruses in a particular geographical location. Isolation is also carried out when DFA results in an ambiguous result. Either the mice inoculation technique (MIT) or the rapid tissue culture infection test (RTCT) is performed (Koprowsky 1996; Webster and Casey 1996).

Mouse Inoculation Test

Mouse inoculation test is performed on a litter of 2-day-old newborn mice or 3–4 weeks old mice. The suspected brain material is homogenized, and a 10% suspension (w/v) prepared. The supernatant of a 10% homogenate of brain material is inoculated intra-cerebral to the mice, and they are observed for 28 days. Depending on the incubation period, after about 5–7 days the mice exhibit typical signs characteristic of rabies-like hunching of the back, ruffling of hair, dragging of hind limbs followed by complete paralysis of hind limbs followed by forelimbs. Confirmatory diagnosis is by extracting the brain from these mice and subjecting it to DFA. A positive test yields a large amount of virus from a single mouse brain. The major disadvantage of animal inoculation tests is the time required before a diagnosis can be made. Thus, alternatively, cell culture facilities, if available shall be preferred (OIE 2012).

Rapid Tissue Culture Infection Test (RTCT)

The isolation of viruses in cell culture is faster and yields results in 48 hours. The neural origin cell lines are most suitable for rabies virus isolation, and murine neuroblastoma cell line Neuro-2a is the most commonly used one. Baby hamster kidney (BHK 21) cells can also be used for isolation (Fig. 1.4). The cells are grown in a shell vial or 96-well plates, and then the specimen is inoculated and incubated for 24–48 h. Further, after decanting the cell culture media, the vials/plates are fixed with acetone and then subjected to direct fluorescent antibody assay (DFA). Recently, the fixed and street viruses were isolated in a human embryonic kidney cell line (HEK 293) which was found to be as sensitive and specific as the Neuro-2a cell line (Madhusudana et al. 2010).

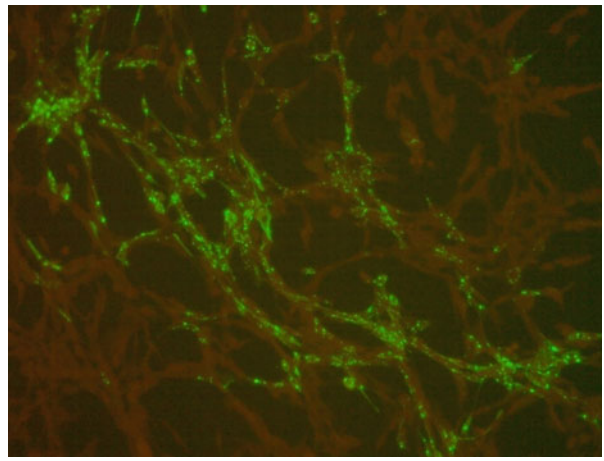

Fig. 1.4 Rabies virus (PV-3462) propagated in BHK21 confirmed by DFA (200×)

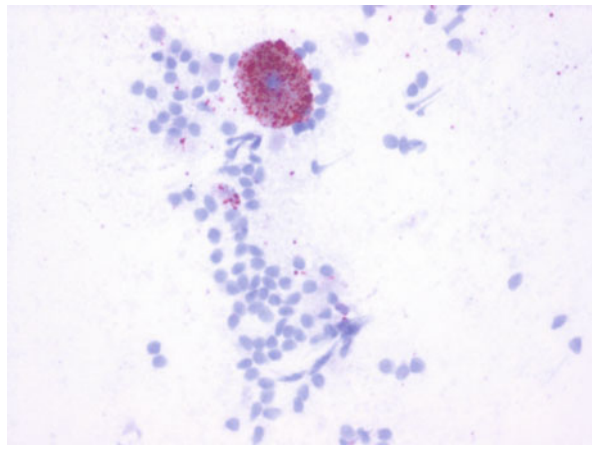

Fig. 1.5 Rabid animal brain impression tested by dRIT (200×)

1.9.5.4 Direct Rapid Immunohistochemistry Test (dRIT)

The Center for Disease Control and Prevention (CDC) in Atlanta, the USA developed dRIT, which is one of the most significant developments for the diagnosis of rabies. This test too detects the N protein of rabies virus in brain impressions of suspected animals. Buffered formalin is used for fixing the smear which is further treated with a cocktail of highly concentrated and purified biotinylated monoclonal antibody (to N protein). This is followed by streptavidin peroxidase and indicator system (H_2O_2 and aminoethyl carbazole). The aggregates of viral clusters are seen as brick red clusters which are present within the neuron, along the axons and scattered all over the brain impression (Fig. 1.5). No such brick red inclusions are seen in negative brain impressions (Fig. 1.6).

This test procedure is of one-hour duration, and the results can be interpreted using an ordinary light microscope and thus is applicable in field conditions. It has

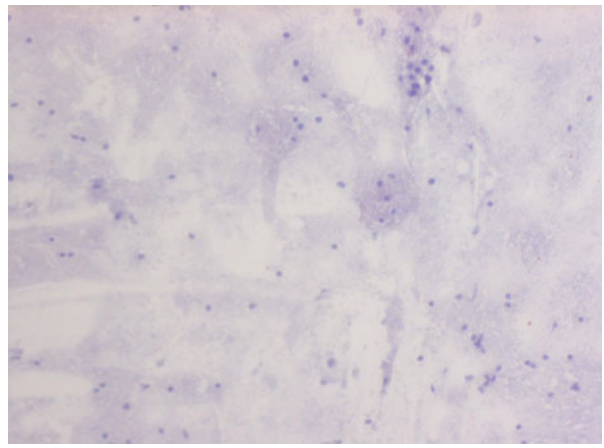

Fig. 1.6 Non-rabid animal brain impression tested by dRIT (200×)

been evaluated in various countries and has been found to be 100% as sensitive and specific as DFA. This user-friendly, cost-effective test will be of immense use particularly for developing countries in improving rabies epidemiologic surveillance independent of the expensive fluorescent microscope and where cold storage facilities may not be available (Isloor et al. 2017). A study indicating 100 percent correlation between DFA and dRIT was conducted and the dRIT was found to be suitable in the prevailing conditions in India (Nithin Prabhu et al. 2014). The WHO recommends further application of dRIT as an alternative to the DFA for decentralized laboratory-based surveillance (Isloor et al. 2017).

1.9.5.5 Lateral Flow Assay (Immunochromatography)

Lateral flow assay is an immunochromatographic test which is simple and rapid. This test kit developed using monoclonal antibodies recognizes the rabies virus nucleoprotein. It has been evaluated as a rapid screening test for rabies (Tajunnisa et al. 2018). This assay is an immunodiagnostic test, which yields rapid results in field conditions through detection of RABV antigen in post-mortem samples without the need for laboratory equipment. In brief, the detector antibodies are attached on a membrane at two different zones, and when the processed specimen is added to the device at the given slot, it results in the development of coloured lines indicating the presence of viral antigen (Kang et al. 2007). In case of rabies virus-positive brain samples, coloured lines are seen in both “C” (Control) and “T” (test) zones, whereas only “C” zone shows the colour development in case of negative sample (Fig. 1.7). Furthermore, this test could also be successfully employed for detection of rabies virus in the cell culture (Sharada et al. 2015).

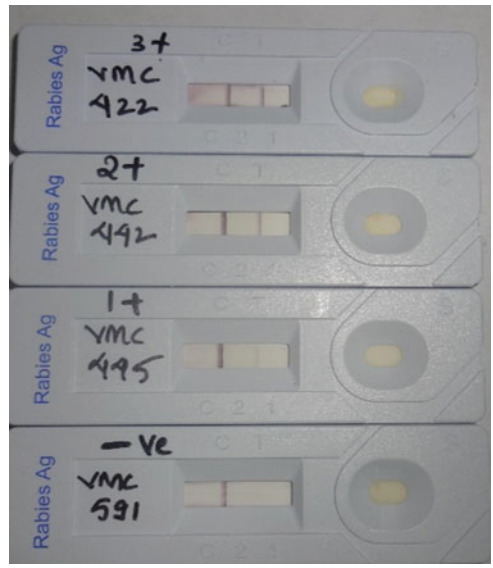

Fig. 1.7 Lateral flow assay of rabies positive and negative brain sample suspension

1.9.5.6 Other Antigen Detection Assays

Other less common antigen detection assays which are less commonly used are

- A rapid sandwich ELISA is used for detecting lyssaviruses belonging to all seven genotypes which circulate in Europe, Africa, Asia and Oceania (Mani and Madhusudana 2013; Xu et al. 2007, 2008).
- Dot-blot immunoassay employed for brain tissues (Madhusudana et al. 2004) and
- An enzyme immunoassay (EIA) which is employed for rapid diagnosis in humans and animals (Vasanth et al. 2004).

1.9.5.7 Nucleic Acid Detection Techniques

In the recent past, molecular tools have become widely accepted and used in the diagnosis of rabies. Particularly, the polymerase chain reaction (PCR) which involves nucleic acid amplification has revolutionized the diagnosis of rabies in both animals and human beings. The antemortem detection of rabies has been significantly effective with this approach (Nath et al. 2017). Presently, various PCR based assays are developed for antemortem as well as post-mortem rabies diagnosis. Most of such PCR versions involve the amplification of the nucleoprotein (N) gene since it is highly conserved (Mani and Madhusudana 2013).

Reverse Transcriptase PCR (RT-PCR)

The gel-based reverse transcriptase PCR (RT-PCR) assays are also being used for detecting the rabies viral RNA in clinical samples (Coertse et al. 2010; Crepin et al. 1998; Dacheux et al. 2008; Nadin-Davis 1998; Macedo et al. 2006; Biswal et al. 2012). The amplicons generated particularly by targeting N, G and G-L intergenic sequences in these assays have been further sequenced for characterizing the virus and to study its phylogeny (Pramina et al. 2016; NithinPrabhu 2015; Veeresh et al. 2013, 2015). However, these assays are prone to the risk of cross-contamination, which is a major drawback inhibiting the routine use of this assay for rabies diagnosis (Mani and Madhusudana 2013).

Real-Time PCR

The real-time PCR reduces the risk of cross-contamination and is used to detect and quantify the genome copies. The SYBR Green approach of real-time PCR is used for antemortem diagnosis of rabies (Nagaraj et al. 2006; Nath et al. 2017) and also for detecting lyssaviruses (Hayman et al. 2011). High specificity was observed with the TaqMan real-time PCR assays (Mani et al. 2013; Nadin-Davis et al. 2009; Wakeley et al. 2005; Hughes et al. 2004; Wacharapluesadee et al. 2008), which also enables a wide detectable range. Compared to the traditional nested RT-PCR, this was found to be 100 times more sensitive (Wakeley et al. 2005).

Other Molecular Based Assays

For detection of the rabies virus in the antemortem samples like saliva and CSF, automated NASBA (Nucleic Acid Sequence-Based Amplification) is found to be highly sensitive than the conventional PCR. This assay is easier and yields rapid results (Wacharapluesadee and Hemachudha 2001). Another amplification-based assay is the loop-mediated isothermal amplification (LAMP) which does not require a thermal cycler for rabies diagnosis (Muleya et al. 2012; Boldbaatar et al. 2009; Tajunnisa 2017). As this test is independent of expensive thermal cycling, it can be employed for field-based or less sophisticated laboratories (Fooks et al. 2009).

The viral nucleic acid detection tests can be employed for diagnosis of rabies from both antemortem and post-mortem samples since it can be performed on any of the biological samples like saliva, CSF, skin biopsy, tears, hair follicles, urine, brain tissue, etc. (Mani and Madhusudana 2013). These assays are particularly helpful in diagnosis from decomposed samples and also archived samples (Mani et al. 2013; Araújo et al. 2008; David et al. 2002; Johnson and Fooks 2005). Furthermore, these assays also aid in epidemiological studies in addition to retrospective diagnosis. The real-time PCR is helpful in estimating the presence and load of viral RNA in the samples. It also enables the study of disease progression and the efficacy of various

therapeutics in experimental approaches (Nadin-Davis et al. 2009; Maier et al. 2010).

LN 34 Real-Time PCR Assay

The conventional DFA test is dependent on the quality of the antibody conjugates, the experience of the laboratory personnel involved, an expensive fluorescent microscope, and the specimen quality. Recently, LN34 pan-lyssavirus real-time RT-PCR assay has been developed. This assay can detect viral RNA in deteriorated tissues and the diverse genera of lyssavirus and is highly sensitive. The assay was evaluated across the Americas, Europe, Africa and Asia in 14 laboratories wherein 2978 samples (1049 DFA positive) were tested. This could detect the viral RNA in formalin-fixed brain tissues, in deteriorated samples, frozen, archived samples in addition to fresh samples with high specificity (99.68%) and sensitivity (99.90%) in comparison to the DFA. There were very fewer variations in repeatability/reproducibility. Samples which were not testable by DFA or which yielded inconclusive results could be clearly identified by LN34 assay. Further false positives and negatives in DFA were also detectable demonstrating the use of this assay for improved rabies diagnosis and also surveillance (Gigante et al. 2018).

One of the limitations of molecular assays is the need for stringent quality control and the lack of a universal protocol and also international standards. Furthermore, the molecular assays are not practically feasible for regular post-mortem diagnosis, especially in developing countries. The immunological assays described above can be employed for routine purposes (WHO 2013).

1.9.5.8 Demonstration of Antibodies

The rabies virus neutralization test particularly FAVN or RFFIT are the referred tests to assess the neutralizing antibodies although they have certain limitations (Sunilkumar et al. 2016). As an alternative, various types of ELISA (enzyme-linked immunosorbent assay) are also used since they are safe, simple, safer and rapid. Besides, these assays do not require the handling of live virus and thus also high-containment facilities. The ELISA results are found to correlate well with the results of RFFIT results (Servat et al. 2007; Muhamuda et al. 2006; Shyamsundar et al. 2014; Sharada et al. 2016; Santosh 2017). An ELISA based on N and G protein Mab was developed for the detection of rabies antigens aiding in antemortem diagnosis (Muhamuda et al. 2007). The Platelia Rabies II, a second-generation ELISA kit was developed for detecting the antibodies against the glycoprotein in serum samples and also CSF samples. This ELISA was evaluated (Shyamsundar et al. 2014) and found to correlate well with RFFIT and therefore suggested for use in laboratories where cell culture facilities are not available (Feyssaguet et al. 2007). However, ELISA is less sensitive compared to the neutralization tests (Cliquet et al. 2004; Servat and Cliquet 2006; Welch et al. 2009). Recently, electrochemiluminescence based ELISA

has shown high sensitivity in detecting rabies glycoprotein antibodies in serum samples (Ma et al. 2012). A rapid neutralizing antibody detection test (RAPINA) was developed based on immunochromatography. This is an easier assay for semi-quantitative detection of neutralizing antibodies (Nishizono et al. 2012). Latex agglutination test, a field test is also available for detecting the antibodies against rabies (Madhusudana and Saraswati 2003). A recombinant antigen-based double sandwich ELISA was developed for detection of antibodies in serum samples (Yang et al. 2006). Furthermore, Nimmagadda et al. (2010) have reported the use of a recombinant antibody-based ELISA for quantifying rabies viral glycoprotein in human rabies vaccines (Nimmagadda et al. 2010). Such assays may be of immense promise in diagnosis of rabies in future.

Rabies Diagnosis in Animals: Recent OIE Initiatives in India

In India, the facilities for diagnosis of rabies are available in selected metropolitan cities depriving the rural areas. Considering the need, in 2013, the Commonwealth Veterinary Association (CVA) in collaboration with the Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU) and Crucell, Holland, established a state-of-the-art rabies diagnostic laboratory, at the Veterinary College, Bangalore. This has all facilities for early diagnosis of rabies and is the first of its kind in the Indian subcontinent. In 2016, under the OIE Twinning Programme, this laboratory has been twinned with the OIE Reference Laboratories for rabies in the UK, based at the Animal and Plant Health Agency (APHA), UK, and the Centers for Disease Control and Prevention (CDC) in Atlanta, the USA, and have been awarded a four-year OIE Twinning Project with the mandate of building rabies diagnostic capabilities at the KVAFSU/CVA/Crucell Laboratory. Acting as joint parent laboratories, APHA and CDC address the objectives of the OIE Twinning Project as follows: (a) to increase awareness, knowledge and preparedness for rabies through sharing of information, transfer of technology and the provision of knowledge management skills; (b) to enable a common methodology of rabies diagnosis in Indian provincial laboratories, that comply with OIE standards and (c) to establish an OIE Reference Laboratory for rabies in India, to ensure an expanded network of OIE reference laboratories and to act as a focal point by providing technical support to other laboratories for rabies control in the Indian continent. The team at the Veterinary College, Bengaluru receives and tests rabies submissions from several sources, including Department of Animal Husbandry and Veterinary Services of State Governments, Disease Investigation Sections, Veterinary colleges, Institute of Animal Health and Veterinary Biologicals and Private Veterinary Practitioners. Approximately, 120 samples are received per year, of which approximately 60% are positive for rabies. The majority of samples are from dogs, although other species tested include domesticated and wild animals (Rahman and Isloor 2018).

1.10 Control

The current human rabies prevention protocol in human beings is based on the guidelines of WHO. This involves post-exposure prophylaxis (PEP) wherein administration of a rabies vaccine regimen and rabies immunoglobulin is practised. These methods are almost 100% efficacious and prevent deaths caused by rabies virus if employed properly in time post-exposure. Even in spite of the availability of effective PEP, still there is a large number of untreated human rabies virus exposures leading to death (Sudarshan and Ashwathnarayana 2010). In the USA, though rabies was very much prevalent until 1945, canine rabies was brought under control with effective vaccination campaigns in 1945 and Canada by 1950s. Because of the public health significance of rabies, the Bangladesh government has initiated several strategies to eliminate rabies. The World Animal Protection (WAP) implemented anti-rabies vaccination programme the "Red Collar" programme in Cox Bazar area, Bangladesh. In Dhaka, during 2015, the OIE vaccine bank provision was availed, and yet another successful anti-rabies vaccination programme was conducted. With the implementation of these two programmes, the incidence of rabies in these areas reduced significantly. In Sri Lanka during 1975, the National programme to eliminate rabies was started with the aim of elimination of rabies in 2020. The impact of this control programme was tremendous as there was reduction in the incidence of rabies-related human deaths from 377 in 1973 to 24 in 2015. Majority of the human deaths (75%) were due to dogs (Animal Health Bulletin 2015). The recent dog vaccination programmes carried out in cities of Karachi and Lahore have been reported to be successful (Salahuddin et al. 2016).

1.10.1 Control of Rabies in India: A Veterinary Perspective

In India, recent trends have increased the usage of PEP. The nerve tissue vaccine in all the countries in the Indian subcontinent is phased out. In 2006, the Government of India approved and began subsidizing the use of intradermal rabies vaccination in public hospitals. Furthermore, initiatives such as World Rabies Day launched in 2007 have contributed to greater care-seeking behaviour. The control of rabies in animal reservoirs depends on control of rabies in dogs. To achieve this, a co-ordinated multipronged approach involving various agencies is necessary. In India, despite all the progress, rabies is still a major concern of public health importance. The reduction of rabies deaths is influenced by intersectoral efforts by government and non-governmental organizations, awareness and access to medicines. Given this, a National Rabies Control Programme (Zoonosis Division) was implemented in the 12th Five Year Plan of the Government of India (2012–2017) with 69 million USD earmarked for rabies initiative activities over 5 years (Government of India Planning Commission 2011). The program has two components, namely human and animal component. Human component is being implemented in

all the states and UTs. National Centre for Disease Control is the nodal agency for the human component of the program. The strategy for the human component is training of health professionals; implementing the use of intra-dermal route of inoculation of cell culture vaccines; strengthening surveillance of human rabies; information education and communication; laboratory strengthening. Whereas the animal component was pilot tested in the Haryana and Chennai. The Animal Welfare Board of India, Ministry of Environment and Forests was the nodal agency for the animal component. The strategy for the animal component included population survey of dogs; dog population management and mass vaccination of dogs. In this context, since 2014, Mission Rabies has been working with other agencies to implement a state-wide rabies control program in Goa state. The component consisted of mass dog vaccination, canine rabies detection and education in schools. Due to these efforts, the deaths in human beings significantly reduced from 17 in 2014 to 5 in 2015 and 1 in 2016 (Gibson et al. 2017). Such pre-exposure mass vaccination programmes along with birth control programmes need to be implemented to tackle overpopulation of street dogs through the involvement of governmental and non-governmental organizations, municipal corporations and animal welfare organizations. Other approaches envisaged are registration of owned pet dogs and their vaccination against rabies every year, compulsory pet licensing, increasing the public awareness (through educational institutions, media and co-operation) about the benefits of successful mass anti-rabies vaccination. India is primarily a rural oriented country; most of the rabies cases are reported from the rural areas which in general are deprived of the implementation of rabies control programmes employed in urban India. Given this, there is need to evolve a programme for implementation/strengthening of rabies control in vast rural India with emphasis on regular booster vaccination and seromonitoring vaccinal anti-bodies in both domestic as well as street dogs. To implement this throughout country, skilled manpower is achieved through upgrading skills of vets/para vets/ dog catchers through training programmes, and importantly logistic support laboratories are needed.

1.10.2 Animal Birth Control Programme (ABC) in India

The stray dog population in India is estimated to be about 25 million (Sudarshan and Ashwathnarayana 2010), and this makes national vaccination program a herculean task. Although several NGOs initiated dog vaccination programs, these vaccination rates are a small fraction of the 70% coverage required for dog rabies control (Chatterjee 2009). The elimination of the dogs by culling is ineffective as it is replaced by inward migration of new dogs. International agencies such as WHO, FAO and OIE support this position. India has supported dog control by humane approaches for several years by promoting the Animal Birth Control (ABC) programme. Initially, this concept was developed in Chennai. Later, sustained ABC-AR programmes were started in Jaipur. The purpose of this programme is to

reduce the number of street dogs by employing a humane approach and in turn, reduce the number of rabies cases. Although, several Indian cities have taken up ABC programme, in many instances it has not been a sustained programme. The Government of India in 2001 passed the Dog Control ABC Rules making it illegal to kill a dog unless it was terminally ill or badly injured.

1.10.3 Requirement of Vaccine

Globally, vaccine manufacturers produce vaccines to cater to the requirement of about 28 million rabies post-exposure prophylactic (PEP) courses in countries endemic for rabies including Asia, Eastern Mediterranean region and Africa. The rabies vaccine availability in urban localities in Asia and Africa has resulted in increased usage of PEP even to persons without risk of developing rabies (Hampson et al. 2015). To meet this demand, a vaccine bank for canine rabies was set up by OIE in 2012 to assist countries in Asia (such as Afghanistan, Laos and Myanmar) or strengthening vaccination programmes in Philippines, Sri Lanka and Vietnam (Dehove 2015; OIE 2012). Currently, the production capacity of major vaccine manufacturers is estimated to be approximately 100 million doses (CDC 2011). Given this scarcity of vaccine in India, intradermal vaccination (IDRV) is recommended by the WHO and has been practised recently due to its cost-effectiveness and high immunogenicity. In Himachal Pradesh, the cost-effectiveness of recent low-cost intra-dermal (IDRV) anti-rabies (both pre- and post-exposure) vaccination protocol in domestic bovines has been investigated.

1.10.4 Oral Vaccines Used for Rabies Control

Initially, the preventive measures to control rabies in its reservoir comprised of culling fox by various approaches but this did not result in preventing transmission of rabies. The effective approach to control rabies in wildlife was to employ oral vaccination by using vaccine baits with the attenuated anti-rabies liquid vaccine. An approximately 60–70% coverage of bait based oral rabies vaccination in the fox population resulted in breaking the transmission chain. Because of this experience, it is aimed to employ this approach in control of rabies in free-ranging dogs also. Furthermore, oral rabies vaccines can be easily administered and thereby could overcome the logistical problems in rabies control, especially in developing countries. In dogs, among various candidates of bait based oral vaccine, the attenuated rabies vaccine strain SAG-2 was reported to induce protective immunity. However, importantly, live oral vaccines must be safe as they could pose a risk in young/immune depressed individuals. Nevertheless, SAG-2 has confirmed its safety in target as well as non-target species. Oral bait vaccination in the wildlife has eliminated red fox rabies in certain areas. In a recent study carried out in Goa,

India, several benefits using the oral bait method were identified. The operational cost per dog "vaccinated" for the oral method was estimated at Rs. 39 per dog, as compared to Rs. 140/- per dog using CVR (Catch, Vaccinate and Release) approach.

1.10.5 Enhancement of Immune Surveillance through Seromonitoring of Rabies Vaccinal Antibodies in Dogs

The anti-rabies vaccination programmes, preferably mass vaccinations need to be evaluated through seromonitoring of vaccinal antibodies by the WHO/OIE approved tests such as RFFIT or FAVN for testing the vaccinal performance, to detect the protective level of antibodies, to decide on the need and doses of post-exposure prophylactic vaccination and making provision for exporting dogs to selected countries. Considering the success of mass vaccination campaigns in controlling canine rabies, effective vaccination campaigns were planned and canine rabies was eradicated from the USA, Canada, Western Europe, Malaysia, Japan and a few Latin American countries. This approach needs to be adopted in Asian countries where canine rabies is highly prevalent. However, limited availability of facilities to conduct RFFIT, given its need for handling the live virus, cell culture facility, fluorescent microscope and expertise, is a major constraint in seromonitoring of rabies vaccinal antibodies on regular basis.

1.11 Future Perspectives

The target of "Elimination of Canine mediated human rabies by 2030" is set by the international agencies WHO, OIE, FAO and Global Alliance for Rabies Control (GARC) for Africa, Asia and Latin America. The main objective of this rabies elimination program in human beings includes control, prevention and subsequent elimination of rabies in dogs by successive annual mass vaccination covering at least 70% of the dog population (WHO 2013, Report of the Global Conference, 2015 and WHO, 2016). Also, various measures should be taken up to control rabies. People should be made aware through public education campaigns, particularly in rural areas. There has to be a regular supply of vaccines and anti-rabies immunoglobulin in all hospitals and primary-health-care centres. Primary health care personals should be well trained to administer appropriate prophylaxis, including intradermal anti-rabies vaccination. The primary school curriculum shall cover rabies awareness among students. Ensure that all dogs are vaccinated and stray animals sterilized. Rabies should be incorporated into a "One health programme" at all levels. Further, a significant role is being played by the NGOs in addressing rabies. The initiatives of World Rabies Day, by the GARC, throws light on the need to systematically address rabies worldwide and provides impetus for local governments to act. Success stories

from GARC projects in Philippines and Indonesian communities can be the guidance for local initiatives in the Indian subcontinent and turn achieve the global goal of "Dog mediated human rabies elimination by 2030".

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Conflict of Interest There is no conflict of interest.

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Chapter 2
Monkeypox Virus

Nikola Sklenovská

Abstract *Monkeypox virus* is a member of the *Orthopoxvirus* genus in the *Poxviridae* family. Monkeypox virus causes monkeypox, an emerging zoonotic disease recognized as the most important orthopoxvirus infection in humans in the smallpox post-eradication era. Many animal species, including rodents and monkeys, can transmit the virus. However, the animal reservoir has not been identified yet. Human-to-human transmission exists, and the longest reported chain of transmission is six generations. The clinical presentation of monkeypox is very similar to the presentation of smallpox, i.e. the febrile prodrome is followed by a skin eruption period. Lymphadenopathy is a typical sign of monkeypox. The case fatality of monkeypox depends on the virus clade, and it falls between 1% and 11%. Monkeypox was reported in 11 countries of Central and West Africa. The disease was also exported outside of the African continent to the USA, the UK, Israel, and Singapore. The frequency and geographical spread of human monkeypox cases have increased in recent years, with little understanding of the disease's emergence, epidemiology, and ecology. Monkeypox can be diagnosed by polymerase chain reaction performed on lesion specimens. Serological tests and antigen detection do not provide a definitive diagnosis given the orthopoxvirus serological cross-reactivity. Modified vaccinia Ankara vaccine was recently approved in the USA for monkeypox prevention in adults at high risk of infection. There is currently no specific treatment for monkeypox infection.

Keywords Monkeypox · Orthopoxvirus · Emerging infectious diseases · Zoonosis · Disease outbreaks · One health

Abbreviations

CA	Central African
CEV	Cell-associated virion

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dsDNA	Double-stranded DNA
DRC	Democratic Republic of the Congo
EEV	Extracellular enveloped virion
EV	Enveloped virion
IEV	Intracellular enveloped virion
IMV	Intracellular mature virion
ITR	Terminal inverted repetition
mRNA	Messenger RNA
MPXV	Monkeypox virus
MPXV-ZAI	Monkeypox virus Zaire-96-I-16 Strain
MV	Mature virion
MVA	Modified vaccinia Ankara
OPV	Orthopoxvirus
PCR	Polymerase chain reaction
VZV	Varicella-zoster virus
WA	West African
WHO	World Health Organization

2.1 Preamble

Monkeypox virus (MPXV) was discovered and described in the Statens Serum Institut (Copenhagen, Denmark) in 1958 when two outbreaks of pox-like disease were observed in cynomolgus monkeys. The institute was receiving a continuous supply of monkeys from Singapore, which was used for polio vaccine research and production (Magnus et al. 1959). Subsequently, multiple other laboratory outbreaks of monkeypox were recorded in Europe and the USA in captive monkeys imported from Asia (Arita et al. 1972). Seroprevalence studies in Asia did not find evidence of monkeypox on the continent (Arita et al. 1972). Later it was suggested that grivets (MPXV-susceptible monkeys also exported in large scale to Europe and North America) could have been the source of infection of Asian monkeys during co-transportation (Jezek and Fenner 1988). The first human monkeypox case was reported in August 1970 in the remote village of Bokenda, in the equatorial province of the Democratic Republic of the Congo (DRC) (Ladnyj et al. 1972).

With the eradication of smallpox in 1980 and subsequent cessation of smallpox vaccination, monkeypox has emerged as the most important orthopoxvirus pathogenic for humans. Monkeypox was considered a rare sporadic zoonotic disease with a limited capacity to spread between humans in the past (WHO 1984). However, the number of reported cases and their geographical range has increased after the cessation of the smallpox vaccination and the disease can be life-threatening in the DRC and other countries in West and Central Africa (Meyer et al. 2002; Rimoin et al. 2010). Additionally, multiple exportations of the virus outside of Africa in the past years have highlighted its global importance.

2.2 Classification

MPXV is a member of the genus *Orthopoxvirus* (OPV) and the family *Poxviridae*. MPXV is one of the five OPV species pathogenic for humans, together with variola virus, the causative agent of smallpox, now eradicated in nature, cowpox virus, camelpox virus, and vaccinia virus (Shchelkunov et al. 2005).

Poxviruses infect most vertebrates and invertebrates, causing a variety of diseases of veterinary and medical importance. The family *Poxviridae* is divided into the subfamily *Chordopoxvirinae* whose viruses infect vertebrates and the subfamily *Entomopoxvirinae* which infect insects. The subfamily *Chordopoxvirinae* is divided into 11 genera, one of which is OPV (Table 2.1). All OPV species, except variola virus which is an exclusively human pathogen, have animal reservoirs and are therefore classified as zoonotic pathogens.

2.3 The Virus

2.3.1 Morphology

Monkeypox virus, together with other poxviruses, is considered one of the largest and most complex viruses (Ferreira Barreto-Vieira and Monika Barth 2015). They are brick-like shaped particles with a size ranging from 220 nm to 450 nm in length and 140 nm to 260 nm in width (Jahrling et al. 2007, pp. 215–240); therefore, MPXV is large enough to be discerned by light microscope, with its ultrastructure resolvable via electron microscopy. However, higher magnification provided by electron microscopy is needed to resolve ultrastructure (Moss and Damon 2013). The orthopox virion consists of four major elements—core, lateral bodies, outer membrane, and the outer lipoprotein envelope. The central core contains the viral

Table 2.1 Classification of orthopoxviruses

Family	*Poxviridae*
Subfamily	*Chordopoxvirinae*
Genus	*Orthopoxvirus*
Species	*Camelpox virus*
	Cowpox virus
	Ectromelia virus
	Monkeypox virus
	Raccoonpox virus
	Skunkpox virus
	Taterapox virus
	Vaccinia virus
	Variola virus
	Volepox virus

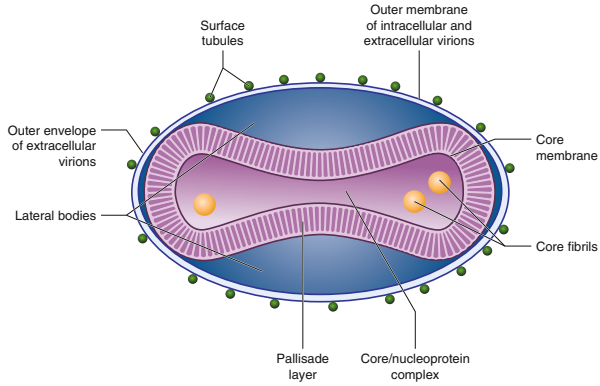

Fig. 2.1 Schematic representation of a poxvirus particle. Adapted from Principles of Molecular Virology, 6th Edition (p. 46), by Alan J. Cann, 2016, UK: Elsevier. Copyright 2016 by Elsevier. Adapted with permission

double-stranded DNA (dsDNA) and core fibrils, and it is surrounded by a tightly arranged layer of rod-shaped structures known as palisade layer. The central core, palisade layer, and the lateral bodies are enclosed together by the outer membrane that is composed of many surface tubules (Fig. 2.1). Spontaneously released virions often have the outer lipoprotein envelope, while virions released by cellular disruption lack this envelope (Appleyard et al. 1971; Ladnyi et al. 1988). A mature virion contains at least 80 viral proteins (Resch et al. 2007).

2.3.2 Genome

The monkeypox genome (Fig. 2.2) is a large (197 kbp) single linear molecule of dsDNA, which is among the largest of all viral genomes (Moss and Damon 2013). Each end of the genome contains identical but oppositely oriented terminal reads with a size of about 6 kbp (Shchelkunov et al. 2002) with a set of short tandem repeats (Wittek and Moss 1980) and terminal hairpin loops (Baroudy et al. 1982). The genome consists of about 190 nonoverlapping open reading frames (>180 bp long) containing 60 or more amino acid residues. Of these, four are present within the inverted terminal repetition (Seet et al. 2003; Shchelkunov et al. 2002). The guanine and cytosine content of MPXV DNA is low, about 31.1% (Shchelkunov et al. 2001). Two distinct genetic clades of MPXV have been characterized including the West African (WA) and the Central African (CA) clade (Likos et al. 2005).

Sequencing of the whole genome of many OPVs has revealed a high degree of homology in the genes located centrally, and high variability in the genes located terminally on both sides of the genome. Conserved OPV genes are mostly involved in essential viral functions like replication and virion assembly (Seet et al. 2003), and the variable OPV terminal reads are likely to contribute to the virulence of different

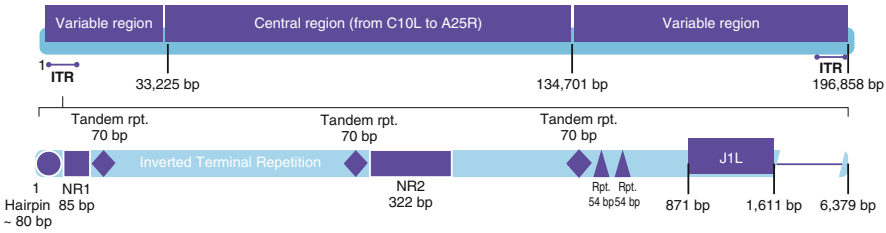

Fig. 2.2 Schematic genome representation of Zaire-96-I-16 (MPXV-ZAI) strain isolated during the 1996 outbreak of monkeypox in Zaire. The whole genome consists of 196,858 bp with the central genomic region comprising of 101,476 bp (Shchelkunov et al. 2001). Both terminal variables end (right end longer than the left one) include a 6379 bp terminal inverted repetition (ITR) (Shchelkunov et al. 2002) with approximately 80 bp long hairpin loop (Chen et al. 2005a), 70 bp or 54 bp short tandem repeats and unique ITR sequences NR 1 and NR 2 and the coding region (Shchelkunov et al. 2002). Adapted from "Human monkeypox and smallpox viruses: a genomic comparison" by Shchelkunov et al. (2001), FEBS Letters, 509, pp. 66–70. Copyright by John Wiley & Sons, Inc. and from "Analysis of the Monkeypox Virus Genome" by Shchelkunov et al. (2002), Virology, 297, pp. 172–194. Copyright by Elsevier. Adapted with permission

OPVs (Afonso et al. 2002; Chen et al. 2005b; Goebel et al. 1990; Tulman et al. 2006). Many terminal genes contribute to immune evasion by interfering with signaling, presentation, and recognition of antigens and apoptosis (Barry et al. 2004; Seet et al. 2003).

2.3.3 Replication Cycle

The replication cycle (Fig. 2.3) of poxviruses, unlike most DNA viruses, occurs in the cytoplasm of the host cell (Buller and Palumbo 1991). Poxviruses enter cells by a multistep process consisting of attachment, hemifusion, and core entry that can occur at the plasma membrane or after endocytosis (Moss 2016) The exact mechanism used by poxviruses to enter cells depends on its infectious form—mature virion (MV) with single outer membrane or extracellular enveloped virion (EV) which has an additional membrane with a different protein composition. For EV form, the external EV-specific membrane is discarded exposing the underlying MV membrane, which then fuses with the cell. Although MV is more abundant, EV is specialized for cell-to-cell spread largely by its long, mobile, projections that are formed by actin polymerization which adhere to the cell surface (Moss 2016; Moss and Damon 2013).

The mature virion undergoes the first uncoating during its entry, and once in the cytoplasm, the virus releases prepackaged viral proteins and enzymatic factors that disable cell defenses and stimulate expression of early genes. This is followed by a synthesis of early messenger RNA (mRNA) by viral DNA-dependent RNA polymerase. Translation of early mRNA facilitates the second uncoating process, DNA replication, and production of intermediate transcription factors. In the following stage, intermediate mRNA is transcribed and translated to induce the expression of

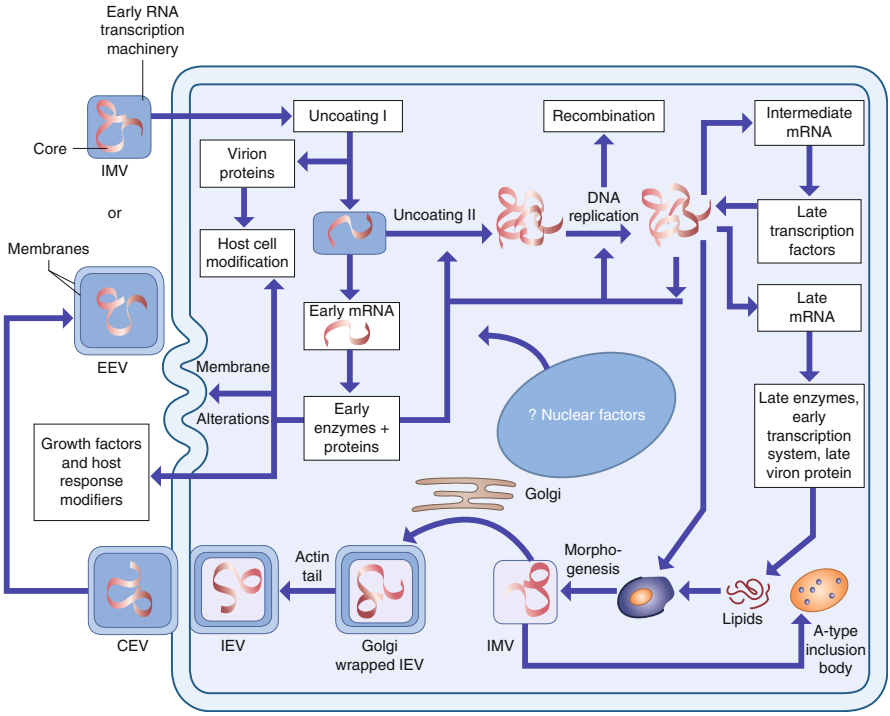

Fig. 2.3 Schematic representation of a poxvirus life cycle (Bray and Buller 2004). After the virion binds and fuses with the host cell membrane, the viral core is released into the cytoplasm of the host cell. Enzymes and factors carried within the core initiate transcription. Most virions stay in the cytoplasm as intracellular mature virions (IMVs) and end up encased within the protein matrix of scabs. The rest of the virions acquire an additional envelope (intracellular enveloped virions, IEVs) and are moved and attached to the host cell membrane. These cell surface-associated enveloped virions (CEVs) are responsible for the cell-to-cell spread of the virus, whereas extracellular enveloped virions (EEVs) can participate in systemic spread of the virus. Virus- and host-encoded proteins on the surface of CEV and EEV protect them against complement activation. Reprinted from "Looking Back at Smallpox" by Bray and Buller (2004), 38, pp. 882–889. Public domain

late mRNAs and its translation into structural proteins and non-structural proteins (enzymes and early transcription factors). The translated proteins get assembled alongside DNA concatemers formed during the early stage of replication and get packed into immature virions that develop into intracellular mature virions (IMVs). IMVs lack an outer membrane and are infectious only when they are released by cell disruption. IMV particles which did not end up encased within the protein matrix of cytoplasm become the intracellular enveloped virions (IEVs) by acquiring a second membrane (Bray and Buller 2004; Hiller and Weber 1985; Roberts and Smith 2008). Those migrate to the inner cell membrane with the help of microtubules and fuse with it, forming cell-associated virions (CEVs), which trigger actin polymerization and formation of filaments that help CEVs to leave the cell. The CEVs which have left the cell are called extracellular enveloped virions (EEVs) (Roberts and Smith 2008).

Both intracellular and extracellular virions play an important role in the pathogenesis. The intracellular virions (IMV and IEV) and CEVs are responsible for the spread of the virus from cell to cell, while EEVs are important for the systemic spread of the virus within the infected organism (Pauli et al. 2010).

2.4 Clinical Profile

Most people infected with MPXV are symptomatic, but subclinical (asymptomatic) infection can occur (Jezek et al. 1986, 1987c). It was suggested that subclinical infections could constitute up to 30% of all monkeypox infections (Jezek and Fenner 1988). Limited information is available regarding the incubation period of MPXV in humans, although recent analysis suggests 5–13 days (Nolen et al. 2016). The longest documented incubation period was roughly 17 days (Breman et al. 1980). However, a maximum incubation period of 21 days has been assumed for extra caution. The incubation period, disease presentation, severity, and duration can also be influenced by the route of infection. For example, infection via bites can result in a shorter incubation period, absence of a distinct febrile stage, and more severe illness than non-invasive exposures (Reynolds et al. 2006). The clinical presentation of monkeypox closely resembles that of smallpox, although it is clinically less severe. The major difference distinguishing monkeypox from smallpox is the occurrence of lymph node enlargement.

The monkeypox disease in humans can be divided into two periods, the prodrome and the rash period. The prodrome is defined by fever, headache, chills and/or sweats, sore throat, muscle ache, lack of energy, and lymphadenopathy (Nalca et al. 2005). The rash period usually manifests 1–3 days after the onset of fever and lymphadenopathy, and is characterized by a few to several thousand lesions (Jezek et al. 1987d). The lesions appear simultaneously and evolve at about the same rate. The lesions progress from macules to papules, vesicles, pustules, and finally to crusts. Their distribution is mainly peripheral but can cover the whole body during a severe illness (Fig. 2.4). Depending on the severity of the illness, it takes about 2–3 weeks for the lesions to dry and desquamate (Ladnyi et al. 1988). Patients vaccinated against smallpox with vaccinia vaccine have significantly less lesions than non-vaccinated (Jezek and Fenner 1988).

Patients often experience gastrointestinal symptoms such as nausea, vomiting, diarrhea, and loss of appetite. Oral and alimentary tract lesions can be apparent. Skin perturbation from the rash can lead to secondary bacterial infection (common) and dehydration. Ocular infections with MPXV and secondary bacterial infections can also occur and often render the patient's eye swollen, red, sensitive to light, and can lead to loss of vision. The respiratory tract can also be affected; patients can present with coughing, difficulty breathing, or bronchopneumonia. Other complications include encephalitis and sepsis (Reynolds et al. 2017). A case fatality ranges between 1 and 11% in unvaccinated patients (Jezek et al. 1987d; WHO 1997), and

Fig. 2.4 Typical clinical presentation of human monkeypox in a 7-year-old female child, Sankuru District, Democratic Republic of Congo. Reprinted from "Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo" by Anne W. Rimoin et al. 2010, PNAS, 107(37), pp. 16262–16267. Copyright by Proceedings of the National Academy of Sciences of the United States of America. Reprinted with permission

is generally higher in cases infected with the CA clade of the virus than with the WA clade.

Monkeypox can clinically resemble various rash illnesses which need to be considered during differential diagnosis. This includes smallpox (eradicated in nature), measles, bacterial skin infections, scabies, syphilis, medication-associated allergies, and chickenpox. The latter, chickenpox, also known as varicella (caused by varicella-zoster virus, VZV), is most commonly confused with monkeypox (up to 50% of cases in some outbreaks) (Jezek et al. 1988b; Meyer et al. 2002) because of the similarities in the clinical presentation of the two diseases. Unlike the varicella lesions, the lesions of monkeypox appear simultaneously (varicella lesions appear gradually) and they concentrate on the face, arms, and legs but can cover a whole body (varicella lesions appear mainly on the trunk of the body) (Heymann et al. 1998). Monkeypox lesions are hard, deep, and well-circumscribed, while varicella lesions are superficial with irregular borders (McCollum and Damon 2014). Furthermore, chickenpox has a shorter and milder prodrome and clinical course, lymphadenopathy is infrequent, and death is extremely rare (Breman 2000; Jezek et al. 1988b). However, it was recently reported that a large proportion of varicella patients in the DRC presented with non-typical varicella rash and clinical signs and symptoms (Leung et al. 2019). Coinfections with both MPXV and VZV have been reported several times (Hutin et al. 2001; Meyer et al. 2002; Morier 2014; Rimoin et al. 2007). The role of the VZV in MPXV epidemiology is not clear.

2.5 Pathobiology

The disease pathobiology is only partially described and is predominantly based on animal studies. Black-tailed prairie dogs (*Cynomys ludovicianus*) have been shown to mimic the human disease better than other models, experiencing a prolonged incubation period and development of skin rash (Hutson et al. 2009).

A model of MPXV pathogenesis is depicted in Fig. 2.5. MPXV is first detected at the local site of infection (through respiratory, percutaneous, or per mucosal exposures) and is associated with an intense inflammatory response characterized by cell necrosis, phagocytosis, vasculitis, and local replication of MPXV (Cho and Wenner 1973). This is followed by the virus multiplication occurring in the regional lymphatics and then in the bloodstream leading to transient primary viremia. Following this, the virus multiplies in the spleen, liver, bone marrow, and other reticuloendothelial organs (Moss and Damon 2013) but it can also be detected in other organs like the small intestines (Hutson et al. 2015). After this, a secondary viremia period ensues, followed by the seeding of other organs leading to clinical signs of disease including characteristic disseminated cutaneous lesions. Monkeypox antibodies can be detected at the same time or shortly after the cutaneous lesion presentation.

In the prairie dog model, day 12 post-infection seems to be a pivotal time associated with unexpected deaths, uniform antibody production, and peak virus levels. Furthermore, this was also the only point at which viable virus was recovered from blood samples (Hutson et al. 2015).

Histopathological changes, both intracellular and in tissues, attributable to viral infection exhibit around day 6 in affected organs (Hutson et al. 2015). Cytoplasmic inclusion bodies are a typical intracellular histopathological feature of orthopoxvirus infections. Two morphologies manifest, A-type inclusion bodies, where virions are clustered within an intracytoplasmic structure, and B-type inclusions (Guarneri bodies), which are perinuclear and contain the viroplasm and maturing viral particles (Moss and Damon 2013). Some tissues also show prominent histopathological

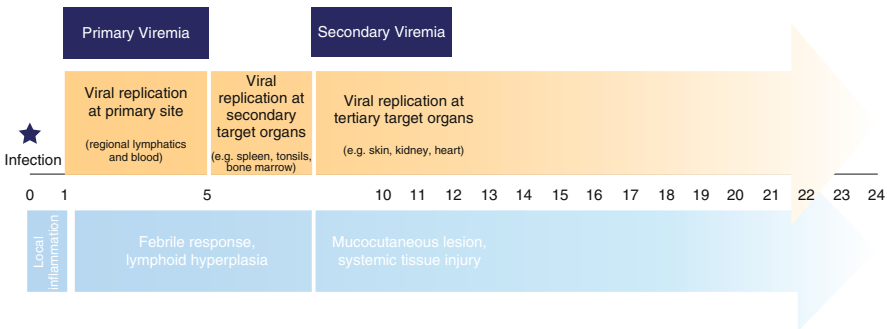

Fig. 2.5 Model of monkeypox virus pathogenesis. Adapted from "Comparison of Monkeypox Virus Clade Kinetics and Pathology within the Prairie Dog Animal Model Using a Serial Sacrifice Study Design" by Hutson et al. (2015), BioMed Research International, Volume 2015, Article ID 965710, 19 pages. Copyright Christina L. Hutson et al. Adapted under the Creative Commons Attribution License

changes at this stage of infection. Spleen samples typically exhibit neutrophil infiltration within the red pulp and increased apoptotic or necrotic cells. Conversely, the liver only tends to show the minimal trend of increased apoptotic cells even though these tissues generally have similarly high loads of virus in infected animals (Hutson et al. 2015).

While the two clades of MPXV, the CA and the WA clade, show similar onset and range of clinical symptoms in the prairie dog animal model, they show certain pathological differences. Generally, the CA MPXV spreads more rapidly, accumulates to greater levels in tissues, and causes greater morbidity in animals compared to the WA MPXV (Hutson et al. 2010, 2015).

2.6 Epidemiology

2.6.1 Prevalence and Incidence

Surveillance activities for monkeypox are not well established, and limited information is available on the prevalence and incidence of the disease. To identify the prevalence of MPXV infection of humans, serological studies of persons without vaccination scars were carried out in the DRC, Republic of Congo, Ivory Coast, and Sierra Leone in 1981. Of all 10,300 sera samples, 15.4% tested positive for orthopoxvirus of which 0.71% tested positive for MPXV. Later follow-up showed that some samples were taken from vaccinated individuals making the results inconclusive (Jezek and Fenner 1988).

The average annual cumulative incidence for inhabitants living in forested areas of the northern DRC between 1981 and 1986 was 1.58 per 10,000 population (Jezek and Fenner 1988). A study in the Sankuru Province (DRC) between November 2005 and November 2007 showed an average annual cumulative incidence of 5.53 per 10,000 (2.18–14.42). This study suggested a 20-fold increase in monkeypox in the same health zone from the 1980s (Rimoin et al. 2010). The most recent analysis of data from the DRC between 2010 and 2015 identified an average annual cumulative incidence of 0.13 cases per 10,000 inhabitants (Mandja et al. 2019).

Seasonal patterns of infections are undetermined: data between 1970 and 1980 suggested January–March (Breman et al. 1980); data between 1981 and 1986 (the 6-year cumulative) suggested June–August (Jezek and Fenner 1988); data between 2000 and 2009 suggested July–September; while between 2010 and 2015 suggested January–March (Mandja et al. 2019).

2.6.2 Sex and Age

Human MPXV infections have been reported to affect all age groups. Between 1980 and 1986, however, 52% were between the ages of 0 and 4 and 37% were between

the ages of 5 and 9. This age pattern may reflect the decrease in the collective immunity after the cessation of the smallpox vaccination. During the same period, there were more males (58%) than females (42%) among the primary cases, and more females (57%) than males (43%) among the secondary cases (Jezek and Fenner 1988). This pattern was likely caused by the social roles linked with gender (e.g., males are more often exposed to animals and females to a sick family member) (Jezek and Fenner 1988; Quiner et al. 2017). More recent data from Nigeria between September 2017 and September 2018 showed that persons with confirmed MPXV infection were between 2 days and 50 years (median 29 years) and majority were males (69%) (Yinka-Ogunleye et al. 2019). This shift towards older age might reflect the further decrease in immunity against OPVs.

2.6.3 Geographical Distribution

Monkeypox most commonly occurs in areas covered by rainforest in Central and West Africa. This type of habitat has been identified as suitable for the transmission of the virus by ecological niche models built based on the association of reported cases and potential risk factors including environmental conditions (e.g., location, temperature, precipitation, vegetation indexes from satellite imagery, etc.) (Ellis et al. 2012; Nakazawa et al. 2015).

Analysis of historical data showed that most monkeypox cases are reported in small villages in tropical rain forests which are, however, not closely surrounded by high forest on all sides. A common situation is that they consist of groups of houses along roads through the forests surrounded by agricultural areas (consisting of gardens and secondary forest) and primary rain forest close by. Each of the three zones (settlement, an agricultural area, and forest) has a characteristic fauna (Khodakevich et al. 1987a, b). Monkeypox cases, however, have also been recorded in urban areas of Africa (Yinka-Ogunleye et al. 2019).

Cases of confirmed human monkeypox in Africa were reported from the DRC, Republic of Congo, Cameroon, Central African Republic, Nigeria, Liberia, Ivory Coast, Gabon, Sierra Leone. Additionally, monkeypox has been imported to Benin (Beninese infected in Nigeria) (Breman et al. 1980) and to South Sudan (movement of people from DRC) (Nakazawa et al. 2013). The virus was also exported outside of the African continent to the USA in 2003 via infected animals from Ghana (Reed et al. 2004). Finally, multiple infected travelers from Nigeria were confirmed in the UK (Vaughan et al. 2018) and Israel (Erez et al. 2019) in 2018 and Singapore in 2019 (Fig. 2.6).

After the first human case of monkeypox was described in 1970, a total of 59 cases of monkeypox have been confirmed in West and Central Africa till 1980 (Jezek et al. 1987b). After the declaration of smallpox eradication in 1980, monkeypox was designated as the most important orthopoxvirus infection in humans in the post-smallpox eradication era resulting in establishing enhanced monkeypox surveillance by the World Health Organization (WHO) in the DRC between 1981 and

Fig. 2.6 Countries which reported confirmed cases of monkeypox in humans

1986 (WHO 1980). During this time, 338 confirmed monkeypox cases were identified in the DRC, and much of the current knowledge on monkeypox was obtained during this time. The total number of confirmed monkeypox cases in West and Central Africa between 1970 and 1986 was 404 (Jezek and Fenner 1988). The number of reported cases has dramatically decreased after the intensified surveillance was discontinued (Table 2.2).

Since its discovery, there have been several prominent MPX outbreaks. A prolonged, relatively large outbreak of 511 suspected cases was reported in DRC in 1996–1997 (WHO 1997) but a substantial proportion might have been chickenpox cases (WHO 1997). The longest chain of transmission was recorded in the Republic of Congo in 2003, accounting for seven viral transmission generations (Learned et al. 2005). In South Sudan in 2005, monkeypox was thought to have expanded outside of its traditional ecology when it was recorded in a dry savannah environment for the first time (Formenty et al. 2010) but it was likely an importation from the DRC (Nakazawa et al. 2015).

An increase in monkeypox geographical range and number of cases has been observed in recent years. The DRC has reported more than 1000 suspected cases per year since 2005 (Durski et al. 2018). Outbreaks were reported in Sierra Leone (2014), Liberia (2017), and Nigeria (2017) after 40 years since the first and only occurrence. The most recent outbreak in Nigeria in 2017 was the biggest outbreak of the West African clade ever documented. The number of monkeypox cases is likely underestimated due to limited specific surveillance and laboratory capacity in forested areas of West and Central Africa.

2.6.4 Host Species

Monkeypox is a zoonotic disease for which the natural reservoir that maintains the virus in nature is not known. Many animal species have been identified as animals that are susceptible to the virus, mainly rodents and non-human primates, listed in Table 2.3 (Reynolds et al. 2019a). Non-human primates are generally accepted as incidental hosts with no critical role in the maintenance of the virus in nature due to the low OPV seroprevalence in these animals. Squirrels (*Funisciurus* spp.), giant pouched rats (*Cricetomys* spp.), and African dormouse (*Graphiurus* spp.) and possibly other forest rodents are considered to be the most likely reservoir hosts based on evidence obtained from multiple fields and laboratory investigations (Doty et al. 2017). The virus has only been isolated twice from a wild animal, a rope squirrel (*Funisciurus anerythrus*) in the DRC (Khodakevich et al. 1986) and a sooty mangabey (*Cercocebus atys*) in Ivory Coast (Radonić et al. 2014).

Table 2.2 Number of suspected and confirmed human cases of monkeypox between 1970 and 2019

Country	Year	Total number of cases (confirmed cases)	References
Democratic Republic of the Congo	1970–1986	386 (386)	Jezek and Fenner (1988)
	1997	511 (?)	WHO (1997)
	After 1997	Not fully enumerable	–
Central African Republic	1984	6 (6)	Khodakevich et al. (1985)
	2001	4 (4)	CDC (2015a)
	2010	2 (2)	Berthet et al. (2011)
	2015–2016	12 (4)	Kalthan et al. (2016)
	2016	26 (3)	WHO (2016)
	2017	? (3)	WHO (2017b)
	2018–2019	34 (25)	WHO (2019b)
	2018	6 (6)	Besombes et al. (2019)
Cameroon	1979	2 (1)	Eozenou (1980)
	1989	1 (1)	Tchokoteu et al. (1991)
	2018	7 (1)	Sadeuh-Mba et al. (2019)
Nigeria	1971	2 (2)	Breman et al. (1980)
	2017–2018	276 (122)	Yinka-Ogunleye et al. (2019)
Ivory Coast	1971	1 (1)	Breman et al. (1980)
	1981	1 (1)	Merouze and Lesoin (1983)
Liberia	1970	3 (3)	Lourie et al. (1972)
	1970	1 (1)	Lourie et al. (1972)
	2016–2017	16 (2)	WHO (2018)
Sierra Leone	1970	1 (1)	Lourie et al. (1972)
	2014	1 (1)	Reynolds et al. (2019b)
	2017	1 (1)	Ye et al. (2019)
Gabon	1987	4 (1)	Meyer et al. (1991)
	1991	?	Heymann et al. (1998)
Benin	1978	1 (1)	Breman et al. (1980)
Republic of Congo	2003	12 (3)	Learned et al. (2005)
	2009	10 (2)	Reynolds et al. (2013)
	2017	88 (7)	Doshi et al. (2018)
	2019	9 (2)	WHO (2019b)

(continued)

Table 2.2 (continued)

Country	Year	Total number of cases (confirmed cases)	References
South Sudan	2005	49 (10)	Formenty et al. (2010)
USA	2003	72 (37)	Reed et al. (2004)
UK	2018	1 (1)	Vaughan et al. (2018)
	2018	2 (2)	Vaughan et al. (2018)
Israel	2018	1 (1)	Erez et al. (2019)
Singapore	2019	1 (1)	WHO (2019a)

2.6.5 Transmission

Monkeypox virus can be transmitted both from animal to human (primary transmission) and from human to human (secondary transmission). The virus can enter the body through broken skin (even if not visible), mucous membranes (eyes and mouth), and the respiratory tract. Primary animal to human transmission results from direct contact with body fluids, lesion material, or respiratory droplets (the latter being the least efficient) of infected animals (Hutson et al. 2011, 2013). Viral shedding via urine and feces has also been documented and may represent another exposure source (Hutson et al. 2015). Secondary human-to-human transmission is associated with direct contact with body fluids and lesion material of infected persons. Respiratory transmission also occurs through direct contact with large respiratory droplets after prolonged face-to-face contact. Transmission can also occur through virus-contaminated objects, such as bedding and clothing (Formenty et al. 2010; Nolen et al. 2015). Transmission of the virus from infected pregnant women to the fetus has been described. Limited information is available on the impact of human MPXV infection on pregnancy outcomes with vertical transmission; however, case studies of miscarriage and fetal death exist (Mbala et al. 2017). Patients are infectious from the onset of the illness (fever), and the lesions contain infectious virus through all stages until the crusts separate and a fresh layer of skin forms. This can take up to 4 weeks.

During 1980–1986, up to 70% of human infections were caused by primary transmission from animals. The main presumptive risk factor for primary transmission is close contact with infected animals when hunting (Quiner et al. 2017). Secondary transmissions were more common in persons without a history of smallpox vaccination and those living in the same household. Among household contacts of monkeypox cases in the DRC, there was an observed attack rate of 1.3% for individuals vaccinated against smallpox versus 9.3% for unvaccinated individuals, and 11.7% for age group 0–4 years (7 times higher) (Jezek et al. 1988a). A more recent study showed an attack rate within households to be 50% (Nolen et al. 2016).

Models which used data from 1981 to 1986 calculated the human-to-human transmission reproductive rate (R_0) to be 0.8 predicting that the disease would not be able to sustain human infections without repeated zoonotic introductions (Fine et al. 1988; Jezek et al. 1987a). However, these older models may no longer provide

Table 2.3 Animal species susceptible to monkeypox virus infection

Order	Family	Species	References
Didelphimorphia	*Didelphidae*	*Monodelphis domestica*; *Didelphis marsupialis*	Hutson et al. (2007)
Eulipotyphla	*Erinaceidae*	*Atelerix* spp.	Hutson et al. (2007)
Lagomorpha	*Leporidae*	*Oryctolagus cuniculus*	Marennikova and Seluhina (1976)
Macroscelidea	*Macroscelididae*	*Petrodromus tetradactylus*	Doty et al. (2017) and Hutin et al. (2001)
Pilosa	*Myrmecophagidae*	*Myrmecophaga tridactyla*	Peters (1966)
Rodentia	*Chinchillidae*	*Chinchilla lanigera*	Hutson et al. (2007)
	Cricetidae	*Sigmodon hispidus*	Reynolds et al. (2019a)
	Dipodidae	*Jaculus* spp.	Hutson et al. (2007)
	Gliridae	*Graphiurus* spp.	Doty et al. (2017), Earl et al. (2015), and Hutson et al. (2007)
	Muridae	*Mus musculus*; *Mastomys natalensis*; *Oenomys hypoxanthus*; *Rattus norvegicus*	Americo et al. (2010), Doty et al. (2017), Earl et al. (2015), Reynolds et al. (2012), and Reynolds et al. (2019a)
	Nesomyidae	*Cricetomys* spp.	Doty et al. (2017) and Hutson et al. (2007)
	Sciuridae	*Cynomys ludovicanus*; *Funisciurus anerythrus*; *F. isabella*; *F. lemniscatus*; *F. congicus*; *Heliosciurus gambianus*; *H. rufobrachium*; *Protexerus strangeri*; *Marmota monax*; *M. bobak*; *Spermophilus tridecemlineatus*; *Sciurus vulgaris*; *Xerus* sp.	Doty et al. (2017), Falendysz et al. (2014), Hutin et al. (2001), Hutson et al. (2007), Jezek and Fenner (1988), Khodakevich et al. (1986), Marennikova et al. (1989), Reynolds et al. (2010), and Sbrana et al. (2007)
	Hystricidae	*Atherurus africanus*	Jezek and Fenner (1988)
Primates	*Callitrichidae*	*Callithrix jacchus*	Peters (1966)
	Cercopithecidae	*Cercocebus galeritus*; *C. atys*; *Macaca irus*; *M. mulatta*; *M. fascicularis philippinensis*; *Cercopithecus petaurista*; *C. ascanius*; *C. mona*; *C. neglectus*; *C. pogonias*; *C. aethiops*; *C. nictitans*; *C. hamlyni*; *Semnopithecus* spp.; *Colobus badius*	Arita et al. (1972), Arita and Henderson (1968), Breman et al. (1977a), Breman et al. (1977b), Gispen et al. (1976), Jezek and Fenner (1988), Peters (1966), Radonić et al. (2014), and Sauer et al. (1960)

(continued)

Table 2.3 (continued)

Order	Family	Species	References
	Hominidae	*Gorilla* sp.; *Pan troglodytes*; *Pongo pygmaeus*	Arita et al. (1972), Arita and Henderson (1968), and Peters (1966)
	Hylobatidae	*Hylobates lar*	Peters (1966)
	Cebidae	*Saimiri sciureus*	Peters (1966)
	Lorisidae	*Perodicticus potto*	Jezek and Fenner (1988)
Carnivora	*Procyonidae*	*Nasua nasua*	Hutson et al. (2007)
	Felidae	*Felis* spp.	Jezek and Fenner (1988) and Khodakevich et al. (1987b)
Artiodactyla	*Suidae*	*Sus scrofa*	Hutin et al. (2001)

an accurate representation of the epidemic potential of the virus. This may be due to changes within human or zoonotic populations, including the spread of HIV/AIDS, altered access to health care facilities, altered population age structure of the population, ecologic disturbance, and others (Antia et al. 2003). Nonetheless, a more recent model did not suggest any changes in monkeypox transmissibility (Blumberg and Lloyd-Smith 2013) but acknowledges that more surveillance data is required for a reliable assessment of changes in transmissibility of monkeypox (Blumberg et al. 2014).

2.6.6 Genetic Characterization of MPXV

Two genetic clades of MPXV have been characterized, including the WA and the CA clade. The two clades are geographically separated and have defined epidemiological and clinical differences. The WA clade demonstrates a case fatality of between 0 and 6%, and limited human-to-human transmission has been documented (Breman et al. 1980; Yinka-Ogunleye et al. 2019). In comparison, the CA clade mortality can be as high as 11% (Jezek et al. 1987d), and up to 17% in children (Breman et al. 1980). Human-to-human transmission up to six sequential events (seven when including the primary transmission from animal to human) has been observed (Learned et al. 2005). The WA clade has been reported in Nigeria, Liberia, Ivory Coast and Sierra Leone, while the CA clade in Gabon, Cameroon, the Republic of Congo, and the DRC (Chen et al. 2005b; Jezek et al. 1987d; Likos et al. 2005; Sbrana et al. 2007).

Sustainability maps for the MPXV transmission produced by using ecological niche modeling suggested the Cameroon Highlands as a break in the distribution of suitable environmental conditions for the MPXV transmission. This partition of the MPXV geographic range coincides with the WA and CA clades (Ellis et al. 2012). This theory was supported by the analysis of many genomic sequences from MPXV

isolates covering the known geographic distribution of MPXV. However, it is not clear whether the presence of a river (Cross or Sanaga River), change in elevation, or change in the dominant vegetation cover is involved in the genetic differentiation of MPXV (Nakazawa et al. 2015).

2.7 Laboratory Diagnosis

Historically, poxviruses used to be diagnosed based on their biological properties through virus isolation assays. The morphology of viral pocks produced on a chicken embryo chorioallantoic membrane or the reproductive ceiling temperature in a cell culture allowed identification of particular poxviruses. However, these methods are laborious, time-consuming (because they require virus isolation and propagation), and are restricted to well-equipped laboratories (Jezek and Fenner 1988; Lewis-Jones 2004). Similarly, negative-stain electron microscopy was widely used for the diagnosis of viruses before the development of molecular techniques, but given the similar morphological characteristics of OPVs, the differentiation of species within the genera is not possible (Ferreira Barreto-Vieira and Monika Barth 2015; Kurth and Nitsche 2007). Lesion material is the most suitable specimen for the abovementioned techniques.

Confirmation of the MPXV infection is best done by polymerase chain reaction (PCR) as it is the only method which can differentiate between the orthopoxvirus species. The large central genomic region is highly conserved among OPV isolates which explains the significant degree of cross-reactivity in various tests, whereas terminal regions are much more variable which makes them ideal targets for PCR-based techniques. Genes often targeted for monkeypox diagnosis are hemagglutinin (Ropp et al. 1995), the acidophilic-type inclusion body gene (Meyer et al. 1997), the *crmB* gene (Loparev et al. 2001) envelope protein gene (*B6R*) (Li et al. 2006), *B7R* gene (Shchelkunov et al. 2011), and the tumor necrosis factor binding protein gene (Davi et al. 2019).

The most suitable specimen is lesion material—biopsy, roof, fluid, or crust depending on the rash stage. The timing and duration of viremia are variable, and results are often inconclusive. Therefore, the collection of blood is not recommended for diagnostic purposes.

Protein-based methods detecting different antigens from clinical samples were developed (Czerny et al. 1989; Hughes et al. 2014; Johann and Czerny 1993; Stern et al. 2016b) but they are less sensitive than PCR and do not permit differentiation of OPV (Pauli et al. 2010). Nevertheless, protein-based methods are usually robust and well-adaptable for field use. There were two systems developed for detection of orthopoxviruses: Tetracore Orthopox BioThreat® (Townsend et al. 2013) and ABICAP immunofiltration system (Stern et al. 2016a).

When no virologic specimen is available, serologic diagnostic methods are very useful for retrospective analysis. The most commonly used serologic test for poxvirus diagnosis (not specific to MPXV) is antibody-capture enzyme-linked

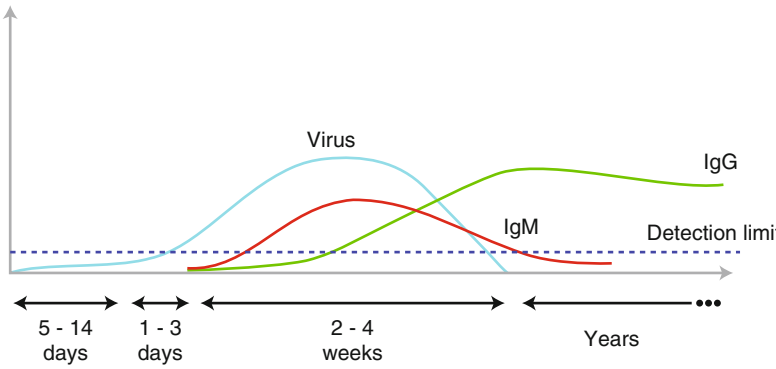

Fig. 2.7 Schematic representation of the relevant diagnostic markers. Virus: present in the blood from the end of the incubation period, through a febrile stage and the beginning of rash stage; in the oral mucosa from lesions which typically appear as the febrile stage is ending and the rash is beginning; in the lesions throughout all rash stages. IgM: appears soon after the rash onset and rises for about 2 weeks before declining and disappearing within a year. IgG: appears soon after the rash onset, rises for about 6 weeks, and lasts for decades

immunosorbent assay. The kinetics of an antibody response varies from person to person and can be dependent on smallpox vaccination history. The optimal time for collecting serum for IgM detection is between 4 and 56 days post-rash onset. This antibody response typically rises during the first 2 weeks of rash illness before eventually waning within a year. IgG titers will rise as antibody production switches from the acute to memory phase. IgG appears soon after rash onset, rises for up to 2 months and antibodies may remain observable for a lifetime. Serum collection for IgG detection should occur 2 weeks or more after rash onset (Karem et al. 2005).

While there are numerous diagnostic tests for clinically relevant infectious diseases, there are no commercially available laboratory assays for monkeypox, including on-site diagnostic tests (Stern et al. 2016a). Routine MPXV specimen preparation, pathological and molecular diagnostic tests should be conducted in BSL-2 facilities with BSL-2 work practices, while culturing MPXV specimens should be carried out in BSL-3 facility (CDC 2015b; Jezek and Fenner 1988; Tian and Zheng 2014) (Fig. 2.7).

2.8 Control Measures

2.8.1 Prevention

Orthopoxviruses induce cross-reactive antibodies that protect against infection from other orthopoxvirus species. Live vaccinia virus vaccine (first generation), which was used during the smallpox eradication program, was estimated to be 85% effective against monkeypox infection (Fine et al. 1988). The vaccination was ceased after smallpox eradication was declared in 1980, causing the proportion of

the unvaccinated population to rise. This first generation of vaccinia vaccine can cause serious adverse events and is contradicted in pregnant women, immunocompromised people, and people with a history of eczema (Lane et al. 1970).

Improved manufacturing procedures allowed the development of the second, third, and fourth generation vaccinia vaccines with reduced side effects and simplified administration. They were developed to be used in the case of the natural or deliberate reemergence of smallpox. The major challenge is that no new developments can be evaluated against naturally occurring smallpox. One example of the second-generation vaccines is ACAM2000, a live attenuated vaccinia vaccine administered by bifurcated needle (like the first generation), only approved in the USA. LC16m8 is an attenuated replication-competent third-generation vaccinia vaccine, immunogenic after a single dose with a good safety profile licensed in Japan (Kenner et al. 2006). Another third-generation vaccine is modified vaccinia Ankara (MVA) requiring a two-dose administration by injection which was approved in the European Union (marketed as IMVANEX) and Canada (marketed as IMVAMUNE) for smallpox (Overton et al. 2018). MVA is also approved by the US Food and Drug Administration (marketed as JYNNEOS) for prevention of smallpox and monkeypox in adults determined to be at high risk for the infection. This makes MVA the first approved vaccinia vaccine for monkeypox, although its approval is based on survival data obtained in lethal MPXV challenge studies in non-human primates (BavarianNordic 2019). MVA's effectiveness, immunogenicity, and safety are also being evaluated in healthcare personnel at risk of monkeypox infection in the DRC (Petersen et al. 2019). The fourth generation of vaccinia vaccines (gene-based and protein-based) is still in development phase (Buchman et al. 2010; Hooper et al. 2004).

For the general public, there is no vaccinia vaccine available, but vaccine stockpiles are maintained by several countries and WHO (WHO 2017a). There has been no formal study on post-exposure use of vaccinia vaccine for monkeypox infections, but it has been used for this purpose in the cases of imported monkeypox to UK (Vaughan et al. 2018) and Singapore (WHO 2019a).

Given the lack of approved vaccines for monkeypox, the only prevention of this disease involves education for health workers (Bass et al. 2013) and education of the population at risk on the dangers of contact with sick or dead animals which could carry the virus (Jezek and Fenner 1988). The awareness-raising should mainly focus on how to recognize the disease and how people can protect themselves from the infection.

2.8.2 Treatment

To date, there is no approved treatment for MPXV infections. Therefore, treatment is symptomatic and supportive. However, several investigational antivirals demonstrate activity against MPXV in vitro and animal model systems (Yu and Raj 2019). These include cidofovir (Andrei and Snoeck 2010), brincidofovir (Lanier et al. 2010), and tecovirimat (Berhanu et al. 2015; Yang et al. 2005), but none was

evaluated in a clinical trial. Tecovirimat is approved by the US Food and Drug Administration for the treatment of smallpox.

The mechanism of action of cidofovir is through the inhibition of viral DNA polymerase. The same is true for brincidofovir, which is a modified cidofovir, lacking nephrotoxicity and being orally available. Instead, tecovirimat targets a specific viral product blocking the release of intracellular virus from the cell.

2.9 Zoonotic and Transboundary Threat

Monkeypox has been, until recently, considered a rare zoonotic disease. Nevertheless, we have seen an increase in the number of reported cases and expansion in the geographical range in the last few years (Sklenovska and Van Ranst 2018). This is probably caused by a myriad of factors like the reduced immunity since the cessation of smallpox vaccination, better means of diagnosis and stronger surveillance systems, and other environmental and social factors whose scope is not fully understood. Climate change and deforestation might be increasing the risk for contact between humans and infected animals, but also the displacement of populations or necessity might drive people into the bush looking for potentially infected meat.

Currently, monkeypox is a public health concern in various countries of Central and West Africa, with a seemingly increasing trend which cannot be explained solely by improvements in surveillance (Mandja et al. 2019). MPXV was exported outside of the African continent for the first time to the USA in 2003 through the infected African rodents. This was followed by reports of 4 independent infected travelers from Nigeria to UK (2), Israel (1), and Singapore (1) in 2018 and 2019, of which one involved a secondary transmission to a health worker. These examples illustrate how globalization, animal trade, and travel increase the transboundary threat of monkeypox.

The threat of monkeypox would be expected to increase in the following cases: an increase in virulence (both naturally (Blumberg and Lloyd-Smith 2013; Shchelkunov et al. 2005) or via genetic engineering (Jackson et al. 2001)), the virus spilling into more widely distributed taxa (Reynolds et al. 2012) or introduction in other continents (Rimoin et al. 2010). That is why MPXV belongs to the "biosafety level 3" category, the "high threat" biodefense category in the EU (Tian and Zheng 2014) and why it is on the list of select agents in the USA (FSAP 2017).

2.10 Conclusion and Prospects

Monkeypox virus is an emerging pathogen causing a disease of epidemic potential about which much is still unknown. Health workers are often not aware of the existence of monkeypox and its characteristics, laboratory capacity in the affected countries is limited, and there is no systematic surveillance mechanism to report

monkeypox, leaving significant gaps in our understanding of the disease epidemiology and burden.

At the same time, cases of monkeypox in humans have been increasing, which is probably driven by a combination of environmental and anthropogenic factors. Climate change, deforestation, and war, among others, result in more frequent contact of people with infected wildlife. Additionally, vaccination against smallpox with vaccinia vaccine was ceased in 1980, which is still causing an increasingly growing proportion of the population to become vulnerable to MPXV and other orthopoxviruses.

The recent approval of the MVA vaccine for monkeypox prevention by the US Food and Drug Administration is a significant milestone, but no monkeypox-specific treatment options are approved, and clinical guidelines do not exist. Symptomatic and supportive treatment is currently the only care a patient can receive; however, experimental evidence of the efficacy of several compounds against MPXV infection seems promising.

Considering the perceived public health importance of monkeypox in affected countries on the one hand, and the lack of understanding and means to prevent and control it on the other, it is clear that monkeypox needs to receive more attention. Awareness-raising, surveillance strengthening, and diagnostic capacity building are some of the most important activities to improve the detection, treatment, and limit further spread of the virus. Furthermore, research activities to generate knowledge and guide further improvement in prevention and control of monkeypox are needed. This includes clinical trials to further test modern vaccinia vaccines and antivirals for monkeypox.

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Chapter 3
Nipah Virus

Shailendra K. Saxena, Vimal K. Maurya, Swatantra Kumar, and Madan L. B. Bhatt

Abstract Nipah virus (NiV) is one of the newly emerging paramyxoviruses affecting both humans and animals. NiV is known as 'deadly virus' and is on the top of the list of ten severe diseases identified by World Health Organization that have very high potentials for forthcoming outbreaks. NiV outbreaks have been documented in Southeast Asia mainly in Malaysia, Singapore, Bangladesh and India. From 1998 to 2018, more than 643 cases of NiV human infections were reported with a high case fatality rate. The NiV infection has an incubation period between 4 and 21 days, and the common symptoms are: rapid onset of fever, headaches, drowsiness, convulsion, disorientation, myalgia and respiratory perplexity. There is no licenced antiviral drug or vaccine available to cure NiV infection, and the available treatment is only based on intensive supportive care. Although mammalian cell-derived NiV like particles (VLP) and Canarypox virus-based vaccines have shown protection against NiV infection in various animal models, which may act as a prophylactic vaccine for human. In the same way, antiviral drugs such as ribavirin, favipiravir, GS-5734, chloroquine and monoclonal antibodies are found to be effective against NiV in various animal models. This article summarizes some of the available therapeutics and treatment regimens based on modern, complementary and alternative medicines for the cure of NiV infection.

Keywords Nipah virus · Outbreaks · Ribavirin · Favipiravir · Complementary and alternative medicines

Abbreviations

CFR	Case fatality rate
JE	Japanese encephalitis
NiV	Nipah virus

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RdRp RNA-dependent RNA polymerase
VLP Mammalian cell-derived NiV like particles
WHO World Health Organization

3.1 Prologue

Nipah virus (NiV) is one of the newly emerging paramyxoviruses affecting both humans and animals. NiV is mainly transmitted through bats, pigs and from one infected person to another with extreme case fatality rate (Ang et al. 2018). In humans, NiV is transmitted via aerosol route, or else direct contact with NiV infected person, contagious body fluids or other secretions. Taking infected (NiV contaminated) date palm sap is the foremost source of NiV infection in Bangladesh and India (Weatherman et al. 2018). Fruit-eating bats belong to *Pteropus* genus, and family *Pteropodidae* are natural hosts of the virus (Fig. 3.1). The NiV infection has incubation period between 4 and 21 days, and the common symptoms are: high fever, headaches, drowsiness, convulsion, disorientation and these symptoms may develop into a coma within 24–48 h (Bellini et al. 2005). NiV infection in human may also result in severe symptoms as meningoencephalitis, inflammation of blood vessel walls (systemic vasculitis) and severe respiratory perplexity in infected individuals with very high mortality rates. Moreover, NiV survivors are usually suffering from long-term neurological complications (Tan et al. 2002; Centers for Disease Control and Prevention 2018).

3.2 Genomic Characterization and Replication of Nipah Virus

NiV is an RNA virus responsible for lethal encephalitis like symptoms in animals and humans. The genome size of NiV is 18,246 nucleotides long encoding six genes and six proteins namely nucleocapsid protein (N), phosphoprotein (p), matrix protein (M), fusion protein (F), glycoprotein (G) and large RNA polymerase (Fig. 3.2) (Kulkarni et al. 2013). Three additional accessory proteins are coded by the NiV P gene, namely V, W and C proteins (Chan et al. 2001). The V and C protein are the major determinants of pathogenesis, whereas W protein is known for the modulation of host inflammatory immune response that determines the severity of NiV infection. The G and F protein are involved in the induction of neutralizing antibodies as well as responsible for virus internalization. Similarly phosphoprotein has an important role in NiV replication, whereas nucleocapsid and matrix proteins serve as virulence factors (Cox and Plemper 2017). To understand the complete pathobiology of NiV infection, the various host cells were investigated. Alteration in the symptoms and severity of infection in animals and humans are associated with

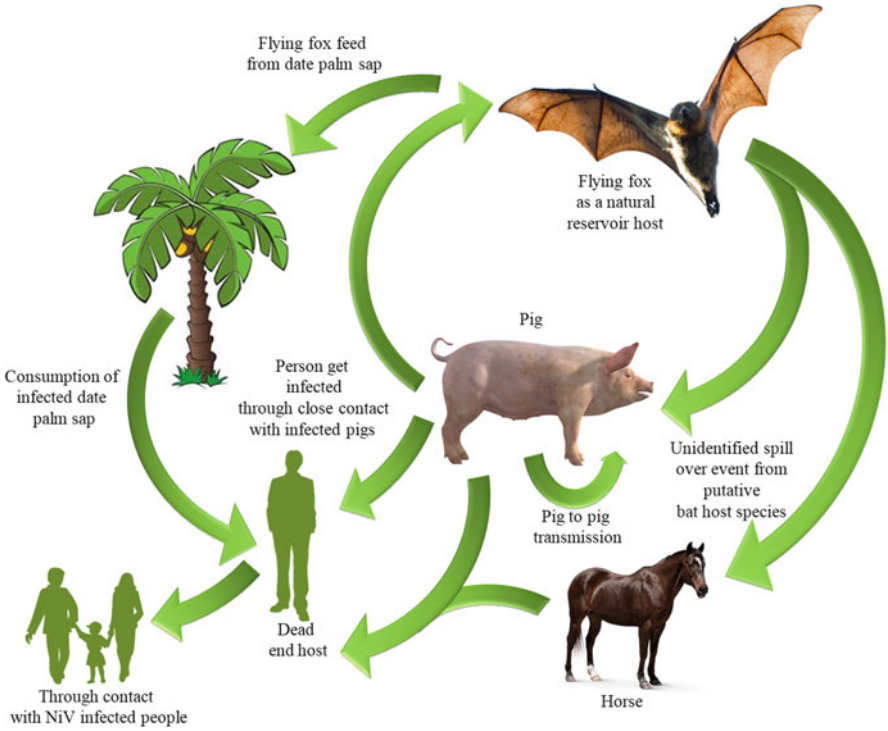

Fig. 3.1 *Nipah virus transmission*: The natural reservoir of the Nipah virus (NiV) is the fruit-eating bats (flying fox). Humans are the dead-end host which may get infected through close contact with infected pigs. Ingesting of infected or contaminated date palm sap is involved in NiV transmission to the dead-end host. Pigs and horses both acts as spill over host in the NiV transmission. Other people get infected with close contact with NiV infected individuals

the extent of NiV replication. Human lung fibroblasts are the main site of replication for NiV. This virus also infects the human monocytes, but this may result in a low level of infection. Blood–brain barrier (BBB) can be breached by NiV and infect brain cells which may result in a high copy number of intracellular virus RNA with the low release of extracellular progeny virions. Similar to all paramyxoviruses, replication of NiV transpires in the cytoplasm of the infected cell (Lamb and Kolakovsky 2001). The entry of NiV in the cell is mediated by G-protein via binding with virion at the cell surface receptor as ephrin B2. After internalization, the release of viral genome into the cytoplasm occurs where genomic RNA converted into mRNAs by viral polymerase (Diederich and Maisner 2007). Translation of mRNAs for both surface glycoproteins (G and F) exhibits occurs at ribosomes in the endoplasmic reticulum (ER). After the translation, both glycoproteins are co-translationally introduced into the ER membrane and transported via the secretory pathway to the cell surface. Later in infection, M, N, P and L proteins of NiV are synthesized (Chang et al. 2006). Eventually, all the newly synthesized RNAs become encapsidated by the N proteins leading to the formation of

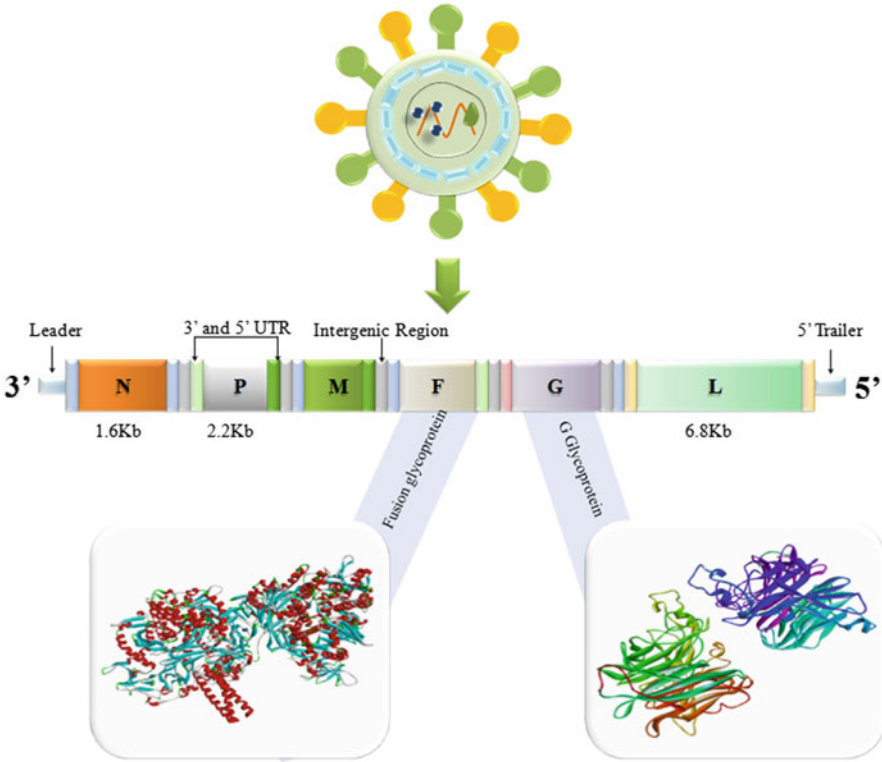

Fig. 3.2 *Nipah virus genome*: The genome size of NiV is ~18 Kb nucleotides long encoding six genes and six proteins namely glycoprotein (G-protein), nucleocapsid protein, phosphoprotein (p), fusion protein (F), matrix protein and large RNA polymerase. The G and F protein is responsible for the induction of neutralizing antibodies as well as involve in virus internalization

ribonucleocapsids (RNPs). Ultimately, the ribonucleoprotein and viral membrane proteins as G, F and M complexes are orchestrated at the cell surface resulting in viral budding and release of progeny virions (Fig. 3.3) (Jensen et al. 2018).

3.3 Epidemiology and Outbreaks of Nipah Virus

Infectious diseases are the leading cause of global public health concern. The outbreak of any infectious diseases is always linked with serious social, political and economic imbalance (Rabinowitz and Conti 2013). NiV was first identified in humans and pigs in Kampung Sungai Nipah, a village of Malaysia in 1998–1999 where 265 patients were infected, and about 40% of them died from serious nervous disease (Goh et al. 2000). The outbreak was initially considered as a result of the Japanese encephalitis virus infection due to similarity in neurological

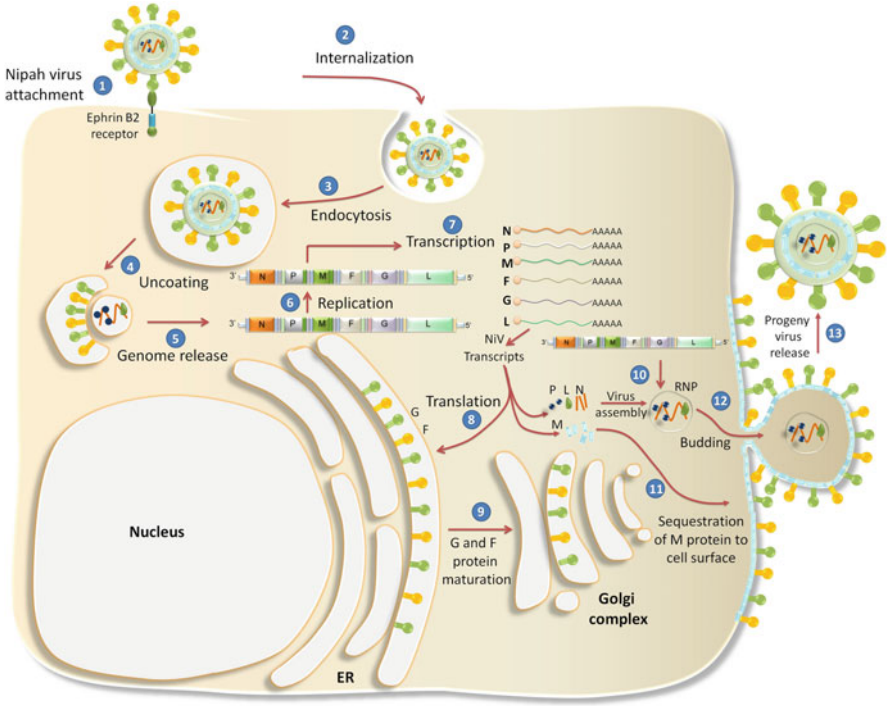

Fig. 3.3 *Nipah virus replication:* 1, 2. Nipah virus (NiV) infection initiates upon attachment and internalization of the NiV mediated by Ephrin B2 receptor; 3. NiV internalization occurs via endocytosis mechanism; 4, 5. uncoating of endocytic membrane leads to the release of NiV genome into the cytoplasm; 6, 7. the viral genome gets replicated that results in the generation of NiV transcripts; 8. these transcripts gets translated into G and F proteins at the rough endoplasmic reticulum; 9. these proteins gets transported to golgi complex for maturation; 10. in addition, NiV genome, P, L and N protein together form ribonucleocapsid (RNPs) along with; 11. sequestration of M protein to the cell surface receptor that causes: 12. budding of RNPs and 13. progeny virus release

complications but it was later recognized as NiV encephalitis. The major epidemics of NiV have been reported in Southeast Asia predominantly in Malaysia, Singapore, Bangladesh and India. Ingestion of contaminated date palm sap by NiV infected bats was the foremost cause of the infection in Bangladesh in 2004. Human-to-human transmission has also been reported in India (Sazzad et al. 2013). In 2001, the first major epidemic occurred in Siliguri (West Bengal), India, where 66 people were infected and out of which 45 deaths were reported (Harit et al. 2006). A second outbreak takes place at Nadia in West Bengal in 2007 where five cases of Nipah virus were reported with 100% case fatality (Krishnan 2007). Recently, Nipah virus emergence occurred in Kozhikode and Malappuram districts of Kerala (May 2018) where 19 peoples were infected with NiV, out of 19 reported cases 17 people died, from the two affected districts (Table 3.1).

Table 3.1 Nipah virus encephalitis associated morbidity and mortality in the Southeast Asia Region (1998–2018) [Adapted from WHO (2007), Ching et al. (2015)]

Month/Year	Locations	Country	Cases	Deaths	Case fatality rate (CFR) (%)
May 2018	Kozhikode and Malappuram	India	19	17	89.47
Feb 2015	Nilphamari, Magura, Ponchoghor, Faridpur, Naugaon, Rajbari	Bangladesh	9	6	66.66
May 2014	Tinalon, Midtungok (Senator Ninoy Aquino, Sultan Kudarat)	Philippines	17	9	52.94
Feb 2014	Manikganj, Magura, Chapai Nawabganj, Rangpur Shariatpur, Kushtia, Rajshahi, Natore, Faridpur, Dinajpur, Naogaon	Bangladesh	18	9	50
May 2013	Gaibandha, Jhinaidaha, Kushtia, Magura, Mymensingh, Naogaon, Natore, Manikganj, Nilphamari, Pabna, Kurigram, Rajshahi, Rajbari	Bangladesh	24	21	87.50
Feb 2012	Joypurhat, Natore, Gopalganj, Rajshahi, Rajbari	Bangladesh	12	10	83.33
Jan–Feb 2011	Lalmonirhat, Dinajpur, Comilla, Nilphamari, Rangpur	Bangladesh	44	40	90.90
Feb–Mar 2010	Faridpur, Gopalganj, Madaripur, Rajbari	Bangladesh	16	14	87.50
Jan 2009	Gaibandha, Rangpur, Nilphamari, Rajbari	Bangladesh	4	1	25
Apr 2008	Rajbari, Faridpur	Bangladesh	7	5	71.42
Feb 2008	Manikganj	Bangladesh	4	4	100
Apr 2007	Nadia	India	5	5	100
Apr 2007	Naogaon	Bangladesh	3	1	33.33
Mar 2007	Kushtia, Natore, Pabna	Bangladesh	8	5	62.50
Jan–Feb 2007	Thakurgaon	Bangladesh	7	3	42.85
Jan–Mar 2005	Tangail	Bangladesh	12	11	91.66
Apr 2004	Faridpur	Bangladesh	36	27	75
Jan 2004	Rajbari	Bangladesh	31	23	74.19
Jan 2003	Naogaon	Bangladesh	12	8	66.66
Apr–May 2001	Meherpur	Bangladesh	13	9	69.23
Jan–Feb 2001	Siliguri	India	66	45	68.18
Mar 1999	Singapore	Singapore	11	1	9.09
Sept 1998–Apr 1999	Perak, Selangor, Negeri, Sembilan states	Malaysia	265	105	39.62
		Total	643	379	58.94

3.4 Management of Nipah Virus Infection

3.4.1 Diagnosis

NiV infection can be identified by various methods in both humans and animals, such as virus isolation, serologic tests and nucleic acid amplification. Differential diagnosis from another viral encephalitis primarily as Japanese encephalitis, bacterial meningitis and herpes simplex encephalitis is crucial for the early detection of the virus during infection (Kaku et al. 2012). NiV diagnosis can be performed by virus isolation via cell culture, serum neutralization, ELISA, PCR, immunofluorescence assay. Fatal cases of NiV infection can be confirmed by immunohistochemistry of tissues (Wang and Daniels 2012).

3.4.2 Preventive and Therapeutic Approaches

Early detection of infection in hosts is the only way of restricting NiV. During the outbreaks, proper barrier nursing techniques and standard infection control practices are also crucial in the restriction of person-to-person transmission (Satterfield 2017). The passive immunization via human monoclonal antibody targeting NiV glycoprotein has been evaluated in the post-exposure therapy in the various animal models and found to be effective. There is no licensed antiviral drug or vaccine available to cure NiV infection. Most of the hopeful vaccines are in the pre-clinical stages and have been tested in various animal models. Mammalian cell-derived NiV like particles (VLP) including G, F and M protein have been used to develop a vaccine. VLP-based vaccine shows protection against NiV infection in the hamster model and has the potential to become a prophylactic vaccine for human (Walpita et al. 2017). Vaccine vectors based on canarypox virus comprised of ALVAC-F gene which encodes for NiV fusion protein or the glycoproteins (ALVAC-G) have been used to immunize pigs (Teigler et al. 2014). Still, the most deliberately tested vaccines are soluble glycoprotein (sG) based vaccine which demonstrates cross-protection against both NiV and HeV (Mungall et al. 2006). Thus, the available treatment is based on only intensive supportive care mainly based on management of the high fever, neurological and respiratory symptoms in the NiV infected individuals. **Ribavirin** is a guanosine analogue which has antiviral activity against both NiV and HeV in vitro. Ribavirin was firstly used during the Malaysian outbreak with 36% reduction in mortality (Snell 2004). **GS-5734** is an adenosine nucleoside analogue that exhibits good efficacy in animal models and currently under phase 2 clinical trial for Ebola virus treatment (Lo et al. 2017). A recent study shows that **GS-5734** has antiviral activity against broad range of viruses belonging to *Filoviridae, Paramyxoviridae* and *Coronaviridae* families. Recently, a purine analogue namely **Favipiravir** (T-705) is approved for the management of novel and reemerging influenza virus in Japan. This drug is currently under phase 3 clinical trial in the

USA for the treatment of influenza. Favipiravir targets and inhibits viral RNA-dependent RNA polymerase (RdRp) and demonstrates in vitro antiviral activity against an extensive range of RNA viruses including flaviviruses, alphaviruses, arenaviruses, enteroviruses, norovirus, filoviruses, bunyaviruses and rhabdoviruses. Some recent studies show that favipiravir inhibits the replication and transcription of Nipah and Hendra virus at micromolar concentrations. Favipiravir is also used to treat Ebola virus infection, and it is reported that favipiravir treatment reduces the mortality in Ebola patients having low to moderate viral load (Dawes et al. 2018). **Chloroquine** (anti-malarial drug) was found to inhibit the maturation process of NiV, although no clinical benefit has yet been observed (Pallister et al. 2009). **Monoclonal antibodies** work as post-exposure prophylaxis and show efficacy in animal models by targeting the envelope protein of NiV. However, their effectiveness in human infection needs to be investigated (Geisbert et al. 2014). Fusion protein-based **peptides derived from the C-terminal heptad repeat (HRC)** prevent fusion and virus internalization process into the host cells by inhibiting the synthesis of the fusogenic six-helix bundle and reduce mortality during NiV infection in various animal models (Porotto et al. 2010).

3.4.3 Complementary and Alternative Medicines (CAM) for Nipah Virus Infection

Treatment with CAM may provide a substitute approach for a supportive therapeutic strategy for the prevention of infection. No specific antiviral drug or effective vaccines are available for the management of NiV infection. Therefore, the treatment is completely based on sign and symptoms arise during the infection. Initially, infected patients should take plenty of fluids to maintain fluid and electrolyte balance. Paracetamol is the drug given for the management of moderate to high fever, whereas other non-steroidal anti-inflammatory drugs (NSAID) such as aspirin is contraindicated in any NiV infected patient due to its potential complications. Similarly, patients with signs of respiratory distress, tachypnea, oxygen saturation less than 90% and dyspnea should provide oxygen therapy.

In the same way, for the management of neurological complications anticonvulsants may be the drug of choice. Intravenous diazepam, phenobarbitone, phenytoin or levetiracetam may be given in standard recommended doses. Mannitol could be provided in case of increased intracranial tension (Clinical management protocol for Nipah virus disease 2018). Homoeopathic prescriptions such as belladonna, calcarea carb, nuxvomica and hyoscyamus help to cure a wide range of symptoms like moderate to high grade fever, headache, inflammation, vomiting, respiratory complications, sensorium, convulsion, myalgia and fatigue (Manchanda et al. 2015). So, these homoeopathic drugs might be beneficial in the symptomatic management of NiV disease. In Ayurveda, several natural products are used for the treatment of viral diseases. Glycyrrhizin obtained from G. glabra family (*Leguminosae*) inhibits the

replication of several viruses (influenza, hepatitis C and human immunodeficiency virus) in vitro (Wolkerstorfer et al. 2009; Sasaki et al. 2002–2003). In the same way dandelion and standardized elderberry liquid extract demonstrate the antiviral property against influenza (A and B) virus in vitro by decreasing its polymerase activity as well as the nucleoprotein RNA level (Krawitz et al. 2011).

3.5 Conclusions and Future Perspectives

Nipah virus comes into view as a newly emerging virus just 20 years back and its continuous outbreaks resulted in high mortality rates both in humans and animals in India, Bangladesh, Malaysia and Singapore. Pteropus bats are the NiV reservoir which is geographically widespread in Southeast Asia. Therefore, the potential for forthcoming outbreaks to occur in new areas remains substantial. To combat the NiV infection, some of the necessary steps should be taken such as urgent development of rapid molecular diagnostics and specific therapeutics and preventive strategies. To promote the early detection of NiV in the areas of known or potential henipavirus spillover risk, surveillance programs should be initiated. In the past recent years, the development of effective and specific therapeutics against NiV helps in controlling the infection during the outbreaks. Membrane targeting strategy by antiviral peptides exhibits a novel approach towards the development of fusion antivirals. Licencing the safe and effective multivalent vaccines for use in humans that protect against both NiV and HeV should be generated where broader population-based vaccination strategies may be applicable. The inter-institutional and international harmonization among human–animal virologists and ecologists helps to understand the transmission cycle and virus replication in the host system. At the same time, educating the peoples about individual and food hygiene may help to prevent NiV infection.

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Chapter 4
Animal Caliciviruses

Souvik Ghosh, Yashpal Singh Malik, and Nobumichi Kobayashi

Abstract The family *Caliciviridae* consists of an expanding list of small, non-enveloped, positive-sense, single-stranded RNA viruses. The International Committee on Taxonomy of Viruses (ICTV) has recognized at least five genera (*Lagovirus*, *Nebovirus*, *Norovirus*, *Sapovirus* and *Vesivirus*) within the family *Caliciviridae*, while several other viruses remain to be classified. Caliciviruses have been detected in a wide variety of terrestrial and marine host species. Among them, human noroviruses, a major aetiological agent of outbreaks of viral gastroenteritis, have been studied extensively worldwide. On the other hand, studies on caliciviruses in other host species are relatively limited in scope and number, and the available information is scattered. Caliciviruses cause significant mortality and morbidity in various animal host species and have been associated with a broad range of disease syndromes. Moreover, there is evidence on the zoonotic potential of some of the animal caliciviruses. This chapter is a comprehensive and updated review of various animal caliciviruses. Salient aspects of calicivirus infection in different animal host species, such as virus diversity, epidemiology including zoonosis, pathogenesis, clinical signs, necropsy findings, treatment, control and preventative strategies, have been discussed.

Keywords Caliciviridae · Feline calicivirus · European Brown Hare Syndrome · Lagovirus · Norovirus · Nebovirus · Rabbit haemorrhagic disease · Sapovirus · Vesivirus · Vesicular exanthema in swine · Virulent-systemic disease in cats

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Abbreviations

EBHSV	European brown hare syndrome virus
FCV	Feline calicivirus
FCV-VSD	Feline calicivirus virulent-systemic disease
FSG	Chronic lympho-plasmacytic gingivitis/stomatitis
gRNA	Genomic RNA
ICTV	International Committee on Taxonomy of Viruses
ORF	Open reading frame
NoV	Norovirus
RHDV	Rabbit haemorrhagic disease virus
SaV	Sapovirus
sgRNA	Subgenomic RNA
VES	Vesicular exanthema in swine
UTR	Untranslated region

4.1 Prologue

The first evidence of a disease caused by caliciviruses dates back to 1932 when these viruses were detected in epizootics of vesicular disease of pigs in California, USA (Traum 1936; Smith et al. 1998). This disease, known as vesicular exanthema of swine (VES), appeared to be clinically indistinguishable from the foot-and-mouth (FMD) disease (caused by a picornavirus), although immunologic studies failed to associate VES with the FMD virus. In 1968, the VES virus was identified as a small, icosahedral RNA virus (Wawrzkiewicz et al. 1968). In 1957, feline caliciviruses (FCV), an important aetiology of respiratory illness in cats, were first isolated in cell culture (Fastier 1957). In humans, caliciviruses (the Norwalk virus) were first associated with an outbreak of diarrhoea in humans in Ohio, USA in 1968 (Kapikian et al. 1972). Eventually, the Norwalk virus was discovered in 1972. In the same year, the first calicivirus (San Miguel sea lion virus) was isolated from a marine animal (sea lions) (Smith et al. 1973, 1998).

The Norwalk virus, FCV, San Miguel sea lion virus and VES virus were initially thought to be members of the family *Picornaviridae* (Melnick et al. 1974). However, based on differences in structure, replication and physiochemical properties between caliciviruses and picornaviruses, the International Committee on Taxonomy of Viruses (ICTV) recommended the removal of caliciviruses from the family *Picornaviridae*, and in 1979, a new virus family, the family *Caliciviridae*, appeared in the third report of the ICTV (Cooper et al. 1978; Matthews 1979). Since then, the family *Caliciviridae* has expanded significantly, and currently includes five genera and several unclassified viruses (Clarke et al. 2012). Although hepatitis E viruses (HEVs) were found to share structural similarities with caliciviruses, the ICTV does not include HEVs in the family *Caliciviridae* (Green et al. 2000).

Table 4.1 Classification, diversity and host range of caliciviruses

Genus[a]	Virus species[a]/diversity	Host
Vesivirus	*Feline calicivirus*	Cats Also detected in dogs, lions and tigers
	Vesicular exanthema of swine virus (this group includes the swine vesicular exanthema virus and vesiviruses detected in various other host species including marine life)	Cattle, cetaceans, horses, humans, ocean fish, pigs, pinnipeds, primates, rabbits, reptiles, skunks, walruses
	Canine calicivirus strain 48, mink calicivirus and calicivirus strain 2117 and 2117-like viruses (isolated from Chinese hamster ovary cells) have been proposed as new species	Dogs (canine calicivirus strain 48) Mink (mink calicivirus) Strain 2117 and 2117-like viruses are of unknown origin. Viruses showing sequence homology to strain 2117 have been detected in dogs
Norovirus	*Norwalk virus* Seven genogroups (GI–GVII), and a tentative new genogroup from a sea lion >40 genotypes within genogroups	Bats, cattle, cats, dogs, humans, lions, mouse, pigs, porpoises, primates (rhesus macaques), rats, sea lions, sheep, wild birds
Sapovirus	*Sapporo virus* 19 genogroups (GI–GXIX) 51 genotypes within genogroups	Bats, chimpanzees, dogs, foxes, humans, lions, mink, pigs, rats, sea lions, spotted hyenas
Lagovirus	*European brown hare syndrome virus* *Rabbit haemorrhagic disease virus*	European rabbits, hares
Nebovirus	*Newbury-1 virus* (includes two major phylogenetic clades, Nebraska-like and Newbury-1-like) Two new species/genotypes (strain Bo/DijonA216/06/FR and Kırklareli virus) have also been proposed	Cattle
Proposed genera/novel/unclassified caliciviruses (host species/source)		
Bavovirus (chicken), *Minovirus* (baitfish), *Nacovirus* (chicken, goose and turkey), *Recovirus* (rhesus macaques), *Salovirus* (Atlantic salmon), *Sanovirus* (goose), *Secalivirus* (sewage), *Valovirus* (pigs)		

[a]Based on the ICTV 9th report (Clark et al. 2012)

Members of the family *Caliciviridae* have been detected in many terrestrial and marine host species (Table 4.1). Among them, human noroviruses, a major aetiological agent of outbreaks of viral gastroenteritis, have been studied extensively worldwide (Bányai et al. 2018; de Graaf et al. 2016). On the other hand, studies on caliciviruses in other host species are relatively limited in scope and number, and the available information is scattered. Caliciviruses cause significant mortality and morbidity in various animal host species and have been associated with a broad range of disease syndromes, including abortions, encephalitis, gastroenteritis, haemorrhages, hepatic necrosis, limping syndrome, pancreatitis, pneumonia, myocarditis and pericarditis, upper respiratory tract illness, vesicular disease, virulent-systemic infections and sudden death. Moreover, there is evidence on the zoonotic potential of some of the animal caliciviruses. This chapter is a comprehensive and updated review of various animal caliciviruses.

4.2 Virus Taxonomy

The ICTV has recognized five genera within the family *Caliciviridae*: *Lagovirus*, *Nebovirus*, *Norovirus*, *Sapovirus* and *Vesivirus* (Clarke et al. 2012). In addition, several unclassified novel caliciviruses/calici-like viruses have been proposed as distinct genera, such as *Bavovirus* from chickens, *Minovirus* (strain FHMCV-2012 from baitfish in the USA), *Nacovirus* in turkeys, chicken and goose, *Recovirus* (Tulane virus (TV) isolated from faeces of captive juvenile rhesus macaques (*Macaca mulatta*)), *Salovirus* from Atlantic salmon, *Sanovirus* from goose, *Secalivirus* from sewage samples and *Valovirus* (St-Valérien-like caliciviruses from pig faeces in Canada) (Wolf et al. 2012; Mor et al. 2017; Day et al. 2010; Liao et al. 2014; Farkas et al. 2008; Mikalsen et al. 2014; Wang et al. 2017; Ng et al. 2012; L'Homme et al. 2009).

4.3 Virus Structure

Caliciviruses are non-enveloped viruses with a diameter of 27–40 nm (Green 2013). A unique morphological feature seen in some caliciviruses is the presence of 32 cup-shaped depressions on the surface of the viral capsid, forming the basis of naming the virus family *Caliciviridae* ('Calici' derived from the Latin word 'calix' meaning cup or goblet) (Clarke et al. 2012).

The calicivirus capsid is composed of ninety dimers of the single, major capsid protein, VP1, that are arranged on a T = 3 icosahedral lattice (Green 2013; Clarke et al. 2012). The VP1 protein has two domains; the variable externally exposed protruding (P) domain and the relatively conserved internal shell (S) domain. The P domain is further subdivided into P1 and P2 subdomains (Green 2013; Rocha-Pereira et al. 2014). The hypervariable region in the P2 subdomain interacts with the receptors/co-receptors (histoblood group antigen and sialic acid-containing glycosphingolipids) on the surface of host cells and is an important determinant of antigenic diversity of caliciviruses (Tan and Jiang 2010).

Calicivirus virions also contain a minor basic protein, VP2, in association with the VP1 S domain at the inner surface of the viral capsid (Green 2013; Goodfellow and Taube 2016; Vongpunsawad et al. 2013). The VP2 protein is believed to enhance the stability of VP1 and is essential for the production of infectious virions (Sosnovtsev et al. 2005; Vongpunsawad et al. 2013).

As caliciviruses lack a lipid envelope, they are relatively stable in the environment, and many strains exhibit resistance to inactivation by chemicals (ether, chloroform and mild detergents) and heat (Clarke et al. 2012).

4.4 Virus Genome Organization

Caliciviruses possess a positive sense, single-stranded, polycistronic RNA genome (genomic RNA, gRNA) that is ~7.3 to 8.5 kb in length (Green 2013). The 5′-end of the viral RNA is covalently linked to the nonstructural protein VPg (viral protein, genome-linked), while the 3′-end is polyadenylated (Alhatlani et al. 2015; Goodfellow and Taube 2016) (Fig. 4.1). A 3′-co-terminal subgenomic RNA (sgRNA), shorter version of the gRNA, is also synthesized during virus replication (Goodfellow and Taube 2016). The sgRNA retains many of the features of the gRNA, such as covalently linked to VPg protein at the 5′-end, and a short conserved 5′-untranslated region (UTR). The overall gene order of caliciviruses appears to be conserved, with the nonstructural and structural coding regions located towards the 5′- and 3′-terminal, respectively, of the viral genome (Goodfellow and Taube 2016) (Fig. 4.1).

Caliciviruses have 2–4 open reading frames (ORF). In genera *Norovirus* and *Vesivirus*, ORF1 encodes a polyprotein that is co- and post-translationally cleaved into 6 or 7 nonstructural proteins (p48 [NS1/2], NTPase [NS3], p22 [NS4], VPg [NS5], a viral protease [Pro, 3C-like, NS6] and a viral RNA-dependent RNA polymerase [RdRp, NS7]), while ORF2 and ORF3 of the sgRNA encode the VP1 and VP2, respectively (Royall and Locker 2016). On the other hand, in *Lagovirus*, *Nebovirus* and *Sapovirus*, the coding region of VP1 is located at the 3′-end of ORF1, while ORF2 of the sgRNA codes for VP2. Therefore, VP1 is encoded as a part of a polyprotein (ORF1) as well as a single protein (sgRNA) in these viruses (rabbit haemorrhagic disease virus and some sapoviruses) (Alhatlani et al. 2015; Goodfellow and Taube 2016).

In addition to core proteins, murine noroviruses encode a virulence factor 1 (VF1) protein (encoded by a 4th ORF located within the VP1 coding region), and feline calicivirus encodes a leader capsid peptide (LC) (encoded by the 5′-end of ORF2) (Abente et al. 2013; McFadden et al. 2011). A third ORF that is located within the 3′-terminus of ORF1 has been identified in some human and bat sapovirus strains (Oka et al. 2016; Yinda et al. 2017).

Recombination and mutation are the key mechanisms of genetic diversity of caliciviruses and have been documented in all the five recognized genera (Bull and White 2010; Clarke et al. 2012).

4.5 Virus Replication

Calicivirus attachment and entry into host cells is initiated through the binding of the viral VP1 protein to surface receptors on permissive cells (Tan and Jiang 2010). Most caliciviruses appear to bind to carbohydrate receptors on host cellular membranes, such as histoblood group antigens (HBGAs), heparan sulphate and sialic acid (in murine norovirus and feline calicivirus) (Ghosh et al. 2018; Tan and Jiang 2010).

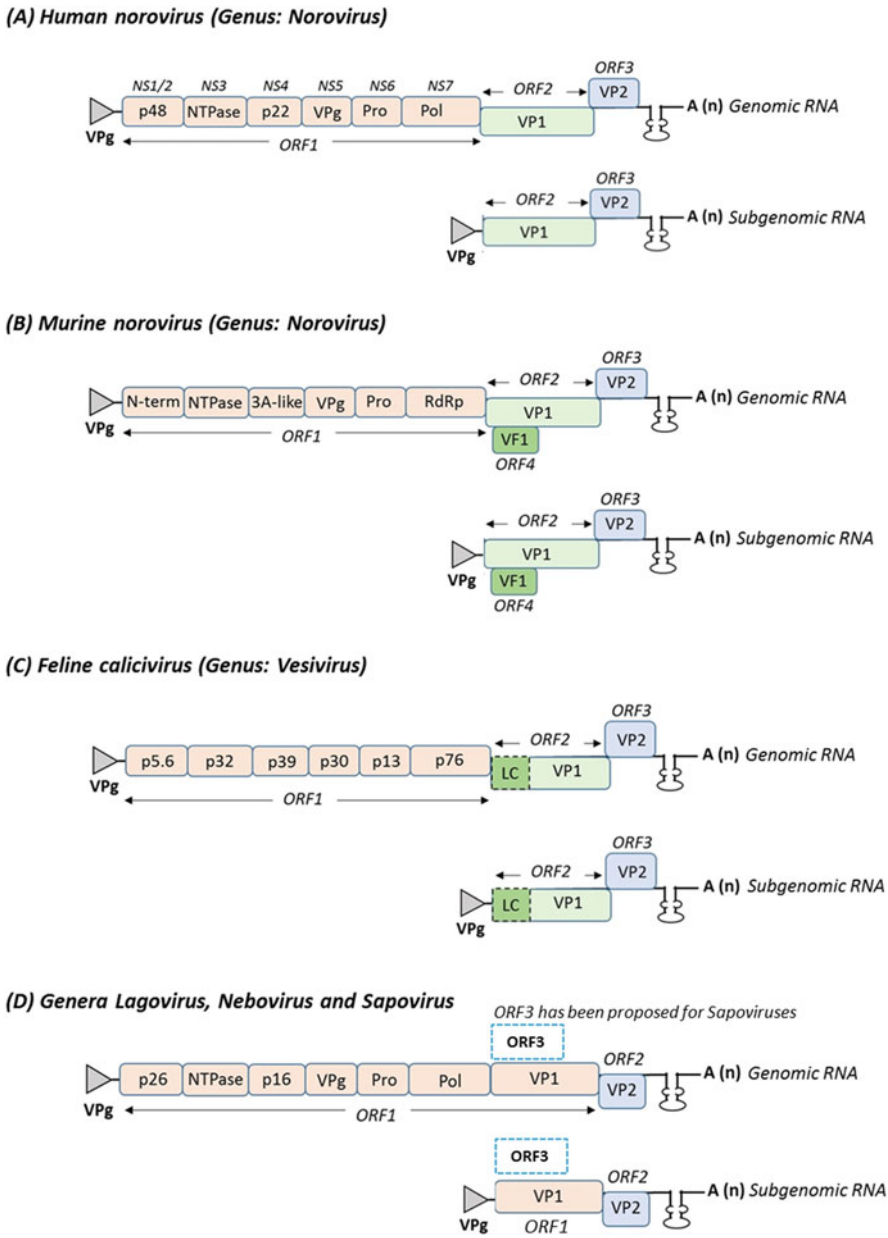

Fig. 4.1 The organization of the viral RNA genome (genomic RNA and subgenomic RNA) in different genera of the family *Caliciviridae*. In addition to core proteins, murine noroviruses encode a virulence factor 1 (VF1) protein, and feline calicivirus encodes a leader capsid peptide (LC). A third open reading frame (ORF) has been identified in some bat and human sapovirus strains

Also, the junctional adhesion molecule-1 (JAM-1), a member of the immunoglobulin (Ig) superfamily, has been shown to function as a receptor/co-receptor for feline calicivirus and Hom-1 vesivirus (Makino et al. 2006; Sosnovtsev et al. 2017). Although internalization events of caliciviruses remain to be properly elucidated, MNVs have been found to depend on cholesterol and dynamin in a clathrin- and caveolae-independent pathway (Gerondopoulos et al. 2010; Perry and Wobus 2010).

Translation and transcription of calicivirus genome occur in the cytoplasm of the host cell. After uncoating and disassembly, the virus-encoded VPg protein that is covalently linked to the 5′-end of viral genome recruits host translational factors to mediate the translation of the parental viral RNA (Goodfellow and Taube 2016). The ORF-1 codes for the polyprotein that is co- and post-translationally cleaved into precursors and products preferably by a virus-encoded protease (3CLpro/NS6). Viral RNA replication occurs in close association with rearranged intracellular membranes (derived from the endoplasmic reticulum, or Golgi apparatus) and involves synthesis of negative-sense RNA from the parental viral RNA, which in turn serves as the template for generation of VPg-linked new positive sense gRNAs and sgRNAs. These new gRNAs and sgRNAs participate in additional rounds of translation, yielding high levels of viral proteins including VP1 and VP2. Mechanisms involved in virus encapsidation and release are not clear. Apoptosis, as well as persistent infection of permissive cells, has been reported (Goodfellow and Taube 2016; Karst and Tibbetts 2016).

4.6 Genus *Vesivirus*

Phylogenetically, members of the genus *Vesivirus* constitute a distinct cluster within the family *Caliciviridae* (Clarke et al. 2012). Based on phylogenetic clustering patterns, subtle differences in viral genomic structure and host specificity, the ICTV has further classified genus *Vesivirus* into at least two major taxonomic groups/species: (1) *Feline Calicivirus* and (2) the *swine vesicular exanthema virus*, and vesiviruses detected in marine life and other host species (cattle, horses, humans, primates, rabbits, reptiles, skunks and walruses) (Clarke et al. 2012; Smith et al. 1998). In addition, canine caliciviruses (GenBank accession no. AB070225), mink caliciviruses and calicivirus strain 2117 and 2117-like viruses (caliciviruses of unknown origin isolated from Chinese hamster ovary cells, and recently, vesiviruses from dogs with acute hemorrhagic gastroenteritis exhibiting sequence homology to strain 2117) have been proposed as members of the genus *Vesivirus* (Clarke et al. 2012; Oehmig et al. 2003; Renshaw et al. 2018).

4.6.1 Feline Calicivirus (FCV)

Feline calicivirus is a highly infectious, ubiquitous pathogen of cats (Gaskell et al. 2011). Depending on the tropism and virulence of FCV strains, infected cats may exhibit a wide range of clinical syndromes, such as inapparent infections, mild or acute self-limiting oral and upper respiratory tract disease, chronic gingivitis or faucitis, limping syndrome, severe pneumonia and virulent-systemic disease (FCV-VSD) (Gaskell et al. 2011; Radford et al. 2007, 2009).

4.6.1.1 Virus Diversity

FCV strains exhibit high genetic diversity, attributed to mutations and recombination events, and are considered to constitute a diverse genogroup with little evidence for sub-species clustering (Afonso et al. 2017; Pesavento et al. 2008). Viral quasispecies formation has been reported for FCV (Radford et al. 1998). Significant associations between FCV phylogeny and the pedigree status of sampled cats have been shown (Spiri et al. 2016). Although genetically divergent FCV strains show antigenic differences, they also exhibit some cross-reactivity, allowing their classification into a single diverse serotype, and forming the basis of FCV vaccines (Afonso et al. 2017; Coyne et al. 2012; Povey and Ingersoll 1975; Radford et al. 2009; Scherk et al. 2013). However, the widespread use of FCV vaccines coupled with a high diversity of FCV strains raises possible concerns on the efficacy of broadly cross-reactive vaccines against emerging vaccine-resistant strains (Afonso et al. 2017; Pesavento et al. 2008; Scherk et al. 2013).

4.6.1.2 Epidemiology

FCV infects cats as well as other members of the family *Felidae* (Gaskell et al. 2011). FCV is widely distributed in cat populations, with higher prevalence rates (up to 40%) in larger groups of cats, such as in colonies or shelters, and lower prevalence rates (~10%) in privately owned or small groups of cats (Bannasch and Foley 2005; Helps et al. 2005; Radford et al. 2007, 2009). Infection is generally more common in younger animals (Gaskell et al. 2011).

Cats primarily acquire infection by coming in direct contact with infected oral, nasal or ocular secretions, either from acutely infected animals or from clinically recovered carrier cats (Gaskell et al. 2004, 2011; Radford et al. 2007, 2009). Aerosols do not appear to be important in the transmission of the virus (Gaskell et al. 2011; Scherk et al. 2013). Indirect transmission of FCV can occur through contaminated fomites, or personnel in confined environments, such as cat shelters (Gaskell et al. 2011; Radford et al. 2007).

Although the acutely infected cats are the most common sources of infection, the FCV carrier state has been detected in approximately 10% of household and 25–75%

of shelter/colony cats (Coyne et al. 2006a, b; Gaskell et al. 2011). Following recovery, most cats appear to shed the virus for 30 days, while the carrier cats continue to persistently or intermittently shed virus beyond this period, even lasting for several years in a few cases (Gaskell et al. 2011; Radford et al. 2009).

FCV and FCV-like viruses have also been reported in dogs, lions and tigers (Harrison et al. 2007; Martella et al. 2002; Kadoi et al. 1997; Tian et al. 2016).

4.6.1.3 Pathogenesis and Clinical Disease

Cats usually acquire FCV infection through the oral, nasal or conjunctival routes (Radford et al. 2009). The virus replicates primarily in the oral and respiratory tissues. However, strains may have a predilection for other tissues and have been detected in viscera, faeces and occasionally in urine (Radford et al. 2007). Depending on differences in tropism and virulence of FCV strains as well as other factors, such as age and immune status of the host, presence of concurrent disease/s, environment, population density and husbandry practices, FCVs have been associated with a wide spectrum of clinical syndromes in cats, ranging from inapparent infection to severe systemic disease (Pesavento et al. 2008). Maternal antibody titres may persist for 10–14 weeks and protect kittens during the first weeks of life but may interfere with vaccination, while virus-neutralizing antibodies appear around a week after infection (Johnson and Povey 1983; Radford et al. 2009).

Oral and Upper Respiratory Tract Infection Most FCV strains induce a mild clinical syndrome characterized by fever, oral ulceration and mild acute respiratory disease (Radford et al. 2007, 2009). Oral ulceration, especially on the margins of the tongue, is the most consistent lesion of FCV infection (Gaskell et al. 2011). These ulcers begin as vesicles, which eventually rupture, followed by necrosis of the overlying epithelium and infiltration of neutrophils at the periphery and the base. Healing is completed in 2–3 weeks. Because of oral ulceration, cats show hypersalivation and anorexia. Upper respiratory tract disease in FCV positive cats often involves co-infection with other viral and bacterial pathogens (Fernandez et al. 2017; Gaskell et al. 2011; Helps et al. 2005). Although sneezing, conjunctivitis and ocular and nasal discharges are observed, these signs are less prominent compared with feline herpesvirus-1 infection (Gaskell et al. 2011). Clinical signs can be severe in young kittens following the decline of maternal antibodies (Scherk et al. 2013).

Lower Respiratory Tract Infection Although pulmonary lesions are rare in typical FCV infections, primary interstitial pneumonia characterized by severe diffuse alveolar damage have been reported with the more virulent FCV strains (Pesavento et al. 2008).

Limping Syndrome Some FCV isolates have been associated with transient febrile lameness syndrome in cats (Dawson et al. 1994; Radford et al. 2007, 2009). The lameness in cats may or may not occur in conjunction with clinical signs of FCV oral and respiratory disease and has also been observed following vaccination with live

vaccines. Clinical signs in cats consisted of pyrexia, lethargy and lameness. Acute synovitis with thickened synovial membrane and increased amounts of synovial fluid has been observed in the FCV-infected joints. FCV antigen has been detected in macrophage-like cells from the joint lesions. Most cats were found to make a complete recovery in 24–48 h.

Virulent-Systemic Disease (FCV-VSD) The FCV-VSD is the most severe clinical form of FCV, characterized by a systemic inflammatory response syndrome, disseminated intravascular coagulation, multiple organ failure and high mortality rates (~50%) (Pesavento et al. 2008; Radford et al. 2007, 2009; Willi et al. 2016). Epizootics of FCV-VSD were first reported in the USA, and subsequently, in European countries (Coyne et al. 2006a, b; Pedersen et al. 2000; Willi et al. 2016). Most FCV-VSD outbreaks are nosocomial infections that occur in multi-cat environments, especially after the introduction of cats from large rescue colonies into a new population. The FCV-VSD isolates reported so far exhibit distinct genetic backgrounds and neutralization patterns, and attempts to identify genetic markers unique to these strains have remained inconclusive (Willi et al. 2016). FCV-VSD appears to be more severe in adults than in kittens. Clinical signs vary and include fever, facial and paw oedema, oral ulceration and upper respiratory infection, variable ulceration and alopecia on the nose, lips and ears, around the eyes and on the footpads, jaundice, severe respiratory distress, ecchymosis, epistaxis and bloody faeces (Gaskell et al. 2011; Radford et al. 2007, 2009; Willi et al. 2016). Necropsy findings may include bronchointerstitial pneumonia, hepatic necrosis, pancreatitis and pericarditis. Vaccination with current FCV vaccines may not offer complete protection against FCV-VSD strains.

Chronic Lympho-Plasmacytic Gingivitis/Stomatitis (FSG) FCV has been detected in a high proportion of cats with chronic gingivostomatitis complex (Radford et al. 2009). Although the FSG syndrome could not be reproduced in experimental FCV infections so far, it is believed to result from feline immune responses to FCV antigens and/or other pathogens (Harley et al. 1999; Nakanishi et al. 2018; Poulet et al. 2000).

4.6.1.4 Diagnosis

Although FCV diagnosis may be attempted on clinical signs, such as oral ulceration, there is a poor correlation between the presence of virus and clinical signs, as evidenced from the asymptomatic carrier states, and therefore, any FCV positive result by a diagnostic test should be treated with suspicion (Radford et al. 2009). On the other hand, FCV-VSD can be diagnosed by clinical signs, rapid spread, high mortality rates and genome sequencing-based confirmation of the same FCV strain from several infected cats (Radford et al. 2009; Willi et al. 2016).

FCV replicates in cell lines of feline origin and can be isolated from conjunctival, nasal and oropharyngeal swabs (Radford et al. 2009). However, viral isolation is time-consuming and may fail due to the presence of small number of viruses in

samples, loss of stability of viruses during transit or in vitro neutralization of viruses. Molecular diagnostic assays are more sensitive than virus isolation and include RT-PCR and RT-qPCR assays that can detect viral RNA in ocular and oropharyngeal swabs, blood, skin scrapings and lung tissues (Meli et al. 2018; Radford et al. 2009; Wilhelm and Truyen 2006). However, because of the high genetic diversity of FCV, RT-PCR assays may fail to detect positive cases. Serological assays are not useful for diagnosis as FCV antibodies are widespread in cats due to natural infection and vaccination (Gaskell et al. 2011).

4.6.1.5 Treatment, Control and Prevention

Treatment of FCV infection is primarily symptomatic and supportive and depends on the clinical presentation of the disease. Good nursing care, fluid therapy, feeding blended warm food, use of mucolytic drugs or nebulization with saline to clear and hydrate air passages may bring relief to affected cats. Broad-spectrum antibiotics that can penetrate effectively into the respiratory tract may be used to prevent secondary bacterial infections.

Currently, there is no licensed antiviral for the treatment of FCV. Ribavirin was shown to inhibit virus replication in cell culture but is toxic to cats (Povey 1978). Parenteral administration of virus-specific antiviral phosphorodiamidate morpholino oligomer (PMO) has been shown to increase survival rates in treated cats compared to untreated cats during FCV-VSD outbreaks (Smith et al. 2008). Recombinant feline interferon-ω (rfeIFN-ω) was shown to be effective against FCV in vitro, but topical administration of rfeIFN-ω did not improve outcome in acute upper respiratory disease caused by FCV (Ballin et al. 2014). Interferons have also been used to treat chronic gingivostomatitis complex (Hennet et al. 2011). Combination therapy using short interfering RNAs (siRNAs) was shown to inhibit FCV replication in vitro (McDonagh et al. 2015). Fexaramine has been found to block the entry of FCV in cultured cells (Kim and Chang 2018).

All cats should be vaccinated against FCV (Scherk et al. 2013). Both modified-live vaccines (administered parenterally or intranasally) and inactivated vaccines (generally adjuvanted, parenteral administration) are available. Majority of the FCV vaccines are combined with FHV-1 vaccines or may include additional other antigens. Both modified-live and inactivated vaccines have been shown to provide reasonable protection against oral and upper respiratory disease. However, FCV-VSD has been reported in vaccinated cats (Willi et al. 2016). None of the current FCV vaccines can prevent infection or viral shedding. Vaccination can be initiated as early as 6 weeks; however, in some countries, the 1st dose is administered at 8–9 weeks of age. Vaccination should be repeated every 3–4 weeks (2–3 weeks in shelters) until 16–20 weeks of age (Radford et al. 2009; Scherk et al. 2013). Revaccination should take place after 1 year, followed by a booster dose every 3 years (1 year in high-risk areas). Inactivated vaccines are recommended for pregnant queen cats and immunocompromised cats. There is no guarantee that vaccination will offer life-long protection against different strains of FCV.

In addition to vaccination, proper management and hygiene practices, such as disinfection of premises (bleach, potassium peroxymonosulfate, chlorine dioxide), adequate ventilation, low relative humidity, optimal temperatures, strict quarantine measures and low stress levels, may help control FCV disease (Gaskell et al. 2011; Radford et al. 2009).

4.6.2 Vesicular Exanthema in Swine (VES)

VES is an acute febrile disease of pigs that is clinically indistinguishable from foot-and-mouth disease, vesicular stomatitis and swine vesicular disease (Radostits et al. 2007).

4.6.2.1 Virus Diversity

Phylogenetically, the causative virus, vesicular exanthema in swine virus (VESV), is grouped with the marine vesiviruses (Clarke et al. 2012). At least 17 antigenic types of VESV have been reported in pigs since 1972 (Radostits et al. 2007). Following inoculation, marine vesiviruses such as the San Miguel sea lion virus isolated from sea lions and fur seals have been shown to induce vesicular exanthema-like disease in pigs (Smith et al. 1973, 1998). These observations suggested that VESV or progenitor strains may have a marine host reservoir, and that feeding of contaminated marine meat or garbage containing marine products may have caused the initial epizootics of VESV in pigs, followed by a pig-to-pig transmission through raw garbage feed (Smith et al. 1998).

4.6.2.2 Epidemiology

In most countries, VES is a notifiable disease (Radostits et al. 2007). VES was first reported in Orange County, California, in 1932, followed by a series of outbreaks in California between 1930 and 1940 (Smith et al. 1998). By 1953, VES was detected in all major swine-production areas (41 states) in the USA, prompting the federal government to enforce strict eradication measures, with emphasis being laid on feeding cooked garbage to swine. In 1959, the virus was officially declared eradicated from the USA. Outside the USA, isolated outbreaks have been reported from Iceland and the Hawaii Islands (Radostits et al. 2007).

Healthy pigs acquire infection by coming in direct contact with vesicular fluid, oral and nasal secretions, faeces, urine (not before 12 h before vesicles appear, and 1–5 days afterwards) and vesicle coverings from diseased animals, or through consumption of contaminated, uncooked pork or marine meat/products (Horak et al. 2016; Radostits et al. 2007). The virus can persist in frozen meat and is generally stable in the environment.

4.6.2.3 Pathogenesis and Clinical Disease

VESV has been associated with high morbidity (90%), but low mortality in pigs (Horak et al. 2016). The incubation period usually ranges from 1 to 3 days, depending on the virulence of the viral strain (Radostits et al. 2007). Acquisition of infection is followed by a viraemia that commences 48 h before vesiculation and may last for 72–84 h.

Vesicles appear within the oral cavity, on the tongue, snout, teats and udder, and feet (between the claws, and on heel bulbs and coronary bands) (Horak et al. 2016; Radostits et al. 2007). Early-stage vesicles are small, thick-walled and contain a small amount of fluid (Gelberg and Lewis 1982; Knowles and Reuter 2012). Around 2 days post-infection, these vesicles mature into large, thin-walled structures containing increasing amounts of clear fluid. Vesicles rupture after 24–48 h of appearance, leaving raw, eroded areas. The vesicular stage of the disease is accompanied by a febrile condition (40.5–41 °C) that drops once the vesicles rupture (Gelberg and Lewis 1982). Sometimes, secondary vesicles may result from fluids released by the rupture of primary vesicles. Ulceration is usually seen 4–7 days post-infection (Horak et al. 2016).

Epithelial lesions include vesiculation, necrosis, sloughing and mild scarring. A large number of inflammatory cells are seen around blood vessels in the dermis. Lymph nodes may show focal necrosis and oedema. In uncomplicated cases, healing is completed in 1–2 weeks. Infection during late pregnancy may result in abortions, and lactating sows may go dry. Myocarditis, encephalitis and diarrhoea have also been occasionally observed in pigs. Anti-VESV antibody levels have been shown to increase dramatically 3 days post-infection, peaking at 7–10 days post-infection, and have been detected up to six months post-infection (Gelberg and Lewis 1982; Horak et al. 2016; Knowles and Reuter 2012).

4.6.2.4 Diagnosis

VES is clinically indistinguishable from foot-and-mouth disease, vesicular stomatitis and swine vesicular disease (Radostits et al. 2007). Viral titres have been found to be highest in the gross epithelial lesions (Gelberg and Lewis 1982). VESV can be isolated from mammalian cell cultures (African green monkey kidney or porcine kidney cells) and cause rapid and destructive cytopathic effects in cultured cells. VES diagnostics include electron microscopy, RT-PCR/RT-qPCR assays that detect viral RNA and detection of anti-VESV antibodies using serological assays such as complement fixation, virus neutralization (VN) and enzyme-linked immunosorbent assay (ELISA) (Horak et al. 2016).

4.6.2.5 Treatment, Control and Prevention

There is no treatment or licensed vaccine for VES. An outbreak situation should be immediately reported to regulatory authorities. Measures to control an outbreak include implementation of strict quarantine measures, culling of infected animals and disinfection of premises (2% sodium hydroxide solution) (Radostits et al. 2007). Pork, marine meat and garbage should be properly cooked (100 °C for 30 min) before fed to pigs (Horak et al. 2016).

4.6.2.6 Marine Vesiviruses

In 1972, the first vesivirus (San Miguel sea lion virus) was isolated from a marine animal (sea lions) (Smith et al. 1973). Thereafter, marine vesiviruses have been detected in several species of pinnipeds and cetaceans as well as in ocean fish and found to induce natural infections in terrestrial animals, such as cattle, horses, humans (blisters on hands and feet), pigs (VES) and skunks, and in reptiles (Horak et al. 2016; Prato et al. 1974; Smith et al. 1980, 1998).

4.7 Genus *Norovirus*

Noroviruses (NoVs) are recognized as the most common cause of outbreaks of non-bacterial gastroenteritis in humans (Bányai et al. 2018; de Graaf et al. 2016). As a result, human NoVs have been extensively studied worldwide. On the other hand, to date, studies on prevalence, diversity and impact of NoVs in animals are relatively limited. Based on previously published data, NoVs do not appear to cause severe clinical disease in animals. However, considering the zoonotic potential of NoVs, studies on animal NoVs have implications for public health (de Graaf et al. 2016; Scipioni et al. 2008).

4.7.1 Virus Diversity

Although NoVs show high genetic diversity and have been detected in a wide variety of mammalian host species, the genus Norovirus consists of a single viral species, the Norwalk virus (Clarke et al. 2012). Based on phylogenetic analysis of the complete deduced amino acid sequences of VP1 capsid protein, NoVs have been classified into at least seven genogroups, designated as GI–GVII, and a tentative new genogroup from a sea lion (Teng et al. 2018; Vinjé 2015). NoV genogroups further segregate into more than 40 genotypes (Bodnar et al. 2017). Inter-genogroup, inter-genotype and intra-genotype recombination events have been observed in NoVs,

especially between ORF1 and ORF2, resulting in the emergence of novel recombinant viruses (Ludwig-Begall et al. 2018). Considering the high frequency of recombination events, a dual classification system has been proposed for NoVs that includes both ORF1 (RdRp) and ORF2 (VP1) sequences (Kroneman et al. 2013).

4.7.2 Epidemiology

NoVs are highly infectious, as a few viral particles have been shown to induce clinical disease in the host (de Graaf et al. 2016). In both humans and animals, NoVs are mainly transmitted by the faecal–oral route (de Graaf et al. 2016; Scipioni et al. 2008). Other routes of transmission include exposure to aerosolized vomitus particles or through contaminated food and water. NoVs are ubiquitous and have been reported in various animal species, and in wild birds.

NoVs belonging to GI, GII and GIV are known to infect humans (de Graaf et al. 2016). Although porcine NoVs share the same genogroup, GII, as human NoVs, porcine NoVs belong to genotypes GII genotype 11 (GII.11), GII.18 and GII.19, which are different from those of human GII strains (Scheuer et al. 2013). However, recently, human GII.1 and GII-like strains have been reported in pigs (Scheuer et al. 2013; Sisay et al. 2016). Bovine and ovine NoVs were shown to belong to GIII (Di Felice et al. 2016; Wolf et al. 2009). GIV, GVI and GVII genogroups have been reported in canines, while feline NoVs belong to GIV and GVI (Bodnar et al. 2017; Di Martino et al. 2016). GV NoVs have been found in murine and rats (Scipioni et al. 2008; Summa et al. 2018). Human GI, GII.3 and GII.4 NoV strains have been detected in wild birds (Summa et al. 2018). A GIV.2 NOV strain was found in a 4-week-old lion cub that died of severe haemorrhagic enteritis (Martella et al. 2007). NoV strains belonging to GI, GII and possibly GIV were detected in captive rhesus macaques (Farkas 2016). NoVs have also been detected in bats, porpoises and sea lions (de Graaf et al. 2017; Kocher et al. 2018; Teng et al. 2018).

4.7.3 Pathogenesis and Clinical Disease

Although the pathogenesis of NoVs in different animal species remains to be properly elucidated, NoV infection has been reported in animals with and without diarrhoea (Scipioni et al. 2008; Sisay et al. 2016). Experimental infection in gnotobiotic calves resulted in non-haemorrhagic enteritis, mild diarrhoea, transient anorexia and xylose malabsorption, associated with necrosis of the intestinal epithelium and villous atrophy (Di Felice et al. 2016; Scipioni et al. 2008). Bovine NoVs are believed to facilitate or complicate gastroenteritis in young animals (Scipioni et al. 2008).

The prevalence of NoVs in diarrheic dogs has been estimated to vary from 2.1% to 40% (Bodnar et al. 2017). Canine NoVs have been often detected in co-infection

with other pathogens, indicative of synergism in the development of clinical disease (Martella et al. 2011). NoVs have been associated with outbreaks of gastroenteritis in cats (Pinto et al. 2012). Gross lesions (severe haemorrhagic enteritis, haemorrhages in the intestinal lymph nodes, marked dehydration) and histopathological lesions (marked alteration and erosion of the intestinal mucosa, villi depletion and haemorrhagic infiltration) have been observed in a lion cub that tested positive for NoVs (Martella et al. 2007).

Following infection with murine NoVs, immunodeficient mice exhibited clinical signs of encephalitis, vasculitis in cerebral vessels, pneumonia and hepatitis, while wild-type, immunocompetent mice appeared to be asymptomatic (Scipioni et al. 2008).

4.7.4 Diagnosis

Electron microscopy, serological assays (ELISA), and RT-PCR/RT-qPCR have been used for the detection of NoV in faecal samples (Scipioni et al. 2008). However, the high levels of antigenic and genetic diversity among NoV strains may influence the sensitivity of diagnostic assays. Murine NoVs, and recently, human NoV strains have been successfully propagated in cell cultures and enteric organoids (de Graaf et al. 2016; Ettayebi et al. 2016).

4.7.5 Treatment, Prevention and Control

There is no licensed drug or vaccine for NoV infections in animals. Treatment is mainly supportive and symptomatic and aimed at controlling diarrhoea.

4.7.6 Norovirus Zoonosis

Although NoVs are generally considered to be host species specific, the zoonotic potential of these viruses has been a subject of interest (Scipioni et al. 2008). Under experimental conditions, human GII.4 NoVs were shown to induce diarrhoea in gnotobiotic calves and piglets (Scipioni et al. 2008). Moreover, GII.4-like NoVs have been detected in bovine and porcine faecal samples, and a retail raw pork sample (Mattison et al. 2007). Recently, human GII.1 strains have been detected in pigs in Ethiopia (Sisay et al. 2016). Antibodies against human NoVs have been found in pigs (Scipioni et al. 2008).

Human GII.4 and GII.12 strains have been reported in dogs, while antibodies to canine NoVs have been detected in humans (Mesquita et al. 2013; Summa et al. 2012). A GIV.2 NoV has been detected in a lion cub (Martella et al. 2007). Human

GI, GII.3 and GII.4 NoV strains have been found in wild birds (Summa et al. 2018). GI.1 and GII.7 strains were detected in captive rhesus macaques (Farkas et al. 2016). Bat NoVs are antigenically similar to human NoVs (Kocher et al. 2018).

In addition to interspecies transmission events, the possibility of generation of interspecies recombinant NoV strains cannot be excluded (Ludwig-Begall et al. 2018). Both human- and animal-derived NoVs have been found in shellfish, suggesting the potential for interspecies recombination events, as evidenced by detection of feline GIV.2_GVI.I NoV strain FNoVM49 near an oyster farm in Japan (Costantini et al. 2006; Ludwig-Begall et al. 2018).

4.8 Genus *Sapovirus* (SaV)

Sapovirus was first identified by electron microscopy of a human faecal sample in 1976 and was initially known as 'typical human caliciviruses' or 'Sapporo-like' viruses (Oka et al. 2015). In 2002, the ICTV assigned these viruses to the species *Sapporo virus* within genus *Sapovirus* in the family *Caliciviridae*. SaVs have been detected in various mammalian species and are associated with gastroenteritis in humans and pigs.

4.8.1 Virus Diversity and Host Range

Members of the genus *Sapovirus* form a distinct phylogenetic clade within the family Caliciviridae (Oka et al. 2016). Although the SaV virus genome contains two ORFs, a third ORF that is located within the 3′-terminus of ORF1 has been identified in some human and bat SaV strains (Oka et al. 2016; Yinda et al. 2017) (Fig. 4.1). Recombination events are frequent in SaVs (Oka et al. 2015, 2016).

Based on differences in VP1 sequences, SaV has been classified into at least 19 genogroups (GI–GXIX), which further segregate into 51 genotypes (Li et al. 2018). Genogroups GI, GII, GIV and GV have been shown to infect humans. Majority of the porcine SaVs are classified as GIII. Other genogroups (GV–GXI) have also been reported in pigs. SaV strains belonging to different genogroups have also been detected in other animal species, such as GI in chimpanzees, GII and GXV in rats, GV in sea lions, GXII in mink, GXIII in a dog and GXIV and GXVI–GXIX in bats (Li et al. 2018).

Novel SaVs that are phylogenetically distinct from previously reported SaV strains have been found in spotted hyena, African lion and bat-eared fox (Olarte-Castillo et al. 2016). By phylogenetic analysis, some porcine, rat and simian SaV strains were found to cluster with human GI, GV and GII strains, raising concerns on the zoonotic potential of SaVs (Li et al. 2018; Oka et al. 2016; Yinda et al. 2017).

4.8.2 Porcine Sapovirus

4.8.2.1 Epidemiology

Porcine SaVs have been reported in Africa, Asia, Europe, North and South Americas (Proietto et al. 2016; Sisay et al. 2016). The virus does not appear to show any seasonality and has been detected in pigs at different times of the year (Proietto et al. 2016). The faeco-oral route appears to be the major mode of transmission of SaVs in pigs.

4.8.2.2 Pathogenesis and Clinical Disease

Porcine SaVs have been detected in animals with and without diarrhoea, and in co-infection with other porcine viruses, such as rotavirus, porcine epidemic diarrhoea virus and kobuvirus (Kuroda et al. 2017). A correlation between specific SaV genogroups and the prevalence, age distribution and pathogenicity of the virus in pigs has been suspected (Kuroda et al. 2017; Proietto et al. 2016). The prevalence of SaVs appears to be higher in younger pigs (aged 2–8 weeks) compared to older animals (Kuroda et al. 2017; Reuter et al. 2010).

Gnotobiotic pigs were found to exhibit mild and moderate diarrhoea with viral shedding following experimental inoculation of tissue-culture-adapted and wild-type SaV strains, respectively (Proietto et al. 2016). Mild to severe villous atrophy in the duodenum and jejunum, villous fusion, exfoliation and loss of enterocytes, infiltration of mononuclear cells and high numbers of virus-positive enterocytes in the proximal small intestine have been observed in the experimentally infected pigs. Diarrhoea and villous atrophy have also been induced in gnotobiotic piglets (aged 4–6 days) by intravenous inoculation of SaVs; however, the systemic disease has not been documented in these animals (Guo et al. 2001).

In addition to diarrhoea, vomition has been observed in a porcine SaV outbreak in China. A subclinical infection has been reported in pigs (Zhang et al. 2008). There appears to be no information on SaV-related case fatalities or harmful effects of the virus on growth and reproduction in pigs (Proietto et al. 2016).

4.8.2.3 Diagnosis

In most studies, RT-PCR, and more recently, RT-qPCR have been used to detect SaV RNA in faecal samples (Li et al. 2018; Proietto et al. 2016). Serological assays, such as immunofluorescence and immunohistochemistry, can detect SaV antigens in tissues. Antigen-ELISA using antiserum from pigs and guinea pigs as capture antibodies and antibody-ELISAs using recombinant virus-like particles (VLPs) have been developed. Porcine SaVs can be propagated in porcine kidney cell cultures (Oka et al. 2016).

4.8.2.4 Treatment, Prevention and Control

There is no licensed drug or vaccine for SaV infections in pigs so far. Sick pigs should be quarantined (Proietto et al. 2016). Treatment is aimed at restoring fluid and electrolyte balance and providing supportive care. Potential vaccine strategies against porcine SaVs include a PSaV-C-TC strain-based live-attenuated oral vaccine and oral VLP-based vaccines (Guo et al. 2001; Hansman et al. 2005; Proietto et al. 2016). The virus is potentially vulnerable to disinfection with acetic acid, glutaraldehyde, sodium hydroxide, sodium hypochlorite and Virkon-S® (Proietto et al. 2016).

4.9 Genus *Lagovirus*

Members of the genus *Lagovirus* cause a contagious, fulminating, lethal disease in domesticated and wild European rabbits (*Oryctolagus cuniculus*), incurring significant losses to the economy (rabbit meat and fur industry) and negatively impacting the ecology (Abrantes et al. 2012).

4.9.1 Virus Diversity

Phylogenetically, viruses of the genus *Lagovirus* form a distinct clade within the family *Caliciviridae* (Clarke et al. 2012). At least two species, the *rabbit haemorrhagic disease virus* (RHDV) affecting the European rabbits and *European brown hare syndrome virus* (EBHSV) affecting brown, mountain and Italian hares (*Lepus europaeus, L. timidus and L. corsicanus*), have been recognized by the ICTV. Recently, a new classification system has been proposed where RHDV- and EBHSV-related viruses would constitute two separate genogroups within a single species of lagovirus, *Lagovirus europaeus* (Le Pendu et al., 2017).

RHDV and EBHSV viruses are closely related with regard to virus morphology and antigenicity, clinical disease, pathological lesions and mortality rates and show ~70% sequence homology (Abrantes et al. 2012; Esteves et al. 2015). Phylogenetically, pathogenic RHDV strains are classified into the classic genogroups G1-5, the antigenic variant RHDVa/G6 and RHDV2/RHDVb (Esteves et al. 2015). RHDV2 strains cluster between the pathogenic and non-pathogenic strains and show a nucleotide sequence diversity of ~15% with G1–G6 strains. Recombination events are frequent in RHDV (Lopes et al. 2017).

4.9.2 Epidemiology

RHDV was first reported in European rabbits in China in 1984, and thereafter, spread worldwide (Abrantes et al. 2012). The disease is endemic in most parts of Europe, Asia, and parts of Africa, Australia and New Zealand. EBHS was first detected in Sweden in 1980, followed by other European countries (Abrantes et al. 2012). Both pathogenic and non-pathogenic strains that are related to, but genetically divergent (~20% sequence diversity) from pathogenic RHDV strains have been reported in rabbits.

Two different hypotheses have been proposed on the origin of pathogenic RHDVs in European leporids: (1) pre-existing non-pathogenic viruses may have evolved into pathogenic strains, and (2) interspecies transmission of viruses from host species (native or previously introduced) that are sympatric with European leporids, such as the cottontail (*Sylvilagus floridanus*), and both versions are not mutually exclusive (Esteves et al. 2015). Faeco-oral route is considered as the preferential mode of transmission in natural RHDV infections (Abrantes et al. 2012). However, the virus can also be transmitted through aerosol routes, indirectly through contaminated fomites, and by mechanical vectors such as birds, humans, insects, rodents and scavenging animals. Carnivores, such as dogs, foxes and wolves, are believed to serve as passive carriers of RHDV (Di Profio et al. 2018).

4.9.3 Pathogenesis and Clinical Disease

RHDV causes a fatal disease in adult rabbits, while young animals (<4 weeks) appear to be resistant to the virus (Abrantes et al. 2012). However, RHDV2 has been shown to induce clinical disease and death in young rabbits (Dalton et al. 2012). The incubation period of RHDV generally ranges from 1 to 3 days. Rabbits die within 12–36 h after the appearance of fever.

Three different clinical conditions have been observed with RHDV infections in rabbits (Abrantes et al. 2012). The peracute form is characterized by sudden death without the appearance of clinical symptoms. In the acute form of the disease, clinical symptoms may include anorexia, apathy, congestion of conjunctiva, neurological signs (excitement, opisthotonos, paralysis and ataxia). Occasionally, respiratory signs (tracheitis, dyspnea and cyanosis) accompanied with a foamy and bloody discharge from nasal orifices, bleeding from nose, discharges from eyes and ocular haemorrhages have been observed. The subacute condition has a similar clinical presentation as the acute form but is less intense, and most rabbits survive.

RHDV appears to primarily target the liver (severe necrotizing hepatitis), lung (hyperaemia, pulmonary oedema, intra-alveolar and perivascular haemorrhages) and spleen (splenomegaly). Extensive disseminated intravascular coagulation (DIC) induces haemorrhages in various organs and is typically the cause of death of the animal (Ueda et al. 1992). Virus infection coincides with the depletion of B and T

lymphocytes in the liver and the spleen, facilitating the fatal progression of the disease in 2–3 days (Abrantes et al. 2012; Marques et al. 2010).

4.9.4 Diagnosis

The sudden death of a large number of rabbits after a short period of fever, and characteristic hepatic necrosis and haemorrhages in various organs during necropsy may indicate RHDV (OIE 2015). Laboratory diagnosis is based on samples obtained from the liver (has the highest titre of virus in per-acute and acute cases), spleen or blood. Serological assays include haemagglutination (first line of test used for routine laboratory diagnosis), detection of viral antigen by ELISA on 10% liver homogenates, immunostaining and detection of antibodies by indirect, or competitive ELISA (OIE 2015). Western blotting is recommended when HA or ELISA results remain inconclusive. RT-PCR/RT-qPCR-based detection of viral RNA is a rapid and sensitive diagnostic assay (CFSPH 2016).

4.9.5 Treatment, Control and Prevention

There is no treatment available for rabbits showing clinical signs of RHDV (Abrantes et al. 2012). However, passive immunization with hyperimmune antiserum may prevent death and confer short duration protection to rabbits showing subclinical or no clinical signs and has been successfully used during emergencies (CFSPH 2016). Eradication of RHDV in domestic rabbits is possible by depopulation, disinfection, surveillance, quarantines and immunization (OIE 2015). However, if the virus is circulating in wild rabbits, then eradication is not feasible, and in those areas, RHDV can be controlled and prevented by implementing strict biosecurity measures including sanitation, disinfection, quarantine, use of sentinel seronegative rabbits, maintaining closed colonies and vaccination (Abrantes et al. 2012; OIE 2015; CFSPH 2016).

Inactivated, adjuvanted vaccines prepared from tissue (liver) suspensions of experimentally infected rabbits are commercially available. In endemic areas, the 1st dose of the vaccine should be followed by a second dose after 2 weeks, and the annual booster dose (OIE 2015). If there are no reports of RHDV in the farm or area, vaccination may be limited to only breeding rabbits. RHDV/RHDVa vaccines offer poor cross-protection against RHDV and are not effective in preventing infection and losses due to clinical disease (OIE 2015). A parenteral, recombinant vaccine (Nobivac Myxo-RHD) based on the expression of RHDV VP60 protein by modified myxomaviruses is commercially available (www.msd-animal-health.ie/products_roi_vet/myxorhd/overview.aspx).

4.10 Genus *Nebovirus*

Members of the genus Nebovirus have been detected only in bovines so far, and phylogenetically, form a distinct cluster within the family *Caliciviridae* (Clarke et al. 2012). The 9th report of the ICTV has recognized one species, the Newbury-1 virus that consists of viruses belonging to major clades, Nebraska-like and Newbury-1-like (Clarke et al. 2012; Oliver et al. 2006; Park et al. 2008). However, recently, two new species within genus Nebovirus have been reported from cattle (Alkan et al. 2015; Kaplon et al. 2011).

Neboviruses have been detected in faeces of diarrheic calves from different parts of the world, such as Brazil, Germany, France, Italy, Korea, Tunisia, the UK and the USA, and in healthy calves (Cho et al. 2018; Gomez and Weese 2017). Following experimental infection, both the prototype strains, Nebraska and Newbury-1, induced diarrhoea in gnotobiotic calves, and pathological lesions, such as villous atrophy and desquamation, were observed in the small intestine (Cho and Yoon 2014). Neboviruses have been detected in co-infection with other bovine enteric pathogens (Gomez and Weese 2017). The role of nebovirus in bovine diarrhoea, and potential as a zoonotic pathogen remains to be elucidated (Cho et al. 2018; Gomez and Weese 2017).

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Conflict of Interest None

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Chapter 5
Avian Influenza Virus

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Abstract Avian influenza is a disease caused by influenza A virus (IAV) that mainly affects domestic poultry but poses a serious zoonotic threat due to direct transmission from poultry to mammals including human beings. While the high pathogenic avian influenza (HPAI) mainly caused by H5 and H7 subtypes of IAVs lead to high mortality, the low pathogenic avian influenza (LPAI) caused by all the 16 haemagglutinin subtypes lead to high production losses. Wild aquatic birds serve as reservoir hosts as the virus cause productive subclinical infections in them. Reported for the first time in 1878 in Italy, the IAVs have so far caused three pandemics in humans. The H5N1 virus currently circulating for over two decades throughout the world has caused outbreaks in over 60 countries including India. LPAI viruses are transmitted amongst terrestrial poultry via respiratory droplets and aerosols and the HPAI viruses are transmitted via faecal route. Pathogenesis of IAVs is markedly different between wild water birds, terrestrial poultry and humans. Clinical diagnosis of AI is very difficult and often confused with other respiratory diseases of poultry. Diagnosis of AI involves isolation, identification and characterization of the virus. Current molecular techniques particularly the RT-PCR and real-time RT-PCR are recommended for rapid AI diagnosis. Effective control programs for avian influenza in poultry farms or its spread between farms can reduce the loss due to the disease by a minimum of 75%. The various control measures along with their advantages and disadvantages are discussed in detail in this chapter.

Keywords Avian influenza · Poultry · High pathogenic avian influenza (HPAI) · Low pathogenic avian influenza (LPAI) · RT-PCR · Real-time RT-PCR

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5.1 Prologue

Avian influenza (AI) is a disease affecting domestic poultry (chickens, ducks, turkeys, etc.), leading to huge economic loss to the poultry industry. The disease also affects wild and pet birds. Occasionally, human beings and other mammals are also affected. Depending on its severity in poultry, the disease is categorized into low pathogenic AI (LPAI), which cause mild disease and express few or no clinical signs and the highly pathogenic AI (HPAI) characterized by sudden onset of severe clinical signs and high mortality rate reaching up to 100% (Suarez 2000). While the clinical signs of HPAI in chickens include gasping, greenish diarrhoea, swollen head, discoloration of head and shank and rapid death, turkeys and other poultry species display nervous signs.

The disease is caused by influenza A virus (IAV), a member of the family *Orthomyxoviridae*. The virion is in either spherical or filamentous form with an average diameter of 100 nm for spherical particles and excess of 300 nm length in the filamentous forms. The ratio of surface glycoprotein spikes of AIV, viz. haemagglutinin (HA) and neuraminidase (NA), is approximately 4:1. Due to the point mutations (antigenic drift) in these two proteins, 16 HA (H1–H16) and 9 NA subtypes (N1–N9) have been detected in birds. The newly found H17N10 and H18N11 subtypes in bats (Tong et al. 2012, 2013) are yet to be reported in avian species. Many different combinations of HA and NA are possible (i.e., H5N1, H6N2, H3N8, etc.). As these surface glycoproteins are antigenically distinct, there is little or no cross-protection between different HA or NA subtypes. The eight genome segments of 13.5 kb long single-stranded, negative-sense RNA genome of AIV are designated as HA, NA, matrix (M), non-structural (NS), nucleoprotein (NP), polymerase basic (PB) 2, PB1, polymerase acidic (PA). While all subtypes of viruses are low pathogenic, so far, barring a few exceptions, the HPAI viruses are either H5 or H7 subtypes only. The emergence of H5N1, H5N8, H5N6 and H7N9 subtypes has raised global concerns due to high mortality in poultry and limited human-to-human transmission raising a pandemic concern.

Wild aquatic birds are the natural reservoir host of the virus with low or no clinical signs of infection and shed the virus to the environment. Majority of viruses cause mild or asymptomatic infection in these species of birds. Majority of the AIV subtypes have been isolated in *Anseriformes* (ducks, geese, swans, etc.) and *Charadriiformes* (gulls, shorebirds, etc.) orders. The transmission of AIV from wild birds to domestic poultry primarily occurs through direct or indirect contact with infected birds.

The HPAI poses a serious threat to the poultry industry, public health and economy globally. For the purpose of international trade, the World Organisation for Animal Health (OIE) defines AI as an infection of poultry caused by H5 and H7 viruses or any IAV which cause $\geq$75% mortality in intravenous pathogenicity index (IVPI) test or an IVPI of $>$1.2 or H5 and H7 viruses that have multiple basic amino acids (arginine/lysine) at the cleavage site of HA even if they do not satisfy the two criteria. Between January 2013 and November 2018, a total of 68 countries have

reported HPAI outbreaks in domestic birds at least once resulting in loss of approximately 122 million birds. While the outbreaks occurred in Asia, Africa, Europe and North America, it is almost absent in Central and South America (OIE 2015, situation report). The impact of HPAI on poultry industry is enormous. The cost of the 2006 H5N1 outbreaks in India is approximately Rs. 2200 crores. The virus occasionally jumps to human beings raising a pandemic concern. As of 21 September 2018, 860 human infections of H5N1 virus have been reported to WHO from 16 countries and territories with 454 fatalities (accessed on 31 October 2018).

In general, transmission of AIV to humans is limited and its replication is not efficient. This species barrier is due to the variation in the presence of sialic acid (SA) receptor in their upper respiratory tract. While avian species predominantly contain α2-3-linked SA receptor recognized by the HAs of AIV in their upper respiratory tract, the humans have α2-6-linked SA receptor recognized by the HAs of human influenza. Apart from humans, AIVs have also been isolated from other mammalian species such as pigs, horses, dogs, cats, tigers, etc. H5 and H7 subtypes of AIV have acquired mutations in HA that can have affinity for human receptors. Human cases due to various AIV subtypes H5N1, H5N6, H6N1, H7N3, H7N7, H7N9, H9N2 and H10N8 have been reported. Humans get infection by direct contact with infected birds and environmental contamination. However, human-to-human infection with these subtypes has not been effectively achieved.

5.2 History

AI is not a new disease. HPAI (formerly known as fowl plague) was first recorded in Italy in 1878 by Perroncito (reported by Lupiani and Reddy 2009). In 1880, Rivolta and Delprato differentiated fowl plague from the acute form of fowl cholera, based on clinical and pathological properties. In 1901 another HPAI outbreak was reported in Italy, which subsequently spread to Austria and Germany and later to France and Belgium. In Germany, the virus spread to other parts following the abrupt closure of the 1901 Brunswick poultry show due to detection of sick birds and sending them back to their place of origin, thereby spreading the disease. The disease became endemic in Italy and Central Europe and disappeared in mid-1930s. In the USA, the first HPAI outbreak was reported in 1924 and then in 1929. There are only two reports of HPAI outbreaks in England during 1922 and 1929. By 1930, fowl plague was considered to have been reported in Austria, Switzerland, France, Belgium, The Netherlands, England, Egypt, China, Japan, USA, Argentina and Brazil as reported by European Commission (Sanco/B3/AH/R17/2000).

In 1934, Burnet and Ferry used embryonated chicken eggs for titration of both Newcastle disease and HPAI viruses. In 1936, Burnet was successful in growing influenza virus in embryonated chicken eggs. The agglutinating property of red blood cells was demonstrated by Hirst in 1941, and Lush in 1942 showed that both avian influenza and Newcastle disease viruses could able to agglutinate red blood cells of chickens.

Table 5.1 History of avian influenza

Year	Events
1878	First description of fowl plague (current HPAI)
1901	Identification of HPAI as virus
1931	First influenza virus isolated in pig
1918	Human pandemic (subtype involved H1N1)
1941	Haemagglutination by influenza virus
1955	HPAI virus identified as influenza A virus
1970	Systematic surveillance of influenza viruses in wild birds initiated
1981	The name HPAI is proposed to replace fowl plague in the 1st International Symposium on Avian Influenza
1999	H9N2 virus-infected human
1997	H5N1 virus-infected human
2003–present	H5N1 virus spreads through Asia, Europe and Africa

Early studies provided an operational definition of the virus as filterable agents. Centanni demonstrated the filterability of the fowl plague virus in 1901. In 1955, the true nature of the fowl plague virus was identified as influenza A virus by Werner Schafer while working at Max Planck Institute, Germany.

The first isolation of an IAV from wild birds (common terns, *Sterna hirundo*) was done in 1961 in South Africa, where an HPAI virus (H5N3 subtype) was detected (Webster et al. 1992). Systematic avian influenza surveillance was initiated in the 1970s to demonstrate the widespread distribution of influenza viruses in wild bird population (Webster et al. 1992). Since then, influenza viruses have been isolated from at least 105 wild bird species from 26 different families (Olsen et al. 2006). These surveillance studies have identified 16 HA and 9 NA subtypes with a 103 HA and NA combination out of 144 possible combinations (Alexander 2007; Munster et al. 2007). During 1959 and early 2012, 30 epizootics of H5 and H7 HPAI have been recorded all over the world. The largest amongst them is the H5N1 HPAI outbreak which began in China in 1996 and is continuing.

In the last 100 years, there have been three influenza human pandemics—1918 (subtypes involved H1N1), 1957 (H2N2) and 1968 (H3N2), which was accounted for the death of millions of people around the globe (Table 5.1).

5.3 Host Range

The IAV host-range restriction is a multigenic trait, which includes surface glycoprotein genes, proteins associated with viral replication and proteins counteracting the host responses. Even though some strains of avian influenza viruses can cause spillover infections with clinical sequelae in horse, cat, donkey, dog, ferret, stone marten, mink, palm civet, marine mammals and other mammalian species, birds are

the most commonly affected species. Wild aquatic birds including waterfowl and shorebirds appear to be the reservoirs for the influenza A viruses and can carry all of the known subtypes (Kawaoka et al. 1988). Amongst the cage birds, passerine birds are the most affected and psittacine birds are the least or rarely affected species with influenza viruses. Currently, several clades and subclades of HPAI H5N1 viruses are circulating and affecting poultry. The H5N1 viruses infect many bird species in addition to poultry. Most HPAI H5N1 viruses circulating currently have been isolated from *Anseriformes* birds, particularly of the families Anatidae (ducks, geese and swans) and *Charadriiformes* (gulls, terns and shorebirds). Several strains of HPAIV H5N1 can cause productive subclinical infections in domestic ducks, which serve as reservoir of the virus leading to perpetuation in poultry populations. Fatal H5N1 infections have been reported in many mammalian species such as tigers and leopards in zoological parks, stone marten, cats, captive palm civets, donkeys, dog, etc. Human beings are accidental hosts as the species-specific transmission barriers are high (Lvov et al. 1978; Englund et al. 1986; Webster et al. 1992).

5.4 Global Scenario

HPAIVs are widely distributed all over the world. Outbreaks of avian influenza have occurred at irregular intervals in all the continents. Till date, H5 and H7 influenza subtypes have caused most of the outbreaks of HPAI in domestic poultry. HPAI has been present for almost 100 years and was endemic in some parts and occurred regularly in others (Alexander 2000). Until 1999, HPAI was thought to be a relatively rare disease, and only 17 outbreaks were reported globally during 1959–1998; however, since 1999 the global outbreak occurrence has increased significantly (Capua and Alexander 2007). Out of 17 outbreaks of HPAI in poultry, 12 and 5 outbreaks were in chickens and turkeys, respectively. Nine out of seventeen outbreaks were due to H7 subtype and eight due to H5 subtype. In England, during 1991 a single flock was affected with the HPAI outbreak (Alexander et al. 1993). However, avian influenza virus spread to large geographical area in USA during 1983, Mexico in 1994 (Villarreal and Flores 1997) and 1995 in Pakistan (Naeem 1998). In 1983, H5 outbreak in Pennsylvania affected three species, viz. chickens, turkeys and guinea fowl (Beard 1989).

In Hong Kong, highly pathogenic avian influenza, H5N1 outbreak occurred first time during March–May 1997, with mortality reaching up to 100%. The human infection of H5N1 virus was also detected in Hong Kong live bird markets, and entire poultry population was slaughtered to contain the infection (Claas et al. 1998). The precursor to the 1997 H5N1 virus was identified in Guangdong province of China, in 1996, where it resulted in geese mortality (Chen et al. 2011; Webster et al. 1992). The 1997 H5N1 virus was of a reassortant nature, including HA gene from domestic geese (A/goose/Guangdong/1/96), NA gene from H6N1 virus, from teals (A/teal/Hong Kong/W312/97), and the segments for the internal proteins, from low pathogenic avian influenza H9N2 virus (Xu et al. 1999; Hoffmann et al. 2000; Guan

et al. 2002). Outbreaks due to different genotypes of the H5N1 virus have been reported in different countries including South East Asia (2004–2006) (Cauthen et. al. 2000; Sharshov et al. 2010).

Highly pathogenic avian influenza, H5N1 virus emerged in Asia during 2003 and spread to domestic poultry population at a very fast rate. The HPAI outbreaks decreased in spring of 2004, but in summer the virus reappeared in several countries in Asia. The currently circulating strains of H5N1virus in Asia are genetically distinct from the 1997 human virus isolated in Hong Kong. HPAI H5N1 viruses were mainly restricted to Southeast Asia during 1997–2005, but after infecting the wild birds in Qinghai Lake, China, H5N1 started expanding its geographical coverage and global spread. Till date, the H5N1 virus has been isolated from more than 60 countries across the globe and caused human infection in 17 countries resulting in 455 deaths.

5.5 Indian Scenario

Type A influenza viruses of avian origin are becoming a major public health problem in the country. In 2003, H9N2 LPAI virus was isolated from the chickens for the first time from Haryana and Punjab (Nagarajan et al. 2009) and subsequently, the virus has so far been isolated from many states. Till January 2006, India was free from HPAI disease. In February 2006, the outbreak of H5N1 HPAI was recorded in the commercial poultry population with a history of severe mortality in the Navapur taluka of Nandurbar district in Maharashtra (Pattnaik et al. 2006). Since then several outbreaks of H5N1 virus have been reported continually in many states of the country. Genetic and molecular characterization of these viruses has revealed that while the H9N2 viruses isolated in India have acquired the ability to bind to human receptors, the H5N1 viruses still have only avian receptor specificity (Tosh et al. 2008; Nagarajan et al. 2012; Tosh et al. 2016). Phylogenetic analyses have revealed that while all the H9N2 viruses from 2003 to till 2018 belonged to G1 lineage, multiple clades of H5N1 viruses have circulated at various time points in India. While clade 2.2 viruses circulated from 2006 to 2010, they were replaced by clade 2.3.2.1a since 2011. In the year 2014, a new clade of H5N1 viruses, viz. 2.3.2.1c, has caused outbreaks in Kerala, Chandigarh and Uttar Pradesh. These viruses have not been reported since March 2015. In October 2016, a new H5N8 subtype of HPAIV belonging to clade 2.3.4.4 was isolated from wild birds in zoological parks, wetlands and domestic poultry (Nagarajan et al. 2017). This virus was last isolated in India in the year 2017. Recent outbreaks of H5N1 viruses in 2018 in the states of Bihar, Odisha and Jharkhand belonged to clade 2.3.2.1a only. Apart from these, H6N2 viruses have been reported from Assam and Kerala states, and phylogenetic analysis indicated that the viruses are yet to be adapted to poultry and might have been introduced independently (Kumar et al. 2018).

These outbreaks have resulted in severe economic loss due to heavy mortality, culling operations, destruction of feed and egg, drop in meat and egg consumption, a

reduced price of chicken and egg and also due to ban on exports. India has a diverse ecological setup and hence the survival of avian influenza virus in different regions will have a varied pattern. Consequently, any biosecurity control measure in one region may not be effective in the other. Many states, particularly the northeastern region of the country share a large international border, and this poses a serious threat of introduction of newer strains of avian influenza viruses in the country either by migratory birds or movement of poultry across the border.

5.6 Transmission in Birds

Wild water birds of orders *Anseriformes* (ducks, geese and swans) and *Charadriiformes* (gulls, terns and shorebirds) are the natural reservoirs of influenza A viruses, and they can be infected with viruses of different haemagglutinin (HA) and neuraminidase (NA) subtypes (Webster et al. 1992; Fouchier et al. 2005). Influenza A viruses of 16 (HA 1–16) and 9 (NA 1–9) subtypes and most of HA/NA combinations have been reported in the wild bird reservoirs (Krauss et al. 2004; Huang et al. 2012). In wild birds, influenza viruses preferentially infect epithelial cells of the intestinal tract and are excreted in high concentrations in their faeces. Transmission is mainly through the faecal–oral route, which may represent an efficient way to transmit viruses between water birds, by excreting the virus via faeces into the surface water (Webster et al. 1992).

Avian influenza viruses of different strains (H5, H6, H7 and H9 viruses) have affected terrestrial poultry and became endemic in poultry population (Chu et al. 2011; Hooper and Selleck 2003). Low pathogenic avian influenza (LPAI) viruses mainly infect respiratory epithelial lining cells (Tumpey et al. 2002). Therefore, LPAI viruses can also be transmitted amongst terrestrial poultry via respiratory droplets and aerosols.

5.7 Transmission in Non-human Mammals

HPAI H5N1 viruses can infect several mammalian species, mainly classified in the order *Carnivora*. The avian influenza spread of the virus amongst carnivores might be attributed to predatory habits, the H5N1 virus has been isolated from the meat of infected animals (Kuiken 2004) and ingestion of H5N1 virus-infected meat can result in infection (Amonsin et al. 2006). Captive tigers (*Panthera tigris*) and leopards (*Panthera pardus*) died of H5N1 infection in Thailand zoos after an outbreak of H5N1 virus in wild birds during 2003 and 2004 (Keawcharoen et al. 2004; Thanawongnuwech et al. 2005; Desvaux et al. 2009). An outbreak of H5N1 during 2009 in the Phnom Tamao Wildlife Rescue Centre in Cambodia caused 100% mortality in lions (*Panthera leo*), Asiatic golden cats (*Catopuma temminckii*) and clouded leopard (*Neofelis nebulosa*) (Reperant et al. 2009). H5N1 viruses have also

been detected in dogs, cats (*Felis catus*), Owston's palm civets (*Chrotogale owstoni*), stone marten (*Mustela foina*) and an American mink (*Mustela vison*) (Short et al. 2015). Also LPAI viruses of H3, H4, H7 and H10 subtypes have been detected in harbour seals causing disease and mortality, the exact transmission route between seals is not known but it is most probably respiratory route during resting of seals on land. LPAIVs have also been isolated from different species of whales (Nidom et al. 2010). In Indonesia during a surveillance study from 2005 to 2007 H5N1 viruses were isolated from domestic pig farms. The HA and NA genes of these viruses clustered with H5N1 viruses circulating in domestic poultry (Subbarao et al. 1998). These studies highlighted that pigs can be infected with avian H5N1 viruses.

5.8 Transmission in Humans

Human infections with avian influenza viruses are rare; however, recently, avian influenza viruses of the H5N1 and H7N9 subtypes have infected many people in Asia and Africa with case fatality rates of approximately 60% and 30%, respectively. There have been few reports of isolated human infections with several other subtype viruses, namely H9N2, H6N1, H7N7, H10N8, H7N2 and H7N3 (reviewed by Neumann and Kawaoka 2015). The transmission of highly pathogenic avian H5N1 viruses to humans was first reported in 1997 in Hong Kong during an outbreak of H5N1 in poultry (Kandun et al. 2006). A few cases of suspected human-to-human transmission have been reported. However, the sustained transmission has not been reported (Nicholls et al. 2007). The receptor specificity of influenza viruses is governed by the haemagglutinin protein, which can bind to either α2,6-linked sialic acid or α2,3-linked sialic acid, or both. In humans, α2,6-linked sialic acids are expressed in ciliated epithelial cells (in the upper respiratory tract, trachea and bronchus), while type II pneumocytes within the alveolus express predominantly α-2,3 linked sialic acids (Shinya et al. 2006; Herfst et al. 2012). Attachment of H5N1 viruses to ciliated epithelial cells in the upper respiratory tract is essential for their efficient transmission amongst the humans as assessed by airborne transmissibility of reassortant H5N1 viruses amongst ferrets (Wang et al. 2007) even though the role of other factors cannot be ruled out.

5.9 Immunopathology of Avian Influenza

Host–pathogen interactions are crucial in the outcome of infectious diseases. The pathogen recognition receptors (PRRs) recognize the invading pathogens and triggers downstream immune-related genes and sets up an immune response against the pathogens (Neumann and Kawaoka 2015). The H5N1 virus-associated immune response includes the regulation of pro-inflammatory cytokines, antiviral cytokines

and interferons (IFNs) (Sladkova and Kostolansky 2006). There are marked differences in the pathogenesis of avian influenza virus between wild water birds, terrestrial poultry and humans. Although HA majorly determines virulence of AIVs, optimal combination of internal genes is essential for maximum expression of virulence (Bosch et al. 1979). The nasal epithelium is infected with HPAIV after inhalation of infectious virions and within 24 h nasal mucosa is infiltrated with inflammatory cells and virus could be demonstrated in the capillary endothelial cells. While the macrophages and heterophils are crucial for initial replication and the spread of HPAIV, virus replication within endothelial cells and spread through the vascular or lymphatic systems is also crucial. This viraemia allows dissemination of HPAIV and sets in replication in different systems such as in brain, skin and visceral organs. Avian influenza-associated pathology is mainly attributed to direct tissue damage, indirect effect of cell mediators and microthrombosis associated ischaemia (Swayne and Halvorson 2008). In chickens infected with H5N1 virus, rapid increases in mRNA transcripts of antiviral (IFNα and IFNβ) and pro-inflammatory (IL6, IL8, IL15 and IL18) cytokines were observed at 12 hpi as compared to pre-infection levels. The cytokine mRNA expression levels peaked at 24 hpi; however, the levels downregulated at 32 hpi, prior to death (Suzuki et al. 2009). Similarly, chickens infected with H5N1 virus had rapid increases in mRNA levels of the pro-inflammatory cytokines IFN-γ, IL1β and IL6 in different tissues (Burggraaf et al. 2014; Kuribayashi et al. 2013). Inflammatory cytokines are less influenced with H5N1 virus infection in ducks than chickens; however, TLR7 is upregulated ducks. On the contrary to chickens, H5N1 infected ducks showed little or no change in IFN-γ, IL1 β, IL6 and IL18 cytokine expression when compared to uninfected controls at 24 hpi (Burggraaf et al. 2014). The robust induction of *RIG-1* immune response in ducks may contribute to their relative resistance to H5N1 infection as compared to chicken (Barber et al. 2010). H5N1 infection in mice suggested that TNF-α may result in morbidity, while IL-1 may play an important role in clearance of H5N1 virus (Szretter et al. 2007). In humans infected with H5N1 virus peripheral blood lymphocyte counts showed low total and CD3+ lymphocyte counts and inverted CD4+/CD8+ ratios of cells. Increased serum/plasma levels of pro-inflammatory cytokines and chemokines, i.e. hypercytokinaemia, are mainly responsible for the disease pathogenesis. Chemokines IP-10, MIG and MCP-1 were elevated in patients died of avian influenza. Similarly neutrophil chemoattractant IL-8 levels were also elevated in H5N1infected patients. Plasma levels of IL-10, IL-6 and IFN-γ were elevated in H5N1 infected patients (de Jong et al. 2006). Human monocyte-derived macrophages (hMDM) infected with H5N1 virus showed elevated cytokine production than H1N1 virus supporting the important role of macrophages cells in the hypercytokinaemia in the lungs (Cheung et al. 2002). Studies in human cell model myeloid dendritic cells and plasmacytoid dendritic cells also suggested contribution of pro-inflammatory responses in avian influenza pathogenesis (Sandbulte et al. 2008).

5.10 Diagnostics

The clinical diagnosis of AI is very difficult and often confused with other respiratory diseases of poultry due to the lack of pathognomonic clinical signs and variation in different avian hosts. Conventionally, diagnosis of AI involves isolation, identification and characterization of the virus (Alexander 2008). This method is successful and remains the method of choice, at least for the initial outbreaks. However, the conventional tests are time taking, thereby, delays the implementation of stamping out policies (applicable for OIE notifiable AI). For the implementation of the control policies there is a demand for rapid results from the laboratory. Therefore, the current molecular techniques particularly the RT-PCR and real-time RT-PCR are being recommended by OIE for rapid diagnosis of AI. As all IAVs have antigenically similar nucleoprotein and matrix antigens, these are targets for serological tests for influenza A group-specific test (OIE 2015). Therefore, testing sera using antibody detection methods may supplement diagnosis.

5.10.1 Identification of the Agent

5.10.1.1 Virus Isolation

Virus isolation is the 'gold standard' used for diagnosis of the first clinical case. Isolates obtained are useful for further characterization of the virus. AIV grow well in the allantoic cavity of embryonated chicken eggs and agglutinate RBCs (haemagglutination). Tracheal and cloacal (or faces) samples from live birds or pooled organ samples from dead birds are inoculated into the allantoic cavity of 9- to 11-day-old embryonated chicken eggs and incubated at 37 °C for 2–7 days. Allantoic fluid collected from the eggs containing dead/dying embryos and all other eggs after the incubation period is tested for the presence of haemagglutinating activity. It must be remembered that virus isolation yields positive results only when the infectious virus is present in the sample. So specimens not properly stored or shipped may yield a false-negative result. Diagnostic laboratories with biosafety level 3 capacity are required for isolation of virus. The method used for determination of strain virulence for birds is either by intravenous inoculation of virus to chickens (intravenous pathogenicity index test) or determination of amino acid sequence of HA1–HA2 cleavage region. A table is available on the OFFLU website (http://www.offlu.net/fileadmin/home/en/resource-centre/pdf/Influenza_A_Cleavage_Sites.pdf) listing the reported HA1-HA2cleavage site for H5 and H7 LPAI and HPAI viruses based on deduced amino acid sequence.

5.10.1.2 Rapid Detection of Viral Antigens

There are several commercial ELISA kits available to detect the presence of IAVs. These tests may directly detect IAVs from oropharyngeal/throat/nasal swabs, cloacal swabs and faeces. The main advantage of these tests is the speed in which it detects (15 min) the presence of IAV; however, the limited sensitivity for detection may result in false-positive or -negative results. Therefore, the tests should only be interpreted on a flock basis and not as an individual bird test. The rapid tests do not require BSL-2 or BSL-3 facilities and have been designed to be performed under field conditions by non-laboratory-trained persons.

5.10.1.3 Molecular Techniques to Detect Viral RNA

Presence of AIVs is demonstrated by detecting direct RNA using PCR tests viz., reverse transcriptase PCR (RT-PCR) and real-time RT-PCR (RRT-PCR). In conventional RT-PCR, the matrix or the nucleoprotein genes which are highly conserved across all IAVs are targeted for type A detection and HA and NA genes are used for subtype identification. In a conventional RT-PCR, the size of the amplified product is measured by visualization after separation by gel electrophoresis. However, the preferred molecular test for the detection of IAV in most of the reference laboratories is real-time RT-PCR. In real-time RT-PCR, an internal fluorescent probe is used to monitor the PCR amplified product directly on a computer screen. The test is high sensitivity and specificity similar to virus isolation. However, one of the problems is high genetic variability of the virus, which may produce false-negative result due to change in the binding regions of primers and probes. Protocols, primers and probes can be accessed for detection of notifiable AI (H5 and H7 subtypes) from the OFFLU website (http://www.offlu.net/). A BSL-2 level laboratory facility is required for performing PCR on direct clinical samples.

5.10.2 Serological Diagnosis

Serological diagnosis is important when clinical samples for virus isolation/detection are not available or when the laboratory does not have resources. The serological diagnosis of AI can be performed using different methods viz., agar gel immunodiffusion (AGID), ELISA and haemagglutination inhibition (HI) tests.

5.10.2.1 Agar Gel Immunodiffusion (AGID) Test

The AGID test is used to detect antibodies against type A group-specific antigens, namely nucleocapsid and matrix proteins. Therefore, the AGID test can be used as a

group-specific test to detect the presence of antibodies to any IAV. The test is considered positive when the precipitin line of the known positive control is continuous with the line between the antigen and the test sample. The visibility of the precipitin line is dependent on the concentrations of the antibody present in the sample. The results can be obtained within 24–48 h and this test is recommended by OIE as a preliminary screening of serum samples of poultry. However, this test may not be useful in case of avian species which do not produce precipitating antibodies such as ducks.

5.10.2.2 Haemagglutination Inhibition Test

The HA protein of AIV has the property to agglutinate RBCs (haemagglutination) from several species including chicken. Specific antibodies to the antigenic sites on the HA molecule prevent or inhibit the haemagglutination reaction. The haemagglutination inhibition (HI) test can thus be used to identify the antibodies to a specific subtype of AIVs when reference AIV is available. The HI titre is considered positive if there is inhibition at a serum dilution of 1/16. Therefore, paired (acute and convalescent) sera should be used for diagnosis. However, in an HPAI outbreak situation antibody to H5N1 HPAI subtype is absent or very low. Results of HI can be obtained within two to three days, making the test suitable for most epidemiological investigations.

5.10.2.3 Competitive Inhibition ELISA

Commercial ELISA (enzyme-linked immunosorbent assay) kits that detect antibodies to nucleocapsid protein of AIV are available. The kits are in indirect and competitive formats to detect type A influenza virus-specific antibodies. The principle of the test involves competition between antibody present in the test serum and anti-AIV monoclonal antibody-conjugate for binding to the antigenic sites of AIV nucleoprotein or haemagglutinin pre-coated onto a microtiter plate. The residual enzyme activity will thus be directly inversely proportional (per cent inhibition) to the anti-AIV antibodies present in the serum. Haemolytic or bacteria-contaminated samples may result in a false-positive reaction. It is important to follow the guidelines recommended by the manufacturers of the kit for optimum performance.

5.11 Prevention and Control

Any prevention and control program of avian influenza should aim to reduce its incidence in poultry and the likelihood of direct or indirect contact of humans with infected birds. This will not only prevent the human cases but will also minimize the chances of mammalian adaptation and emergence of a human pandemic virus.

Effective control programs for avian influenza in poultry farms or its spread between farms can reduce the loss due to the disease by a minimum of 75%. The outbreaks of LPAIVs, especially H5 and H7 subtypes have become a trade barrier which disrupts the poultry trade in exporting countries because of the concern that these viruses might mutate to become highly pathogenic. Many alternative measures have experimented worldwide for control of avian influenza-like vaccination, treatment with antiviral drugs, molecular approaches that control the expression of host genes such as avian cytokines either through recombinant DNA technology or RNA interference, selection and breeding of naturally resistant birds and production of transgenic birds.

5.12 Biosecurity

A set of management practices followed to reduce the possibility of introduction and spread of disease-causing microbes onto and between sites is called biosecurity. Farm biosecurity aims to protect the farm produce from all manner of invasive species, be they vertebrates, weeds, insects or pathogens. The term 'biosecurity' is often used in the discussion on HPAI control since it is most usefully applied to this disease. Its application on a poultry farm may vary in extent from an ideal state of affairs in which all measures with cost-intensive infrastructure are in place to strictly prevent incursion and spread of disease to a simple package of farm practices without additional physical inputs and investment to reduce the risk of incursion of disease to a bearable level. In its application at farm level, biosecurity includes to those measures that should be taken to minimize the risk of incursion of disease into individual production units *(bioexclusion)* and the risk of outward transmission *(biocontainment)* and onward transmission through the market chain.

5.13 Stamping Out

Outbreaks of HPAIVs in poultry are controlled using stamping out strategy which is a reliable and proven method. This strategy involved creating awareness through education of stakeholders (like poultry farmers, veterinary professionals, consumers and bird handlers), implementation of farm biosecurity measures, development of rapid diagnostics and implementing surveillance programs and elimination of infected birds. The major advantage of stamping out policy is a reduction of the time required for eradication of virus infection. However, with the industrialization of the poultry rearing, implementation of the stamping out policy for the control of outbreaks of avian influenza virus involved culling of large number of apparently healthy birds which are likely to have been exposed either by close proximity or through fomites apart from infected birds to break the chain of transmission. There are also concerns of permanent loss of native breeds of chicken adapted to the local conditions.

5.14 Vaccination

The concept of using large scale vaccination for control of avian influenza in poultry was not explored before its successful implementation during the 1994–1995 outbreak of HPAI H5N2 virus in Mexico. Subsequently, the vaccination has been used by Pakistan to control an outbreak of H7N1 outbreak in its poultry. Since the start of the ongoing panzootic outbreak of H5N1 virus in 2003, several countries have used emergency, prophylactic or routine vaccination as a tool for control of H5 and H7 viruses. These include national or routine vaccination in Bangladesh, China, Egypt, Indonesia and Vietnam, emergency vaccination in Cote d'Ivoire, Israel, North Korea, Pakistan, Russia and Sudan and preventive vaccination in France, Kazakhstan, Mongolia and the Netherlands. The aim of vaccination programs was to increase the resistance of the poultry population to the AIVs targeted.

Nearly 95.5% of the vaccines used in poultry are inactivated vaccines, and the remaining 4.5% are live virus-vectored vaccines (Swayne and Halvorson 2008). The virus used for inactivated vaccine production might be whole wild virus or reverse genetics engineered virus. The virus vectors used for live vaccines are Poxvirus vectors (Vaccinia virus, Canarypox and Fowlpox virus), Alphavirus vectors (Semliki Forest virus, Sindbis virus and Venezuelan equine encephalitis), Herpes virus vectors (Duck enteritis virus, Infectious laryngotracheitis virus, Pseudorabies virus and equine herpesvirus-1), Vesicular stomatitis virus vectors, Newcastle disease virus vectors, Baculovirus vectors, Parainfluenza-5 virus vectors, Adenovirus vectors and Adeno-associated virus vectors. A fowl pox vectored vaccine for H5 subtype of AIV (TROVAC-H5) has been used for large scale vaccination in Mexico, Guatemala and El Salvador and is approved for emergency use in the USA. The HA and NP are the major antigens used for vaccination in virus vectors. Various researchers have reported use of NA and matrix and different combinations of all the four antigens with varying results.

The advantages of control AIV in poultry through vaccination are:

a. Reduction in the viral shedding leading to a decrease in environmental contamination and increase in the resistance of the bird to the virus infection leading to reduced morbidity, mortality and bird-to-bird viral transmission.
b. Possibility of mass vaccination administered through the spray, drinking water or other methods especially with virus-vectored vaccines which can also induce mucosal and cellular immune responses.
c. Application of DIVA (differentiate infected from vaccinated animals) strategy to identify infected birds, especially for trade purposes.

However, the major disadvantages of the vaccination include:

a. The vaccination may not induce sterile immunity as it may not prevent virus shedding but protect the birds from clinical disease. This will result in 'silent' circulation of the virus amongst vaccinated birds and environmental contamination.

b. Limited or no heterotypic cross-protection amongst the various subtypes or even between antigenically different strains within the same subtypes (like different clades of H5N1 viruses).
c. Virus-vectored vaccines have significantly lower efficacy in protecting the birds with active immunity against AIV or viral vector.
d. Even though vaccination is highly effective against the antigenically related virus, faster emergence of antigenic variants through higher rates of antigenic drift necessitates a periodic update of the vaccine strain. Further, the presence of maternal antibodies, infection with immunosuppressive pathogens or mycotoxins, ineffective maintenance of cold chain (in case of live virus-vectored vaccines) interfere with the effectiveness of even the antigenically related virus vaccines.
e. Interference in routine serological surveillance as DIVA requires advanced diagnostic methodologies.
f. The immune responses to vaccination in species other than chicken like ducks, geese, turkeys, zoo and/or exotic birds are yet to be fully elucidated. There are reports of differences in the immune responses to vaccination even within the same species (i.e., Muscovy vs. Pekin ducks).

While non-endemic countries use stamping out policy, vaccination is used in a very few endemic countries. Any policy leading to the vaccination use must include an exit strategy as the country is not considered disease-free until the last vaccinated bird was slaughtered. Factors related to vaccine coverage, quality of vaccine manufacturing, updating of vaccine strain at regular intervals, proper handling and/or administration should be considered for effective implementation of the vaccination program. Care must also be taken in making assumptions such as how long vaccine-induced immunity is likely to last and frequency of vaccination. However, any policy decision to implement or not to implement should always be dynamic and should be taken after consideration of socio-economic cost: benefit ratio and public health risk.

5.15 Antivirals

M2 blockers and neuraminidase inhibitors (NAIs) are the two major types of antivirals used for control of AIV in poultry. They are used for either prophylaxis or therapeutic treatment in birds. Other drugs that are currently under experimental stage mainly for use in humans and not in poultry include Favipiravir (T-705) and Laninamivir which target polymerase and synthetic derivatives that target sialic acid receptors.

Amantadine hydrochloride and rimantadine are two M2 blockers which are commonly used. Amantadine is one of the cheapest anti-influenza drugs and its use for control of avian influenza in poultry has been documented in some countries especially in China. However, the emergence of stable and irreversible amantadine-

resistant strains with no loss of pathogenicity is a major disadvantage in the usage of this drug in poultry for control of avian influenza. Further, it was also observed that the resistant strains became dominant and often replaced wild type strains (Bean and Webster 1988; Bean et al. 1989). Hence, in 2005, international organizations banned the extra-label use of amantadine in poultry. However, the emergence of amantadine-resistant strains may not always be associated with the drug usage since such resistance could be acquired by stable mutation of the virus in M2 gene (Tosh et al. 2011).

Oseltamivir (Tamiflu®) and zanamivir (Relenza®) are the two major NAI drugs that are currently used mainly in humans. Oseltamivir is currently the drug of choice, and oseltamivir-resistant H5N1 strains have been isolated from avian species and humans. However, mass usage of oseltamivir to control avian influenza in poultry has not been documented so far probably because the drug is extremely expensive and limited in supply. Hence, such emergence of resistant strains might be due to a natural mutation process and may not be associated with its usage in poultry.

Antiviral effects of various plants or their derivatives/extracts such as Eugenia jambolana, Acacia arabica var. indica, Ocimum tenuiflorum, Azadirachta indica, NAS preparation, a Chinese herbal medicine, eucalyptus and peppermint essential oils preparations, lyophilized green tea by-product and green tea extract have been reported by several researchers (Shang et al. 2010; Barbour et al. 2011; Shaukat et al. 2011; Sood et al. 2013). However, all the experiments have been carried out either in tissue culture or in embryonated chicken eggs, and their effectiveness in control of avian influenza under field conditions is yet to be documented.

5.16 Recombinant DNA Technologies

Recently, China has authorized the use of recombinant chicken α-interferon protein produced by cloning the gene into Escherichia coli in the prevention and treatment of H9 subtype of avian influenza virus in poultry. The purified protein could be administered orally to poultry through the drinking water. Experimentally, live attenuated *Salmonella enterica* serovar Typhimurium expressing ChIFN-α alone or in combination with ChIL-18 administered orally reduced clinical signs induced by H9N2 virus and virus shedding (Rahman et al. 2012). Similar results have also been reported for recombinant fowlpox virus (rFPV) vaccine expressing both the HA gene (H9, H5 or H7 subtypes) and ChIL-18 with the induction of significantly higher humoral and cellular mediated immune responses (Chen et al. 2011).

Direct injection of the plasmid DNA causes the expression of the protein encoded antigen in the inoculated cell and produces either humoral or cellular immune response just like a live virus infection. DNA immunization was found to be more effective in cross-protection against different strains within the same subtype of influenza A virus compared to the inactivated vaccine (Kodihalli et al. 2000). Vaccination using DNA constructs has many advantages in terms of storage, immune response elicited and transportation. DNA vaccination overcomes the

limitations of both safety and steric hindrance and can be a viable alternative to conventional methods for antisera production for diagnostic purposes. Factors influencing immune responses to DNA include dose of DNA, route of immunization, age of the bird, immunization schedule and adjuvants used.

5.17 RNA Interference (RNAi)

The RNA interference (RNAi) is a process of inhibition of gene expression or virus replication by double-stranded RNA duplexes through sequence-specific degradation of homologous mRNA through small interfering RNAs (siRNA). siRNAs targeting conserved regions in influenza viral genome like NP or PA or PB1 can be effective regardless of subtype/serotype variations and antigenic drift and shift. The inhibition of the viral replication by siRNA correlates inversely to time post virus challenge but directly to the dose of the siRNA (Behera et al. 2015).

5.18 Selection for Natural Disease Resistance

Indigenous chicken breeds are generally considered to be more resistant to common poultry diseases compared to their commercial counterparts, and variation in the susceptibility to avian influenza has been reported between different breeds of chicken (Suba et al. 2015). Similarly, different species of ducks exhibit different susceptibility or resistance to avian influenza virus infection. Mallard ducks are reported to be highly resistant while the dabbling ducks are found to be susceptible. In chickens, resistance to AIV infection has been attributed to the presence of B21 *MHC class I* haplotype in chickens (Boonyanuwat et al. 2006), a nonsynonymous single nucleotide polymorphism (G to A) at position 2,032 in in allele A of Mx gene resulting in S631N mutation of Mx 1 protein in chickens and ducks (Ko et al. 2002) and retinoic acid-inducible gene I (RIG-I) gene in ducks. Other genetic markers reported are cyclophilin A, ISG15, viperin, heat shock cognate protein 70 (Hsc70) and Ebp1 and/or ErbB3-binding protein (Abdelwhab and Hafez 2012). However, their utility for selection of birds or production of transgenic chickens is questionable as there are contradictory reports on their efficiency to confer AIV disease resistance.

5.19 Transgenic Chickens

Genetic modification of the chicken germplasm by the introduction of genes that confer resistance either through RNA interference or direct DNA injection or oncoviral vector infection has been reported. Lyall et al. (2011) reported suppression of avian influenza transmission in genetically modified chickens expressing short-

hairpin RNA decoy that inhibits and blocks polymerase proteins of influenza viruses. However, none of the transgenic chickens survived the HPAI virus challenge. Further, a lot of challenges such as introduction of multiple decoys that will prevent frequent emergence of mutants that will make the transgenic chicken vulnerable, consumer preference, safety issues, etc. have to be overcome before transgenic chickens being considered as a viable alternative for control of avian influenza.

5.20 Conclusion

Avian influenza is a major economic and zoonotic threat which also threatens the food security of many nations who depend on poultry meat as a cheaper protein source. Hence, training of human resources, creation of awareness amongst consumers, the establishment of diagnostic facilities, defined policy measures including administrative structure to deal with outbreaks and scientifically designed surveillance program are essential for preparedness and early diagnosis of the AIV infections. While the HPAI viruses receive more attention due to their devastating nature, the LPAI viruses also deserve the same attention as they can cause not only huge production losses to the poultry industry but may silently spread the infection amongst humans. The ongoing outbreak of H7N9 LPAI virus in China is an eye-opener in this context. Recent human infection of H5N1 virus in Nepal indicates the risk posed by the H5N1 virus in South Asia and also in countries where the virus is still being reported.

Contrary to the population view, vaccination is not a magic bullet which offers a one-stop solution for the problem of AIVs. It should be combined with other measures especially biosecurity and culling and should be backed by an effective surveillance program with policies in place for continuous monitoring and implementation of control measures. Every country should decide on the control measures (one or combination of many) to be adopted for AIVs after ascertaining their prevalence and socio-economic impact and public health threat posed by them.

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Chapter 6
Pandemic Influenza A Virus (pH1N1)

Shailendra K. Saxena, Vimal K. Maurya, Swatantra Kumar, and Madan L. B. Bhatt

Abstract The latest flu-pandemic caused by influenza A (H1N1) pdm09 (pH1N1) has taken several hundred lives. Influenza virus contributes to respiratory diseases that lead to the nasal secretions, barking cough, decreased appetite, etc., human beings serve to be the dead-end hosts for the virus. The seasonal reassortment and regeneration of virus contribute to chronic infections, which cannot be treated and leads to drug-resistant strains and antigenic shift that is involved in viral entry, spread and tissue tropism. Various antiviral drugs and vaccines are undergoing clinical trials to fight against the virus. For the treatment of infection antiviral drugs like zanamivir and oseltamivir are given to the patients within 48 h of symptom initiation. The main objective nowadays is the search for alternative vaccines that can effectively combat the reasserted virus. Therefore, this article emphasizes on the availability of vaccines and antiviral drugs which can be used to prevent viral infections during the severe outbreaks.

Keywords Flu-pandemic · Influenza virus · Antiviral agent · Vaccines

Abbreviations

ARIs	Acute respiratory infections
DC-Chol/DPPC	Cationic liposomes comprising cationic compound neutral phospholipids
IFITM3	Interferon-inducible trans-membrane protein family membranes 3

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6.1 Prologue

The worldwide recurring outbreaks of influenza, leads the significant morbidity and mortality with variable severity. Influenza virus leads to 2,90,000–6,50,000 fatalities annually and 3 to 5 million serious cases of infections globally (Omoto et al. 2018). Moreover, epidemics are triggered by newly emerging viruses with catastrophic global impact. Influenza is one of the leading causes of lung infections specially acute respiratory infections (ARIs) in humans as compared to the other viruses such as respiratory syncytial virus, adenovirus, rhinovirus, enterovirus, etc. (Dziąbowska et al. 2018). Influenza virus disease contributes to clinical signs such as fever, sore throat, headache, body pain, myositis, emphysema, joint pain, fatigue, secondary renal failure, pneumatoceles and diarrhoea resulting in severe fatality. Pigs are the major influenza A reservoirs, which act as a middle host both for transmission of the interspecies and for the genetic reassortment of viruses. Therefore, pigs may be potential source of risk to human and avian influenza viruses and pandemics too. Co-infection was observed in pigs by both human and avian influenza viruses (Neumann and Kawaoka 2015). The important reservoirs for influenza viruses are waterfowls and wild boars. Influenza viruses can transmit their distinct alleles to new mammalian hosts, such as the recent appearance of influenza in bats in Central America (Venkatesh et al. 2018).

Influenza is also known as "mother of all pandemics". Among all subtypes, influenza A has been associated with the major worldwide outbreaks (Paules and Subbarao 2017). In the last century, four major outbreaks of influenza have been reported: swine influenza (H1N1) in 2009, Hong Kong influenza (H3N2) in 1968, Asian flu (H2N2) in 1957 and Spanish flu (H1N1) in 1918 (Gagnon et al. 2018). Although the annual vaccination is recommended and antiviral drugs are available, but both have several limitations. The various drawbacks associated with the vaccines are: slow manufacturing and short duration of protection, antigenic changes over time and lack of cross-reactivity and poor immunogenicity in certain populations. Similarly, antiviral agents are also associated with high drug resistance due to reassortment and regeneration of the virus (Sherman et al. 2019).

6.2 Epidemiology

The H1N1 epidemic firstly appeared in Spain in 1918, resulting in millions of fatalities. Several outbreaks have been recorded later due to influenza virus in 1968, 1998, 2009, etc. Recently, the World Health Organization (WHO) GISRS/NIC laboratories tested more than 46,002 specimens from 13 to 26 May 2019 (data as of 07-06-2019); for influenza virus detection, 5285 were found to be positive, of which 3157 (59.7%) were typed as influenza A and 2128 (40.3%) as influenza B. Of the sub-typed influenza A viruses, 620 (30.5%) were influenza A (H1N1) pdm09 and 1414 (69.5%) were influenza A (H3N2). Of the characterized B viruses, 34 (3%) belongs to the B Yamagata lineage and 1104 (97%) to the B-Victoria lineage (Fig. 6.1). Summary of influenza virus detections from influenza laboratory

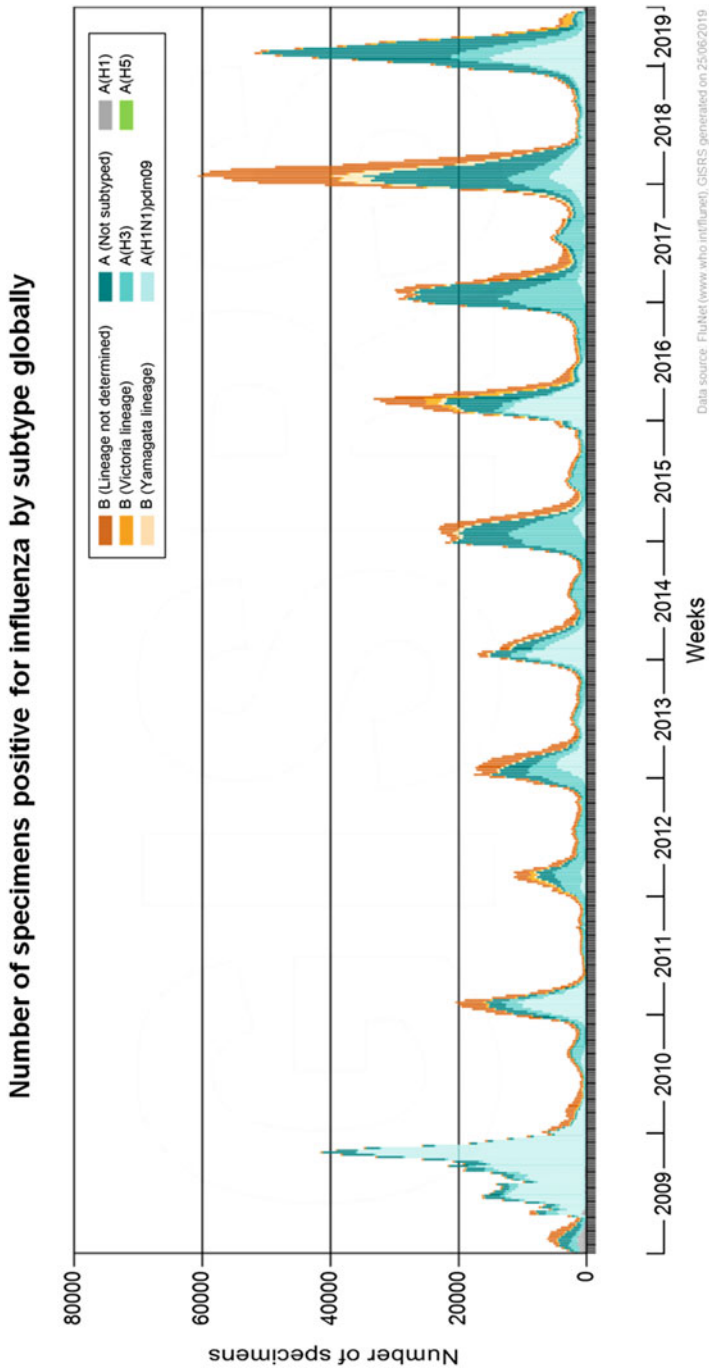

Fig. 6.1 Global circulation of influenza viruses 2009–2019

surveillance information in India during 2009–2019 (as on 26 May 2019; source WHO) has been presented in (Fig. 6.2). Antigenic drift results in mutation in the genome of the virus making it efficient in pathogenesis. The adult population is 90% more common in fatalities, suggesting an increase in immune system dysregulation in conjunction with age (Flu Net 2019).

6.3 Structure of Influenza Viruses

Influenza A virus has a diameter of about 80–120 nm and belongs to the family *Orthomyxoviridae*. The virus genome size is ~13.5 kb (Saxena et al. 2009). The influenza RNA genome is divided in 8 segmented negative sense RNA strands, encoding 11 distinct proteins, i.e. envelope proteins (HA and NA), matrix proteins (M1and M2), non-structural proteins (NS1 and NS2) and viral RNA polymerases (PB2, PB1, PB1-F2, PB and PA) are important for replication of viruses and pathogenesis (Fig. 6.3) (Saxena et al. 2012). Influenza viral infections take place due to the evolution of new viral strains arising from the reassortment of haemagglutinin (HA) and neuraminidase (NA) viral proteins. Both the proteins are mainly responsible for the pathogenesis of the virus by facilitating its internalization and replication in host cells. Modification of viral genome and cellular adaption is the key feature of the virus which leads to severe infections. The significant viral changes in the new host systems rely on the host cell tropism, distribution and viral infection (de Silva et al. 2012).

6.4 Molecular Mechanisms and Ramification

Various influenza viruses have distinct antigenic features for binding to the host's sialyl moiety. Some strains are capable of binding to both glycan bonds, which makes them more virulent and causes intestinal diseases such as diarrhoea (Schrauwen and Fouchier 2014). Mutations in the HA virus region may change the host binding affinity of the virus via distinct strain antigenic shift which contributes to the differential pathogenesis. Various studies have been performed to decipher the internalization mechanism of the virus. HA protein enables the binding of host sialic acid receptor with virus, and this binding complex internalized into the host cell via endocytosis (Sriwilaijaroen and Suzuki 2012). After the entry of virus into the host cells, viral mRNA releases and viral genomic ssRNA synthesis starts by using host cellular machinery. Then it is assembled, matured and eventually produced by budding from host cell membranes in progeny virions (Long et al. 2019).

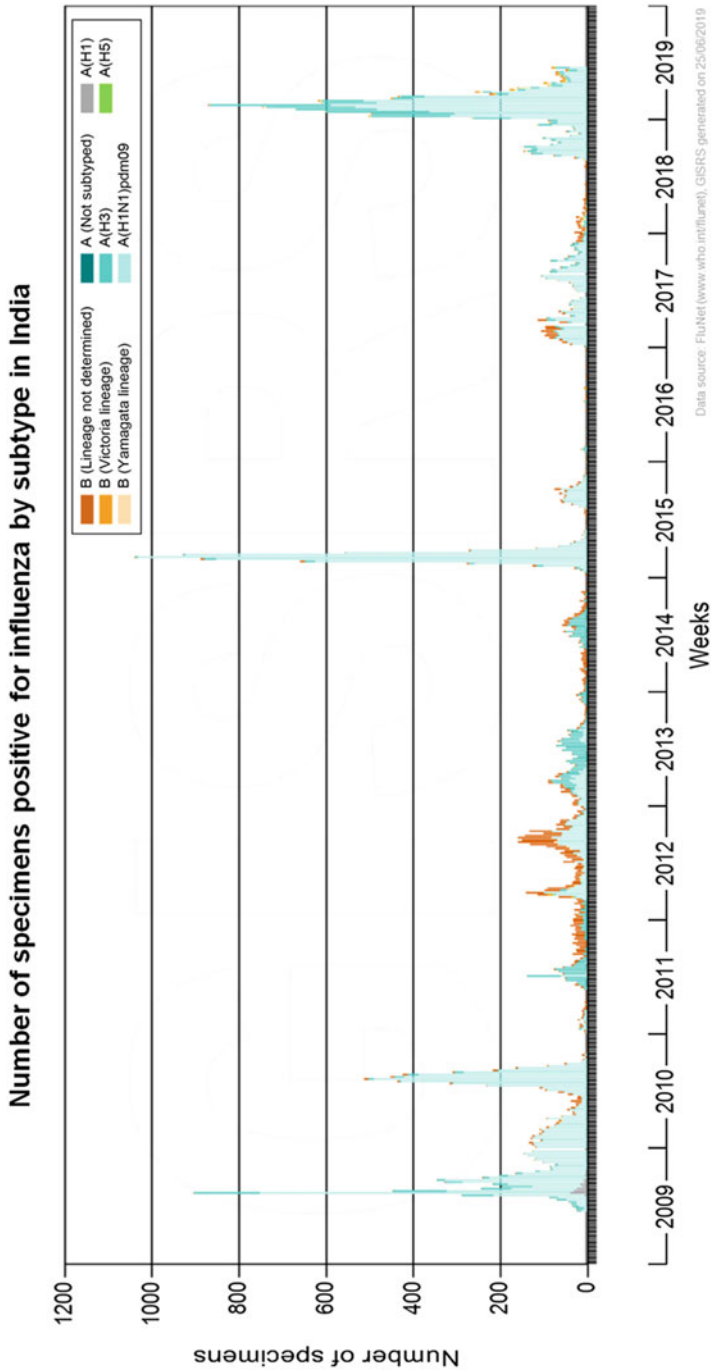

Fig. 6.2 Circulation of influenza viruses in India (2009–2019)

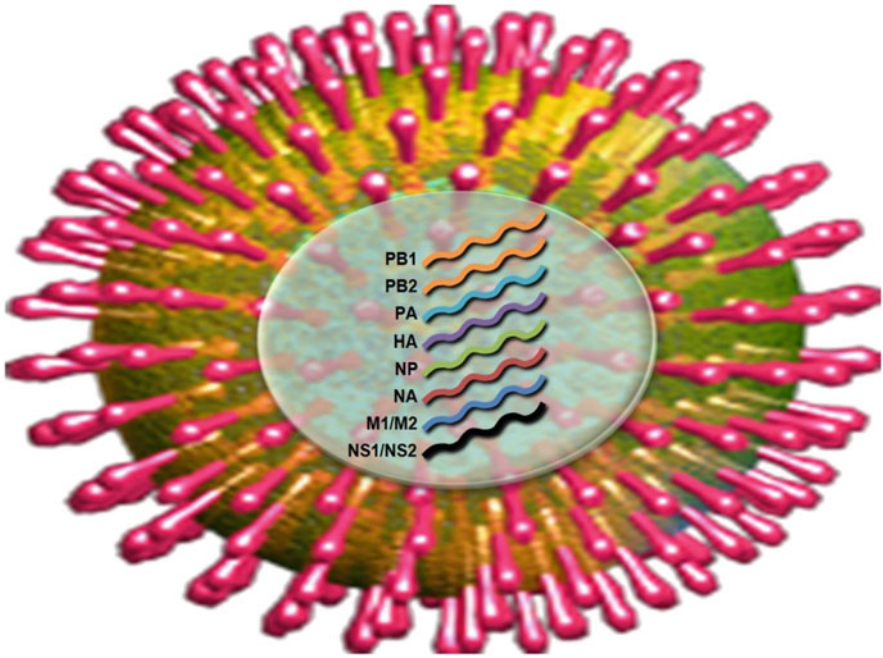

Fig. 6.3 Structure of influenza virus. Influenza virus is a segmented RNA virus possessing eight (single) segmented negative sense RNA strands. Segmented genome encodes eight structural proteins and at least two non-structural proteins. Envelope proteins (HA and NA), viral RNA polymerases (PB2, PB1, PB1-F2, PB and PA), matrix proteins (M1and M2) and non-structural proteins (NS1 and NS2)

6.5 Treatment

There are presently two treatment options suggested for influenza: vaccination and use of antiviral drugs. Antivirals can be used either to avoid or manage individuals who have become infected with influenza. For the successful management of infection, antiviral treatment should begin within 48 h of first symptoms (Barik 2012). NA inhibitors (zanamivir and oseltamivir) and M2 ion channel blockers (rimantadine and amantadine) are presently accessible for the treatment of influenza. NA inhibitors block the viral infection by inhibiting the release of new viral particles from the host cells. The antagonists of M2 ion channel are used to inhibit the viral replication by preventing the virus nucleus being uncoated within the host cell (Duwe 2017). Interferon-inducible trans-membrane protein family membranes 3 (IFITM3) is shown to be a prospective candidate for restricting influenza infections demonstrated by recent studies (Anafu et al. 2013). A broad-spectrum antiviral agent is urgently needed because influenza strains are resistant to present antiviral drugs (Table 6.1).

Table 6.1 Antiviral medications recommended for treatment of influenza (adapted from CDC)

Antiviral agent	Effective against	Recommended dose for adults	Adverse drug reactions
Oral oseltamivir	Influenza A and B	75 mg twice daily	Nausea, vomiting, headache. Postmarketing reports of serious skin reactions and sporadic, transient neuropsychiatric events
Inhaled zanamivir	Influenza A and B	10 mg (two 5-mg inhalations) twice daily	Risk of bronchospasm, especially in the setting of underlying airways disease, sinusitis and dizziness. Postmarketing reports of serious skin reactions and sporadic, transient neuropsychiatric events
Intravenous peramivir	Influenza A and B4	(13 years and older) one 600 mg dose, via intravenous infusion for a minimum of 15 min	Diarrhoea. Postmarketing reports of serious skin reactions and sporadic, transient neuropsychiatric events
Oral baloxavir	Influenza A and B6	(12 years and older) 40 to <80 kg: One 40 mg dose; >80 kg: One 80 mg dose	None more common than placebo in clinical trials
Amantadine	Influenza A	Capsule/tablet, syrup; 100 mg amantadine hydrochloride, twice a day	Mostly discontinued due to resistance; may be recalled in future epidemics
Laninamivir	Influenza A, B (for example, H1N1, H3N2)	Single inhalation (20 or 40 mg)	Similar to oseltamivir. Approved in Japan, but not yet in the USA

6.6 Prevention

Vaccines are the most efficient way of avoiding infectious diseases. There are safe and efficient vaccines that have been used for over 60 years against influenza (Rémy et al. 2015). Developing and producing new influenza vaccines are vital elements for the health care professionals in case of an extensive seasonal and pandemic response to influenza (Buckland 2015). It is necessary to develop potential candidate for vaccines strategy against the H1N1 sequence-based target. The current H1N1 vaccines were designed based on HA and NA has shown to be ineffective due to the antigenic shift and virus reassortment. A universal vaccine against influenza can be developed by targeting the potent epitopes of viral nucleoproteins (Sautto et al. 2018). Microneedle-based vaccines are the most recent and promising strategy, where the microneedles coated with inactivated influenza virus provides lifelong protection, via inducing humoral and cellular immune responses (Song et al. 2010). In combination with the subunit vaccinations, adjuvants are also essential to cause adequate immediate responses. DC-Chol/DPPC (cationic liposomes comprising cationic compound neutral phospholipids) have demonstrated potent

immunogenicity against H1N1 due to the physicochemical property of cationic liposomes which are needed for effective adjuvanticity in subunit vaccines (Barnier Quer et al. 2012). MF59—an adjuvant vaccine—is also more immunogenic, providing constant virus protection. Adjuvant monovalent vaccines are based on nanoparticles that use poly(d), l-lactic-co-glycolic acid (PLGA) and toll-like receptors (TLR) that provide effective protection against H1N1 infection (Pati et al. 2018). According to the immunization practices advisory committee on 20 June 2018, the general efficacy of the 2017–2018 flu vaccine against influenza A and B viruses is estimated at 40%. Similarly, as per the CDC reports 2018, it is suggested that the available vaccines only have 25% protection against A (H3N2), 49% protection against influenza B and 65% protection against A (H1N1) against various influenza strains (Centers for Disease Control and Prevention (CDC) 2019).

6.7 Conclusions and Future Perspectives

Influenza has been causing human morbidity and mortality through the routine seasonal spread and worldwide pandemics for a long time. H1N1 swine flu is a subtype of influenza A virus which is mainly responsible for upper and lower respiratory tract infections in humans. Combined with the assortment of its various genomic sections and mutation rate of the viral RNA genome encourages antigenic variety and new subtypes, enabling the virus to escape vaccines and become resistant to antiviral drugs. Therefore, the novel anti-influenza therapies with new targets have high significance during the future influenza outbreaks. Further, various control measures like using hygienic masks from protection of infected aerosols, keeping infected individuals under medical guidance and isolating them from immunocompromised non-infected individuals and preventing the mass gathering of infected individuals can minimize the risk of disease transmission.

The emergence of various influenza strains has been reported globally due to the rapid globalization, climate shifts and reassortment of viral strains. Therefore, a global alert scheme that can forecast the likelihood of a pandemic in future by its statistical analysis is highly essential. The implementation of best practices in patient care, prevention, diagnosis and selection of antiviral drugs may help to combat the viral infection. The sequence-based drug targeting approaches should be modified while developing newer drugs or vaccines. Similarly, to manage the emerging and re-emerging viral infections, the harmonization among the researchers, doctors, policymakers, virologists, drug designers and the local population is necessary.

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Chapter 7
Buffalopox Virus

Amit Kumar, Gnanavel Venkatesan, and Raj Kumar Singh

Abstract Buffalopox is a highly contagious, emerging and re-emerging zoonosis that affects buffaloes with occasional involvement of cows and humans. Since the first recorded incidence of buffalopox infection from undivided India in 1934, several Indian states and other countries of Indian subcontinent have reported regular outbreaks. The aetiological agent, buffalopox virus (BPXV) was later isolated and confirmed as a separate entity from the vaccinia virus or cowpox virus. BPXV is considered as an Indian variant of vaccinia like viruses (VLVs). It shares genetic, biological and serological identity with VACV. In animals, the infection is characterized by localized pock lesions on teats and udder with complications of mastitis and is often associated with high morbidity and productivity losses. Rarely, a generalized form involving several body parts is also observed. Epidemiologically, the disease spreads through milkers with zoonotic involvement. The disease has been declared as important occupational zoonosis by Joint FAO/WHO Expert Committee on Zoonosis. Present-day human population born after discontinuation of smallpox vaccination program lacks antibodies to orthopoxvirus infections like BPXV. Recently, concurrent involvement of buffaloes, cows and human in India and nosocomial infections in burn patients in Pakistan raises serious economic and public health concerns. There is a need for detailed systematic study on viral epidemiology along with whole-genome sequencing of BPXV isolates from different geographical areas and development of rapid, specific and sensitive diagnostics for confirmatory diagnosis of BPXV. Here, epidemiology, clinical aspects of BPXV infection and diagnostic procedures along with control measures are described.

Keywords Buffalopox virus · Diagnosis · Epidemiology · Vaccinia-like-virus · Zoonosis

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7.1 Prologue

Buffalopox is a contagious emerging and re-emerging zoonosis affecting buffaloes with occasional involvement of cows and humans. The first recorded incidence of buffalopox disease was reported from undivided India (Sharma 1934). In the early 1950s, buffalopox infection in animals was believed to be caused by vaccinia virus (VACV) or cowpox virus (CPXV) because of its similar clinical proximity, giving rise to synonyms like variola, variola-vaccinia, cowpox-variola, etc. to describe the disease. Later, the definitive isolation of the causative agent, buffalopox virus (BPXV), was confirmed as a separate entity from North India (Singh and Singh 1967). Further biological, physicochemical and serological characterization of BPXV revealed it as a distinct entity, most closely related to VACV (Singh and Singh 1967; Kataria and Singh 1970; Baxby and Hill 1971; Lal and Singh 1973). Later, electron microscopic studies were undertaken which also demonstrated the resemblance of BPXV with VACV (Bloch and Lal 1975; Sehgal et al. 1977). To cultivate the virus, researchers used chorioallantoic membrane of chick embryo and several tissue cultures (Lal and Singh 1977). Apart from natural infection, the experimental host range of BPXV in different farm and laboratory animals has been determined (Singh et al. 1996). Since 1934, the zoonotic nature of BPXV has been demonstrated. Some buffalopox outbreaks have been contemporary to vaccinia virus vaccination programs and smallpox outbreaks, while other buffalopox outbreaks continued even after the cessation of the anti-smallpox vaccination program. The origin of BPXV is still obscure, for which several hypotheses have been proposed. 'Vaccine-escape hypothesis' suggests that during the initial production of VACV vaccines in the buffalo-calf skin in India and Indonesia, VACV probably got adapted to buffaloes via several passages to become a separate entity which is now naturally maintained in a transmission cycle involving buffaloes (Hobday et al. 1961). Thus, BPXV is considered as an Indian vaccinia-like virus (VLV). Similar outbreaks caused by other VLVs, namely Cantagalo virus, Aracatuba virus, Guarani virus, etc., have been reported in dairy cows and humans in Brazil (Kroon et al. 2011). The disease is prevalent in many states of India, Pakistan, Nepal, Bangladesh, Egypt, Indonesia, Italy and Russia (Singh et al. 2007b). Buffalopox infection in animals is characterized mainly by localized pock lesions on teats and udder with complications of mastitis and is often associated with high morbidity and productivity losses in terms of decreased milk yield, severe mastitis, hide damage and reduced draught capacity of the affected animals (Bhanuprakash et al. 2010). The disease has been declared as an important occupational zoonosis by joint FAO/WHO Expert Committee on Zoonosis (FAO/WHO 1967). Apart from frequent outbreaks in buffaloes in the Indian subcontinent, simultaneous involvement of cows and human in recent outbreaks is of economic and public health concern. Present-day human population born after cessation of smallpox vaccination program lacks protective antibodies and is, therefore, more susceptible to orthopoxvirus (OPV)

infections like BPXV and other Brazilian VLVs. In this chapter, BPXV has been described with reference to molecular characterization, antigenic properties, epidemiology, host range, pathogenesis, clinical disease, zoonosis, diagnosis and prevention strategies.

7.2 Buffalopox Virus

The aetiological agent, BPXV, is classified as a vaccinia-like virus or variant of VACV in genus *Orthopoxvirus* (OPV) of the subfamily *Chordopoxvirinae* in the *Poxviridae* family (King et al. 2012) based on its biological, physicochemical and serological properties (Singh and Singh 1967; Kataria and Singh 1970; Baxby and Hill 1971; Lal and Singh 1973). The BP4 (Hisar) strain isolated by Singh and Singh (1967) has been regarded as the reference virus strain for BPXV.

7.2.1 Morphology and Physicochemical Characteristics

The mature virion of BPXV resembles VACV in electron microscopic studies with the size of 280–330 nm × 200–250 nm (Bloch and Lal 1975). The developmental forms are similar to those of VACV. The virion is brick-shaped with complex symmetry and produces small, abundant, irregular, eosinophilic and granulated intra-cytoplasmic B-type inclusions (Singh and Singh 1967). The virus shows resistance to the ether but is susceptible to heat, acid, bile salts, chloroform and pH (Singh and Singh 1967; Lal and Singh 1977).

7.2.2 Genome Organization

BPXV is presumed to have a similar genomic organization to that of VACV, i.e., double-stranded DNA genome of ~200 kbp size with covalently closed termini. Till date, the complete genome sequence of BPXV is not available. Whole-genome RFLP (restriction fragment length polymorphism) analysis did not reveal a significant difference between VACV and BPXV (Dumbell and Richardson 1993). Although BP4 strain differed from other 12 BPXV isolates from Maharashtra state (India) in above study. A number of BPXV genes encoding for structural, non-structural and host-range proteins have been characterized, namely VACV homologues of H3L, A27L, D8L (Singh et al. 2006b), B5R (Singh et al. 2007b; Bera et al. 2012), K1L (Barua et al. 2011), H4L (Singh et al. 2007a), C17L/B23R (Singh et al. 2008) and E3L, K3L, C7L (Bera et al. 2012). These genes showed 98–99% nucleotide and amino acid sequence identity to VACV. The D8L protein showed K163T substitution in all BPXV isolates as compared to VACV. C18L gene

encoding ankyrin-repeat protein is used specifically to differentiate BPXV from VACV and other OPVs, as BPXV shows only <77% nucleotide and <67% amino acid identity with VACV. BPXV C18L gene encodes only 50 amino acids length protein as compared to 150 amino acids in VACV and shows separate clustering from VACVs (Singh et al. 2008). Sequence analyses of haemagglutinin (HA) gene of isolates from cows in India confirmed the aetiological agent to be BPXV with a higher sequence identity with modified vaccinia Ankara strain than Brazilian VLVs (Yadav et al. 2010). Brazilian VLVs characteristically show a deletion of six codons at position 248–253 in HA protein (Kroon et al. 2011). Whole-genome sequencing of BPXV will provide a clearer picture of origin, evolution and virulence determinants.

7.2.3 Antigenic and Serological Properties

Numerous antigens of BPXV have been recognized by conventional serological tests such as protein soluble antigens (LS antigens), nucleoprotein antigens (NP antigens), haemagglutinin (HA) and factors responsible for infectivity (Kataria and Singh 1970; Baxby and Hill 1971; Lal and Singh 1973). Virion polypeptides of different purified BPXV isolates using SDS-PAGE gel electrophoresis are 15–26 (14.2–347 kDa size) (Maan and Kalra 1995; Singh et al. 2006a) depending on the specific isolate. Western blot analysis revealed 15 immunodominant proteins specific to BPXV (Anand Kumar and Butchaiah 2004).

BPXV shows serological cross-reactivity with other OPVs especially VACV by serum neutralization test (SNT) (Singh and Singh 1967; Kataria and Singh 1970), immunodiffusion (Lal and Singh 1973), double immunodiffusion (Baxby and Hill 1971) and complement fixation test (Kataria and Singh 1970; Lal and Singh 1973). BPXV shows no cross-reactivity with fowlpox virus, swinepox virus and sheeppox virus (Lal and Singh 1973). BPXV shows haemagglutination activity with sheep, rabbit, guinea-pig and chicken RBCs (Baxby and Hill 1971) but does not agglutinate RBCs of camel, buffalo calf and goat (Kataria and Singh 1970).

7.3 Epidemiology of Disease

7.3.1 Geographical Distribution

Since 1934, buffalopox has been reported from several Indian states including Maharashtra, Haryana, Punjab, Uttar Pradesh, Gujarat, Andhra Pradesh, Karnataka, Kerala, etc. (Singh and Singh 1967; Dumbell and Richardson 1993; Venkatesan et al. 2010; Gurav et al. 2011; Goyal et al. 2013) (Table 7.1). In the Maharashtra state of India, outbreaks of buffalopox were reported from 1976, 1985–1987, 1992–1996, 2003, 2008–2009 (Table 7.1). Dhule, Kolhapur, Beed districts of Maharashtra state

Table 7.1 Major Buffalopox outbreaks in India in chronological order

Year of outbreak	Place	Comments	Zoonotic involvement	Reference
1934	Lahore (then, undivided India)	Buffaloes and cows with generalized infection along with localized infection in humans	Yes	Sharma (1934)
1935	Bhiwani (then, Hisar district), Haryana (then Punjab)	Buffaloes with eye and ear lesions	No	Bhatia (1936)
1952	Guntur, Andhra Pradesh	Generalized infection in buffaloes with high infection rate, no disease in in-contact cows	No	Ramakrishnan and Ananthapadmanabhan (1957)
1966	Hisar, Haryana	Generalized lesions in buffaloes with localized lesions on hands in humans	Yes	Singh and Singh (1967)
1975–1976	Aarey Milk colony, Goregaon, Bombay	Several outbreaks with high morbidity in animals involving death of buffalo calves, localized lesions in humans	Yes	Mathew et al. (1978)
1976	Dhule, Maharashtra	Animals from several villages showed localized lesions, fever and localized lesions in humans	Yes	Ghosh et al. (1977) Sehgal et al. (1977)
1978	Bareilly, Uttar Pradesh	Large population of buffaloes affected with localized lesions on ear, eyes and udder; humans with localized infection	Yes	Mehrotra et al. (1981)
1978	Bithari Chainpur, Bareilly district, Uttar Pradesh	Large population of buffaloes affected with ear and/or eye lesions with secondary complications like otorrhoea and conjunctivitis and high morbidity rate	No	Mallick and Dwivedi (1982)
1986	Rethoura, Bareilly district, Uttar Pradesh	Large population of buffaloes affected with ear or eye lesions, low morbidity rate	No	Mallick (1988)
1985–1987	Ratnagiri, Beed, Dhule, Pune, Sholapur districts of Maharashtra	18 scab samples collected	No	Dumbel and Richardson (1993)

(continued)

Table 7.1 (continued)

Year of outbreak	Place	Comments	Zoonotic involvement	Reference
1992–1996	Dhule, Jalgaon, Beed districts of Maharashtra	Udder and teats lesions in buffaloes, cows; localized lesions in humans	Yes	Kolhapure et al. (1997)
1997	Thotlavalluru, Krishna district (Andhra Pradesh)	Buffaloes with atypical form involving only brisket region		Babu et al. (1998)
1997	Nasik, Maharashtra	Both localized and generalized skin lesions in animals, localized lesions in young humans not vaccinated for smallpox	Yes	Raut et al. (1997)
2003	Aurangabad, Maharashtra	Lesions on udder, teats, hindquarters; local lesions on forehand, fingers, fever, lymph-adenopathy in humans	Yes	Singh et al. (2006c)
2006	Thotapalli Gudur, James Garden, Nellore (Andhra Pradesh)	Localized lesions in buffalo calves along with human cases	Yes	Bhanuprakash et al. (2010)
2006	Sardar Krishinagar, Gujarat	Localized infections in humans	Yes	Bhanuprakash et al. (2010)
2008–2009	Solapur and Kolhapur districts, Maharashtra	Lesions on udder and teats in buffaloes with human involvement	Yes	Gurav et al. (2011)
2009	Kolhapur, Maharashtra	Large population of buffaloes affected with lesions on udder and teats, localized lesions in humans	Yes	Venkatesan et al. (2010)
2011	Batnor, Meerut (Uttar Pradesh)	Simultaneous localized infection of buffaloes, cows and humans with corneal opacity in two humans	Yes	Goyal et al. (2013)
2014	Hisar	Laboratory-acquired infection in human	Yes	Riyesh et al. (2014)

(India) have been endemic regions for buffalopox. Dhule, being a large cattle market, acts as foci for the spreading of many infectious diseases including buffalopox into nearby areas. Outbreaks of buffalopox have also been reported from many other countries of the world including Pakistan (Maqsood 1958), Egypt (Tantawi et al. 1979), Italy (Oreste and Sabastini cited by Hutyra et al. 1946), Russia (Ganiev and Ferzaliev 1964), Indonesia (Mansjoer 1951), Nepal and Bangladesh (Singh et al. 2007). The disease is presently associated with increasing outbreaks in buffaloes (Venkatesan et al. 2010). Four outbreaks of BPXV have been reported in India between 2006 and 2008 (Bhanuprakash et al. 2010).

7.3.2 Host Range

Naturally, water buffaloes are the primary host for BPXV with occasional involvement of cows and humans (Ghosh et al. 1977; Yadav et al. 2010; Gurav et al. 2011). Cows and buffaloes in the same herd can be infected simultaneously (Yadav et al. 2010; Goyal et al. 2013). The disease has not been reported from African buffalo (*Syncerus caffer*) (Singh et al. 2012). Experimentally, a wide host range involving buffalo calves, cow calves (Singh et al. 1996), rabbits (Singh et al. 1996), guinea pigs (Singh and Singh 1967; Singh et al. 1996; Kumar et al. 2016), BALB/c and Swiss white infant mice (Dogra et al. 1978; Singh et al. 1996; Kumar et al. 2015) are susceptible, whereas adult mice (BALB/c and Swiss white), hamsters, sheep, goat and fowl are refractory to buffalopox infection (Singh and Singh 1967; Singh et al. 1996). Transmissibility of BPXV between multiple species, including buffaloes, cows and humans, may lead to the emergence of BPXV with altered pathogenicity.

7.3.3 Transmission

The disease is reported to occur in sporadic and epidemic forms in domestic/commercial farms. The spread of BPXV into naive areas is predominantly associated with animal movement through trade. Animal-to-animal transmission of BPXV usually occurs through close contact, especially during calf feeding, contaminated milkers' hands and milking machine. Mechanical transmission by flies and insects has also been suspected (Muraleedharan et al. 1989). Animal handlers and milkers get the infection while milking or close contact with lesions, which is further spread to naïve animals of the same farm or distinct farms. Human-to-human transmission of BPXV has been suspected in India (Singh et al. 1996; Kolhapure et al. 1997) but the same has been confirmed in Brazilian VLVs (Oliveira et al. 2014). BPXV (Singh et al. 2006c) and Brazilian VLVs (Abrahao et al. 2009b) have been isolated from milk indicating possible food-borne transmission. Till date, reservoir host/s for BPXV is still not known. Although some rodent species have been suspected to be involved in the transmission of BPXV in Namakkal district of Tamil Nadu state,

India (Nedunchelliyan et al. 1992). Peridomestic rodents have been suspected of transmitting the bovine vaccinia infection from wild to domestic animals in Brazil (Abrahao et al. 2009a). Further, the circulation of BPXV in wild animals in India still needs to be explored.

7.4 Immunopathobiology

Pathogenesis of BPXV has been experimentally studied in susceptible hosts like rabbits, suckling mice and buffalo calves. After intradermal inoculation, the virus undergoes local multiplication at the site of entry in the skin and moves to regional lymph node followed by WBC-associated primary viremia (Rana et al. 1985; Chandra et al. 1985). Following viremia, virus disseminates to target organs, namely lungs, liver and spleen. Multiplication of virus in target organs causes secondary viremia, which is responsible for secondary lesions in the gonads, intestines, stomach, kidney, etc. Suckling mice following inoculation show symptoms like paralysis of hind legs, staggering gait and circling followed by death (Dogra et al. 1978). Usually, generalized skin lesions are not seen in rabbits, guinea pigs and suckling mice. Following experimental inoculation in buffalo calves, symptoms like lacrimation, mucopurulent nasal discharge and diarrhoea were observed. After the appearance of primary skin lesion, the virus is detected in the regional lymph node on the 2nd day followed by viremia at 4th day and multiplication in target organs at 5th day. Secondary viremia starts at 6th day causing a secondary rash, between day 6th–8th on the lips, tongue, neck, perineum region and around the nostrils and eyes. The experimental infection had a course of 13–15 days (Rana et al. 1985). Antibodies are detected after 12 days following experimental infection. Both humoral and cellular immune responses have been shown to play a role in buffalopox infected rabbits (Chandra et al. 1990), mice (Kaushik and Pandey 1980) and guinea pigs (Kalra et al. 1976). Role of antibodies in poxvirus control and recovery from secondary infection is well known. Passive transfer of anti-BPXV serum (Kaushik and Pandey 1980), anti-A27L serum (Kumar et al. 2015), anti-H3L serum (Kumar et al. 2016) and combined A27L and H3L antibody cocktail (Kumar et al. 2017) has shown 87%, 60%, 80% and 100% protection, respectively. Antibodies to intracellular mature virion (IMV) proteins like A27L and H3L probably directly neutralize the initial virus inoculum as well as progeny IMV virions released from lysed cells and also inhibit maturation of IMV to intracellular enveloped virion (IEV). Complete cross-protection has been demonstrated in buffaloes between BPXV and VACV (Ramakrishnan and Ananthapadmanabhan 1957) and BPXV and CPXV (Tantawi et al. 1979).

7.5 Clinical Disease in Animals

Buffaloes of all ages and both the sex are affected, but the clinical disease is more severe in young and old animals. After an incubation period of 4–6 days (Ghosh et al. 1977), animal shows anorexia, the rise in temperature and lacrimation, followed by the development of skin lesions. Clinically, buffalopox occurs in two forms, namely mild localized and severe generalized. A severe generalized form involving several body parts like udder, teats, vulva, thigh and abdomen (Ramakrishnan and Ananthapadmanabhan 1957; Sreemannarayana and Ramachandraiah 1999) is rare and uncommon nowadays. Buffalopox occurs mainly in localized mild form affecting mainly udder and teats (Fig. 7.1), sometimes inguinal region and thighs in milking animals (Sharma 1934; Singh and Singh 1967) or parotid region, ear and eyes in draught animals (Venkatesan et al. 2010). Lesions on udder and teats lead to thickening of teats, stenosis of teat ducts followed by secondary bacterial complications. Approximately 50% of buffalopox affected animals develop mastitis leading to a reduction in milk yield. Severe cases may involve a permanent reduction in milk yield (Singh et al. 2006c). Suckling calves may contract the disease from the affected dam and develop lesions around muzzle including purulent gingivitis. After the involvement of eyes and ears, frequent complications like otorrhoea, conjunctivitis and corneal opacity are observed (Venkatesan et al. 2010). An atypical form involving only brisket region has been reported in BPXV outbreak in Thotlavalluru village of Krishna district in Andhra Pradesh, India (Babu et al. 1998). Pock lesions pass through successive stages such as roseolar, papular, vesicular, pustular and desquamative. Temperature returns to normal after pustules subside. Recovery usually takes 3–4 weeks in uncomplicated cases. Recovered animals show life-long immunity. Morbidity in affected animals is as low as 5–10% to as high as 65–70%. Death is seen sometimes in calves (Mathew et al. 1978).

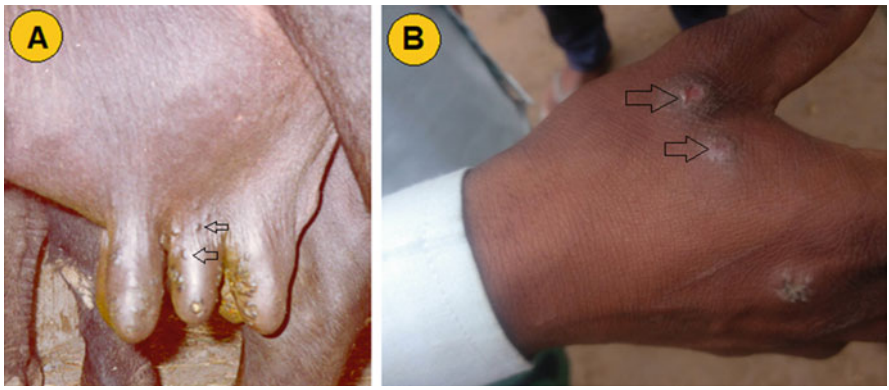

Fig. 7.1 Localized lesions of buffalopox infection. (**a**) Teat lesions in buffalo (**b**) lesions on hand in human (indicated by arrows)

7.6 Buffalopox in Humans

The joint FAO/WHO Expert Committee on zoonosis has listed buffalopox as an important emerging, re-emerging zoonosis. Humans in close contact with infected animals like milkers, animal attendants, veterinarians, laboratory personnel handling the virus, etc. are particularly susceptible to buffalopox infection. Milking of infected animals is one of the major modes of spread. After an incubation period of 3–19 days, affected humans show symptoms like fever, general malaise, regional lymphadenopathy followed by appearance of localized pock lesions on forearms, forehead and face (Kolhapure et al. 1997; Venkatesan et al. 2010; Gurav et al. 2011; Goyal et al. 2013) (Fig. 7.1). The generalized BPXV infection in humans has not been reported except one case of an immunodeficient patient with a history of Darier's disease in Telangana, India (Prasad et al. 2009). Zoonotic outbreaks are frequent in endemic regions of Maharashtra state (India), namely Dhule, Beed, Jalgaon, Nasik, Kolhapur and Aurangabad districts of Maharashtra (Dumbell and Richardson 1993; Kolhapure et al. 1997; Singh et al. 2006c; Venkatesan et al. 2010; Gurav et al. 2011). In recent years, it has been observed that buffalopox is running a more severe course in humans, especially those who have not been immunized for smallpox in the past. During 1992–1996 outbreak of buffalopox in Dhule (Maharashtra state, India), in addition to affected humans and in-contact milkers, neutralizing antibodies against BPXV were also detected in young individuals who have neither history of clinical pox disease nor history of contact with buffaloes and never been vaccinated against smallpox (Kolhapure et al. 1997; Venkatesan et al. 2010). In some outbreaks, a few children who had no previous contact with infected animals showed clinical manifestations raising suspicion for possible man-to-man transmission (Kolhapure et al. 1997; Gurav et al. 2011). BPXV infection in buffaloes, cows and humans simultaneously in the same space and time has been reported in Meerut (Uttar Pradesh, India) in 2011 (Goyal et al. 2013). The outbreak also involved two human cases with corneal opacity. There has also been a report of nosocomial infections of buffalopox from Karachi, Pakistan, in burn patients and paramedical staff which proved an efficient mode of indirect transmission of an OPV (Zafar et al. 2007). Laboratory-acquired BPXV infection has been seen on both smallpox vaccinated (Baxby and Hill 1971) and non-vaccinated humans (Riyesh et al. 2014).

7.7 Diagnostics

Although clinical characteristics of buffalopox might be helpful in symptomatic diagnosis, clinical lesions of various other vesicular diseases such as cowpox, pseudocowpox and bovine herpesvirus mammillitis may potentially mislead the diagnosis. Laboratory investigations are, therefore, essential for establishing a definitive diagnosis. Buffalopox infections can be diagnosed by conventional, serological (antigen and antibody detection) and molecular techniques. Suitable specimens like

scabs, vesicle fluids, skin biopsy tissues and serum can be collected. After making 10% (w/v) suspension of scab material using phosphate-buffered saline (pH 7.4), homogenate suspension is freeze-thawed three times followed by clarification at 1500 × g for 10 min. The supernatant is filtered through 0.45 μm membrane filters after addition of antibiotics such as penicillin and streptomycin and stored for further use either for DNA extraction or isolation of the virus in cell culture.

7.7.1 Conventional Diagnosis

Conventional techniques like electron microscopy by negative staining of vesicular fluid, or micro-sectioning of scab; virus isolation in embryonated eggs via chorio-allantoic membrane (CAM) route, cell culture, inoculation into experimental animals like rabbits or infant suckling mice have been used for primary diagnosis (Singh et al. 2007a). By electron microscopy, it is easy to differentiate OPVs from other viruses, but differentiation at species level within OPVs is not possible. On the chorioallantoic membrane of embryonated chicken eggs, BPXV produces two types of non-haemorrhagic pocks 48–72 h post-infection—greyish flat type and white raised type (Singh and Singh 1967). BPXV replicates in a wide range of cell cultures including Vero, BHK_{21}, RK-13, primary hamster kidney cells, HeLa cells, CEF, pup kidney cell culture, etc. Vero cells are commonly used for isolation of BPXV. In cell culture, rounding, clumping and increased refractivity of infected cells is seen at 24 hpi (hours post-infection) followed by pin-point degenerative foci at 42 hpi, and microplaque formation followed by complete detachment after 72–96 hpi (Fig. 7.2). Clinical samples may require two blind passages to give cytopathic effect (CPE). The virus produces plaques on RK-13 and CEF cells.

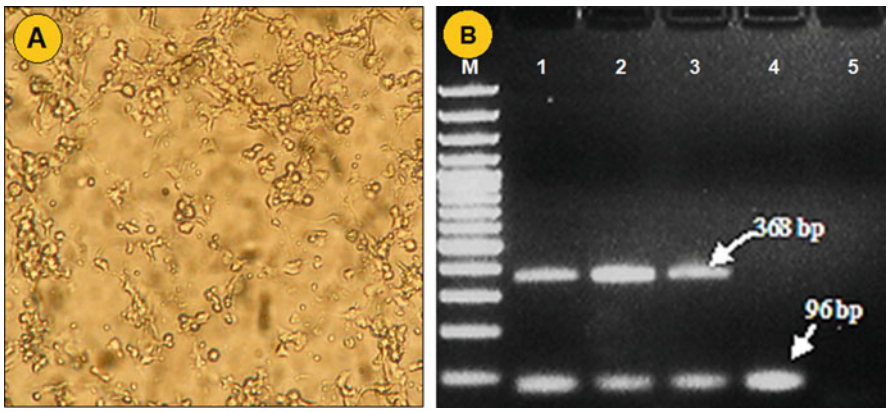

Fig. 7.2 (**a**) Cytopathic effect of BPXV 48 h post-infection showing rounding, clumping of infected cells. (**b**) Duplex PCR based on C18L and DNA polymerase genes (Singh et al. 2008) showing 368 bp amplified product specific for BPXV and 96 bp amplified product specific for OPV. Lane 1: BPXV-Vij96 (vaccine virus); Lane 2: BPXV-Pune (buffalo); Lane 3: BPXV-Pune (human); Lane 4: Camelpox virus; Lane 5: No template control

7.7.2 Antigen Detection

Serological tests like agar gel immunodiffusion (AGID) (Paul et al. 1984) and counter-immunoelectrophoresis (CIE) using a reference antigen and its hyperimmune serum can also be used. CIE test identifies either unknown soluble antigen in suspected pox lesions or antibody present in the serum samples using standard/reference positive serum or antigen, respectively. Fluorescent antibody test (Kaushik and Pandey 1981), immunoperoxidase test (IPT) (Grover et al. 1980; Ghildyal et al. 1986; Mohanty et al. 1989c) and immunoenzymatic (Grover et al. 1980; Kaushik and Pandey 1981) techniques can be employed for detection of BPXV in cell culture or formalin-fixed tissues. Serological assays like AGPT, CIE and IPT tests fail in the accurate diagnosis of the disease because of antigenic cross-reactivity with other OPVs. Moreover, these techniques are less sensitive, laborious and time-consuming.

7.7.3 Antibody Detection

Serum neutralization test (SNT) and whole antigen-based indirect ELISA have been developed for detection of buffalopox antibodies in experimentally infected rabbits (Ghildyal et al. 1986) and vaccinated/infected buffaloes (Mohanty and Rai 1990). Whole antigen-based indirect ELISA has been found to be more sensitive than SNT and CFT. The whole antigen-based ELISA and SNT require handling of the live virus which is risky, laborious and uneconomical to produce the diagnostic whole viral antigen in bulk posing a public/biosecurity threat. A recombinant antigen-based diagnostic assay will be a better alternative for reliable post-outbreak sero-surveillance in the countries where BPXV or VACV-like agents are endemic. Positive serological findings in humans have to be carefully interpreted in light of previous smallpox vaccination. Due to the advancement in gene expression technology, the production of recombinant viral proteins has become relatively easier and more efficient. In the past, various VACV proteins have been expressed in different expression systems such as bacterial, mammalian, yeast and insect cells to assess potential diagnostic and immunoprophylactic potential. Two IMV proteins of BPXV, namely A27L and H3L, have been expressed in the prokaryotic system and evaluated for their diagnostic potential in ELISA (Kumar et al. 2015, 2016).

7.7.4 Molecular Diagnosis

PCR assays targeting HA (Ropp et al. 1995; Damaso et al. 2007) and ATI genes (Meyer et al. 1997) are used for the detection and differentiation of OPVs. Several molecular tests have been described for the detection and differentiation of BPXV.

TaqMan probe-based quantitative real-time PCR targeting C18L gene and duplex PCR based on C18L and DNA polymerase genes can be used to differentiate BPXV from other OPVs (Singh et al. 2008). C18L and DNA polymerase genes based duplex PCR amplifies 96 bp amplicon for all OPVs and 368 bp amplicon only BPXV (Fig. 7.2).

RAPD-PCR can also be used for differentiation of BPXV (Singh et al. 2007a). However, these techniques need specialized equipment leading to the high cost of diagnosis at field conditions. Therefore, there is an urgent need for sensitive, specific, rapid and user-friendly diagnostic tool like loop-mediated isothermal amplification (LAMP) assay in less equipped field diagnostic laboratories for timely identification of BPXV infection and its differentiation from other OPVs in the target population to initiate control measures quickly.

7.8 Prevention and Control

India and other South Asian countries are endemic to buffalopox. BPXV affected animals should be segregated from apparently healthy animals with separate arrangement for milking of infected animals by separate attendants. The difficulty in restricting animal movement especially between villages during trade, socio-cultural issues and economic considerations restricts efficient control of BPXV in India. Application of biosecurity measures such as segregation of infected animals, use of disinfectants in sheds, etc. is the only means of containing the spread of infection. Uncomplicated buffalopox infections are self-limiting with a course of 3–4 weeks, but animals need to be treated symptomatically along with treatment for secondary bacterial infections. For teat/other skin lesions, antiseptic lotions/ointments can be applied, whereas mastitis cases are treated with parenteral antibiotics. Local antibiotic ointment for eye and/or ear lesions along with parenteral antibiotics is generally used to control secondary infections. Human cases are treated with antipyretics, anti-inflammatory drugs and broad-spectrum antibiotics. Efficacy of certain drugs effective against OPV infections, viz. ST-246, cidofovir, STI-571, CMX001, etc., needs to be evaluated for BPXV.

Initially, the vaccinia lymph vaccine has been tried for BPXV (Mathew et al. 1978). Later, homologous ethylamine, formalin and beta-propiolactone inactivated BPXV vaccine was tried, but unsuccessful with lack of protection in rabbits and buffaloes (Mohanty et al. 1989a). Protection by using CEF attenuated buffalopox virus BP4 strain (passage-70) in rabbits (Dogra and Sharma 1981) and Vero cell-adapted buffalopox virus BP4 strain (passage-40) in buffaloes (Mohanty and Rai 1989) has been demonstrated. In Pox Virus Laboratory, IVRI Mukteshwar (India), a live attenuated vaccine has been developed using Vero cell-adapted BPXV (Vijayawada 96 strain, passage-50). This vaccine has been found to be safe and potent in in-house trials. This vaccine needs further large-scale field validation. However, there is a need for alternative immunoprophylactic strategies involving the use of DNA and subunit vaccines because of safety and zoonotic potential of the virus. Out

of ~200 proteins encoded by VACV, few proteins including H3L, A27L, B5R, D8L and L1R proteins have been shown to induce neutralizing antibodies. Several VACV proteins have been expressed in different expression systems and tested for immunogenicity and protective efficacy in animal models, individually or in combination (Hooper et al. 2003; Berhanu et al. 2008). Antibodies against surface proteins from both IMV and extracellular enveloped virion (EEV) forms are important for protective immunity. So, combined subunit vaccine using different IMV and/or EEV proteins in the form of polyvalent formulations might be effective for better protection than individual proteins. Immunogenicity of two IMV proteins of BPXV, namely A27L and H3L, has been evaluated in laboratory animals (Kumar et al. 2015, 2016). However, work on BPXV and other VLVs proteomics is scanty. Further, the nature of immunogenic viral proteins involved in eliciting immune response of these viruses in farm animals and human is yet to be known.

7.9 Conclusion

Buffalopox is an emerging and re-emerging zoonosis of public health importance. The possibility of human-to-human transmission may be a serious concern to veterinarians and WHO. Considering the zoonotic significance and widespread incidence of buffalopox infection in the Indian subcontinent, a detailed systematic study is warranted to carry out epidemiological investigations and appropriate control measures. Whole-genome sequencing of BPXV isolates from different geographical area will provide a clear picture of the origin of BPXV.

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Chapter 8
Animal Rotaviruses

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Abstract Rotaviruses (RVs) are ubiquitous and remain the major cause of acute viral gastroenteritis in young animals, bird species and children worldwide. The disease is acute, occurs predominantly in intensively reared animals and characterized by a short incubation period, anorexia and diarrhoea. Post-infection immunity and immune system and intestinal microbiome maturation make immunocompetent adults of different species resistant to clinical RV disease. RVs of groups A, B, C, E, H, I and J have been detected in sporadic, endemic or epidemic infections of various mammalian species, whereas RV strains of groups D, F and G are only found in poultry, such as chickens and turkeys. Recently identified novel RVs in sheltered dogs in Hungary and bats in Serbia are tentatively identified as group I and J, respectively. Historically, diagnosis of RV infections relied on conventional techniques such as isolation in cell culture, electron microscopy, electropherotyping and

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various serological tests. Presently, RT-PCR assays and molecular typing using sequencing or genomic hybridization techniques are used predominantly for RV diagnosis and classification. Because RVs are endemic in most animal populations and exhibit extreme genetic diversity due to frequent mutations and re-assortment events, available RV vaccines are only marginally efficient, and eradication of the pathogen remains a challenge. Thus, a better understanding of the historic and current prevalence and genetic diversity of animal RVs in different geographic regions, disease pathogenesis, available control strategies and zoonotic potential is needed. This knowledge will lead to the development of more optimal strategies to manage RV diarrhoeal disease in animals, birds and humans.

Keywords Animal rotaviruses · Porcine rotaviruses · Bovine rotaviruses · Wildlife rotaviruses · Avian rotaviruses · Epidemiology · Genetic variability · RV pathogenesis · Vaccines · Diagnosis · Zoonotic potential

8.1 Prologue

Rotaviruses (RVs) are a major cause of acute enteric disease in the young of many mammalian and avian species and children worldwide (Estes and Greenberg 2013). The disease is characterized by a short incubation period, anorexia and diarrhoea. Usually, the adults of different species acquire post-infection immunity and have a mature immune system which makes them immunocompetent and resistant to clinical diseases. However, rotavirus group B (RVB) infections are shown to be more frequently associated with diarrhoea in adults.

The name 'Rotavirus' is derived from a Latin word *rota* (which means wheel), due to its characteristic appearance when observed by negative-stain electron microscopy (EM). Rotavirus infection was first recognized in 1963 in mice, and soon after that, the SA11 (simian agent 11) was isolated from a healthy vervet monkey. In 1969, bovine group A RV (RVA) was isolated in cell culture and confirmed as a cause of calf diarrhoea (Mebus et al. 1971). These murine, simian and bovine agents (RVAs) were later found to share a common group antigen (VP6) and to be morphologically indistinguishable. Human RV was discovered soon after, in 1973, by Bishop et al. (1973), where wheel-shaped particles were observed in electron micrographs of the intestinal mucosa and stool samples of children with acute gastroenteritis (Bishop et al. 1973). Following studies documented the widespread prevalence of RVA infections in young animals, including calves and pigs, and their association with diarrhoea in animals <1 month of age (Malik et al. 2013a, b, c; Woode and Bridger 1975; McNulty et al. 1978; Saif and Jiang 1994). Animal RVs are considered as potential reservoirs for genetic exchange with human rotaviruses. Continuous reassortments event and due to direct transmission, animal rotaviruses have been found to infect humans which leads to the generations of peculiar reassortant strains which includes genes of human origin also.

In 1977 a study from the USA reported avian RV in turkey poults in the USA, and since then different RVs of group A (RVA), D (RVD), F (RVF) and G (RVG) have been described globally (Dhama et al. 2015). Bovine RVs (BRV) were among the earliest RVs to be successfully adapted to serial propagation in cell culture (Mebus et al. 1971) which facilitated research efforts toward their characterization. In 1980 group C RVs were first isolated in piglets and thereafter they have been identified in other animals and humans (Pedley et al. 1986; Saif and Jiang 1994). An RV-like agent was first described in a diarrhoeic pig in the 1980s which was later confirmed as porcine RVB (Bridger et al. 1983; Theil et al. 1986). In addition to pigs, RVB strains have also been detected in cattle (Chang et al. 1997; Tsunemitsu et al. 1999; Ghosh et al. 2007a), lambs (Shen et al. 1993) and rats. Group E RV (RVE) was reported as an atypical porcine RV in UK swine, in a serological survey where a widespread distribution of antibodies against this virus in 10 weeks older pigs was observed (Bridger 1987). Group H RV (RVH) strains were reported from pigs in Japan, Brazil and the USA, where they might be circulating since at least 2002 (Marthaler et al. 2014).

Rotaviruses have a worldwide distribution and commonly affect calves (Mebus et al. 1969a, b), lambs (Snodgrass et al. 1984), piglets, goat kids, foals (Malik et al. 2014; Flewett et al. 1975) and chickens (McNulty, et al. 1980).

8.2 Virus Structure

Complete infectious RV consists of three layers of protein and is termed triple-layered particle (TLP). The innermost layer is formed by 120 molecules of viral protein 2 (VP2) arranged in parallel asymmetric dimers. Five copies of the dimers radiate from the fivefold axis of symmetry to form a decamer, and 12 such decamers form the core protein layer which is uniform except for small pores along the fivefold axis (McClain et al. 2010). The core encloses the viral genome that consists of 11 segments of dsRNA as well as replication enzyme complexes, consisting of VP1 (the RNA-dependent RNA polymerase) and VP3 (the methyltransferase and guanylyltransferase). VP1 protein is modelled as cage-like structure disrupted by four channels which allow for (1) the entry of free nucleoside triphosphates (NTPs), (2) the entry of template ssRNA, (3) the exit of the (+) ssRNA product, and (4) the exit of (−) ssRNA and dsRNA (Estrozi et al. 2013). Rotavirus core is described as the 'molecular machine' owing to its capacity to synthesize capped viral mRNA transcripts.

The viral core is surrounded by 260 trimers of VP6, which is a highly conserved, group-specific viral protein, forms the middle layer and constitutes double-layered particles (DLPs). DLPs are the transcriptionally competent forms of the virus formed during the replication process. The chief protein of rotavirus particle is VP6 (based on weight percent). It plays an essential role in the overall organization of the rotavirus architecture wherein it interacts with the VP7 and VP4 (outer layer proteins), and VP2 (innermost layer protein) (Charpilienne et al. 2002). The middle

layer is covered by outermost capsid protein layer forming a nearly spherical icosahedron which consists of 260 trimers (780 copies) of VP7, decorated by 60 spikes, with each being formed by VP4 trimers (180 copies) to form the TLPs. VP7 is known as calcium-binding protein and comprises two domains: domain I has a disulphide bridge and displays a Rossmann fold, while the domain II possesses three disulphide bridges and displays a jelly-roll b-sandwich fold. To each subunit interface of trimer, two Ca2+ ions are attached. A plate-like trimer is formed when three VP7 subunits interact with each other which sit on top of the VP6 trimers. The N-terminal arms of three VP7 subunits then grasp the underlying VP6 trimers and intrude into the VP4 foot cavity. These interactions among different trimers indicate that the VP4 spikes have to be first attached to the DLPs before the addition of VP7 during virus assembly, and only after the addition of VP7 a shift in the underlying VP6 trimers takes place. The VP4 spike has a distinct structure comprising of two distal globular domains, a central body and an internal globular domain popped inside the VP7 layer in the peri-pentonal channel of the T = 13 icosahedral lattice. X-ray structures of proteolytic fragments of VP4, VP8*, and VP5* reveal strong evidence that the distal globular domain of the VP4 spike is composed of VP8* with the remaining body of the spike consisting of VP5*. Infectivity of rotaviruses increases when there is a proteolytic cleavage of VP4 yielding two proteins, VP5* and VP8* (Dormitzer et al. 2004).

The architecture of RV has a unique feature of the presence of large channels that penetrate through the VP7 and VP6 layers. These channels form a passage to the aqueous materials and biochemical substrates into and out of the capsid. The 132 channels at the fivefold and quasi sixfold positions of the T = 13 lattice are grouped into three distinct types. At the fivefold vertices of the capsid, there are twelve type I channels. 60 type II channels are present at each of the pentavalent locations surrounding the type I channels, and near to which VP4 is attached to VP7 and VP6. Surrounding the icosahedral threefold axes the remaining hexavalent positions on the capsid are occupied by the 60 type III channels (Jayaram et al. 2004).

8.3 Genome Structure and Organization

The RVs genome consists of 11 segments of dsRNA ranging in size from 667 to 3302 nucleotides and molecular weight ranging from 10^5 to 10^6 Da, enclosed within the virus core capsid. An open reading frame (ORF) is present in each RNA segment that encodes viral proteins. The RV genome segments code for both structural proteins (found in the virus particle) and the nonstructural proteins (found in infected cells but absent in mature virion particles). RVs encode for six structural proteins (VP1–VP4, VP6 and VP7) and six nonstructural proteins (NSP1–NSP5/6) (Manson et al. 1983). Except segment 11 which encodes for two proteins as NSP5 and NSP6 (in some serogroups), rest of the genomic segments are monocistronic. All the proteins encoded by rotavirus genes are well established with reviewed properties

Table 8.1 Rotavirus genes and encoded proteins

RNA segment	Size (bp)	Protein	Length (AA)	Location	Function
1	3302	VP1	1088	At the vertices	RNA-dependent RNA Polymerase
2	2690	VP2	881	Forms inner shell of the core	Stimulates viral RNA replicase
3	2591	VP3	835	At the vertices of the core	Guanylyl transferase mRNA capping enzyme
4	2362	VP4	776	Surface spike	Cell attachment, virulence
5	1611	NSP1	495	Nonstructural	Not essential to virus Growth
6	1356	VP6	397	Inner capsid	Structural and species specific antigen
7	1104	NSP3	312	Nonstructural	Enhances viral mRNA Activity and shut-offs Cellular protein synthesis
8	1059	NSP2	317	Nonstructural	NTPase involved in RNA Packaging
9	1062	VP7	326	Surface	Structural and neutralization antigen
10	751	NSP4	175	Nonstructural	Enterotoxin
11	667	NSP5 NSP6	198	Nonstructural	ssRNA and dsRNA binding modulator of NSP2

Table is based on the simian rotavirus strain SA11 (Desselberger 2000; Patton and Spencer 2000). RNA protein-coding assignments differ in some strains

(Table 8.1). Segment 1 encodes the VP1 protein and functions as an RNA-dependent RNA polymerase. VP1 protein is complexed with VP3 protein in the core of the virion. The latter protein is encoded by segment 3. The fourth segment encodes VP4 protein which is an outer capsid protein, plays a major role as neutralizing antigen. Leaving some of the serogroups apart, most of the serogroups of RVs follow the same rule. In RVD, segment 3 encodes the VP4 protein, while segment 4 encodes the VP3 protein; this order is inverted compared with the gene–protein assignment of RVAs (Trojnar et al. 2010).

8.4 Classification

Rotaviruses are dsRNA viruses in the *Reoviridae* family, and each RV is named after the species in which it occurs (Estes and Greenberg 2013). *Reoviridae* family is subdivided into two sub-families, i.e. *Sedoreovirinae* (e.g. genera *Orbivirus*, *Rotavirus*) and the *Spinareovirinae* (e.g. genera *Coltivirus*, *Orthoreovirus*). Rotavirus genome consists of 11 segments of dsRNA encoding 6 structural viral proteins (VP1–VP4, VP6 and VP7) and 5 nonstructural proteins (NSP1–NSP5/6) (Estes

and Greenberg 2013). The intact virus is composed of 3-capsid layers: an inner core, an intermediate capsid and an outer capsid with short radiating spikes. Three types of rotavirus particles visualized under the EM are (1) the complete infectious or triple-layered particles (TLP), (2) the double-layered particles (DLP) and (3) the core or single-layered particles, which harbour double-stranded RNA (dsRNA) genome (Estes and Kapikian 2007).

RVs are classified into ten groups (A–J) based on antigenic relationships of their VP6 proteins, with provisional I and J species recently identified in sheltered dogs in Hungary and bats in Serbia, respectively (Matthijnssens et al. 2011; Otto et al. 2012; Mihalov-Kovács et al. 2015; Bányai et al. 2017). Commonly groups A, B and C (RVA, RVB and RVC) infect humans and animals. Historically, RVA strains were the most prevalent and represented the most significant causes of acute diarrhoea from the public as well as veterinary health perspectives. Based on the outer capsid proteins (VP7 and VP4), which induce neutralizing antibodies, G and P dual typing system has been used to denote strains with a particular genotype (Estes and Greenberg 2013). To date, at least 31 different G- and 44 P-genotypes have been described in both humans and animals for RVAs (Matthijnssens et al. 2009). RVs of different groups are further classified into 'P' and 'G' genotypes based on the sequence identities within VP4 ('P'/Protease sensitive) and VP7 ('G'/Glycoprotein) genes, respectively. In 2008, Rotavirus Classification Working Group (RCWG) extended the dual (G/P) typing system of RVA strains to a full-genome sequence classification system, with nucleotide percent identity cut-off values established for all 11 gene segments, with the notations Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx used for the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 encoding genes, respectively (Matthijnssens et al. 2009; Ciarlet et al. 2008). Subsequently, to maintain the proposed guidelines and the classification system RCWG was formed (Matthijnssens et al. 2009; Ciarlet et al. 2008) which ensure complete and accurate classification of novel RVA strains.

On the contrary, till now only RVA classification has been developed which is being maintained by the RCWG, whereas very little is known regarding epidemiology and disease affliction associated with infection by other RV serotypes in different avian and mammal species. Using similar criteria established for RVA strains, RVCs were classified into nine different VP7 (G) genotypes, and now an 11 genome segment classification system has been adopted for RVC classification. Even higher genetic diversity was reported for RVB strains with 20 G-genotypes identified. Marthaler et al. (2012) findings suggested that porcine RVB strains have been circulating in the USA for a prolonged time (at least since the 1980s) and may be more prevalent than initially thought (Marthaler et al. 2012). This underestimation of RVB prevalence and diversity may be associated with the lack of adequate diagnostic tools, shorter and lower magnitude of the virus shedding as well as uncertain clinical significance for different age groups.

The RVC, RVE and RVH have been detected in the sporadic form in a few mammalian species. However, RVD, RVF and RVG are found only in poultry, such as chickens and turkeys (Kusumakar et al. 2008; McNulty et al. 1978; Martella et al. 2010; Trojnar et al. 2013). RVI and RVJ identified recently in sheltered dogs and

bats in Hungary and Serbia, respectively, although confirmation by the International Committee on Taxonomy of Viruses is pending (Mihalov-Kovács et al. 2015; Bányai et al. 2017).

8.4.1 Pathogenesis

The pathogenesis of RV infection in animals is very similar to that of enteric coronaviruses. The main transmission route is faecal-oral. The outcome of infection in all species depends on the virulence of the RV strain, the quantity of virus ingested, the presence of maternally derived or actively acquired antibodies in the lumen of the gut at the time of exposure, age-related resistance to the disease and animal management practices. Infection occurs shortly after birth, but it usually is subclinical in the presence of colostral antibodies in the gut. However, recent studies in the USA have shown a higher prevalence of RVC compared to RVAs in neonatal diarrhoeic piglets <3 weeks of age, where it appeared to be the only causative agent of diarrhoea that developed in suckling piglets and piglets on milk replacer.

RVs infect mature enterocytes at the tips of the villi of the small intestine (Fig. 8.1). As a result of infection, these cells, which have an absorptive function, are desquamated more rapidly (Fig. 8.1) and replaced by undifferentiated epithelial crypt cells which have a secretory function (Dhama et al. 2015). Due to resultant malabsorption, undigested carbohydrates in the lumen of the colon are fermented by bacteria to short-chain fatty acids, leading to accumulation of a hypertonic solution and subsequent osmotic fluid loss.

As a result of damage to the epithelium, the cellular sodium transport system is also disturbed, resulting in a net flow of fluid from the extracellular space into the

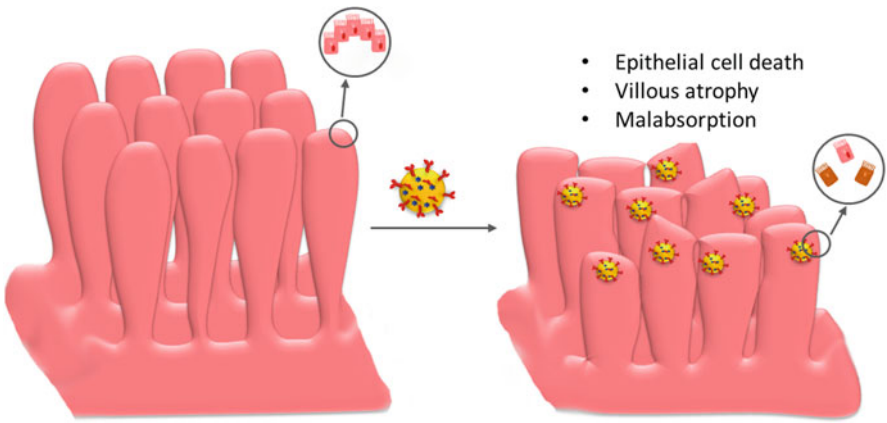

Fig. 8.1 Rotavirus pathogenesis. Rotavirus infection of villous enterocytes results in the cell death, villous atrophy and leads to malabsorption

lumen of the gut. The total faecal output is thus markedly increased with a concurrent loss of Na^+ and Ca^- ions. Affected animals develop dehydration, electrolyte imbalance and concomitant acidosis if appropriate therapy is not used. Inflammatory changes in the intestine may cause hypermotility and aggravate the diarrhoea (Woode 1976). An immune evasion mechanism by RV mediated by downregulation of interferons and other cytokines was suggested based on gene expression profiling using microarrays (Aich et al. 2007). While uncomplicated RV disease is often self-resolved within a few days, immune-suppressive RV mechanisms promote colonization of the bowel by other infectious agents such as Salmonella serovars, clostridia and other bacteria and increase the severity of the condition (Woode and Bridger 1975). Complicated RV diarrhoea increases the risk of death. The extra-intestinal spread of RV was confirmed by detection of RV dsRNA, RV antigen or infectious RV in serum and different organs (Kim et al. 2014; Park et al. 2011a). Although the clinical significance of the extra-intestinal RV infections is not established, RV replication in the liver, the biliary system and the pancreas leads to biliary atresia and pancreatitis in immunocompromised hosts. Vomiting in RV-infected hosts was explained by the fact that RV can infect the enterochromaffin cells in the gut, stimulating the production of serotonin which activates the afferent vagus nerve and stimulates the brain stem structures controlling vomiting.

8.4.2 Clinical Signs

RV diarrhoea is often sporadic or self-resolving in nature because most infections in suckling animals with maternal immunity remain subclinical. However, in situations with limited or no transfer of maternal immunity or after the loss of maternal immunity (weaning) and where other predisposing factors co-occur, the prevalence of disease may reach epidemic proportions. Additionally, maternal immunity does not seem to confer sufficient protection against diarrhoea associated with RVC.

The incubation period in most animals is 18–96 h. Affected animals are initially depressed and anorexic (Woode and Bridger 1975). This is followed by profuse diarrhoea, dehydration, loss of body weight and lethargy. Diarrhoea may persist up to 14 days in piglets and become severe if the animal recovers sufficiently to resume feeding (Woode and Bridger 1975). The colour of the faeces may vary from yellow or brownish-grey to light green and normally does not contain blood or mucus unless secondary bacterial infections occur (Snodgrass et al. 1984). It is usually an afebrile disease unless complicated by a secondary bacterial infection. Affected animals may die as a result of dehydration, electrolyte imbalance and secondary infections. Diarrhoea and stunted growth become noticeable 4 days post-infection with avian RVs (Dhama et al. 2015).

8.4.3 Pathology

Upon necropsy, intestinal walls are thin and filled with yellow fluid. The stomach is often full of undigested milk. Microscopic lesions are generally confined to the villi of the small intestine. The proximal part of the duodenum is generally not affected, and there is a patchy distribution of affected areas throughout the rest of the small intestine. Some variations in RV antigen distribution in different species are reported. Immunofluorescence studies showed that viral antigens are confined to the cells at the tips of villi in the middle and distal small intestine of calves and piglets (Theil et al. 1986) that are shed within 4 days post-infection. In lambs, the antigen is also present in the enterocytes of the large intestine, but it is less abundant than in the small intestine. Within infected cells, the virus is associated with the rough endoplasmic reticulum.

Villi appear blunt, short and fused, giving the mucosa an almost avillous appearance. Shortening of the villi is due to the loss of brush-border columnar epithelial cells that are replaced by cuboidal or squamous cells lacking a brush border from the crypts. Infiltration of mononuclear inflammatory cells may occur in the lamina propria (Mebus et al. 1971).

8.5 Diagnosis

Virus isolation was historically considered the 'gold standard' for detecting viral pathogens in diagnostic samples. However, more rapid and sensitive methods (including ELISA and RT-PCR) became available in the last decades. Cell culture is used to isolate viruses for diagnostic purposes as well as virus propagation for vaccine development or virus genetic characterization. Many cell lines (e.g. MDBK, MA104, TF and PK-15 cells) have been used to isolate RV from animal faecal samples. Viral isolation has three advantages including (a) confirmation of the presence of the infectious virus in a clinical sample, (b) availability of the isolated virus for further genetic, immunity and pathogenesis studies and development of diagnostic kits and vaccine and (c) the method does not require virus/strain reagents. However, many field strains of RV, especially RVB and RVC, do not replicate in most cell cultures. Other disadvantages include low sensitivity, variable permissiveness of cells, dependence on proper collection and storage of samples for virus viability and non-applicability for cytotoxic specimens.

Transmission electron microscopy (TEM) of negatively stained faecal or intestinal content samples is commonly used for visualization of RV particles in the intestinal contents or faeces and RV diagnosis (Saif et al. 1991). This technique has the added advantage of demonstrating other infectious agents in cases of mixed enteric infections. Two different staining techniques (positive and negative staining) can be performed to visualize the target. Additionally, there are direct TEM and immune electron microscopy (IEM) (Saif et al. 1991). Immuno-electron microscopy

has greater sensitivity than direct TEM since the specimen is incubated with an antibody specific for the target virus that agglutinates the virus before staining.

Direct or indirect fluorescent antibody tests can be used to demonstrate antigen in cell culture, faecal smears and histological sections of the intestine (Mebus et al. 1969a, b; Woode and Bridger 1975).

Shared, group-specific protein (VP6)/VP6 antibody are used in commercial enzyme-linked immunosorbent assay (ELISA) kits that can be used to screen large numbers of faecal specimens fast. These kits are available at least for human, murine, porcine, or bovine RV detection. ELISA assay may also be used for serological screening, although high antibody prevalence in most populations negates the diagnostic value of this approach. A rapid, highly specific, and sensitive antigen capture ELISA (AC-ELISA) has been developed for detection of porcine RVA, by using VP6 rabbit polyclonal antibodies (capture antibody) and murine monoclonal antibodies. Similar VP7-specific ELISA was developed for detection and G typing of bovine RVA from beef and dairy calves.

Other tests that were used historically for antigen detection include complement fixation, counterimmuno-electrophoreses, radio-immunoassay and agar-gel diffusion. Polyacrylamide gel electrophoresis (PAGE) of viral RNA extracted from faeces or virus propagated in cell culture was commonly used in epidemiological studies, particularly for differentiating between RV groups and as a rapid means of detecting atypical RVs in faecal specimens (Pedley et al. 1986; Bridger et al. 1983; Chauhan and Singh 1992). However, their use is less common nowadays

Based on the various hypervariable regions of outer capsid genes of RV molecular detection tools—hybridization tests have been developed using labelled cDNA probes that could characterize animal RV strains (Parwani et al. 1996). RT-PCR using validated primers designed from RV genes is currently the most widely used assay for detection of RVs in animals (Lee et al. 2003). Additionally, semi-nested or multiplex RT-PCR has been developed and used (Midgley et al. 2012) for the same purpose. RT-PCR is highly sensitive and specific and is suitable for genotyping RV, and it has become a gold standard for RV diagnostics. Methods like sequencing and oligonucleotide microarray hybridization that are sensitive and capable of discriminating mixed RV infections are also available.

8.5.1 Differential Diagnosis

The etiological diagnosis of neonatal calf diarrhoea is difficult (Tzipori 1985). A variety of infectious agents, including RVs, coronaviruses, enterotoxigenic *E. coli*, and cryptosporidia, may cause diarrhoea in neonatal calves. Laboratory assistance is thus necessary to arrive at a diagnosis. Variation in the frequency of RV and coronavirus detection in beef and dairy calves has been demonstrated. The studies showed that coronavirus are more common in beef calves and RV in dairy animals. The differential diagnoses of enteritis in lambs include colibacillosis, salmonellosis, coccidiosis, cryptosporidiosis and adenovirus infections (Theil et al. 1996).

Diarrhoea as a result of RV infection in foals should be differentiated from that caused by other infections such as *E. coli*, Salmonella serovars, *Rhodococcus equi*, *Actinobacillus equuli* and Clostridium spp., as well as foal-heat diarrhoea, nutritional factors and internal parasites.

The clinical signs, lesions and pathogenesis of porcine RV diarrhoea closely resemble those of porcine transmissible gastroenteritis (TGE), porcine epidemic diarrhoea (PED) or porcine delta coronavirus infection caused by coronaviruses but remain less severe. A multiplex RT-PCR has been developed that is reportedly able to differentiate TGEV, PEDV and porcine RVA. Porcine RV diarrhoea should also be differentiated from that caused by *E. coli*, clostridia, coccidia, cryptosporidia, *Brachyspira hyodysenteriae*, internal parasites and nutritional imbalances.

8.6 Porcine Rotavirus

Of the 9 RV genogroups RVA, RVB and RVC are fairly prevalent and associated with large or isolated outbreaks of diarrhoea in piglets (Bridger et al. 1983; Bridger 1987) (Table 8.1). Reported first several decades ago, porcine RVEs are highly uncommon, and their pathogenesis is not studied (Bridger 1987). A newly defined genogroup RVH was confirmed in diarrhoeic pigs in Japan, Brazil and the USA recently. RVHs were shown to be widespread, and their prevalence was shown to increase with age, while RVH association with diarrhoea in nursing piglets needs further evaluation. Porcine RVAs have been widely recognized and well-studied regarding their pathogenicity, compound epidemiology and high genetic diversity.

Based on the VP7 gene segment analysis a significant genetic diversity has been described recently for RVB and RVC porcine strains. Additionally, RVC was shown to be the most prevalent virus associated with diarrhoea in very young piglets.

RVA infection in pigs has been demonstrated in different age groups throughout the world with or without diarrhoea (Kusumakar et al. 2010; Martella et al. 2010; Ciarlet et al. 2008; Papp et al. 2013). The spatio-temporal fluctuations and re-emergence of certain genotypes like G9 and G1 have been reported, but no evidence of seasonal variation on RVA prevalence has been documented which usually ranges from 3.3 to 67.3% (Collins et al. 2014; Martella et al. 2010; Kim et al. 2014; Midgley et al. 2012), with farm-level prevalence reaching 61–74%. A total of 12 G genotypes (G1 to G6, G8 to G12 and G26) and 16 P genotypes (P[1]–P[8], P[11], P[13], P[19], P[23], P[26], P[27], P[32] and P[34]) have been reported in pigs for RVA (Martella et al. 2010; Collins et al. 2014; Papp et al. 2013) (Table 8.1). The most common genotypes circulating in swine population worldwide have been G3, G4, G5, G9 and G11 coupled with P[5], P[6], P[7], P[13] and P[28] (Matthijnssens et al. 2009; Ciarlet et al. 2008).

Similar to RVA, porcine RVCs have also been reported from all over the world (Kattoor et al. 2017; Pedley et al. 1986, Saif and Jiang 1994). Diarrhoea outbreaks associated with RVCs have been documented in nursing, weaning and post-weaning pigs (Saif and Jiang 1994; Chang et al. 1999), either alone or in mixed infection with

Table 8.2 Porcine RVs of different genogroups

Genogroup	Diarrhoea in adult swine	Diarrhoea in piglets	Faecal shedding	Prevalence	Genotypes circulating in pigs
A	No	Yes	Can last beyond 10 days	High	G1–G6, G8–G12 and G26; P[1]–P[8], P[11], P[13], P[19], P[23], P[26], P[27], P[32] and P[34]
B	Yes	Yes	Shorter	High	G4, G6–G21
C	No	Yes	Can last beyond 10 days	High	G1, G3, G5–G10, G12, G13; P[1]–P[7]
E	No	Yes	N/A	Very low	N/A
H	No	Yes	N/A	High	At least 2 I genotypes

other enteric pathogens. High antibody prevalence of 58–100% demonstrates a very high rate of RVC infection may have been present and has circulated for many decades in porcine herds in developed countries (Saif and Jiang 1994). Studies from the USA and Canada on swine samples have revealed a very high rate of prevalence in very young (78%, ≤3 days old) and young (65%, 4–20 day old) piglets. Prototype porcine RVC strains Cowden and HF were initially assigned to RVC genotypes G1 and G3, respectively. Based on different sequence-based reports, RVC strains have been classified into a total of nine G genotypes (G1–G9), seven P genotypes (P[1]–P[7]) and seven I genotypes (I1–I7) (Marthaler et al. 2014). Moreover, majority of porcine RVCs belong to G1, G3, G5–G9 genotypes and a newly described genotype G10 (Table 8.2), while bovine, human and canine RVCs are classified as G2, G4 and G11 genotypes, respectively (Collins et al. 2014). Recently, two provisional G genotypes (G12 and G13 based on the 86% nucleotide identity cut-off value) have been described (Table 8.2).

Due to a difficult adaptive capability of RVB in cell culture, molecular characterization of RVB strains has been hampered (Saif and Jiang 1994). Furthermore, inadequate and inconstant faecal shedding and instability in faeces were shown for RVBs (Chang et al. 1997). In one of the studies from 2000 to 2007 in Japan, VP7 gene of 38 swine RVB strains was analysed and using 67% and 76% nucleotide cut-off values (66% and 79% on the amino acid level, respectively) 5 genotypes proposed were further divided into 12 clusters. An extensive diversity of porcine RVBs based on the analysis of VP7 gene of 68 RVB strains (collected in 2009 from 14 US states and Japan) was suggested (Marthaler et al. 2012) (Table 8.1). Around 20 G genotypes based on an 80% nucleotide identity cut-off value were described and it also provided the first indication that porcine RVB genotypes may be host species- and region-specific and can be disseminated into 17 tentative G-genotypes. Species wise RVB genotypes distribution has shown that G1, G2 and G3/G5 are only found in rats, humans and bovine species, respectively, whereas common porcine genotypes include G4, G7, G9, G13, G15 and G19 reported from Japan. A very small number of swine RVB strains have been associated with genotypes

G10 and G17 which were reported in the USA. In India, a new G21 genotype has been detected in pigs.

In the beginning, strains ADRV-N, J19 and B219 were identified as three human RVH strains, whereas strain SKA-1 was identified as putative porcine RVH strain during 1997–2002. In 2012, three Brazilian porcine RVH strains BR63, BR60 and BR59 were again reported. Marthaler and colleagues demonstrated a surprisingly high prevalence of 15% of swine RVH strains in comparatively old age piglets of 21–55 days (Marthaler et al. 2014). These reports pointed towards a continuous circulation of porcine RVH strains in the US herds since 2002 and also described their distinct evolution from those of human and porcine RVH strains of Brazil and Japan (Marthaler et al. 2014). The novel RVH strain MRC-DPRU1575 identified in South Africa clustered together with the SKA-1 and known porcine strains from USA and Brazil (based on the available gene segments).

Porcine RVE has been only identified in the UK and Australia roughly three decades ago and therefore further data is required to estimate its epidemiological significance (Bridger 1987).

8.6.1 Zoonotic Potential of Porcine RVs

While, historically, RVs were believed to be host-specific, currently porcine, bovine, ovine, pteropine, rodent, avian and insectivore species are suggested to be sources of zoonotic RV infections (Midgley et al. 2012). Reports of some porcine origin G genotypes, G9 and G12, have emerged from human cases which arise due to gene reassortments (Tsunemitsu et al. 2005; Ghosh et al. 2007b, Matthijnssens et al. 2009). Around 10 G genotypes (G1-5, G9-G12 and G26) and 7 P genotypes (P[4], P[6], P[8], P[13], P[14], P[19] and P[25]) of swine-origin have been identified in humans till date out of which few genotypes like G10, G11, G12, G26, P[13], P[14], P[19] and P[25] are found in Asian or African countries only, while the rest are emerging globally. Histo-blood group antigens (HBGA, ABOH, Lewis) and sialic acids receptors are known to interact with different RVA strains via VP4 gene which may provide further insights into the local distribution and increased zoonotic potential of some RVAs of porcine origin, as similar polymorphic HBGAs are also witnessed in pigs (A and H antigens). These observations may provide insights into why P[6] genotype of certain RVA strains (that recognize H antigen) is more frequently transmitted between pigs and humans in different countries, whereas a potent porcine origin genotype P[19] found in humans continues to be restricted in Indian, Asian and African countries which coincides with distinct polymorphisms in Lewis antigens associated with Caucasian and other populations.

Similar to porcine RVA strains, there is growing evidence of porcine RVC zoonotic potential. There have been few reports of human and bovine RVC where porcine origin genes have been reported. Bovine RVC strain WD534tc has been identified to be of possible porcine origin (Chang et al. 1999), whole genome sequencing and analysis of certain porcine RVC strains from Japan have advocated

towards a close phylogenetic relationship between human and porcine RVC strains. Nevertheless, the hypothesis regarding the possible zoonotic transmission of animal RVCs has also been described in view of increased seroprevalence of RVC in humans and high prevalence of RVC in few particular geographical regions where they may attribute towards <5% of gastroenteritis-associated hospitalizations in childhood. Recently, human-like RVC VP6 and VP3 genes were identified in porcine RVC strains identified in India and Japan, respectively. Though, it is important to note that the limited genetic variability of RVCs in humans contrasts with the high genetic diversity currently seen in pigs.

More recently, RVB strains were identified from sporadic cases of infantile diarrhoea in Bangladesh as opposed to adult diarrhoea cases associated with RVB in China and India. Though, the recently reported Chinese RVB strains differed genetically which suggest that human RVB is different. Medici and colleagues provided limited evidence of the zoonotic potential of some RVB strains wherein they demonstrated a high nucleotide identity between human and porcine NSP2 gene sequences.

Apart from the epidemiological data of porcine RVA, information has been scarce concerning porcine RVB/RVC/RVH which is warranted towards the need to control their regional and global zoonotic spread.

8.6.2 Vaccines and Control Strategies

In livestock, based on the induction of active and passive immunity of herds vaccination strategies are planned. Maternal RV vaccines in the field are influenced by strain, vaccine dose, adjuvant, route of administration, inactivating agent and porcine RV exposure levels. Nevertheless, oral vaccines of attenuated RV vaccines given to piglets and calves were often inefficient (Saif and Fernandez 1996). Due to the ubiquitous and endemic nature of RV infections, there has been a need to boost the lactogenic immunity to provide passive antibodies to the newborn with milk and colostrum.

Due to the replication-independent mechanism of genetically engineered VLP vaccines which allows circumvention of maternal antibodies, they are being used as promising tools to boost antibodies in mammary secretions. However, because of the low protective efficacy of such vaccines, priming with live attenuated RV vaccines was deemed necessary. Yet, field application of G5P[7] (porcine RVA OSU) based vaccines or ProSystem porcine RV vaccine (which contained modified live porcine RVA strains of G4P[6] and G5P[7] genotype combinations) have resulted in circulation of porcine RVA with these genotypes and their latest substitution by G9 and G11 genotypes or reassortant G4 and G5 variants. On the other hand, they could generate herd immunity progressively reducing the prevalence of the G4/G5 porcine RVA genotypes and allowing the spread of novel emerging swine RVAs.

8.7 Bovine Rotavirus

Among different infectious diseases in neonatal calves, diarrhoea is a major concern. The aetiology of diarrhoea in bovines is complex, which often involves many infectious agents and a range of other factors like nutritional, immunological and environmental. Many etiological agents, including *E. coli*, *Clostridium*, *Salmonella*, *Cryptosporidium* and *Coccidia* have been suggested to be associated with calf diarrhoea. Among viral agents, RVs, coronavirus (CoVs), norovirus (NoVs), bovine viral diarrhoea (BVDV) have been described to be the most significant contributors to the diarrhoeal disease complex in young calves. Young calves of 2–10 weeks are susceptible to RV disease, and with age progression, adults develop immunity against the virus. The severity of disease and clinical signs are almost similar in all species with symptoms ranging from asymptomatic to severe enteritis. The earliest known documentation of rotavirus and its symptoms in bovine were reported in 1969 in the USA (Mebus et al. 1969a, b) and consequently they were the earliest known RVs to be adapted in the cell culture system (Mebus et al. 1971). Since then many reports emerged describing RV as the causative agent of calf diarrhoea which suggests its global distribution (Woode 1976; Woode and Bridger 1975; Castrucci et al. 1988; Kapikian 1994; Chauhan and Singh 1996; Vende et al. 1999; Bendali et al. 1999; Pisanelli et al. 2005; Alfieri et al. 2006; Ghosh et al. 2007a; Collins et al. 2014; Malik et al. 2016).

8.7.1 Clinical Manifestations

Usually, those calves which have been exposed to the virus via water, milk and feed display the symptoms of diarrhoea. Virus shedding from infected calves causes environmental contamination which in turn becomes pervasive. Grouping of calves in a smaller area also facilitates the transmission over direct contact. It has been observed that the pregnant cattle used to shed the virus during pregnancy which may act as a source of infection for the neonates. Within the second day of infection infected calves start to shed the virus which usually continues for a week and calves under the age of 3 weeks are more susceptible towards the infection (Gomez and Weese 2017). Usually, the infection ceases after 3 months of age in cattle calves, whereas, however, asymptomatic RV infections have been documented up to 6 months of age in buffalo calves and are common in adult cattle.

Additionally, RVB was shown to be associated with diarrhoeal disease in adult cattle as seen with other species (Chang et al. 1997). The incubation period of RV mediated diarrhoea in calves ranges from 12–24 h which may sometimes goes up to 18–96 h. The disease is usually characterized by complications due to secondary pathogens infection in which mortality rate goes up to 80% but usually described to be around 10–20%. Bovine RVs are universal and cause severe diarrhoea by compromising the absorptive capability of an intestinal surface to cause diarrhoea

(Foster and Smith 2009). The diseased calves display varied clinical conditions characterized by diarrhoea, dehydration, increased salivation, loss of appetite and inability to move. Unless secondary bacterial pathogens are present, the faeces are free from mucus or blood. Due to the reoccurrence of diarrhoea exhibited by less colostrum and fluid intake often leads to the death of the calf. The nature of diarrhoea in calves is often malabsorptive, but few studies have also described that a toxin-mediated secretory factor can also be present (Foster and Smith 2009).

8.7.2 Epidemiology of Bovine Rotaviruses

RVs are classified into 10 groups (A–J) based on antigenic relationships of their VP6 proteins. To date, RVA, RVB and RVC genogroups have been described to cause diarrhoea in bovines with varied clinical manifestations.

8.7.2.1 Bovine Rotavirus A (BoRVA)

Out of the different genogroups of RVs described in bovines, RVA accounts for the majority of infections in cattle and buffaloes. Since its first discovery in 1969, where earliest study recorded a high BoRVA prevalence from the USA (98%) and Italy (90%) (Castrucci et al. 1988; Schlafer and Scott 1979). BoRVA infection has been reported globally, which describes its ubiquitous nature. Apart from the North American continent where it was first recorded, incidence and prevalence of BoRVA infection has been widely described from different European countries like England 67% (Woode 1976), Ireland 91% (Reidy et al. 2006), Netherlands 46% (De Leeuw et al. 1980), Italy 90% (Castrucci et al. 1988), Sweden 43.8% (Svensson 1998), France 45.1% (Vende et al. 1999), Switzerland 46% (Luginbühl et al. 2005), Bulgaria 42% (Kharalambiev et al. 1983). In Asia, BoRVA has been reported in India with varied prevalence ranging from 4.3 to 46% (Chauhan and Singh 1996; Malik et al. 2012; Basera et al. 2010; Niture et al. 2009; Saravanan et al. 2006). The low prevalence reports these studies could be attributed to the detection method used (RNA-PAGE) which is considered as less sensitive compared to RT-PCR assay. BoRVA prevalence in other Asian countries includes Japan 16.7% (Fukai et al. 1998), Turkey 41.2% (Gumusova et al. 2007), Bangladesh 7% (Selim et al. 1991), Sri Lanka 68.5% (Sunil-Chandra and Mahalingam 1994). Reports from Latin American countries include Argentina 62.5% (Garaicoechea et al. 2006), Brazil 17% (Barbosa et al. 1998) and Venezuela 11.7% (Ciarlet et al. 1997). Among the Oceanian continents, BoRVA prevalence was 49% in Australia (Tzipori 1985) and New Zealand 13% (Schroeder et al. 1983).

8.7.2.2 Bovine Rotavirus B (RVB)

Apart from RVA, there have been few reports of RVB circulating in the bovine population. However, RVB has been primarily linked to adult diarrhoeal cases. In bovine species, only a few countries have reported the presence of RVB in cattle. Earliest known documentation of non-group A rotavirus has been described in cattle in 1984 and 1987 (Bridger 1987; Snodgrass et al. 1984). In the year 1991, RVB was reported in herds of cows with winter dysentery along with co-infection of CoV, which were detected by IEM (Saif et al. 1991). However, the same group identified RVB of short genome profile in the year 1996 following their identification through IEM (Parwani et al. 1996). Following these few earlier reports of atypical RVs (non-RVA), reports of RVB detection by RT-PCR in bovines emerged from the USA in the year 1994, 1995 and 1997 (Chinsangaram et al. 1995, 1994; Chang et al. 1997). RVB was first detected from Japan in the year 1999 in adult cows followed by its detection in 2001 and 2005 from dairy herds (Tsunemitsu et al. 1999, 2005; Hayashi et al. 2001). In India, RVB detection in bovines was first documented in 2001 through the atypical pattern on RNA-PAGE and also due to the non-reactivity of the virus with antibodies of RVA (Khurana and Pandey 2001), whereas in 2004 sequence report based on VP7 and NSP5 gene was described followed by its occurrence in the Eastern part of India which were named as 'Kolkata Strains' (Ghosh et al. 2007b). This study also highlighted the interstate transmission of RVB strains from adjoining states of West Bengal, India. Serological detection of RVB antibodies has also been reported from Japan and UK from bovine species (Brown et al. 1987; Tsunemitsu et al. 2005).

8.7.2.3 Bovine Rotavirus C (RVC)

Reports on RVC in cattle have been much less common as compared to RVA and RVB. Primarily, RVC has been associated with diarrhoea in porcine and human species. Earlier studies have reported RVC antibodies in cattle (Bridger et al. 1983; Saif and Jiang 1994; Tsunemitsu et al. 1991). The very first characterization was reported from Japan in 1991 when the bovine strain RVC Shintoku was propagated in MA104 cells which were also confirmed by its peculiar electropherotype pattern of 4-3-2-2 on RNA-PAGE (Tsunemitsu et al. 1991). After that, Mawatari et al. (2004) reported BoRVC in six farms in Japan from 2003 to 2010. This study also described the comparative sequence analysis of VP6 and VP7 gene from six bovine RVC strains detected in studied farms in Yamagata. Dual infection of gnotobiotic calves with RVA and RVC was also reported from the USA (Chang et al. 1999). RVC in cattle has been associated with adult diarrhoea which in turn found to influence the milk yielding capacity, thereby decreasing the milk production (Mawatari et al. 2004). Due to a limited number of reports on bovine RVC from restricted geographical areas, the worldwide distribution of RVC in cattle remains

uncertain. Apart from Japan and the USA, a report from South Korea in 2011 described the prevalence of RVC in diarrhoeic calves (Park et al. 2011a).

8.7.3 Genotype Diversity Among Bovine RVs

Similar to other RVs, interspecies transmission and reassortment events are common and generate diverse genotype combinations of bovine RVs. Bovine RVAs mostly belong to genotype G3, G6, G8 and G10, and P[1], P[5], P[6] or P[11] for VP7 and VP4 genes, respectively (Malik et al. 2013a, b, c). The typical genetic backbone of bovine RVAs consists of I2-R2-C2-M2-A3/A13-N2-T6-E2-H3 types (Fig. 8.2) (Martella et al. 2010). Many unusual genotypes for G types (G1–G6, G8, G10–G12, G15, G21, G24) and P types P[1], P[3], P[5], P[6], P[7], P[10], P[11], P[14], P[17], P[21], P[29], P[33] have also been reported from various countries (Abe et al. 2011; Ghosh and Kobayashi 2011; Malik et al. 2016; Masuda et al. 2014; Midgley et al. 2012; Papp et al. 2013; Park et al. 2011b; Reidy et al. 2006). Many common human genotypes like G1 and G9 have also been described in cattle (Blackhall et al. 1992; Kumar et al. 2018). Apart from human-like genotypes, porcine-like genotypes have also been reported from bovines (Ha et al. 2009; Park et al. 2011b).

8.7.4 Diagnosis

Quick detection of the etiological agent is most important to stop the further spread of the disease. RV diagnosis is made by identification and isolation of the virion in faeces of the diseased animal. Similar to other RVs, historically, isolation of bovine RVA has been achieved in monkey kidney cell line MA104. Immunoperoxidase test (IPT), immunofluorescence test (IFT) and the RNA-PAGE gel have been employed to detect the presence of RVs in the faeces of bovine. Few rapid tests like latex agglutination test (LAT), dot-blot assays have also been developed for the early detection of the virus in field conditions (Chauhan and Singh 1992; Hammami et al. 1990; Pirkooh and Shahrabadi 2015). Enzyme-linked immunosorbent assays (ELISA) have been developed by many research groups which are highly sensitive and specific for the identification of RVs. Antigen capture assays have also been developed by improving the ELISA using type-specific antibodies. Further, the use of multiple antigenic peptides (MAPs) for raising Abs against RV antigen has also been found successful in the development of antigen capture ELISA (Kumar et al. 2016). These antigenic peptides are based on the outer capsid protein VP6 of RVs, which is conserved in different species and are produced in abundance during acute infection. Recently, more sensitive tests like PCR and RT-PCR have been the choice of researchers for the detection of RVs in bovine faeces. RT-PCR assays exploiting the use of the VP7 and VP4 gene-based primers are widely used for the diagnosis of RVs (Malik et al. 2013a, b, c). The manipulation of PCR techniques as semi-nested

Isolate Name	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5	Genotype Constellation
MAJOR GENOMIC CONSTELLATIONS OF BOVINE STRAINS												
RVA/Cow/USA/NCDV/1971/G6P[1]	G6	P1	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Reference Strain
RVA/Cow/VEN/BRV033/1990/G6P[1]	G6	P1	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Reference Strain
RVA/Cow/USA/WC3/1981/G6P[5]	G6	P5	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Reference Strain
RVA/Cow/GBR/UK/1973/G6P[5]	G6	P5	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Reference Strain
RVA/Cow/IND/CR231/39/1994/G6P[1]	G6	P1	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Like
BOVINE ISOLATES WITH REASSORTENT GENES												
RVA/Cow/IND/HR-B91/2011/G1P[11]	G1	P11	I2	-	-	-	-	N2	T6	E2	H3	Bovine-Like Human-Like
RVA/Cow/IND/WB-H2/2011/G9P[X]	G9	-	-	-	-	-	A11	N2	T6	Ex	H3	Bovine-Like Human-Like
RVA/Cow/IND/M-1/2009/G3P[11]	G3	P11	I2	R2	C2	M2	A11	N2	T6	E2	H3	Bovine-Artiodactyl-Human-Simian Like
RVA/Cow/JPN/Azuk-1/2006/G21P[29]	G21	P29	I2	R2	C2	M2	A13	N2	T9	E2	H3	Bovine-Simian-Canine-Feline Like
RVA/Cow/JPN/Dai-10/2008/G24P[33]	G24	P33	I2	R2	C2	M2	A13	N2	T9	E2	H3	Bovine-Simian-Guanaco
RVA/Cow/ZAF/1603/2007/G6P[5]	G6	P5	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine-Antelope-Giraffe-Human Like
RVA/Cow/ZAF/1604/2007/G8P[1]	G8	P1	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Bovine-Antelope-Giraffe-Human Like
RVA/Cow/ZAF/1605/2007/G6P[5]	G6	P5	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine-Antelope-Giraffe-Human Like
OTHER SPECIES REPORTED TO HAVE BOVINE LIKE BACKBONE												
RVA/Giraffe/IRL/GirRV/2008/G10P[11]	G10	P11	I2	R2	C2	M2	A3	N2	T6	E2	H3	Bovine Like Giraffe Isolate
RVA/Human/SWE/1076/1983/G2P[6]	G2	P6	I2	R2	C2	M2	A2	N2	T2	E2	H2	Bovine-Human Like Human Isolate
RVA/Human/EGY/Egy3399/2004/G6P[14]	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	Caprine-Murine-Bovine Like Human Isolate
RVA/Human/IND/N-1/2009/G6P[14]	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3	Bovine-Human-Caprine Like Human Isolate
RVA/Goat/ARG/0040/2011/G8P[1]	G8	P1	I2	R5	C2	M2	A3	N2	T6	E12	H3	Bovine-Camelid-Guanaco Like Goat Isolate
RVA/Goat/BGD/GO34/1999/G6P[1]	G6	P1	I2	R2	C2	M2	A11	N2	T6	E2	H3	Bovine-Human-Caprine Isolate

	Bovine
	Simian
	Porcine
	Human
	Giraffe
	Caprine
	Antelope
	Guanaco
	Canine-Feline
	New Genotypes
	Murine
	Camelids
-	

Fig. 8.2 Comparison of genomic constellations of bovine RVA strains and RVA strains having a bovine-like backbone. Individual gene segments of all strains are colour coded based on the maximum homology with the RVA strains available in the public domain

and multiplex RT-PCR have been developed which helped in the G and P typing of various bovine isolates circulating in the field (Luan et al. 2006). Use of microarrays for characterizing the genotype to know the G and P-type has also been described (Aich et al. 2007).

8.7.5 Prevention and Disease Control

Better management and proper hygiene practices are beneficial to reduce the severity of disease in bovine herds. Support of antibiotics to minimize the secondary bacterial infection followed by the administration of electrolyte and fluid intake may help to save the life of calves. Local and mucosal immunity has to be boosted in cows, which is critical in protecting the calves. Colostrum-derived antibodies are crucial in neutralizing the virus in the neonates (Agrawal et al. 2002). Actively acquired mucosal immunity is considered better in comparison to the immunity provided by colostrum-based antibodies. Immunization of pregnant dams before parturition is recommended to supplement the protection levels in neonates (Barrandeguy et al. 1998). Vaccination of pregnant cows through intra-mammary and intramuscular routes could contribute to considerable increase in the titers of colostrum and serum antibodies (Saif and Fernandez 1996). Administration of artificial colostrum, whey protein and vegetable oils are also given as an alternative measure to strengthen the immunity of calves (Murakami et al. 1986). Chicken egg yolk immunoglobulins have also been found beneficial in defending neonatal calves from RV mediated diarrhoea.

8.8 Avian Rotaviruses

Most significant viruses involved in enteric diseases in avian spp. include rotavirus, astrovirus, calicivirus, adenovirus and coronavirus (Farkas and Jiang 2009). Apart from mammalian spp., rotaviruses (RVs) are an important cause of gastroenteritis in a wide variety of avian species (Guy 1998). The first record of avian RV dates back to 1977, when it was identified as a potential cause of enteritis in turkey poults (Bergeland et al. 1977) and was later identified in chickens (McNulty et al. 1978). Since then, avian RVs have been described in several avian hosts including pheasants, ducks, pigeons, wild birds, etc. (McNulty et al. 1978; Takehara et al. 1991; Legrottaglie et al. 1997). To date, RVs have been reported in many countries including USA, UK, Europe, Russia, Argentina, Brazil, China, Bangladesh and India. In field conditions, avian RVs may induce subclinical manifestations, or they may be associated with diarrhoea, dehydration, anorexia, low weight gain and increased mortality where dehydration is the major contributor to mortality (Tamehiro et al. 2003). Recently, avian RVs have been reported as one of the causes of running and stunting syndrome (RSS), a major syndrome having a destructive

impact on the poultry industry. Rotavirus infection in turkeys was found to be associated with poult enteritis syndrome (PES), along with other enteric pathogens. Although RVs cause enteric disease, they have also been reported from healthy asymptomatic flocks of chickens (Bezerra et al. 2014). Avian RVs evolved early from their mammalian counterparts (Mori et al. 2002; Trojnar et al. 2010). Thus far, avian RVs are under-investigated as compared to mammalian RVs. Limited literature is available on these dsRNA viruses of avians. However, epidemiological studies have shown their presence worldwide. Phylogenetic studies of the available sequences of avian RVs show geographical segregation of different species (Kattoor et al. 2013). An in-silico analysis on avian RVs based on VP6 gene confirmed biasness in the codon usage for the host as well as for geographical locations (Kattoor et al. 2015).

8.8.1 Classification

Based on antigenic relationships of VP6 proteins used to classify RVs, four species (RVA, RVD, RVF and RVG) have been described in poultry (Otto et al. 2006; Johne et al. 2011). Of these, RVD, RVF and RVG are solely found in poultry (Otto et al. 2012). Among avian RVs, most detected groups are RVA and RVD, while RVF and RVG are less frequent. Genetic reassortment occurs within each species of RVs but never between the members of different serogroups. Each serogroup in RVs is considered as a unique species (Estes and Greenberg 2013). Two clades can be constituted based on the phylogenetic analysis of RVs, rotavirus A/C/D/F (Clade1) and rotavirus B/G/H (Clade2) (Kindler et al. 2013) owing to the diverse nature of RVs.

8.8.1.1 Avian Rotavirus A (RVA)

Rotavirus A infections are most common and most abundant among all the avian RVs. These are well characterized as compared to RVD, RVF and RVG infections. Analysis of the VP7 gene of avian RVA facilitated the classification of available strains into five different G genotypes, G7, G17, G18, G19 and G22 (Ursu et al. 2009). As shown by the studies conducted in rats, avian RVs have the capacity to disseminate in various organs, such as the liver, spleen and pancreas; however, the mechanism by which RVA escapes the gastrointestinal tract remains unknown (Crawford et al. 2006). This has been confirmed by a study conducted in 2016, where avian RVAs were detected from pancreas and spleen of broilers with RSS (Nuñez et al. 2016). Although, the reactivity of monoclonal antibodies with VP6 protein indicates a common classification for avian and mammalian RVs (Minamoto et al. 1993), the electrophoretic (PAGE) mobility pattern of genome segments tells a different story. Electropherotype of avian RVA (5:1:3:2) is different from mammalian RVA (4:2:3:2) wherein the major differences have been observed in the fifth

genome segment. In mammalian RVAs fifth segment migrates close to fourth, but in avian RVAs, it migrates close to the sixth segment (Wani et al. 2003). The first avian RV to be sequenced belonged to group A which was isolated from a pigeon (strain PO-13) with a length of 18,845 nucleotides (Ito et al. 2001). So far, only a handful of genome sequences of avian RVAs (as compared to mammalian RVAs) are available which impair the in-depth molecular characterization of avian RVA (Ito et al. 2001; Trojnar et al. 2013). The antigenic structure of NSP4 and VP6 genes of PO-13 (pigeon) strain of avian RVA has been determined using monoclonal antibodies (Minamoto et al. 1993; Borgan et al. 2003). Avian RVAs have been experimentally transmitted to mice, but the natural transmission seems rare with one report of transmission of avian RVA to calf under field conditions (Brüssow et al. 1992). A report (based on the electrophoretic migration pattern of RNA segments) is available describing the presence of mammalian like RVA in chickens suffering from diarrhoea (Wani et al. 2003). Available data suggest that ancestral strain of avian RVA and RVD has undergone reassortment based on NSP1 encoding gene segment as RVD was found to possess RVA like conserved termini (Trojnar et al. 2010; Matthijnssens et al. 2011; Kindler et al. 2013). Thus, a reassortment event can be predicted. Although interspecies transmission and reassortment have been elucidated for avian RVs (Schumann et al. 2009), detailed studies are required to comment on the cross-species transmission of avian RVA as well as to understand the origin of unusual mammalian strains of RVs.

8.8.1.2 Avian Rotavirus D (RVD)

Initially, RVD was described as virus 132 or D/132 in chickens and rotavirus-like viruses (RVLV) in poultry (McNulty et al. 1981; Pedley et al. 1986; Reynolds et al. 1987), based on the electrophoretic mobility pattern (4:2:2:2). Like other RVs, RVD causes diarrhoea, growth retardation, anorexia, etc. However, it also has a role in causing stunted growth, which was described early in 1994 and was further supported by Otto and co-workers in 2006 where they reported the importance of RVD in the pathogenesis of RSS in chicken flocks with severe villous atrophy (Otto et al. 2006; Roth 2016). The occurrence of RVD has also been reported in apparently healthy asymptomatic chickens (Bezerra et al. 2014). Epidemiological studies suggest the presence of RVD in European countries (Germany, Sweden, Scotland, etc.), Egypt, Asia (India, Bangladesh), Brazil and Nigeria (Ahmed and Ahmed 2006; Otto et al. 2012; Hemida 2013; Pauly et al. 2017). Over the last few years, an increase in the frequency of RVD infections has been observed in some geographical locations based on the molecular-based assays (Bezerra et al. 2014; Deol et al. 2017). Moreover, the detection rates of RVD are higher than RVA at some geographical locations (Otto et al. 2012). In turkeys and chickens, RVD has been designated as the most common and the most frequently occurring RV infection, respectively (Otto et al. 2012). Although structurally RVD is somewhat similar to other RVs, but a slight variation is found in gene-protein coding assignments. For example, in RVD, VP4 and VP3 proteins are encoded by segment 3 and 4, respectively, but for RVA

vice versa is true. The molecular studies on RVD are scarce, and only a single whole genome sequence is available for this species (Trojnar et al. 2010). Hence, unlike RVA, no genotype classification system is available for RVD. Maximum no. of the sequence is available for the VP6 gene, based on which geographical segregation of RVD isolates is presumed (Kattoor et al. 2013). However, to confirm such studies, sequencing information on the other genes of RVD is required as well.

8.8.1.3 Avian Rotavirus F (RVF) and Rotavirus G (RVG)

Avian RVF was first described in turkey faeces and RVG in gut contents of chickens from Northern Ireland, based on the electrophoretic (PAGE-electrophoresis) migration pattern of their genome segments (Theil et al. 1986). Electrophoretic mobility patterns of 4:1:2:2:2 and 4:2:2:3 were described for RVF and RVG, respectively. Later, both of these serogroups were identified from turkeys (Kang et al. 1988). Although the association of these serogroups with clinical disease is still unclear, they have been described as one of the causative agents of RSS in broiler chicks (Otto et al. 2006). Epidemiological studies of RVF and RVG in chickens and turkeys have been carried out, where low incidence and frequency was noted, as compared to other avian RVs (RVA and RVD) (Otto et al. 2012). Currently, these groups may be termed as rare RVs in poultry; the reason behind this rationale might be the lack of more robust diagnostic tools. Not very long ago (in 2012), the complete genome sequences of RVF (03V0568-18,341bp) and RVG (03V0567-18,186bp) from diarrhoeic chickens were deciphered, having ORFs for viral proteins VP1-VP6 and NSP1-NSP5 within the 11 segmented genomes. This opened the opportunity to study RVF and RVG at the molecular level. Based on the phylogenetic analysis, RVF belonged with A/C/D clade, and RVG belonged to B/G/H clade (Ogden et al. 2012). These clades are defined based on all the structural proteins (VP1-VP6) and two nonstructural proteins (NSP2 and NSP5) (Kindler et al. 2013).

8.8.2 Pathogenesis

Viruses, bacteria and parasites are other common pathogens detected alongside avian RVs. Avian RVs have been known to be linked with intestinal illness in commercial poultry, but their particular role in the pathogenesis of diseases has not yet known completely (Falcone et al. 2015). RVs mainly infect mature villous epithelium of small intestine, resulting in impaired absorption. Apart from the intestine, avian RVs also multiply in caecum and colon (McNulty et al. 1983). Infection of avian RVs cannot be prevented even in the presence of maternal antibodies, although the latter may delay the establishment of infection in chickens (Yason and Schat 1986). In experimental infection, RVs were found to cause watery diarrhoea in turkey poults, whereas in chickens no clinical diarrhoea was observed. Like RV infection of gnotobiotic pigs, calves, etc., pathological studies on turkey

RVs (in SPF turkey poults) also confirmed the increase in cellular activity in the infected cells (lamina propria) with the predominance of mononuclear cells. In turkeys and chickens, the differences in clinical manifestation of RVs might be due to physiology, immune status, etc., but replication and antibody development strategies in both the species of poultry were described to be similar (McNulty et al. 1983).

8.8.3 Diagnosis

Avian RVs can remain asymptomatic or can cause diarrhoea, dehydration, anorexia, reduction in growth rate, etc. These clinical manifestations are not sufficient to differentiate RV infections from other enteric pathogens. For confirmatory diagnosis, virus detection or viral antigen/antibody detection is required. Apart from these techniques, PAGE (polyacrylamide gel electrophoresis) being rapid and easy was also used satisfactorily in the past, to detect avian RVs and also to classify them based on typical electrophoretic migration patterns. However, distinct patterns may arise because of events like recombination, mutations, etc. Therefore, PAGE cannot be used as a definitive tool for classification of different strains. Apart from EM, virus isolation and PAGE, serological assays including ELISA, latex agglutination test, etc. have been used for detection of avian RVs (Dhama et al. 2015). At present, the most sensitive diagnostic tool for detection of avian RVs is reverse transcriptase PCR (RT-PCR), but only a few protocols are available, mostly for RVA and some for RVD (Table 8.3) (Bezerra et al. 2012). A multiplex RT-PCR has been developed that can differentiate avian RVs from other viruses causing enteric infections (Jindal et al. 2012). From time to time, different detection systems with different sensitivity and specificity were used to know the status of avian RVs which lead to variable estimation of their prevalence, so better optimized molecular assays for all the group of avian RVs should be made available shortly.

8.9 Ovine Rotaviruses

Sheep is one of the essential resources in agriculture worldwide; however, reports about ovine RV strains still scarce. First evidence regarding RV infection in diarrhoeic lambs came from the United Kingdom and Japan (Theil et al. 1995). Since then, several other countries have attempted to characterize RV strains and ascertain prevalence in sheep. So far, RVA and RVB have been the only two groups of RVs that were detected in lambs. In the 1980s and 1990s, RVB was detected in some outbreaks of neonatal diarrhoea among lambs in the USA and the United Kingdom (Snodgrass et al. 1984; Theil et al. 1995). Morbidity in the above

Table 8.3 Primers and probes for RT-PCR and real-time RT PCR amplification of RVD and avian RVA

Name	Gene targeted	Sequences (5′ to 3′)	Amplicon size (bp)	Assay	Reference
RVD					
RD6F RD6R	VP6	GGAGGCGCTGTCTTCAATTGCG TGGCCAATAGTGTGTGGCAGCT	742	RT-PCR	Bezerra et al. (2012)
RD9F RD9R	VP7	ACCATATAGGAGTGACGCACCT AGCCCACCACTTCTTCCAAT	879	RT-PCR	Bezerra et al. (2014)
ARVD6-1F ARVD6-1R ARVD6probe1	VP6	GCGACAACTGAG ACAACTG GGAAGCAGTTGTCATCAAC TTGCATATTAGATTGTCTCGCTGG TGTATA	186	Real time RT-PCR	Otto et al. (2012)
RVDVP6-D-F RVDVP6-D-R	VP6	GCTATACATTTCGCTGCATTTG TGGCCAATAGTGTGTGGCAGCT	185	RT-PCR	Kattoor et al. (2013)
Avian RVA					
RT-1 RT-4	VP6	GGCTTTTAAACGAAGTCTTC GGTCACATCCTCTCACT	1350	RT-PCR	Ito et al. (2001)
NSP4 F30 NSP4 R660	NSP4	GGGCGTGCGGAAAGATGGAGAAC GGGGTTGGGGTACCAGGG ATTAA	630	Multiplex RT-PCR	Jindal et al. (2012)
ARVA6-1F ARVA6-1R	VP6	CACCACGACTTATGCAGAGA CTCCGAATGGATGCT ACTGT	493	RT-PCR	Otto et al. (2012)
ARVA6-9F ARVA6-9R ARVA6probe3	VP6	GAGCAACTATTGATTACTTCATTGA AAAGTTGCCTTARTGCATTAGA AGGAGCTATTCCATTACGTTGAGATTC	114	Real time RT-PCR	Otto et al. (2012)

outbreaks varied between 50% and 100%, and the mortality rate ranged from 10% to 50%. All the samples taken from the infected lambs were positive to RVB.

The following four RVA strains in sheep were characterized in the United Kingdom: G3P[1], G6P[11], G9P[8], G10P[14] (Fitzgerald et al. 1995). In China, exclusively one genotype was found in the examined RVA strains over the years, the G10P[15] (Shen et al. 1993; Chen et al. 2009; Zhang et al. 2011). Out of these strains, two were sequenced entirely (Lamb-NT, CC0812). In Spain, two strains were identified, G8P[14] and G8P[1] which is considered to be the causal agent of an ovine diarrhoeic syndrome outbreak, where the mortality rate was 17% (Ciarlet et al. 2008; Galindo-Cardiel et al. 2011). In India, one exhaustive study (500 samples) revealed numerous circulating RVA G and P genotypes (total 52 strains). Among the two observed G genotypes, G6 was predominant (48%) followed by G10 (36%). The only VP4 gene found was the genotype P[11], and few samples carried mixed genotype G6+G10 (Gazal et al. 2012). In Greece, out of three RVA positive samples, one was G10P[8], and the two others were untyped (Chatzopoulos et al. 2016). The extant characterized ovine RVs show a high genetic heterogeneity, as most strains have their different G and P genotype combinations.

Apart from the genotypes of ovine RVA and RVB strains have been analysed already, several other studies have been focused on attaining information about the prevalence of rotavirus infection in sheep. The RVA detection rates reportedly are highly variable in samples from diarrhoeic sheep. The first large dataset was obtained in North West Spain, where neonatal diarrhoea has been considered as the major health problem affecting lambs. However, this study detected a low prevalence of RVs, 2.1% in diarrhoeic lambs and 6.5% in the case of the outbreaks (Muñoz et al. 1996). Further comprehensive research showed a higher RVA prevalence (60%) in diarrhoeic lambs in Trinidad. A study on the role of RVs in diarrhoea and estimating the successfulness of the used diagnostic tests were conducted in Egypt, where the adjusted prevalence of RVs was 16.1% among the infected lambs (Khafagi et al. 2010). Currently, multiple extensive analyses have been made in India and depending on the diagnostic assay utilized, RVA prevalence varies from 0.3% to 13.2% (Gazal et al. 2012; Singh et al. 2017). In Greece, 2.5% RV prevalence was recorded in two different monitored flocks (Chatzopoulos et al. 2016). Kingdom of Saudi Arabia has few publications concerning the cause of diarrhoea in farm animals. So far, one overall study has revealed a relatively high prevalence of RVs (31.7%) (Shabana et al. 2017).

8.10 Caprine Rotaviruses

Similar to the ovine RVs, detailed information on the epidemiology of caprine RVs is unavailable. RVA and RVB infections were first described in diarrhoeic goats in the 1980s. One of the earliest reports aimed to make preceding characterization efforts of RV strains detected from South Africa. Around this time, another extensive report about RVA infection in livestock came from Trinidad; the results were two

positive RVA samples from four diarrhoeic goat kids (Kaminjolo and Adesiyun 1994). In Spain, several major studies were conducted, where seven goat kids were affected by severe diarrhoea in a dairy herd, and five of them proved to be infected with RVB (Muñoz et al. 1995). Another investigation identified RVA in three goat kids (8.1%), RVB in five goat kids (13.5%) and for the first time RVC in four non-diarrhoeic goat kids (Muñoz et al. 1996). A 2-year study conducted in France examined eight faecal specimens from goat kids, and seven of them were detected positive for RVB (Gueguen et al. 1996). Faecal specimens (8.68%) were found to be RV positive by electrophoresis in a survey handling with high sample numbers ($n = 484$) in Bangladesh, but no further examination was made for specifying them (Dey et al. 2007). In Egypt, overall, 13.2% of goat kids were positive for RV based on parallel diagnostic tests (Khafagi et al. 2010). In Sudan, a wide range of survey regarding rotavirus infection was taken including several livestock species. 21.7% of the sampled goat kids were positive for RVA (Ali et al. 2011). During a large outbreak of enteritis in Turkey, high morbidity (45%) and mortality (28.2%) rates were detected. Commercial ELISA identified RVA in four stools of goat kids (Alkan et al. 2012). Two recent extensive studies were executed in Asia; both had determined the prevalence of rotavirus infection among sheep and goats. The prevalence of caprine RVA was 8% and ~27% in India and Medina, respectively (Singh et al. 2017; Shabana et al. 2017).

G6P[1] genotype combination was detected in two RVA strains in Italy, and G3P [3] was found in a Korean goat (Pratelli et al. 1999; Lee et al. 2003). During an enteritis outbreak in Turkey, one strain proved to carry genotype G8P[1] (Alkan et al. 2012). The first full genomic analysis of caprine RVs were made in Bangladesh, and it revealed genotype G6P[1] RVA strain (Ghosh et al. 2010). In Argentina, several samples were found to be positive for RVA (4/20) and one of them was genotyped as G8P[1] (Louge Uriarte et al. 2014). A study investigating the possible transmission of RVAs among human and domestic animals describes the G6P[1] genotype combination. The whole genome was sequenced for this strain as well (Bwogi et al. 2017). According to GenBank records additional unpublished genotyped RVA strains are available, such as G6P[14] from South Africa, G6P [1] from Turkey, G10P[15] from China and G8 from India. Further record data comes from strains found in Morocco, where two strains are genotyped as G10 or G6 in combination with P[14].

In the case of the RVB strains, one complete and one partial genomic sequences are available in the literature. One of them was obtained from pooled samples that were collected from two diarrhoeic goat kids in Minnesota; the other strain came from a single Californian goat. The whole genome sequence showed the following conserved genome constellation: G3-P[3]-I3-R3-C3-M3-A3-N3-T3-E3-H3 (Chen et al. 2018). The incomplete one presents the same constellation except for the VP1 gene and VP3 gene, which could not be retrieved as the viral read count was low (Shepherd et al. 2018).

8.11 New-World Camelids Rotavirus

The llama and the alpaca are the domesticated species of South American camelids. The others, the guanaco and the vicuna, are the wild-living species. The llamas and guanacos appear to be highly susceptible to RV infection based on the early reports. A serological survey in Argentina showed 87.7% prevalence of RV antibody in the collected llama sera from different provinces. Compared to the RVs, the other investigated viral antibody rates were low (Puntel et al. 1999). In Patagonia, guanacos affected by a severe outbreak of diarrhoea (100% morbidity and 83% mortality) were screened for RV antigen and antibody. Ninety-five percent of the collected serum samples were positive for RV antibodies. Also, two RV strains were isolated from the young guanacos with acute diarrhoea during the sampling time and were determined as RVA (Parreño et al. 2001). The prevalence of RV antibodies was 100% in another study involving 11 wild-born guanacos from Patagonia (Marcoppido et al. 2011). The first studies investigating the cause of diarrhoea in wild vicunas were released relatively late compared to the ones mentioned above. RV antibodies have been seen in free-living vicunas, llamas and domestic cattle. The entire serum sample found to be positive, but RV shedding was not observed. In Peru, alpacas suffering from a diarrhoea outbreak were tested for several infectious pathogens and 32% of the animals were shown to be infected with RV (Rojas et al. 2016a).

G8P[1] and G8P[14] genotype combinations were reported in young guanacos for the first time in Patagonia (Parreño et al. 2004). Seven more RVA strains found in guanacos were found to be genotype G8P[1] in Argentina (Marcoppido et al. 2011). Several RVA strains found in alpacas were described in Peru, such as G8P[1], G8P [14], G3P[14], G3P[11], G3Px-, G3P[40] and G35P[50] (Badaracco et al. 2014; Garmendia et al. 2015; Rojas et al. 2016b). RVA in wild vicuna was typed as G8P [14] (Badaracco et al. 2013). The complete genome of RVA strains found in most of the alpacas and the vicuna has been reported.

8.12 Old-World Camelids Rotavirus

Camels are essential livestock species either in economy or culture in the African, Arabian and Asian (semi-) deserts (Burger 2016). Despite the increased number of statements about the high incidence of diarrhoea that induced mortality among <6-month-old calves, relatively few studies were concerned with the determination of the causative agents.

Each of the published reports originates either from Africa (Sudan, Egypt) or Arabian Peninsula (Saudi Arabia, Kuwait). The first detailed evidence associated with camel diarrhoea was published in Sudan. During a 2-year (2000–2002) surveillance study which covered a wide range of Sudan, faecal and serum samples were collected from diarrhoeic, healthy and recovered calves. The average

prevalence of RVA in faecal samples analysed by several diagnostic tests was ~20% (Ali et al. 2005a, b). The detection rate of RVA was higher in serum samples (48.1%) (Ali et al. 2005a, b). In another study in Sudan, RVA was detected in 6% (3/50) of the samples collected from diarrhoeic camel calves (Ali et al. 2011). One report came from Egypt, where 8 out of 85 faecal samples were positive for RVA by ELISA (Eman et al. 2009). A study from Kuwait during 2008–2010 in five camel farms and a subsequent sample set collected in 2010 were monitored for RVs by antigen detection kits and RT-PCR based on the VP6 gene, respectively. The RV detection rates were 0.2% (1/408) and 7.3% (8/109) (Papp et al. 2012). Six different parts of Saudi Arabia were surveyed in order to ascertain the viral and bacterial agents causing diarrhoea in camel calves. Depending on the diagnostic test utilized, the prevalence of RVA varied from 13.3% to 18.7% (Al-Ruwaili et al. 2012). One other region of Saudi Arabia showed 6% RV prevalence among diarrhoeic calves (El Wathig and Faye 2016). In the Eastern part of Saudi Arabia, RVA was recorded in 10% and 12% of samples detected by IC and ELISA, respectively (El-Sabagh et al. 2017).

So far, characterization of RVA genotypes in camels has been minimal. Sequencing of the VP7 gene revealed genotype G10 in two isolates from Egypt and one strain from Kuwait (Eman et al. 2009; Papp et al. 2012). Partial VP4 sequence of the latter strain was later identified as P[15]. To date, the whole genome sequence of only one African camel RVA strain was described (G8P[11]) (Jere et al. 2014).

8.13 Rotaviruses in Wildlife Hosts

Species, especially domesticated animals, holding a sort of benefit for the nations have been the main focus of RV infection surveys. Although some reports about RV strains in wild hoofed mammals are available. Some of the accessible information arises from unique cases that have occurred in zoos, zoo nurseries or commercial farms of wild animals. Others reported by studies investigating reassortment events among RV strains of different host species. The first evidence in wild ungulates came from a zoo nursery in the USA, where infants (an impala, an addax and a Thomson's gazelle) were infected by a bovine RV (Eugster et al. 1978). In another zoo in Toronto, an exhaustive study was conducted to test sera of several species for RV antibodies (Petric et al. 1981). A review about diseases of farmed wild animals mentioned RV infection either in red deer (*Cervus elaphus*) or wapiti (*Cervus elaphus* subspp.) (Haigh et al. 2002). The whole genome sequence of two RVA strains detected in Slovenian roe deer (*Capreolus capreolus*) were determined (G6-P[15]-I2-R2-C2-M2-A3-N2-T6-E2-H3; G8-P[14]-I2-R2-C2-M2-A3-N2-T6-E2-H3) (Jamnikar-Ciglenecki et al. 2016, 2017). In Korea, out of 60 samples collected from water deer (*Hydropotes inermis*) one was positive for RVs by RT-PCR (Kim et al. 2014). RV strain was reported in a giraffe suffering from acute diarrhoea and was genotyped as G10P[11] and was shown to be closely related to bovine RVA strains (Mulherin et al. 2008). Several years later the whole genome was sequenced

of this giraffe RVA strain (G10-P[11]-I2-R2-C2-M2-A3- N2-T6-E2-H3) (O'Shea et al. 2014). The complete genome of RV strain found in a South African sable antelope was also sequenced (Matthijnssens et al. 2009). In India, several studies have investigated diarrhoeic buffalo calves. In Western India, 12.5% of the stool samples were positive for RV, and all of them characterized as RVA (Niture et al. 2011). A surveillance study in Mumbai demonstrated the prevalence of RVA similar to the abovementioned (11.8%) (Mondal et al. 2013). In studies conducted in North India, RVA was detected in 4.6% or 10.7% of the faecal samples (Manuja et al. 2008). Some of the observed RVA strains in buffaloes were further analysed and revealed the following genotype combinations: G10P[11], G6P[11], G10P[3].

8.14 Conclusion and Future Perspectives

The high diversity and fast evolution rate of RVs indicate a need for continuous research on molecular characterization, geographical distribution and temporal fluctuations of endemic and emerging RVs. There have been few reports of some unusual G and P RVA genotypes with new RV groups being discovered in different geographic locations and the increasing evidence of high RV prevalence points towards the need to update the molecular diagnostic and characterization toolkits to include the novel RV variants which will ensure accurate epidemiological monitoring (Prasad et al. 2005). Discovery of diverse RVs in various wildlife species indicates that they can serve as natural reservoirs further contributing to the genetic diversity of RVs. While zoonotic and inter-species transmission potential has been demonstrated for bovine and porcine RVs, it was not evaluated for other wildlife and livestock species. A better understanding of RV molecular pathogenesis and immunity is needed to optimize the existing vaccines and improve control of RV infections and spread. Recent research on human and porcine RVAs raised the awareness that attenuated replicating RVA vaccines may be contributing directly to the genetic diversity of RVAs (via reassortment between vaccine and wild type strains) and the emergence of novel genetic variants/RV genogroups that can evade herd immunity against the vaccine strains. Thus, alternative approaches including wide-scale use of probiotics or antivirals, to lessen the RV shedding and decrease the environmental contamination, and to ease porcine RV-mediated intestinal damage are needed. Certain genogroups/genotypes have increased ability to re-assort and cross the interspecies barrier more frequently than other therefore additional studies to decipher their role in infection is needed. In addition to the knowledge of interactions between different porcine RV genotypes with the histo-blood group, antigens are also warranted.

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Chapter 9
Capripoxvirus and Orf Virus

Gnanavel Venkatesan, Amit Kumar, V. Bhanuprakash, V. Balamurugan, and Raj Kumar Singh

Abstract Capripox infections of small ruminants, namely goatpox and sheeppox, are OIE notifiable and transboundary animal diseases. Goatpox and sheeppox are prevalent in some parts of Africa, the Middle East, and Asia with occasional outbreaks in regions of Europe. The etiological agents, goatpox virus (GTPV) and sheeppox virus (SPPV), are indistinguishable serologically. However, they are differentiated by some of the molecular techniques. The diseases are characterized by fever, papules, nodular lesions on the skin, and sometimes internal organs and lymphadenopathy with high morbidity and mortality in affected animals. Contagious ecthyma (orf) is an economically important contagious disease of sheep, goat, and other ruminants with worldwide distribution. It is a local eruptive skin disease characterized by proliferative lesions on mouth and muzzle. The disease has zoonotic importance causing localized lesions in humans. The orf virus (ORFV) is the causative agent of this skin infection belongs to the genus Parapoxvirus. Also, it possesses the capacity to re-infect the host due to its epitheliotropic niche and encoded immunomodulators. Goatpox, sheeppox, and orf infections pose serious economic threat to the agricultural sector and livelihood of the farmers in endemic regions with a major impact on international trade. A prompt diagnosis along with well-planned vaccination and effective bio-security measures are main control measures to contain the infection in any endemic region. Development of recombinant protein-based serodiagnostic assays and rapid pen-side diagnostics that allow

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differentiation of GTPV, SPPV, and ORFV is the need of the hour for improved disease control.

Keywords Capripox infections · Goatpox virus (GTPV) · Sheeppox virus (SPPV) · Parapoxvirus · Contagious ecthyma · Genome · Diagnosis · Epidemiology

9.1 Prologue

Sheeppox and goatpox are collectively known as capripox, and orf are the pox viral diseases of mainly sheep and goats with high socio-economic threats (Malik et al. 1997; Bhanuprakash et al. 2011; Kumar et al. 2015; Madhavan et al. 2016). The causative agents, sheeppox virus (SPPV) and goatpox virus (GTPV), belong to genus *Capripoxvirus*, whereas orf virus (ORFV) belongs to genus *Parapoxvirus* of the family *Poxviridae* (Bhanuprakash et al. 2006; Hosamani et al. 2009; Kumar et al. 2015). Under mixed farming, where sheep and goats population are farmed together in a single platform, more than one or two viruses infect the same host in a simultaneous manner. The mixed infections of capripoxviruses (CaPVs) and ORFV or any other infectious agents may increase the severity of either of those infections (Hosamani et al. 2004a; Chu et al. 2011). Although the clinical sheeppox disease has been reported in first century A.D., the virus was first isolated by Borrel in 1902, whereas goatpox was reported from Norway by Hansen in 1879 (Rafyi and Ramyar 1959). Despite orf infection is long known in small ruminants by shepherds but was first described in 1787 by Steeb (Robinson et al. 1982) followed by elucidation of its contagious nature in 1890. Of late in 1923, the etiological agent to be different from vaccinia virus-based on physicochemical and immunological characteristics has been identified. Earlier classification of sheeppox and goatpox (SGP) based on animal species of origin has now been replaced by molecular methods of differentiation (Hosamani et al. 2004a; Venkatesan et al. 2012a, 2014a, b). Both sheeppox (SP) and goatpox (GP) infections are endemic in the Indian subcontinent, Central Asia, Middle East, and Africa, whereas orf is endemic in almost all parts of the world.

SPPV and GTPV cause similar kind of generalized disease showing the pyrexia, oculo-nasal discharge with typical pox nodules on the skin and internal mucosa (Babiuk et al. 2008) and the outbreaks are linked to a significant production loss due to high morbidity, a decrease in weight gain, damage to hide and wool, and trade barrier (Tuppurainen et al. 2017). In contrast to SGP infections, orf is a self-limiting skin disease causing typical localized proliferative cutaneous lesions over mouth and lips in sheep, goats, and also other wild ruminants. Both SP and GP are notifiable to OIE as the morbidity and mortality may be very high, up to 100% in naïve animals (Bhanuprakash et al. 2006). Orf is often considered as opportunistic pathogen associated with other viral diseases, especially *Peste des petits ruminants* (PPR) and Capripox infections (Hosamani et al. 2009).

Further, it is economically significant owing to its endemicity, ability to emerge in other host species, zoonotic potential, and occurrence of mixed infections (Hosamani et al. 2009). The disease has been described under various names viz., contagious pustular dermatitis, contagious ecthyma of sheep, sore mouth, scabby mouth, contagious pustular stomatitis (Nandi et al. 2011). Parapoxviruses (PPVs) including ORFV have unique ability to re-infect the same host, under the encoded immunomodulators subverting the host immune response (Hosamani et al. 2009) leading to short-term immunity as compared to other poxviruses. This book chapter describes the epidemiology of GTPV, SPPV, and ORFV infections and its molecular characterization, antigenic properties, host range, pathogenesis, clinical disease, diagnosis, and prevention/control measures.

9.2 Etiological Agents: Morphology and Genome Organization

SPPV and GTPV belong to genus *Capripoxvirus* of the family *Poxviridae*. Other member of the genus, lumpy skin disease virus (LSDV) affects cattle. Most of the SPPV and GTPV isolate show host-preference (Madhavan et al. 2016). The ORFV is the prototypic member of the genus *Parapoxvirus* belonging to the family *Poxviridae* (Nandi et al. 2011). Other important members in this genus are pseudocowpox virus (PCPV), bovine papular stomatitis virus (BPSV) of cattle, and parapoxvirus of red deer in New Zealand (PVNZ) of which except the last member, all are reported to be zoonotic. CaPVs are brick-shaped with complex symmetry and 300 × 270 × 200 nm size. The CaPV genome consists of covalently linked double-stranded DNA of 150–160 kbp length with inverted terminal repeats at the ends (Tulman et al. 2002). PPVs have several unique characteristics like distinct virion morphology, high G-C content, and presence of genes coding for immunomodulatory proteins. ORFV has a characteristic ovoid shape with 260 × 160 nm size. Genome is linear double-stranded DNA (134–139 kbp) with high G + C (63–64%) content in comparison to other poxviruses and encodes 132 proteins (Delhon et al. 2004).

The genomes of CaPVs appear to be more divergent as seen in orthopoxviruses (OPVs) in both sequence and size towards their termini (Madhavan et al. 2016). The genome possesses highest A-T content (73–75%) among poxviruses and encodes ~150 proteins. Both SPPV and GTPV share 96% nucleotide identity over the entire length of the genome and they share 97% similarity with LSDV (Tulman et al. 2002). All genes that are present in SPPV and GTPV also present in LSDV. However, there are nine genes that are intact in LSDV associated with virulence and host range functions, are fragmented in SPPV and GTPV genomes (Tulman et al. 2002). GTPV is more closely related to LSDV than to SPPV and they might have evolved from a common SPPV like ancestor (Hosamani et al. 2004a; Le Goff

et al. 2009). Several genes including P32, RPO30, and GPCR allow species differentiation of SPPV and GTPV (Madhavan et al. 2016) at molecular level.

In case of ORFV, most of the essential genes are organized in the conserved central region of the viral genome like VACV except VACV D9R and VACV F15R (Delhon et al. 2004). ORFV possesses all structural genes present in VACV except A36R, K2L, A56R, and B5R (Tan et al. 2009). Terminal ends of the genome are reported to be variable and encode for proteins that are involved in host–virus interaction, virulence, and pathogenesis (Hosamani et al. 2009). PPVs have evolved a repertoire of unique immunomodulatory or host range genes that encode factors targeting the host immune system (Bratke et al. 2013). Among these, CBP (chemokine-binding protein), GIF (GM-CSF inhibitory factor), VIR (viral interferon resistance), and dUTPase genes are homologs of VACV C23L, A41L, E3L, and F2L proteins, respectively (Hosamani et al. 2009; Fleming et al. 2015). DNA polymerase gene-based phylogeny of ORFV isolates shows the close genetic relatedness with *Molluscum contagiosum* virus (Fleming et al. 2015).

9.3 Epidemiology

9.3.1 Geographical Distribution

Currently, SGP infections are endemic in entire Southwest and Central Asia, the Indian subcontinent, and Northern and Central Africa. Occasional outbreaks have been reported from regions of Europe like Turkey, Greece, and Bulgaria (Bhanuprakash et al. 2011). Introduction of affected animals through trade is the major mode of spread in naïve areas (Madhavan et al. 2016; Tuppurainen et al. 2017). Capripox is endemic in India and reported in almost all geographical regions of the country (Bhanuprakash et al. 2006). ORFV infections are reported worldwide including American continent, Europe, Australia, the Indian subcontinent (Hosamani et al. 2009; Nandi et al. 2011). Reports have been documented from different states of India, including the North-Eastern region involving sheep and goats (Venkatesan et al. 2018b).

9.3.2 Host Range, Susceptibility, and Transmission

SPPV and GTPV show host specificity with more severe disease evident in the homologous hosts. But, some strains affect heterologous hosts with lesser severe disease (Bhanuprakash et al. 2010). Some strains have been reported to have a natural infection in both species, including SPPV infection in Makhdoom (India) (Bhanuprakash et al. 2010), GTPV infection in China (Yan et al. 2012), and Ethiopia (Gelaye et al. 2016). Some Middle-Eastern strains also have shown equal pathogenicity for sheep and goats (Kitching et al. 1986). CaPV cause systemic infection in

all ages but severe form is seen in the young animals (Bhanuprakash et al. 2006). Exotic breeds including European are more susceptible (Bhanuprakash et al. 2006). Infection is reported throughout the year. Evidence for the existence of CaPVs in wild ruminants is lacking (Tuppurainen et al. 2017). SPPV and GTPV are mainly transmitted by aerosol route, but it also occurs indirectly through other mucous membranes and abraded skin by contaminated feed and wool. Occasionally, the fomites and insects (Kitching and Mellor 1986) play the role in transmission. The virus can remain infective on hair or wool for as long as three months after infection and for a longer period in scabs (Bowden et al. 2008). Carrier stage is not seen in infected animals (Bhanuprakash et al. 2011). Goatpox has also been reported as a mixed infection with PPR or orf simultaneously and by co-infection (Saravanan et al. 2007; Malik et al. 2011).

Orf mainly infects goats and sheep but shows a wider host range to cattle, camelids, seals, reindeer, mule deer, Sichuan Takin, Japanese serows (*Capricornis crispus*), and Japanese deer (*Cervus Nippon centralis*) (Hosamani et al. 2009) and also identified in blackbuck associated with sarcoptic mange (Sharma et al. 2016). Some tentative species like chamois contagious virus, parapoxvirus of Japanese serow, musk ox, camels (Ausdyk virus), reindeer, seal, sea lions (King et al. 2012) and recently, PPV from the horse (Airas et al. 2016) are yet to be classified in the genus. ORFV primarily infects animals less than one year of age. The disease is seen throughout the year. Stressors such as transportation, pregnancy, and other factors act as predisposing factors (Nandi et al. 2011). ORFV is resistant to physical and chemical agents as identified in other poxviruses. It exists for years in dry and extreme cold conditions including dried scab (Venkatesan et al. 2012b). Dried grass or leaves predispose to skin abrasions around lips and mouth through which virus enters. Transmission mainly occurs through direct contact or indirectly through contaminated non-living objects or fomites (Venkatesan et al. 2011). Chronically infected animals may carry the virus and are responsible for reappearance within same flock and transfer between flocks (Hosamani et al. 2009; Nandi et al. 2011).

9.4 Pathogenesis, Immunity, and Clinical Disease

CaPV have tropism for skin, respiratory, and gastrointestinal tracts. Following primary multiplication, the virus disseminates to the blood via the draining lymph nodes. The cell-associated viremia composing of infected monocytes and macrophages leads to settlement of virus in skin and other tissues (Bowden et al. 2008) and virus sheds in conjunctival, nasal, and other secretions (Balinsky et al. 2008). Various virus, host, environmental, and biometeorological factors decide the severity of capripox infection in affected hosts (Bhanuprakash et al. 2006). In the field conditions, incubation of the disease is 6–12 days. Initially, animal shows high fever along with oedema of eyelids, oculo-nasal discharge followed by progressive appearance of skin lesions, especially on wool-free areas. Macules further develop into papules followed by scab formation (Madhavan et al. 2016) with swollen

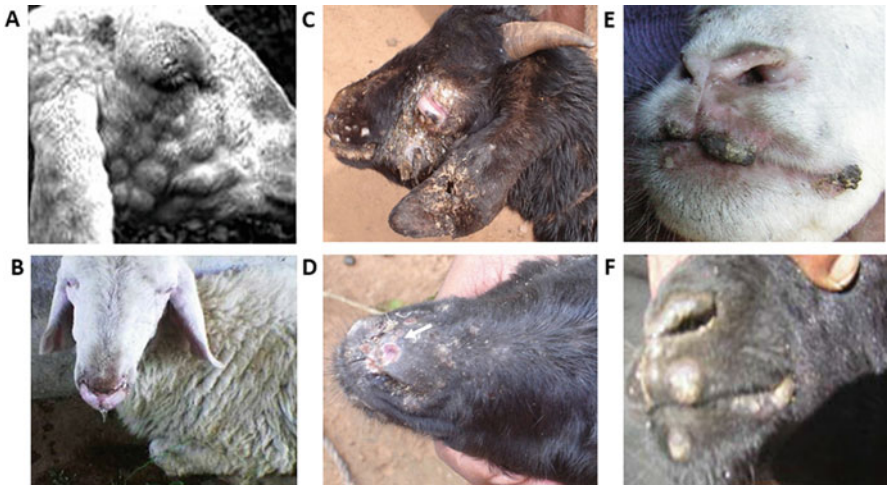

Fig. 9.1 Clinical picture of sheeppox (**a** and **b**), goatpox (**c** and **d**) and contagious ecthyma (**e** and **f**) infections in sheep and goats

pre-scapular lymph nodes. Nodular lesions may also be seen in lungs, digestive organs, liver, and other organs (Bhanuprakash et al. 2006). Visible skin nodular lesions over the head regions and frothy viscous nasal/mouth secretions are observed in sheep following sheeppox infection (Fig. 9.1a, b) and goatpox can cause severe erosions/ulcerations of skin lesions over nasal, ocular, and ear regions in goats which might be complicated with secondary bacterial infections (Fig. 9.1c, d) Virus shedding from mucosa is reported for up to 3–6 weeks following infection (Bowden et al. 2008). Although cell-mediated immunity (CMI) has predominant role following CaPV infection, humoral immunity also plays the part. Low level of neutralizing antibodies may be seen following mild disease or vaccination (Babiuk et al. 2009). Morbidity and mortality in adults may be moderate but, in young animals, these may reach 100% and 95%, respectively. Affected animals may recover in 4 weeks unless the secondary complications appear (Bhanuprakash et al. 2006).

Orf infection mainly starts following abrasions or breaks in the skin around lips and mouth, through which the virus enters and replicate in epidermal keratinocytes of skin as main predilection site (Nandi et al. 2011). Various virus-encoded immunomodulatory factors help the virus to replicate in skin environment. Clinically, orf is characterized by its proliferative skin lesions that may be painful, highly vascularized leading to bleeding. Lesions are seen mainly around mouth and lips either proliferative or nodular type growth in sheep and goats (Fig. 9.1e, f), but rarely seen in other regions (Hosamani et al. 2009). Mouth lesions cause inability to graze and suckle due to which young animals succumb to death causing high mortality in suckling kids and lambs (Venkatesan et al. 2018b). ORF induces short-lived immunity, in which CMI response plays the main role, whereas the role of antibody is unclear (Nandi et al. 2011). Orf in general is mild in affected animals but, severe in lambs and kids due to secondary complications (Hosamani et al. 2009). Both animal

and humans are susceptible to reinfection. Upon reinfection, lesions may be smaller and take less time to heal. Humans especially farmers, veterinarians, and zoo personnel contract the orf infection through direct contact with affected animal or contaminated fomite and is considered as occupational zoonosis (Nandi et al. 2011).

9.5 Economic Impact

Control of capripox and orf is important to boost the small ruminant sector in the developing countries (Babiuk et al. 2008; Nandi et al. 2011) as they cause tangible and intangible losses (Yeruham et al. 2007; Madhavan et al. 2016; Venkatesan et al. 2018b). Besides, they cause trade barrier on animals and their by-products from endemic regions inflicting indirect economic impact. An estimated loss of over INR 105 million in capripox outbreaks occurred in Maharashtra (India) state has been reported with average morbidity and mortality of 63.5% and 49.5%, respectively (Garner et al. 2000). Also, the extrapolated annual loss has been estimated to be INR 1250 million based on this data (Bhanuprakash et al. 2011). Though orf is of mild, it also inflicts economic loss due to severe morbidity in adults and high mortality in young ones and posing trade restriction on endemic countries (Hosamani et al. 2009).

9.6 Diagnostics

A tentative diagnosis of an acute form of SGP can be made based on typical clinical signs consisting of fever, pock lesions, swollen lymph nodes, and pneumonia (Rao and Bandyopadhyay 2000). Similarly, orf can be identified by typical proliferative lesions around mouth region (Hosamani et al. 2009). Nevertheless, the baffling clinical signs with other diseases involving skin, namely foot and mouth disease (FMD), bluetongue (BT), dermatophilosis/streptothricosis, mange, photosensitization, etc. emphasize precise clinical diagnosis, especially in low virulent strains (Bhanuprakash et al. 2006; Hosamani et al. 2009). Therefore, laboratory confirmation of capripox and orf by an array of routine serological and molecular tools is mandatory (Venkatesan et al. 2014a).

9.6.1 Conventional Techniques

Laboratory diagnosis of suspected cases is based on virus isolation, electron microscopy, and serological tests, namely SNT/VNT, FAT AGID, and ELISA (Rao and Bandyopadhyay 2000; Hosamani et al. 2009). Lesions from skin, lung, and lymph nodes collected during the first week of occurrence of clinical signs are preferable for

CaPV isolation and antigen detection, whereas mainly skin lesions around mouth are the main source of ORFV antigen.

1. *Electron microscopy*—Electron microscopy is usually used to detect the virus in tissue samples, but requires expertise and needs to be distinguished from OPVs by immunostaining. Negative electron microscopy readily differentiates ORFV from other poxviruses due to characteristic ovoid shape and criss-cross pattern of the virion (Hosamani et al. 2009).
2. *Virus isolation*—Primary lamb testes (PLT) and lamb kidney (PLK) cells are highly sensitive for primary isolation/adaptation of CaPVs and ORFV. In addition, Vero cells can also be used for CaPV isolation and continuous passaging to attenuate. A cytopathic effect like ballooning, rounding, increased refractivity, detachment, etc. is seen (Madhavan et al. 2016). CaPVs may require several blind passages for the appearance of CPE. Virus isolation of CaPV and ORFV is mostly tiresome and demands scientific skill as well. Laboratory animals do not support the growth of CaPVs (Bhanuprakash et al. 2011). PLK and PLT and OA3.Ts cells are most commonly used for isolation of ORFV (Plowright et al. 1959). Initial rounding, ballooning, and grape like clusters are typical CPE feature of ORFV infection (Nandi et al. 2011).
3. *Antigen detection assays*—Polyclonal antibody based antigen detection assays are common to detect soluble antigens and, therefore, lacks some degree of specificity against CaPV antigen. AGID (Rao and Negi 1997) and counter immunoelectrophoresis (CIE) (Sharma et al. 1988) have been used for diagnosis of capripox and orf, but they show serological cross-reactivity. Immunocapture ELISA using hyperimmune serum against the whole virus (Rao et al. 1997) or recombinant protein (Carn 1995) has been developed for the detection of CaPV antigen.
4. *Antibody detection*—Serological assays like VNT/SNT, AGPT, CIE, latex agglutination test (Rao et al. 1995), indirect fluorescent antibody test (IFA), whole antigen-based ELISA, and immunoblotting (Chand et al. 1994) have been developed but these cannot differentiate among CaPVs. CIE test can be employed to detect ORFV antibodies following infection and vaccination in targeted hosts (Venkatesan et al. 2011).

    i. Serum neutralization test (SNT)—It is the golden standard test for CaPV and ORFV specific antibodies detection and titration. But it is time-consuming, labour-intensive, difficult to interpret and requires handling of live virus (Venkatesan et al. 2018a). However, it is also useful to determine the antigenic relationship between CaPVs and assess the post-vaccination monitoring of antibody status (Bhanuprakash et al. 2006).
    ii. Western blotting using H3L homolog of CaPVs with sera to be tested is both sensitive and specific but tedious and expensive (Chand et al. 1994). It can differentiate CaPVs from ORFV. Western Blot analysis for ORFV is based on the presence of two immunogenic envelope proteins (39 and 22 kDa proteins) from serum samples (Czerny et al. 1997).

iii. ELISA—ELISA can be used to monitor the immune response of vaccinated and infected animals (Hosamani et al. 2004b). Whole inactivated capripoxvirus has been used as an ELISA antigen (Babiuk et al. 2009), but it is tedious and has bio-safety and security issues. Indirect ELISA based on expressed P32 antigen (Heine et al. 1999; Bhanot et al. 2009; Venkatesan et al. 2018a), ORF117 (Dashprakash 2013), ORF 095, and ORF 103 proteins (Bowden et al. 2009) have been developed. But, these assays are unable to detect low titer of neutralizing antibodies following vaccination or mild infection. Recombinant protein based validated immunodiagnostics like ELISA for CaPVs is still under development due to difficulties in selection of single immunodominant capripoxvirus antigen, its efficient expression in a heterologous host system and purification hamper its usage as a diagnostic. Therefore, no validated ELISA is commercially available for the detection of antibodies to CaPVs. There is a need of screening of immunogenic proteins of CaPVs for selection of suitable protein candidate with good expression level, efficient purification along with sensitive detection of low level of antibodies (Madhavan et al. 2016). Whole viruses as well as subunit antigens have been used in ELISA to detect ORFV antibodies significantly (Chin and Petersen 1995).

iv. Diagnostics using specific MAbs can be developed for rapid and sensitive detection of CaPVs antigen/antibody. Anti-ORF086 (Wang et al. 2015) and anti-ORFV059 MAbs (Li et al. 2012) have been reported as potential candidates for developing such diagnostics in past. A lateral flow immunochromatographic assay using two monoclonal antibodies against the ORF011 protein (Zhao et al. 2016) may be more suitable for field-level detection of orf infection.

9.6.2 Nucleic Acid-Based Techniques

In India, frequent outbreaks of SGP with orf often go misdiagnosed or unnoticed (Saravanan et al. 2007; Hosamani et al. 2009). Therefore, it is need of the hour to develop duplex/multiplex PCR targeting the detection of both CaPVs and orf simultaneously in single-tube reaction to enable early diagnosis of them occurring as single or mixed infection (Venkatesan et al. 2014a). These diagnostic tools and techniques can be handful in enzootic or naive regions during clinical surveillance of CaPV and ORFV.

1. *Conventional PCR for diagnosis of individual CaPV and orf infections*—several diagnostic PCR tools have been reported targeting P32 region of CaPV genome for its rapid detection (Ireland and Binepal 1998; Heine et al. 1999) and also DNA polymerase (*DNA pol*) gene has been targeted for PCR in past (Balamurugan et al. 2009). A highly specific semi-nested PCR targeting B2L (Inoshima et al. 2000) and diagnostic PCRs using DNA polymerase gene (Bora et al. 2011),

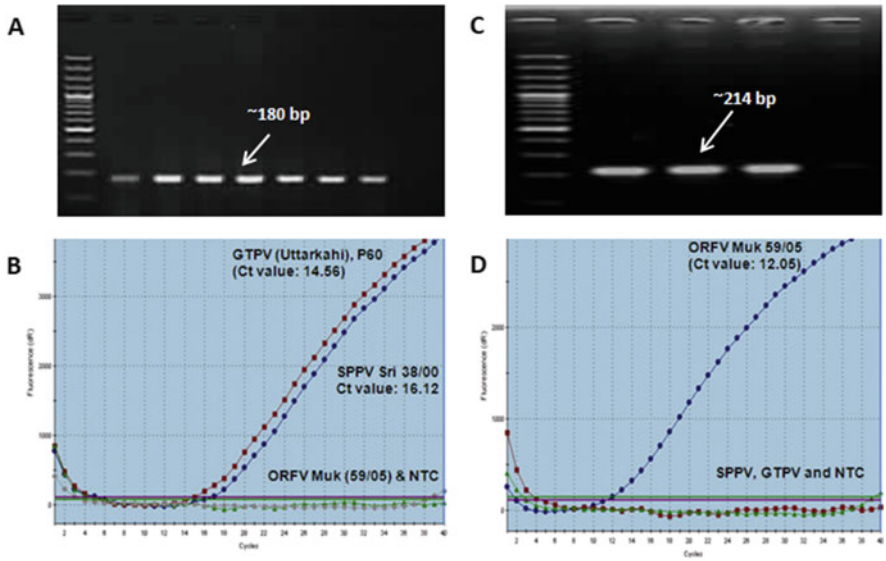

Fig. 9.2 Conventional PCR and TaqMan probe-based real-time PCR assays for rapid and sensitive detection of CaPV (**a** and **b**) and ORFV (**c** and **d**) DNAs

VLTF-1 gene (Kottaridi et al. 2006), VIR gene (Guo et al. 2004), A32L gene (Chan et al. 2009), and F1L gene (Hosamani et al. 2009) has been reported. *DNA Pol* gene-based PCR assays for sensitive diagnosis of CaPV and ORFV, respectively, amplifies approximately 179 bp and 214 bp fragments as observed in agarose gel analysis (Fig. 9.2a,c).

2. *Real-time PCR*—Several TaqMan quantitative polymerase chain reaction (qPCR) assays have been reported for detection of CaPV DNA targeting DNA pol gene (Balamurugan et al. 2009) and poly (A) polymerase (Balinsky et al. 2008). A novel qPCR using snapback primers has been developed for grouping of CaPVs (Gelaye et al. 2013). A genus-specific (Parapoxvirus) TaqMan qPCR has been reported targeting DNA polymerase gene (Das et al. 2016). Further, B2L (Gallina et al. 2006), ORF024 (Du et al. 2013), and DNA polymerase genes (Bora et al. 2011) have also been targeted for the same format. SYBR Green chemistry has also been attempted for the detection of ORFV and other PPVs (Venkatesan et al. 2012b; Zhao et al. 2013; Wang et al. 2017). DNA polymerase gene-based TaqMan probe real-time PCR for rapid, sensitive, and specific detection of CaPV (Fig. 9.2b) and ORFV (Fig. 9.2d) is shown.
3. *Real-time multiplex PCR (mPCR)*—TaqMan probe real-time duplex PCR targeting highly conserved DNA polymerase gene was reported to simultaneous detection of CaPV and its differentiation from ORFV from mixed infections (Venkatesan et al. 2014b). A dual hybridization probe qPCR assay has been reported for grouping of CaPV isolates (Lamien et al. 2011b).

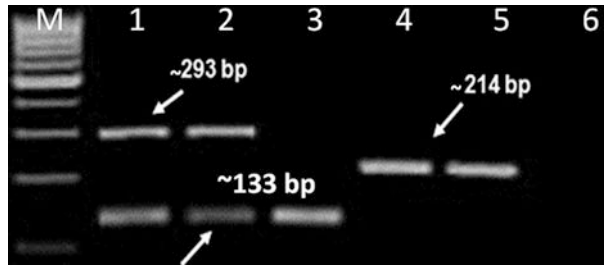

Fig. 9.3 Conventional multiplex PCR showing detection and differentiation of SPPV (293 and 133 bp), GTPV (only 133 bp), and ORFV (214 bp) in a single tube format

4. *Conventional mPCR*—A duplex PCR has been reported for simultaneous detection and differentiation of SPPV and GTPV (Fulzele et al. 2006; Zhao et al. 2017). A multiplex PCR for detection of SPPV and the differentiation of vaccine and field strains has also been reported earlier (Chibssa et al. 2018). Also, two sets of specific primers targeting different genes have been used in past for the same purpose (Zheng et al. 2007; Venkatesan et al. 2014a). RPO30 gene PCR has been developed for differentiation of SPPV and GTPV based on a 21-nucleotide deletion (Lamien et al. 2011a). The conventional mPCR which can amplify two fragments in case of SPPV (293 bp and 133 bp), whereas only one fragment in GTPV (133 bp) and ORFV (214 bp) is shown as Fig. 9.3.
5. *PCR-RFLP for differentiation of SPPV and GTPV*—Significant gene variations among different CaPV members identified by sequence analysis helped to develop a PCR-RFLP strategy in genotyping of SPPV and GTPV. Such a molecular tool using one or two specific restriction enzyme/s has been reported (Hosamani et al. 2004a; Venkatesan et al. 2012a). PCR amplification of P32 region of SPPV and GTPV isolates yield 1027 and 1024 bp amplicons, respectively, and RE digestion of PCR products yielding three fragments (300, 327, 400 bp in SPPV) and two fragments (327 and 697 bp in GTPV) has been reported. In addition, a PCR-RFLP targeting attachment gene PCR product (192 bp) using EcoRI restriction enzyme has been reported that can differentiate SPPV and GTPV upon digestion yielding two fragments of 129 bp and 63 bp for SPPV and whereas, it is single, i.e. 192 bp (Venkatesan et al. 2012a). In the same direction, the genomic RFLP methods using RE enzymes for differentiation of parapoxvirus strains and analysis of heterogeneity for groping of ORFV isolates (Robinson et al. 1982) have been reported.
6. *Isothermal amplification assays*—Among the different isothermal amplification assays, loop-mediated isothermal amplification (LAMP) provides a potential "ASSURED" policy for a diagnostic test to be deployable at field diagnostic settings. The LAMP could produce a highly specific and sensitive reaction (Notomi et al. 2000) which could be performed at a single temperature using a simple heating block and can be developed as POCT (point of care testing) in resource limited field settings (Venkatesan et al. 2015; 2016). LAMP assays targeting P32 (Murray et al. 2013), Poly (A) polymerase (Das et al. 2012), DNA polymerase genes (Venkatesan et al. 2015) of CaPVs have been reported for rapid detection of CaPV and also to differentiate them (Zhao et al. 2014).

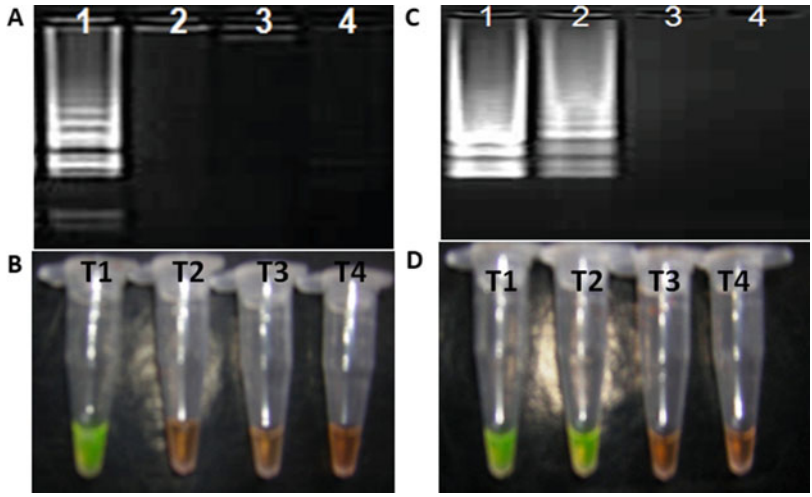

Fig. 9.4 DNA polymerase gene-based LAMP assays for simple and rapid detection of ORFV and CaPV gDNAs [Typical ladder-like pattern in agarose gel analysis and SYBR green I dye-based visual detection of ORFV DNAs (**a** and **b**) and CaPV DNAs (**c** and **d**) by respective LAMP assays] Panel (**a**) and (**b**): Lane 1: ORFV Mukteswar 59/05; Lane 2-3: Negative controls; Lane 4: No template control (NTC). Panel (**c**) and (**d**): Lane 1: SPPV-Srinagar; Lane 2: GTPV Uttarkashi; Lane 3: ORFV DNA, and Lane 4: NTC

These LAMP assays were specific and sensitive comparable to quantitative PCR assays. Similarly, LAMP assays targeting B2L gene (Tsai et al. 2009) and DNA polymerase gene (Li et al. 2013; Venkatesan et al. 2016) have been successfully done to detect ORFV. In similar line to LAMP, another isothermal amplification assay known as recombinase polymerase assay (RPA) using fluorescent probe was reported for rapid detection of ORFV (Yang et al. 2015). DNA polymerase gene-based LAMP assays for simple and fast identification of CaPV by characteristic ladder-like pattern in AGE (Fig. 9.4a) and visual detection using SYBR Green I dye (Fig. 9.4b) and the same pattern of identification for ORFV gDNAs (Fig. 9.4c,d) have been shown.

9.7 Prevention and Control

Active immunization of susceptible hosts against these infections is the best possible economical way of disease control in endemic regions (Madhavan et al. 2016). Vaccines used are either live or inactivated strains of SPPV or GTPV and protect homologous hosts with limited cross-protection potential (Hosamani et al. 2004b). For optimum protection, homologous vaccines are recommended using available indigenous strains (Rao and Bandyopadhyay 2000; Bhanuprakash et al.

2011). Although both inactivated and attenuated vaccines have been used for control of sheeppox, goatpox, and orf infections, live attenuated vaccines are preferred due to its long-lasting immunity.

(i) Live attenuated vaccines—In Africa and the Middle East, Kenyan sheep and goatpox (KSG) O-240 and RM65 strains are commonly used for control of capripox in sheep and goats with satisfactory results (Bhanuprakash et al. 2006, 2011). In India, attenuated SPPV strain (Romanian Fanar) is widely used to control sheeppox (Yogisharadhya et al. 2011). Another indigenous Ranipet strain of SPPV attenuated using ovine thyroid cells and lamb testes cells has been used in Tamil Nadu (Bhanuprakash et al. 2004). Recently, a new indigenous sheeppox vaccine using Srinagar strain of SPPV attenuated in Vero cells is found to be safer and more potent than RF and Ranipet strains (Yogisharadhya et al. 2011; Bhanuprakash et al. 2011). Similarly, a Vero cell-based homologous vaccine for goatpox, developed at IVRI, Mukteswar, is safe and potent inducing a protective immune response in goats on single immunization (Hosamani et al. 2004b). Till date, a vaccine that can confer solid immunity against ORFV infection is not reported. Vaccines derived from sheep ORFV strains may be less efficient to protect the infection in goats or *vice versa* (Musser et al. 2008). Till now, scarification is followed as the preventive measure in many countries to provide immunity. In India, live orf vaccine (Mukteswar 59/05 strain) using PLT cells has been reported as safe, efficacious, and potent in sheep and goats (Hosamani et al. 2009; Bhanuprakash et al. 2011). DNA vaccine expressing ORFV 011 and 059 chimeric proteins has been reported showing great improvement in immunogenicity and potency of vaccination. Therefore, ORF 011 encoding B2L protein can be an appropriate candidate for subunit vaccine development (Zhao et al. 2011).

(ii) Live attenuated combined vaccines—Vaccines using SPPV-RF and GTPV-Uttarkashi strains combined with Peste des petits ruminants (PPRV) Sungri/96 (Hosamani et al. 2006; Chaudhary et al. 2009) have been reported for simultaneous protection against Capripox and PPR in India (Bhanuprakash et al. 2011) as single shot vaccination strategy.

(iii) Recombinant vaccines—Capripox vectored PPR vaccines targeting immunogenic fusion or hemagglutinin genes have been reported as safe and potent against both capripox and PPR (Berhe et al. 2003; Chen et al. 2010). Currently, there are no vaccines that can differentiate infected from vaccinated animals (DIVA) available. For DIVA strategy, a non-essential immunogenic gene needs to be identified and targeted for development of a companion diagnostic test.

9.8 Conclusions and Future Perspectives

SGP and orf pose a socio-economic threat to small ruminants in enzootic countries like India. Possibility of future expansion of their geographical range into naive places due to animal trade is much possible. Mixed or co-infections that may enhance the severity of existing infection are common worldwide and cannot be ruled out during control program strategy. In developing countries like India, occurrence of these mixed type infections in sheep and goats is usually missed or un-diagnosed and no precise diagnostic approach(s) is available to identify them as SPPV or GTPV or ORFV or mixed viruses. In such a condition, early and rapid diagnosis of these targets using PCR as multiplex format in single-tube reaction will be handful (Venkatesan et al. 2014a) during implementation of control program. In addition to prompt and unequivocal diagnosis, a well-organized vaccination and effective bio-security measures are main control elements to contain the disease. Effective live attenuated vaccines, restriction of animal movement, properly implemented mass vaccination programs along with field-deployable diagnostic tests like LAMP assays in closed-tube format are necessary for control strategy. The routinely used conventional serological tests may not be sensitive and specific enough to detect vaccine or disease mounted immune response. Therefore, high-throughput ELISA with improved diagnostic performance is need of the hour. In case of capripox, identification of CaPV isolate(s) that are infective for both sheep and goats may be handful for developing single vaccine for both the species. In future, there is a need to develop DIVA compatible vaccine along with companion test and other molecular epidemiological tools to differentiate SPPV, GTPV, and ORFV in a robust and high throughput manner. Further, genetic characterization of virulence genes of ORFV isolates circulating in a geographical range will help in establishing molecular epidemiology and unravelling the immune evasion mechanisms of ORFV in target species.

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Conflict of Interest Authors declare that there is no conflict of interest.

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Chapter 10
Hepatitis E Virus

Vasileios Papatsiros

Abstract Hepatitis E virus (HEV) is taxonomically classified within *Hepeviridae* family and *Orthohepevirus* genus. Genotypes HEV-1 and HEV-2 infect human, while genotypes HEV-3 and HEV-4 are zoonotic viruses that infect humans, domestic pigs and other animal species (e.g. wild boar, deer). The main route of trans-species transmission is the direct contact with infected animals, as well as via the consumption of HEV-contaminated food products or via the faecal–oral route through drinking of contaminated water. HEV-3 has been detected in pigs around the world (South and North America, Europe, Africa, Asia, and Oceania). HEV-4 has mainly reported in domestic pigs and humans in Asia. Domestic pigs, wild boar, and various species of deer reported to play important role in zoonotic transmission of HEV-3 and HEV-4 from animals to humans. The most important reservoirs of the HEV genotypes are domestic pigs and the most HEV infections in humans are foodborne due mainly to consumption of undercooked meat or meat products (e.g. sausages). The main route of natural HEV transmission in pigs is via the faecal–oral. However, the HEV infection in pig is usually asymptomatic, with low impact on health status. Future studies focus on preventive measures to eliminate the appearance and persistence of HEV in pig farms (including biosecurity and vaccination) are required. Moreover, more studies are needed to investigate deeply the role of wildlife in the epidemiology of HEV infection.

Keywords Hepatitis E virus · HEV · Pig · Human · Pork · Epidemiology · Pathogenesis

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10.1 Prologue

Hepatitis E virus (HEV) is the causative agent of hepatitis E and it is classified into family *Hepeviridae*, which is divided in two genera: *Orthohepevirus* and *Piscihepevirus* (Smith et al. 2014). The genus *Piscihepevirus* includes only *Piscihepevirus A* (cutthroat trout virus), while the genus *Orthohepevirus* is divided in four species (Khuroo et al. 2016; Purdy et al. 2017):

(a) *Orthohepevirus A*, including isolates from such humans, domestic pigs, wild boars, deer, mongoose, rabbits, and camels—Fig. 10.1). Moreover, *Orthohepevirus A* has eight genotypes, five members of them are found to infect humans (Johne et al. 2014).
(b) *Orthohepevirus B*, including three avian isolates (HEV-1, HEV-2, and HEV-3),
(c) *Orthohepevirus C,* including isolates from rats, greater bandicoot, Asian musk shrews, mink, and ferrets, and
(d) *Orthohepevirus D*, including isolates from bats

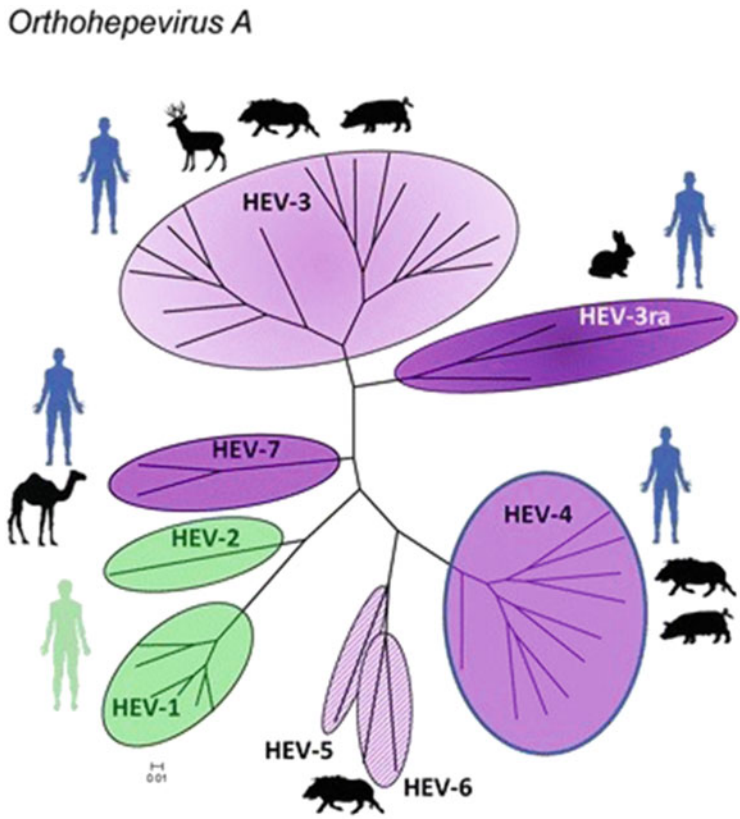

Fig. 10.1 Phylogenetic tree of HEV sequences within the species Orthohepevirus A (adopted from Pavio et al. 2017 and Smith et al. 2016)

HEV is a relatively stable virus, surviving in the gastrointestinal tract environment due to its resistance to gastric secretions and bile salts (Emerson and Purcell 2001). It is a small non-enveloped virus (27–33 nm in diameter) and icosahedral shaped sphere with shaped bumps visible on its surface (Balayan 1997). Its genome consists a single-stranded, positive-sense RNA molecule about 7.5 kilobases (kb) in length, which contains three open reading frames (ORF) (Tam et al. 1991). Based on ORF2 nucleotide sequence analysis, four major genotypes (HEV-1, HEV-2, HEV-3, and HEV-4) have been defined in mammals (Schlauder and Mushahwar 2001).

HEV genotypes 1 and 2 (HEV-1, HEV-2) are reported in humans (Kamar et al. 2017), while HEV genotypes 3 and 4 (HEV-3, HEV-4) are zoonotic viruses, infecting both humans and animals. The main route of trans-species transmission is the direct contact with infected animals, as well as via the consumption of HEV-contaminated food products or via the faecal–oral route through drinking of contaminated water (Colson et al. 2010; Dremsek et al. 2012; Chaussade et al. 2013; Riveiro-Barciela et al. 2015; Guillois et al. 2016). In rabbit species a separate genotype HEV-3 (HEV-3ra) was reported, which also includes a closely related human isolate (Pavio et al. 2017). Furthermore, HEV genotypes 5 and 6 (HEV-5, HEV-6) have been reported in wild boars (Takahashi et al. 2011), while HEV genotypes 7 and 8 (HEV-7, HEV-8) were found in camels (Woo et al. 2016; Lee et al. 2016).

Studies reported the isolation of HEV from various wild and domestic animals, such as domestic pigs, cattle, chickens, sheep, goats, and rodents (Favorov et al. 2000; Meng 2000).

10.2 Epidemiology

10.2.1 Geographic Distribution

The geographical distribution of terrestrial animal reservoirs of HEV is summarized in Fig. 10.2. Pigs, wild boar, and various species of deer are involved in zoonotic transmission of HEV-3 and HEV-4 to humans. However, the role of mongooses, rats, and rabbits in causing human hepatitis E is unclear. Domestic pigs are the most important reservoirs of the HEV genotypes that are capable of infecting humans. In 1997, HEV-3 was first isolated from pigs in USA (Meng et al. 1997) and since then many studies reported high prevalence of HEV (seroprevalences were estimated between 5 and 100%) in pig herds worldwide (de la Caridad Montalvo Villalba et al. 2013; Owolodun et al. 2014; Aniță et al. 2014; Burri et al. 2014; Liu et al. 2015; Merino-Ramos et al. 2016; Thiry et al. 2017a, b), including countries from five continents:

(a) Asia (China, India, Indonesia, Japan, Korea, Mongolia, Philippines, Taiwan, Thailand, and Vietnam),

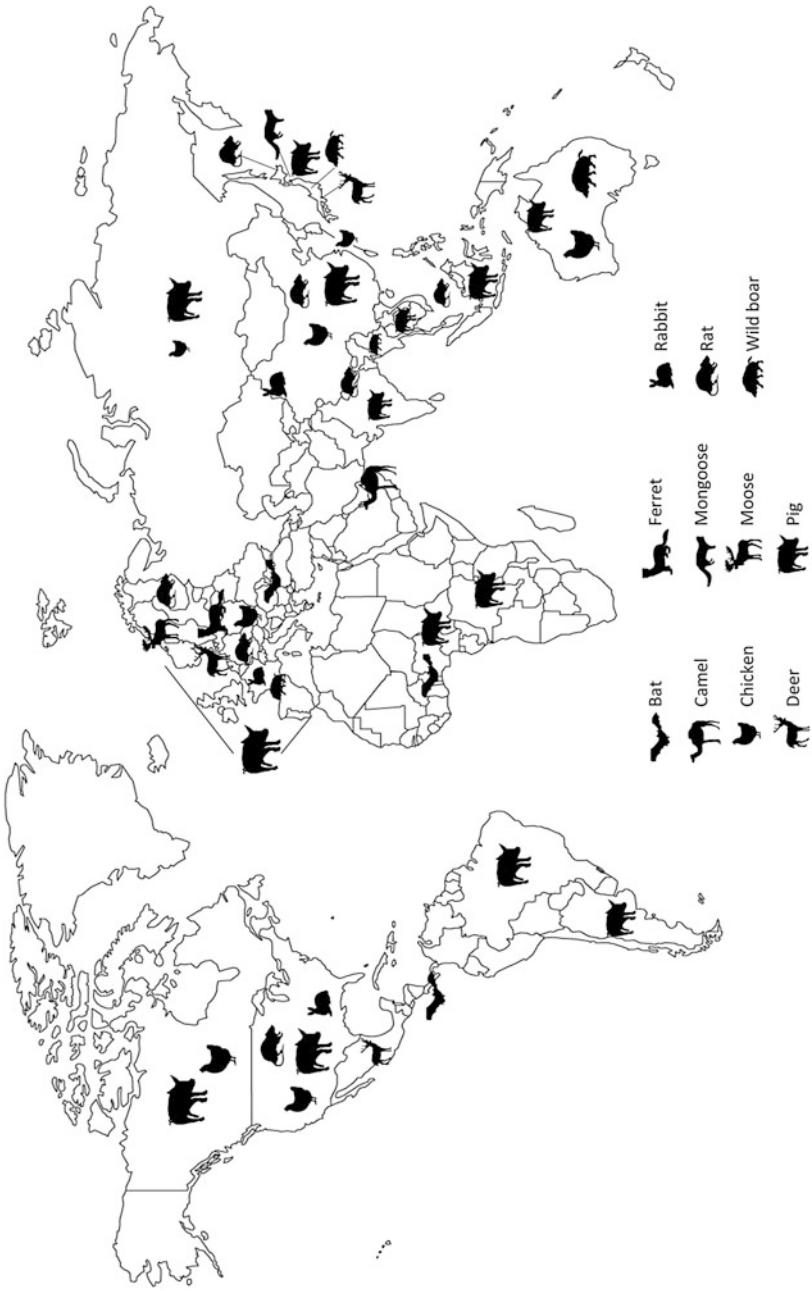

Fig. 10.2 Geographical distribution of HEV3 and HEV4, and novel HEV-like viruses in terrestrial animals (adopted from Sridhar et al. 2015)

(b) South and North America (Argentina, Bolivia, Brazil, Cuba, and Mexico/Canada),
(c) Africa (Cameroon, Democratic Republic of Congo, Nigeria, and Madagascar),
(d) Europe (Belgium, Czech Republic, Finland, France, Germany, Hungary, Italy, the Netherlands, Romania, Spain, Sweden, Switzerland, and the United Kingdom), and
(e) Oceania (Australia, New Caledonia, and New Zeeland).

HEV-3 has been detected in pigs from all aforementioned continents, whereas HEV-4 has mainly been reported in pigs and humans in Asia and recently also in Europe (Hsieh et al. 1999; Schlauder and Mushahwar 2001; Cooper et al. 2005; Thiry et al. 2017a, b; Salines et al. 2017; Pavio et al. 2017).

10.2.2 Transmission of HEV to Humans

Mainly reservoirs for genotypes HEV-3 and HEV-4 are domestic pigs and wildlife (wild boars, sika deer) (Pavio et al. 2015). HEV is transmitted primarily in humans via the faecal–oral route (Purcell and Emerson 2001). Human infections are due mainly to consumption of undercooked meat or meat products (e.g. sausages), direct contact with infected animals, drinking contaminated water and environmental contamination by animal manure run-off (Khuroo et al. 2016).

10.2.2.1 Public Health

HEV is the main causative agent of hepatitis E, which is usually an asymptomatic human liver disease. However, hepatitis E is possible to induce a self-limited acute hepatitis in humans, especially in developing countries, which are characterized by problems of access to water, poor sanitation, and high population density (Purcell and Emerson 2000; Worm et al. 2002; Perez-Gracia and Rodriguez-Iglesias 2003). The genotypes HEV-1, HEV-2, HEV-3, and HEV-4 are frequently associated with clinical cases of acute hepatitis or liver failure, as well as neurological problems. Moreover, human infections from genotypes 1 and 2 (HEV-1, HEV-2) are more associated with high mortality rates in pregnant women and pancreatitis incidence (Lhomme et al. 2012, 2016). During last decade, the reported HEV infections are increasing dramatically, due to more frequent and novel application of diagnostic methods (Aspinall et al. 2015).

Generally, hepatitis E due to HEV-3 and HEV-4 infection is an important zoonosis around the world (Wu et al. 2002; Smith et al. 2014). Sporadic cases of acute and chronic hepatitis E in humans due to HEV-3 infection were reported in non-endemic regions of industrialized countries, where the pig was the major source of infection (Smith et al. 2014). Epidemic forms of hepatitis E were associated with

infection via drinking of contaminated water in developing countries with poor sanitary conditions, as the contamination of water supplies with human faeces remain a common route of HEV spread in these countries (Kamar et al. 2017). Sporadic forms of hepatitis E have been reported between epidemics of disease in these areas or in humans–patients with previous travelling to endemic areas or in humans–patients from industrialized countries, without travelling abroad (autochthonous hepatitis) (Perez-Gracia et al. 2004). Sporadic cases of hepatitis E were associated with the consumption of raw or undercooked meat products (e.g. liver, sausage) from pig or deer (Meng 2011). Large outbreaks of HEV frequently occur in many tropical and subtropical low-income regions, whereas sporadic HEV infections are seen in humans in industrialized countries. HEV sequences isolated from domestic pigs, wild boar, or deer were reported to be closely related to human HEV sequences in many countries worldwide (Meng 2011).

During last years, human HEV-3 infections have been dramatically increasing and the zoonotic transmission from pig to human is a common fact, based on the high sequence identity between isolated strains of human cases and contemporary isolated strains in pigs (Adlhoch et al. 2016). Nowadays, hepatitis E is an important public health concern, as about 20 million new HEV-1 and HEV-2 per year are reporting, including 3.4 million acute cases with 70,000 deaths due to acute liver disease (Rein et al. 2012). For example, studies reported a 10–40% seroprevalence rate of anti-HEV antibodies in many areas of Africa and Asia, while about 80% in Egypt (Kamar et al. 2017).

10.2.2.2 Pork and Meat Products

HEV foodborne infections in humans are caused mainly after consumption of undercooked meat or various meat products, such as sausages (Colson et al. 2010; Guillois et al. 2016). The consumption the parboiled flesh or liver from wild boar, deer, and domestic pigs is associated with autochthonous cases and outbreaks of hepatitis E (Khuroo and Khuroo 2008; Miyashita et al. 2012). Many studies reported detection of HEV-specific RNA in meat and meat products (mainly in liver as well as in sausages with and without liver) worldwide (Yazaki et al. 2003; Feagins et al. 2007; Kulkarni and Arankalle 2008; Pavio et al. 2017). Recently, HEV-contaminated cow milk is reported as a new high risk factor for HEV foodborne infection (Huang et al. 2016) (Table 10.1).

Studies reported a seroprevalence between 2 and 15% of slaughtered pigs, while the detection of HEV in samples from sausages or meat products containing pig liver was higher (especially products prepared with raw pork liver), ranging between 16 and 47%, (Pavio et al. 2014; Di Bartolo et al. 2015; Crossan et al. 2015). For example in Europe, favourite products made from raw pig liver (e.g. fresh sausage made called Figatellu), which are traditionally eaten raw, are considered at high risk of containing HEV (Colson et al. 2010; Garbuglia et al. 2015; Matsuda et al. 2003). Except pig livers, liver from wild boar and deer are also considered at high risk of containing HEV (Tei et al. 2003). However, in a recent study reported that the

Table 10.1 Prevalence of HEV RNA-positive pork, wild boar, and deer meat products

Product	Geographic area (continent, country)	References
Pig—liver	Asia (China, India Hong Kong, Japan, and Thailand)	Li et al. (2009) Kulkarni and Arankalle (2008) Chan et al. (2017) Okano et al. (2014) Ishida et al. (2012) Intharasongkroh et al. (2017)
	North – South America (USA, Canada, Brazil, and Mexico)	Gardinali et al. (2012) Mykytczuk et al. (2017) Leblanc et al. (2010) Wilhelm et al. (2014) Cantú-Martínez et al. (2013) Feagins et al. (2007)
	Africa (Cameroon, Burkina Faso)	de Paula et al. (2013) Traoré et al. (2015)
	Western, Central and South Europe (France, the United Kingdom, The Netherlands, Germany, Czech Republic, Italy, Serbia, Spain)	Di Bartolo et al. (2010) Jori et al. (2016) Rose et al. (2011) Wenzel et al. (2011) Milojević et al. (2019) Bouwknegt et al. (2007) Berto et al. (2012a, b)
Pig—meat	Western, Central, and South Europe (The Netherlands, Czech Republic, Switzerland, Italy)	Di Bartolo et al. (2010) Boxman et al. (2019) Moor et al. (2018)
	South-East Asia (Thailand)	Intharasongkroh et al. (2017)
Sausages and other products (e.g. figatelli) containing or without liver	North – South America (Canada, Brazil)	Heldt et al. (2016) Mykytczuk et al. (2017)
	South Africa (Republic of South Africa)	Korsman et al. (2019)
	Western, Central, and South Europe (France, United Kingdom, The Netherlands Germany, Switzerland, Spain, Italy)	Colson et al. (2010) Hennechart-Collette et al. (2019) Pavio et al. (2014) Szabo et al. (2015) Garbuglia et al. (2015) Boxman et al. (2019) Martin-Latil et al. (2016) Di Bartolo et al. (2015) Giannini et al. (2018) Berto et al. (2012a, b)

(continued)

Table 10.1 (continued)

Product	Geographic area (continent, country)	References
Wild boar—liver	Western, Central, South and East Europe (Belgium, France, The Netherlands Germany, Czech Republic, Hungary, Italy, Romania)	Thiry et al. (2017a, b) Kaba et al. (2010) Anheyer-Behmenburg et al. (2017) Schielke et al. (2009) Schielke et al. (2015) Adlhoch et al. (2009) Kubankova et al. (2015) Forgách et al. (2010) Serracca et al. (2015) Montagnaro et al. (2015) Porea et al. (2018)
	East Asia (Japan)	Sato et al. (2011) Matsuda et al. (2003) Motoya et al. (2016) Sonoda et al. (2004)
Wild boar—meat	Central Europe (Germany)	Anheyer-Behmenburg et al. (2017) Schielke et al. (2015)
Wild boar—Sausages without liver	Western and Central Europe (Belgium, Germany)	Szabo et al. (2015) Thiry et al. (2017a, b)
Deer—liver	Western and Central Europe (Belgium, France, The Netherlands, Germany, Hungary)	Szabo et al. (2015) Thiry et al. (2017a, b) Lhomme et al. (2015) Anheyer-Behmenburg et al. (2017) Forgách et al. (2010) Rutjes et al. (2010)
Deer—meat	Central Europe (Germany)	Anheyer-Behmenburg et al. (2017) Schielke et al. (2015)

prevalence and the amount of HEV RNA in liver samples from deer were significantly lower in comparison to samples from domestic pigs and wild boars (Pavio et al. 2017).

10.2.2.3 Direct contact/Vocational exposure

Direct contact exposure is also reported as a possible route of HEV transmission. Moreover, studies in many countries reported that the vocational exposure of professionals in pig farms (e.g. swine veterinarians, farm workers) with pigs, manure, and sewage is an important high risk factor for HEV infections (Perez-Gracia et al. 2007; Bouwknegt et al. 2008a; Rutjes et al. 2009; Pavio et al. 2017). For example, swine veterinarians and workers in pig farms reported to be 2–5 times more under

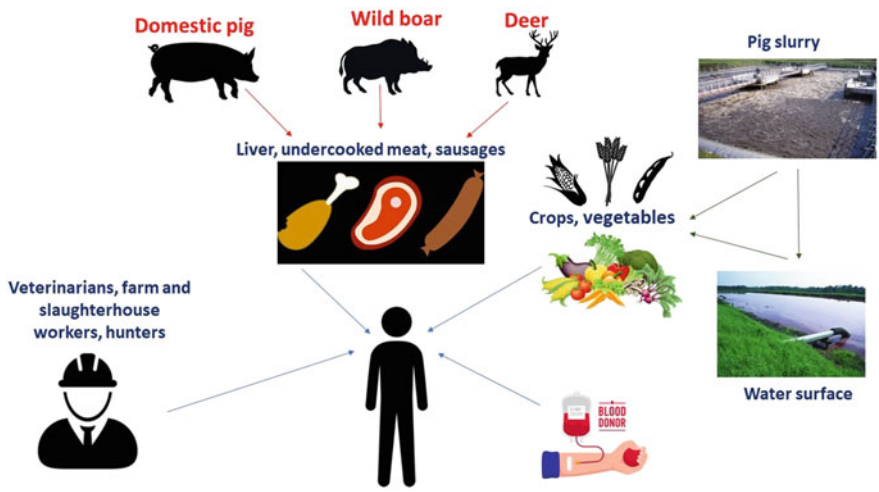

Fig. 10.3 Transmission and exposure routes of HEV infection in humans

the risk to have anti-HEV antibodies in comparison to non-swine veterinarians and the general population (Olsen et al. 2006; Vulcano et al. 2007; Galiana et al. 2008; Bouwknegt et al. 2008b). Moreover, there are reports of HEV transmission due to contact from wildlife to forest workers and hunters, as well as from frequent contact to a pet pig or to pigs at slaughterhouse (Stramer 2014; Juhl et al. 2014; Pavio et al. 2017) (Fig. 10.3).

Recently, HEV transmission from ruminants to farmers (Tritz et al. 2018) and rabbits to humans (slaughterhouse workers) were reported (Geng et al. 2019).

10.2.2.4 Water/Pig Slurry

Hepatitis E is primarily transmitted through the faecal–oral route (Khuroo 1991). Gross faecal contamination of community water supplies has been associated with several outbreaks in developing countries (Khuroo 1980; Naik et al. 1992; Kamar et al. 2017).

The presence of pig manure indicates the potential spread to humans through contact with contaminated crops or in personnel that handle swine manure and spread this waste on agricultural fields (Fernandez-Barredo et al. 2006). Use of pig slurry as pasture can infect agricultural products, such as raspberries, strawberries, and many vegetables used in the salad (Ward et al. 2008; Brassard et al. 2012). Run-off from outdoor pig farms causes contamination of surface water as well as produce receiving surface water (Steyer et al. 2011; Tyrrel and Quinton 2003).

10.2.2.5 Iatrogenic

HEV transmission from blood HEV-infected donors to human by blood transfusion is reported in many studies (Baylis et al. 2012; Hewitt et al. 2014; Gallian et al. 2014; Sauleda et al. 2015; Hogema et al. 2016). Moreover, a case of HEV-7 transmission to human is reported for a liver transplant recipient (Lee et al. 2016).

10.2.3 Transmission of HEV to Pigs

Wild boars are recognized as a potential reservoir of HEV, while HEV is transmitted from them to domestic pigs (Thiry et al. 2016; Schlosser et al. 2015; Jori et al. 2016).

The primary route of natural HEV transmission in pigs is the faecal–oral route, but it may require repeated exposure and high doses of virus (Kasorndorkbua et al. 2004). It is remarkable that the duration of detection of HEV in pig faeces is considerably longer than the duration of HEV viremia (Kasorndorkbua et al. 2004).

Previous studies reported a seroprevalence of HEV in pig between 5% and 100% (Pavio et al. 2017). The prevalence of the virus is depended on the animals age, the kind of tested sample, and the diagnostic method. Usually, HEV infection is detected at an early age after the loss of maternal antibodies. The virus load is high in all ages (weaners, growers, and fatteners), but is reported to be the highest in fatteners. Moreover, the seroprevalence is depended on the production system, as a slightly higher seroprevalence was reported in organic farms compared with conventional and free-range pig farms (Berto et al. 2012a, b). A comprehensive review (Salines et al. 2017) reported that the detection of HEV RNA in pig faeces and serum depends on the pig's age, while the shedding period ranges from 1.5 to 5 months of age (Salines et al. 2017). However, the peak of shedding in faeces happens around 3–4 months of age, whereas the shedding prevalence at slaughter age (around 185 days of age) is possible to be around 6% (Fig. 10.4).

10.3 Pathogenesis

HEV replication occurs mainly in the liver, but the virus can also be detected in other organs, such as small intestine, lymph nodes, and colon (Williams et al. 2001; Ha and Chae 2004). Viraemia is transient (duration of 1–2 weeks), while the peak of viral shedding in faeces occurs 3–8 weeks after weaning (Kantala et al. 2015). The viral shedding in faeces of infected pigs may persist for up to 7 weeks (Pavio et al. 2010). Then it is decreased around 15–18 weeks of age (McCreary et al. 2008), with the appearance of antibodies IgM followed by IgG (seroconversion) (Pavio et al. 2010).

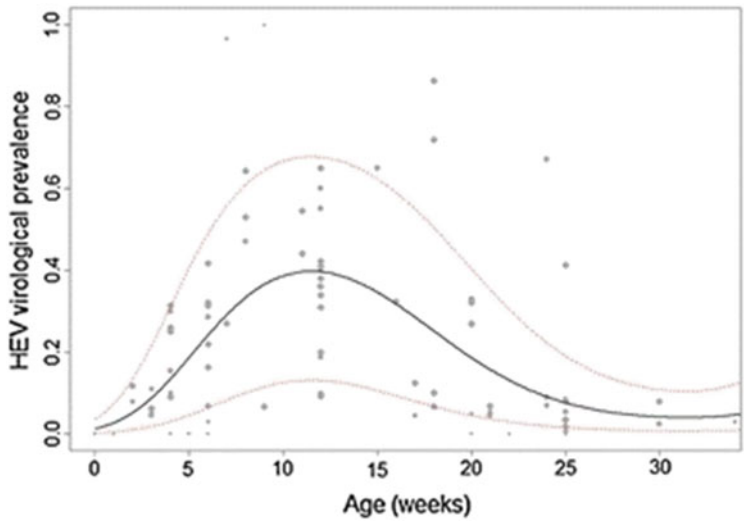

Fig. 10.4 Predicted HEV prevalence in faeces according to animal age (adapted from Salines et al. 2017)

The duration of the immunity acquired after HEV infection is not clear, but re-infection in case of transient decrease of immunity could be possible. A decrease of protection (antibodies or cellular response) over time may happen in older animals and especially in sows (Casas et al. 2011). Many studies reported that the majority of pigs are infected at 8–15 weeks of age, but some of them could be remain positive at slaughter age (de Deus et al. 2008; Meng et al. 1997; Casas et al. 2011). The infection happens at an early age after the loss of maternal antibodies (MAbs), which can be transferred from HEV-Ab positive sows to offspring (Feng et al. 2011). High levels of MAbs are very important for the reduction of prevalence of HEV positive animals (Krog et al. 2019).

10.4 Clinical Signs in Domestic Pig and Wild Boar

HEV infection in pig is usually asymptomatic, without important impact on their health status. HEV replication occurs in the liver and the intestine (Ha and Chae 2004), while it may enhance the clinical performance of disease caused by other porcine viruses, such as porcine reproductive and respiratory syndrome virus (PRRSV) (Salines et al. 2015) or porcine circovirus 2 (PCV2) (Yang et al. 2015; Jäckel et al. 2018). Immune modulatory effects have been reported in cases of PCV2 and HEV co-infection (Jäckel et al. 2018). The aforementioned enhancing activity of the HEV may due to immunosuppressive properties (Cao et al. 2017).

Genotypes HEV-3 and HEV-4 were also reported in wild boars, but without characteristic clinical symptoms in most cases. However, the prevalence of HEV in wild boars is lower than in domestic pigs (Pavio et al. 2017). Furthermore, HEV was detected in species of deer (Neumann et al. 2016; Anheyer-Behmenburg et al. 2017), while other species of animals (e.g. ruminants) are reported to be susceptible to HEV infection (Spahr et al. 2018).

10.5 HEV Monitoring/Prevention

Future studies focus on preventive measures to eliminate the appearance and persistence of HEV in pig farms (including biosecurity and vaccination) are required. Moreover, more studies are needed to investigate deeply the role of wildlife (wild boars, deer, etc.) in the epidemiology of HEV infection.

The prevention of zoonotic HEV infection demands a monitoring system to investigate and prevent the contamination of pork-derived meat products. HEV monitoring activities in the pork production chain are important to be implemented for the following targets:

(a) to maintain a database for the prevalence of HEV and follow-up the prevalence of the different HEV strains;
(b) to investigate in detail the dynamics of HEV infection, as well as their risk factors;
(c) to remove contaminated livers and other high-risk meat products from the food chain;
(d) to inform consumers regarding handling and cooking of high-risk pork-derived meat products (Salines et al. 2017; ANSES 2013).

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Chapter 11
The Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Maged Gomaa Hemida

Abstract The Middle East respiratory syndrome coronavirus (MERS-CoV) is one of the newly identified viral infections affecting humans. It was recently reported in the Arabian Peninsula (AP), particularly Saudi Arabia. The virus primarily causes respiratory failure in affected patients, but can also sometimes cause renal failure. The main reservoir of this virus is dromedary camels. Although a lot is known about the diseases pattern, pathogenesis, and immunity of MERS-CoV infection in humans, very little is known about these parameters in camels. The main goals of this book chapter are to discuss the current understandings about MERS-CoV infection in animals, particularly the dromedary camels. Special attention should be paid to the clinical pattern of MERS-CoV infection in animals, including clinical signs, pathological changes during virus infection, various methods of clinical and laboratory diagnosis, and the recent advances related to the control of MERS-CoV.

Keywords MERS-CoV · Clinical signs · Pathology · Diagnosis · Control · Vaccines

11.1 Prologue

There are 2458 laboratory-confirmed cases of MERS-CoV reported from 27 countries around the globe (till August 20, 2019). The disease was fatal in about 848 cases (34%) (WHO 2019). Unfortunately, until now, there is no effective medication or even vaccine against MERS-CoV. MERS-CoV represents a good example for the One Health concept (Hemida 2019). The virus infects humans and can cause severe illness in some patients, particularly those suffering from chronic diseases and other

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comorbidities such as cancer (Rahman and Sarkar 2019). The main known reservoir for the virus is the dromedary camels (Hemida et al. 2017a). MERS-CoV was isolated from dromedary camels in many countries across the Arabian Peninsula such as Saudi Arabia, UAE, Qatar, and Oman (Hemida et al. 2014a; Nowotny and Kolodziejek 2014; Yusof et al. 2015).

However, there is a scarcity of information about the actual natural infection of MERS-CoV in dromedary camels. The main objective of this article was to highlight the most up-to-date knowledge about MERS-CoV in various species of animals with special attention to dromedary camels.

11.2 Historical Background and Evolution of MERS-CoV

MERS-CoV was first identified in a patient from Saudi Arabia who presented with severe respiratory symptoms and later developed respiratory failure (Zaki et al. 2012). A retrospective study confirmed the detection of MERS-CoV in archived patient samples from Jordan (Hijawi et al. 2013). The virus was called *novel coronavirus* at that time (Zaki et al. 2012). Later on, based on the available epidemiological data, a study group decided to change the name of the virus to 'Middle East respiratory syndrome' (MERS-CoV) (de Groot et al. 2013). Initially, there was a debate about the origin of MERS-CoV. Some studies suggested bats as a potential reservoir for the MERS-CoV (Memish et al. 2013). This study sequenced a short fragment from a coronavirus genome collected from bats in Saudi Arabia and found high similarity to the circulating MERS-CoV strains at that time (Memish et al. 2013). Another recent study was conducted in Africa, in Ghana, where the camels tend to live in close contact with various species of bats. This study failed to detect a similar MERS-CoV in bats in these regions (de Groot et al. 2013). Scientists tried to speculate the potential reservoir of this new coronavirus in the Middle East, especially in the Arabian Peninsula. The first seroprevalence study for MERS-CoV in different livestock animals including dromedary camels was conducted in Oman (Reusken et al. 2013). This study revealed that only dromedary camels carried specific antibodies against MERS-CoV in their sera. Since then there have been quite a few reports of new cases coming from the Arabian Peninsula and other parts of Africa (Ali et al. 2017; Deem et al. 2015; Hemida et al. 2014b; Hemida et al. 2013). On the other hand, there are reports of detection of MERS-CoV nucleic acids in many cases in dromedary camels from both the AP and Africa (Hemida et al. 2017a; Hemida et al. 2014a; Yusof et al. 2015; El-Duah et al. 2019). MERS-CoV detected in archived serum samples, dating back to the 1990s, showed higher seroprevalence of MERS-CoV in the tested specimens (Reusken et al. 2013; Hemida et al. 2014b). This may suggest the possibility of the circulation of MERS-CoV in dromedary camels before its actual emergence in human as announced for the first time in 2012 (Zaki et al. 2012).

11.3 Aetiology, Genome Organization, and Classification of MERS-CoV

MERS-CoV belongs to the genus Betacoronavirus in the family *Coronaviridae* and the order *Nidovirales*. Betacoronaviruses are further divided into four distinct clades labelled as 'A-D' (de Groot et al. 2013). MERS-CoV has been recently classified as one of the members of clade C coronavirus. MERS-CoV genome is a single strand of the positive-sense RNA molecule. The viral genome is close to 30 kilobases in length. The genome is organized as follow (5′UTR, non-structural genes- structural genes- 3′UTR-Poly A tail-3′). The virus genome is characterized by the production of sets of sub-genomic messenger RNAs. The viral genome is flanked by two untranslated regions. Two-third of the 5′ genome is occupied by the non-structural genes called Gene-1. It is composed of two overlapping open reading frames with ribosomal frameshifting site. While, one-third of the genome is occupied by various structural genes, including spike (S), envelope (E), matrix (M), and the nucleocapsid (N). There are some accessory genes scattered across the viral genome (ORF3, ORF4a, ORF4b, ORF5, and ORF8b). One recent study showed that the African lineage of MERS-CoV is distinct from those circulating in the Gulf area. This study showed that African lineages lack ORF4, which is considered to be one of the virulence factors for MERS-CoV. This may explain at least in part why there are no human cases reported in the African countries which have a significant population of dromedary camels (Chu et al. 2018).

11.4 MERS-CoV Receptors and Tropism

MERS-CoV is one of the pneumotropic viruses, which replicates well in the cells of the respiratory tract (Park et al. 2018). It is well known that most coronaviruses utilize their S protein in the process of attachment to the host cells. MERS-CoV uses the S1 protein to attach to the sialic acid in the host cell (Widagdo et al. 2019). Several studies confirmed that dipeptidyl peptidase 4 (DPP4) play an important role in the attachment and tropism identification of MERS-CoV (van Doremalen et al. 2014). This study confirmed the possibility of using the DPP4 receptors in various animal species such as cows, sheep, and goat to establish MERS-CoV infection. This suggests the potential roles of these animals as other possible hosts for MERS-CoV (van Doremalen et al. 2014). However, this study needs further confirmation. Another study showed the involvement of another protein called glucose-regulated protein (GRP-78), in the process of MERS-CoV attachment to the target cells (Chu et al. 2018). This cellular factor may act as a key player in the MERS-CoV tropism and tissue distribution (Chu et al. 2018).

11.5 MERS-CoV Infections in Animals

Some coronaviruses are zoonotic in origin, such as the severe acute respiratory syndrome coronavirus (SARS-CoV). Animals, particularly the Palm Civet cats were the main reservoir for the SARS-CoV (Shi and Hu 2008; Wang and Eaton 2007). Culling of this reservoir and banning its distribution in the public markets contributed substantially in the control and extinction of SARS-CoV (Wang and Eaton 2007). When MERS-CoV emerged, scientists started to look for potential reservoirs in the context of the virus emergence and replication cycle. Several studies were conducted to check various domestic animals that lived in close proximity to confirmed patients, for the presence of MERS-CoV antibodies or nucleic acids (Hemida et al. 2014a; Reusken et al. 2013; Hemida et al. 2014b). Interestingly enough, dromedary camels were the only positive animal for the MERS-CoV (Reusken et al. 2013). Since that time, several research groups started to focus on this line of research and to investigate the roles of MERS-CoV in the transmission cycle of MERS-CoV (Yusof et al. 2015; Xu et al. 2019).

11.6 MERS-CoV in Dromedary Camels

Several studies reported the seroprevalence and virus detection of MERS-CoV in dromedary camels from many countries from Asia and Africa (Yusof et al. 2015; Reusken et al. 2013; Hemida et al. 2014b; Kandeil et al. 2019). Earlier studies showed the high seroprevalence of MERS-CoV in most of the tested camel sera from those two regions in the world. However, animals from Australia were seronegative for the MERS-CoV (Hemida et al. 2014b; Crameri et al. 2015). Another recent study showed the seroconversion of dromedary camels from Canary Island but the absence of virus detection in specimens collected from these group of animals (Gutierrez et al. 2015). MERS-CoV was detected in the body fluids of the infected dromedary camels, especially the nasal and rectal swabs (Hemida et al. 2014a; Yusof et al. 2015; Hemida et al. 2017b).

Meanwhile, high prevalence of MERS-CoV was detected in tissue specimens collected from the upper respiratory tract of dromedary camels (Khalafalla et al. 2015). This was in contrast to absence of the detection of any viral nucleic acids or particles in urine samples collected from camels in Qatar (Farag et al. 2019). It was found that the detection rate of MERS-CoV in naïve camels (less than 2 years old) is much higher than adult animals (Hemida et al. 2017b). This highlights the potential role of the young camels in the transmission of MERS-CoV. Another interesting phenomenon is the susceptibility of dromedary camels to the second round of MERS-CoV infection despite the presence of neutralizing antibodies in their sera (Hemida et al. 2017b). This also explains the high seroprevalence of adult camels to MERS-CoV. Another important study reported the detection of MERS-CoV in apparently normal dromedary camels (Mohran et al. 2016). This highlights the

confirmed roles of dromedary camels in the sustainability of MERS-CoV in the environment. Although the role of camels in the transmission/replication cycle of MERS-CoV was confirmed a while ago, until now, there is no clear evidence of MERS-CoV infection in dromedary camels under the natural conditions.

Some studies reported the results of the experimental infection of dromedary camels with MERS-CoV (Adney et al. 2014). These studies reported the course of the MERS-CoV infection in a limited number of animals and the curve of the virus shedding over the time of the experiments (Adney et al. 2014). According to these studies there were no characteristic clinical signs observed after the experimental challenges with MERS-CoV, except for very mild elevation of the body temperature and some mild nasal discharges (Adney et al. 2014). The nasal discharge ranged from very mild serous exudate to purulent and sometimes haemorrhagic (Adney et al. 2014). According to same study, virus shedding from the nasal secretions started in less than one-week post-inoculation (PI) and lasted for up to 35 days PI (Adney et al. 2014). However, no viral shedding was reported in faecal swabs or urine during infection (Adney et al. 2014). Interestingly, very low virus concentration was detected in the exhaled breath from the infected animals (Adney et al. 2014). Post-mortem inspection of these animals revealed that the viral antigens were detected in the upper respiratory tracts, especially nasal passages, trachea, and nasal turbinate bones by the immunohistochemistry technique (IHC) (Adney et al. 2014). However, no signal was detected on the alveolar epithelium (Adney et al. 2014). These studies were quite useful in the determination of the viral tropism and tissue distribution, which explains the pathogenesis of the viral infection in dromedary camels. Meanwhile, seroconversion of the inoculated animals was observed within 14 days PI (Adney et al. 2014).

11.7 MERS-CoV in Other Members of the Family *Camelidae*

The family *Camelidae* includes dromedary camels, alpaca, and llama. One recent study showed seroprevalence of alpaca kept in close proximity to dromedary camels in Qatar (Reusken et al. 2016). Another study was conducted to check the status of MERS-CoV in dromedary camels, alpaca, and llama. This study found all swabs were negative while some llama were seropositive indicating an active infection among this species of animals in Israel (David et al. 2018). Based on the susceptibility of alpaca to MERS-CoV infection, one study tested the efficacy of a new MERS-CoV-spike based subunit vaccine in both dromedary camels and alpaca. This study showed the induction of a high level of neutralizing antibodies in sera of the challenged animals, which reduced the titre of virus after challenging (Adney et al. 2019a). Recent studies showed that alpaca is susceptible to experimental infection with MERS-CoV (Crameri et al. 2016). The challenged animals shed the virus in

their secretions particularly the nasal tract and showed protection when challenged with the wild virus at the end of the experiment (Crameri et al. 2016).

11.8 MERS-CoV in Other Species of Animals

Earlier studies reported the absence of MERS-CoV antibodies in sera of Bactrian camels from Mongolia (Chan et al. 2015; Miguel et al. 2016). However, one recent study reported high virus shedding in secretions of the MERS-CoV experimentally infected Bactrians (Adney et al. 2019b). This explains the potential role of genetic factors in the susceptibility/resistance of the members of the family *Camelidae* to the MERS-CoV infection. This line of research requires further studies in future. Previous studies showed the absence of any detectable antibodies of MERS-CoV in sera of other livestock animals such as sheep, goat, cattle, horses, and many other animals (El-Duah et al. 2019; Zohaib et al. 2018). This is in contrast to a recent study conducted in Egypt, Tunisia, and Senegal, which showed the detection of specific antibodies in sera collected from of sheep, goat, and donkeys that lived in close contact with camels from these countries (Kandeil et al. 2019). Furthermore, viral nucleic acids were detected in specimens from sheep, goat, and cattle (Kandeil et al. 2019). This line of research requires large-scale studies to understand the dynamics of MERS-CoV among livestock animals in countries where camels live in close contact with these species of animals.

11.9 Animal Models for MERS-CoV

Since the emergence of MERS-CoV in 2012, there was a great demand in finding some potential animal models. Confirmation of the roles of dromedary camels in the transmission cycle of MERS-CoV increased this demand. This is because of the difficulty in access to dromedary camels in North America and Europe. There is also a scarcity of the animal biosafety, level-3 laboratories that are equipped to adopt large animals, especially camels. Initial trials considered the Syrian hamster as a good animal model for the SARS-CoV research (Schaecher et al. 2008); however, it is not a suitable animal model for MERS-CoV (de Wit et al. 2013). The MERS-CoV experimental infection in the golden Syrian hamster was not successful since the virus neither replicates in various body organs nor produces any detectable immune response or antibodies after infection (de Wit et al. 2013). The wild type mouse is non-susceptible to MERS-CoV infection, but recently a MERS-CoV transgenic mouse was developed by the expression of the human DPP4 receptors (hDPP4) (Agrawal et al. 2015). These transgenic mice developed severe clinical signs after the challenge with MERS-CoV. The infected transgenic mice suffered from ruffled fur, severe respiratory manifestations, and finally death, a few days after infection (Agrawal et al. 2015). Both rhesus macaques and marmosets are susceptible to

MERS-CoV infection. Both animals were shown to develop signs similar to humans infected with MERS-CoV, including several lesions on the respiratory tract with pulmonary oedema, haemorrhage, and pneumonia (Yu et al. 2017). Pigs were found to be refractory to MERS-CoV infection. Very low virus titre was detected in challenged animals after infection, suggesting that pigs are not a suitable animal model for MERS-CoV, in contrast to dromedary camels and alpaca.

11.10 Isolation of MERS-CoV

The success of isolating MERS-CoV from clinical specimens collected from human or animal mainly depends on the quality of the collected specimen and its preservation, as well as maintenance, until it is processed in the laboratory. In case of transportation of these specimens from the field to the laboratory, it should be transported on ice and kept cool till it reaches the destination laboratory. Transport medium like foetal bovine sera and antibiotic cocktail should be added to the transport medium containing minimal essential media. Some common cell lines are used in the isolation of MERS-CoV collected from patients or animals, especially Vero cell line (Chan et al. 2014; Park et al. 2016). Successful virus isolation is associated with the induction of cytopathic effects (CPE) on the inoculated cell culture. The observed CPE in case of MERS-CoV isolation is the rounding and detachment of cells from the confluent monolayer sheet within three days from the infection of the cell culture (Chan et al. 2014; Park et al. 2016).

11.11 Laboratory Techniques for the Diagnosis of MERS-CoV

Since the clinical diagnosis of MERS-CoV in dromedary camels is not accurate or even suggestive due to the absence of any pathognomonic clinical signs or post-mortem lesions, laboratory diagnosis is the only way to confirm the presence or absence of MERS-CoV infection in a certain animal. There are two strategies for the diagnosis of MERS-CoV infection in dromedary camels (Fig. 11.1). The first one is the detection of specific viral antibodies in sera of dromedary camels. Several in house and commercial diagnostic antibody detention and titration kits, particularly the enzyme-linked immunosorbent assay (ELISA) are commercially available for MERS-CoV antibodies (Hashem et al. 2019a; Lee et al. 2018; Okba et al. 2019). These tests showed good aptitude in the detection of MERS-CoV in sera of dromedary camels. However, their specificity and sensitivity still need further evaluation. This may be due to the possibility of cross-reactivity that reported between MERS-CoV and other closely related coronaviruses affecting dromedary camels particularly the Bovine coronavirus (Hemida et al. 2014b).

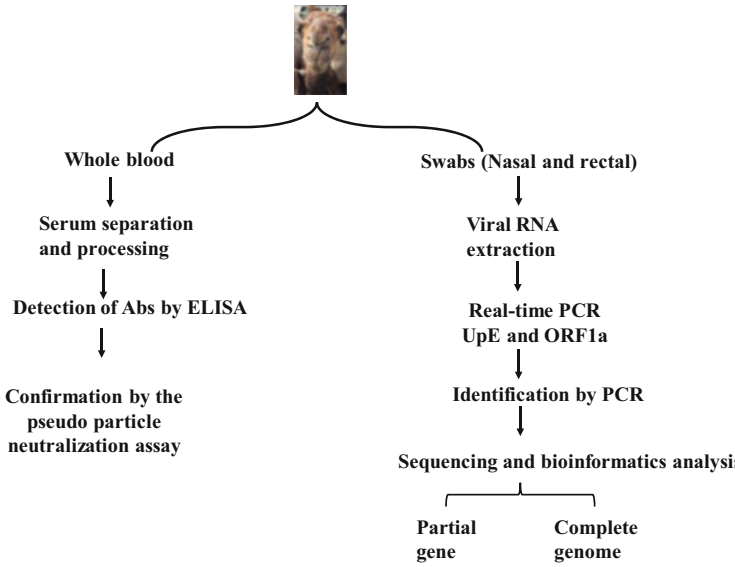

Fig. 11.1 A standard protocol for the laboratory diagnosis of MERS-CoV in animals detection and titration of MERS-CoV antibodies in sera of dromedary camels by commercial or in house ELISA. Confirmation of the ELIA results by the pseudoparticle naturalization assay. Detection and titration of MERS-CoV nucleic acids in swabs (nasal and rectal) collected from animals. Extraction of the viral nucleic acids by standard commercial kits. Detection of the viral nucleic acids by real-time PCR using two targets (up-E and ORF1a). Confirmation of the real-time PCR results by amplification of some viral target genes or the full-length genome by the next-generation sequencing. Revealing the identity of the amplicons can be done by blasting obtained sequences against the available MERS-CoV in the GenBank

Meanwhile, there are some other reliable laboratory accurate techniques used for the detection and quantification of the MERS-CoV specific antibodies in dromedary camel sera. These techniques include, for example, the pseudoparticle neutralization assay (Hemida et al. 2014b). The other strategy is the detection of MERS-CoV infectious virus particles or the viral nucleic acids in the body secretions of the tested animals. The ideal samples for the detecting of the virus or its nucleic acids are the nasal and rectal swabs (Hemida et al. 2014a). There are some quick latex agglutination based tests recently introduced for the quick identification of positive MERS-CoV camels (Kasem et al. 2018). However, these methods are not accurate and prone to false positive/negative results. It is mainly depended on the concentration of the viral antigens in the tested samples. The most accurate technique, considered the gold standard, is the detection of the viral nucleic acids in body secretions by the reverse transcriptase-polymerase chain reactions (RT-PCR) (Hemida et al. 2013). There are many modifications for the RT-PCR technique in the diagnosis of MERS-CoV infection in both humans and animals. One of the robust and user-friendly techniques is the isothermal PCR technique (Shirato et al. 2014). As per the WHO's instruction, confirmation of active MERS-CoV case depends on the testing of

suspected samples by RT-PCR using two different gene targets particularly (up-E and ORF1a) (Corman et al. 2012). The definite diagnosis is the sequencing of the obtained PCR products then blasting the results against the available sequences in the Genebank. Currently, the application of the next-generation sequencing of the partial or the full-length genome is of great value in the diagnosis, classification, and mentoring of changes on the MERS-CoV genomes on the genomic levels (Yusof et al. 2015; Hemida et al. 2013). The application of the IHC techniques to study the antigen localization of the MERS-CoV in various tissues is a good diagnostic approach (Adney et al. 2014).

11.12 Zoonotic Potential of MERS-CoV

MERS-CoV is one of the most important newly emerged viral zoonosis (Azhar et al. 2017). The first observation about the potential zoonotic roles of MERS-CoV was reported in 2014 (Azhar et al. 2014). This study sequenced MERS-CoV from both patients who died from severe pneumonia and from some of the patients' camels that suffered from mild respiratory signs (Azhar et al. 2014). Sequences from both the patient and camels were identical, suggesting the zoonotic transmission of MERS-CoV from camels to human (Azhar et al. 2014). Since that time, there is an ongoing evaluation of the zoonotic potential of MERS-CoV, particularly in the at-risk group of people who come in close contact with dromedary camels on a daily basis. Although some research failed to detect any specific antibodies for MERS-CoV in sera of people in close contact with positive camel herds (Hemida et al. 2015), some other studies reported the seroprevalence of MERS-CoV in people who came in close contact with dromedary camels in Qatar (Reusken et al. 2015). One large-scale serosurveillance study was conducted across Saudi Arabia to investigate the nation-wide prevalence of MERS-CoV in people with a history of close contact experience with dromedary camels. This study showed that 0.15% of tested individuals had antibodies against MERS-CoV (Muller et al. 2015). Another recent study conducted in UAE to assess the risk seroprevalence of MERS-CoV among workers in two slaughterhouses and local camel markets found that around 1.7% of the tested individuals have specific antibodies against MERS-CoV (Adney et al. 2019b). Taking into consideration all these facts, dromedary camels pose some risk to human in the context of MERS-CoV transmission cycle. Further studies are needed to unveil the exact mechanisms of MERS-CoV transmission from camels to humans.

11.13 Control Measures for MERS-CoV

Unfortunately, until now, there is no effective medication or vaccine to treat or prevent MERS-CoV infection in human or animals. The control measures for most viral infections are mainly based on the variability of effective vaccines and the

adoption of high standard hygienic measures in both the health care settings and in the farm animals. Implementation of novel strategies based on the One Health concept will help in the reduction of virus shedding from dromedary camels. This will have great benefits on the spillover of MERS-CoV from dromedary camels to human (Hemida and Alnaeem 2019). One of these approaches is the development of novel vaccines against MERS-CoV in dromedary camels. There is some progress in the development and testing of potential vaccines for MERS-CoV in dromedary camels. Some trials have used various approaches to prepare and test these vaccine candidates in laboratory animals as well as in camels. These approaches include some common vaccine strategies such as DNA vaccines, vector-based vaccines, the subunit vaccines, and the viral-like particles based vaccines (Yong et al. 2019). One recent subunit vaccine showed a high degree of protection and reduction in the time of viral shedding in MERS-CoV challenges alpaca and dromedary camels, respectively (Adney et al. 2019a). Several vaccine candidates used the full-length spike protein or its receptor binding domains (RBD) as a base for the preparation of recombinant vaccines against EMRS-CoV (Song et al. 2013; Volz et al. 2015). Several studies used the adenovirus-based recombinant MERS-CoV-S glycoprotein and proved to induce a high level of potent neutralizing antibodies against MERS-CoV (Hashem et al. 2019b; Jia et al. 2019; Kim et al. 2019). Currently, there are two potential MERS-CoV vaccines which have entered the clinical trials phase—the GLS-5300 and the MERS001 (Xu et al. 2019). The other arm of the control measures against MERS-CoV is the implementation of high degree of biosafety precautions among the at-risk people. Those include people who are in daily close contact with dromedary camels, particularly the camel owners, herders, veterinarians, and slaughterhouse personnel (Hemida et al. 2017a).

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Chapter 12
Japanese Encephalitis Virus

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Abstract Viruses belonging to the genus *Flavivirus* of the family *Flaviviridae* are the established human pathogens and their zoonotic potential has escalated in the last few decades. They are transmitted by vectors and accordingly grouped as tick-borne flaviviruses and mosquito-borne flaviviruses. Viruses transmitted by ticks are closely related species of single sero-complex, whereas mosquito-borne flaviviruses are diverse. Yellow fever virus is the prototype of this genus. There are four important Flaviviruses associated with Japanese encephalitis sero-complex, which causes encephalitis epidemics world over. Examples include Japanese encephalitis, West Nile, Murray Valley encephalitis, and Saint Louis encephalitis viruses. Japanese encephalitis is a leading cause of neurological illness in children's aged below 15 years. It is transmitted by *Culex tritaeniorhynchus* mosquito. JEV is maintained in nature by ardeid birds and pigs (both domestic and wild pigs). There are two enzootic cycles of JEV transmission, i.e. pig-mosquito-pig and bird-mosquito-bird cycle. The ardeid birds are the natural reservoir maintaining the JE virus, whereas pigs are the amplifier host. The disease is endemic in South-East Asian countries, and the highest numbers of deaths are recorded in India. JEV is considered as emerging pathogen due to changing epidemiology. JEV is endemic in 24 countries, and most of them are Asian countries. JE is spreading in new area owing to climate change, expansion of vector range, increase in pig husbandry, and population explosion. Introduction of JE vaccines has curtailed down the incidence of JE to a great extent in several endemic countries. India has also recommended JE vaccination for children in the endemic regions. Although immunization of humans is an effective strategy for JEV prevention, its control is challenging due to the existence of different transmission cycles. Especially, risk from the ardeid birds is unpredictable and cannot be controlled. Few countries have attempted JE

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vaccination in porcine population as a strategy for JE prevention but it is not universally adopted. Co-circulation of different flaviviruses in nature makes the diagnosis and prevention of JE challenging in the endemic countries. In the context of global warming and climate change, it is mandatory to consider JE in One Health paradigm.

Keywords Japanese encephalitis · Japanese encephalitis virus · Ardeid · Encephalitis · Acute encephalitis syndrome · Flavivirus · Zoonoses · *Culex tritaeniorhynchus*

12.1 Prologue

Japanese encephalitis (JE) is an emerging vector (mosquito) borne zoonotic disease caused by the Japanese encephalitis virus (JEV) responsible for encephalitis (acute inflammation of the brain) in horses and humans. Swine act as an amplifier host for JEV, and occationally JEV can cause abortions and stillbirths in pigs. Around three billion people are living in endemic zones and are at risk of JE. JEV is the reason for 35,000–50,000 human cases annually and is responsible for ~10,000 human deaths throughout the globe, though the majority of the cases come from Asian countries (Campbell et al. 2011; CDC 2019). The global incidence of JE is 1.8/100,000 which increases with the inflow of tourists travelling from epidemic areas to the non-endemic area bringing new infections and thus making it an international public health issue (Gao et al. 2019; Campbell et al. 2011). Of the infected cases, approximately 25–30% succumbs to death and about 30–50% people recovered and survived may suffer from permanent neurological sequelae. Living close to paddy cultivation, pig farming, and water lodging are some of the predisposing factors for increased risk of JE in humans. Such factors facilitate the close contact of vector and host. JE is a classic example of Flavivirus infection widespread across the countries of Asia, Western Pacific, and parts of Australia. The disease is hyperendemic in the worlds most populated countries, namely China and India. JE was once considered as a disease of children, but JEV can cause illness and deaths in adults. A survey conducted in Korea revealed that foreign expatriates living in Korea are at more risk of JE with more incidence rate as compared to the native people (Shin et al. 2018). The age factor is crucial in the JE epidemiology and consideration should be given to the adult age group while designing vaccination strategies. It was observed that median age Korean JE infected cases had been increased from 49.8 to 53 years from 2007 to 2015 (Sunwoo et al. 2016). Three JE cases of the US citizens who visited JE endemic countries, viz. Taiwan, China, and South Korea have suffered from clinical JE. All the three US citizens were healthy before their visits to the endemic nations and after return to home country suffered from the disease. Out of them, one died of the disease, and two were completely recovered without any neurological sequelae (Hills et al. 2014). Thus, differential diagnosis of JE is important in the travellers returning from the JE endemic countries.

Similarly, counselling on the prevention and control measures of JE must be done in the travellers. It is well known that JEV epidemiology is changing at a great pace. The complex of multiple factors like introduction in new geographic areas, non-vector risk of spread, age factor, currently available vaccines and vaccination protocol, agricultural practices, migration of birds, etc., makes JE as an important zoonotic disease with potential risk to humans globally (Connor and Bunn 2017).

12.2 Etiology

JE is a zoonotic viral disease of humans and horses. It is caused by the JEV which is a prototype species of JE sero-complex of mosquito-borne flaviviruses. The JEV has an RNA genome which is single-stranded and positive-sense. It is comprised of a short 5′ untranslated region, a single open reading frame (ORF), and a longer 3′ untranslated region. The polyproteins are encoded by the ORF which further cleaved into structural and non-structural proteins by host and viral proteases. Capsid protein (C), pre-membrane protein (PrM), and envelope protein (E) are the three structural proteins, whereas NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 are seven non-structural proteins (Unni et al. 2011; Qiu et al. 2018; Solomon et al. 2003). The JEV belongs to the genus *Flavivirus* of family *Flaviviridae*. The virus is small-sized around 40–50 nm and is enveloped. Structurally it is spheroid with cubical symmetry, and recently, JEV capsid protein crystal structure has also been described (Poonsiri et al. 2019). The positive-sense single-stranded RNA has a genome size of 11 kb with one open reading frame (ORF) that encodes a polyprotein. The genome is covered by a capsid and a host-derived lipid bilayer. The ORF flanked with non-coding region 5′ and 3′ at either side is required for viral replication, transcription, and translation. Envelope protein is a major viral protein playing important role in the virulence, host cell entry, and humoral immune response. NS1 is linked with replication. NS2A is cleaved from NS1 by host proteases, and NS2B is a cofactor of viral serine protease. During virus assembly NS3 acts as a reservoir for viral proteins, NS4 is an important membrane component, and NS5 is an essential component of virus replication complex (Sahoo et al. 2008). Clinically and ecologically, JEV is much closely related to West Nile virus and Saint Louis encephalitis virus. It is assumed that all the closely related flaviviruses must have evolved from the common ancestor's way back some 10–20 thousand years and later separated and adapted different ecological positions (Solomon et al. 2000).

Nucleotide sequencing of the capsid (C), precursor membrane (PrM) and envelope (E) genes, and its further phylogenetic analysis revealed the existence of five genotypes (I, II, III, IV, and V) of JE virus and all these genotypes form a single serotype (Banerjee 1996; Uchil and Satchidanandam 2001; Desingu et al. 2017). These five genotypes have been isolated from different parts of the globe with genotype I being isolated from northern Thailand, Cambodia, and Korea; genotype II from southern Thailand, Malaysia, Indonesia, and Australia. Genotype III from China, India, Japan, Korea, Taiwan, Sri Lanka, Philippines; genotype IV from

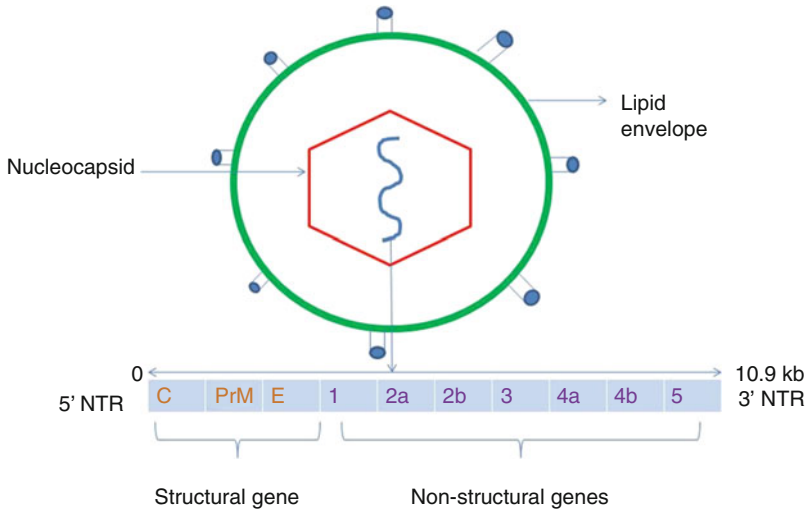

Fig. 12.1 Structure and organization of JE viral genome

Indonesia (Chen et al. 2019) and genotype V from Malaysia (Uchil and Satchidanandam 2001; Solomon et al. 2003). Earlier, genotype III was the most prevalent genotype in humans which is now replaced by genotype I and this shift has been observed in the major JE reporting countries, viz. China, Japan, and Korea (Simon-Loriere et al. 2017; Nga et al. 2004; Wang et al. 2007; Nitatpattana et al. 2008; Pan et al. 2011; Gao et al. 2019). The prevalent genotype I can be further categorized into genotype I-a and genotype I-b clades (Schuh et al. 2014). The earlier dominant genotype III may have less occupancy in Asia as compared to genotype I in present time, but genotype III has spread to other continents like Europe and Africa. Genotypes II and V which were endemic in Malaysia were also spreading to other parts, viz. genotype II to Australia, and genotype V to China and South Korea (Gao et al. 2019). Study on the origin and evolution of JEV suggested that genotype IV has ancient lineage than other genotypes. Similarly, phylogeny of E protein revealed Muar strain as genotype V. It was also stated that JEV genotypes I, II, and III must have diverged recently, while genotype IV diverged 350 (±150) years ago from common ancestors (Solomon et al. 2003) (Fig. 12.1 and Table 12.1).

12.3 History of JEV

The serological surveillance carried out by Mitamura and colleagues in Japan in the 1930s showed that various mammals, such as horses, pigs, goats, rabbits, and sheep, had antibody reactions against JEV (Morita et al. 2015). After that, in the year 1937, the virus was isolated from the brain of a horse suffering from encephalitis in Japan. The role of the mosquito vector (*Culex tritaeniorhynchus*) for JEV transmission was

Table 12.1 Genes and amino acids of the genome of JEV vaccine strain SA-14-14-2

Gene	Nucleotide sequence length (bp)	Amino acid
5′ Non-coding region	95	Nil
Capsid	381	127
Pre-membrane/membrane	501	167
Envelope glycoprotein	1500	500
Non-structural 1	1245	415
NS2a	492	164
NS2b	393	131
NS3	1857	619
NS4a	801	267
NS4b	411	137
NS5	2715	905
3′ Non-coding region	582	Nil
	10,973	3432

documented in the year 1938 (Mitamura et al. 1938; Erlanger et al. 2009). Genetic studies of JEV lead to the finding of its origin to the Malay Archipelago—an area between the mainlands of India, China to Australia. This ancient JE virus then evolved into the many present genotypes and travelled across Asian countries and is putting its foot to European counties also (Solomon et al. 2003; Schuh et al. 2013). Globally, the disease is endemic in parts of China, India, South Korea, Japan, Nepal, Vietnam, Indonesia, Philippians, Taiwan, Sri Lanka and is being reported from many other countries of Asia and spreading to Russia and European countries.

The first extensive research findings on the origin and evolution of JEV was published by Solomon et al. (2003). Their study suggested that JEV genotypes originated from Indonesia and Malaysia regions from the ancestor viruses. It was further stated that South-East Asian regions could be the hotspots for the emergence of viral pathogens. Historically, endemic genotype III has been replaced by genotype I in many parts of the JE endemic regions. Genotype V of JEV is not extensively reported and others that Muar strain isolated in 1952 in Malaysia, it was not reported at other places. The genotype V of JEV was detected in *Culex tritaeniorhynchus* mosquitoes in 2009 from China. This strain was designated as XZ0934. The study suggested the re-emergence of JEV genotype V in Asia (Li et al. 2011). A study from the Republic of Korea has also detected JEV genotype V for the first time in *Cx. Bitaeniorhynchus mosquitoes (*Takhampunya et al. 2011*).* JEV genotype I was found to be the predominant genotype in Asia as studied by Pan et al. (2011). As per this study, JEV genotypes diverged over some time in the following order as: JEV genotype IV, genotype III, genotype II, and genotype I. Gradual increase in the genetic diversity of genotype I is consistent and thus it is a predominant genotype at present in Asia. The estimated years of occurrence of genotypic diversions in the JEV recorded were 1695 years ago, 973 years ago, 620 years ago and 193 years ago in for the JEV genotypes IV, III, II, and I, respectively (Pan et al. 2011). Phylogenetic analysis of JEV genotypes based on the whole genomic sequencing

of all five genotypes revealed that 1930–1960 and 1980–1990 are the periods of peak genetic diversity and after 2000 it remains high (Gao et al. 2015). Clinical illness resembling the JE viral infection dates back in the nineteenth century. It was responsible for summer encephalitis in Japan, and first clinical case of JE was documented in 1871. Recurrent JE outbreaks were recorded during 1930s in Japan almost after every 10 years. In 1935, JEV was first time isolated from the brain which is known as Nakayama strain, a prototype strain of JEV. The role of vectors, reservoirs, and amplifiers was documented in the year 1938 (Tsai 1997).

12.4 Host Range

The reservoir maintenance host of JE virus is ardeid birds, the reservoir amplifier host pigs, and the accidental dead-end host is human and horses. In equid, family donkeys are also susceptible to JEV. Bovine, ovine, caprine, dogs, cats, chickens, ducks, wild mammals, reptiles, bats, and amphibian can get sub-clinical infection, but they probably do not contribute to the spread of JEV (Yang et al. 2011; Xiao et al. 2018). The epidemiology and ecology of JEV are complex, and several epidemics were documented in the absence of amplifying host pigs. JEV has proven hosts as birds as reservoirs, pigs and Ardeidae birds as amplifiers, and human and equines as dead-end hosts. However, serological evidence of JEV antibodies in other animals like cattle, chickens, ducks, bats, small ruminants, dogs and cats, amphibians like frogs, monkeys, raccoons, etc., highlighted the need for exploring the role of animal species other than pigs and birds in the JE transmission (Shimoda et al. 2011; Bhattacharya and Basu 2014). The potential of bats in JEV transmission has already been documented (Mackenzie et al. 2008). Recently, a study from Malaysia has reported high prevalence of JE in dogs and followed by pigs, cattle, cats, and monkeys (Kumar et al. 2018).

12.5 Geographical Distribution

Infection due to JEV has been reported from several countries, viz. China, India, Japan, Bangladesh, Australia, Burma, Indonesia, Vietnam, South Korea, North Korea, Nepal, Sri Lanka, Pakistan, Philippines, Malaysia, Thailand, Taiwan, Timor-Leste, Papua New Guinea, Russia, Saipan, Singapore, Cambodia, Guam, Laos, Brunei. JE is considered as an emerging zoonosis and is rapidly spreading to new regions (Park et al. 2018; Zhao et al. 2018; CDC 2019; Yap et al. 2019). Here we will elaborate the JE scenario in those countries where JE incidences are more or emerging. More than 95% of JEV cases are seen from China and India, the two highest populous countries of the globe. The JE epidemiological pattern is epidemic and endemic in northern and southern parts of the world, respectively. Bangladesh, China, Taiwan, Japan, South Korea, India, Thailand are the countries where the

Fig. 12.2 Global distribution of Japanese encephalitis (Dark areas represent epidemic pattern of JE; light grey colour represents endemic pattern of JE)

epidemic pattern of JE is recorded. While endemic pattern is recorded in countries like Australia, Cambodia, Indonesia, Laos, Malaysia, Vietnam, Sri Lanka, and Timor-Leste (Wang and Liang 2015). As of today, JEV infection has been detected in 27 countries globally (Figs. 12.2 and 12.3).

India JE is endemic to the country and epidemics are reported from most of the Indian states, mainly from plain belts and emerging in hilly states. It is a major paediatric problem with cases of adults also coming forward (Kulkarni et al. 2018; Baruah et al. 2018). First clinical case of JE was reported from Vellore, Tamil Nadu in the year 1955 (Namachivayam and Umayal 1982) and thereafter India recorded the first major outbreak from Burdwan and Bankura districts of West Bengal in the year 1973 with 700 cases taking a toll of 300 human lives (Banerjee 1996). Since then, the virus is active in almost all parts of India, especially from rural parts of the country with regular reports from Uttar Pradesh, Bihar, Tamil Nadu, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Haryana, Kerala, West Bengal, Orissa, Goa, and Pondicherry (Kabilan et al. 2004). Gorakhpur district of Uttar Pradesh is the worst affected district in India. Major outbreaks of JE were reported in the years 1978, 1988, and 2005 with more than 1000 deaths in each of the outbreak (Tiwari et al. 2012). Presently, JE is not only endemic in many areas; it is also spreading to naive non-endemic areas, viz. hill states of North Eastern part of the country. Outbreaks recorded in Malkangiri, Orissa state and Manipur state in 2012 and 2016, respectively, are the examples. In India, 24 states/Union territories have

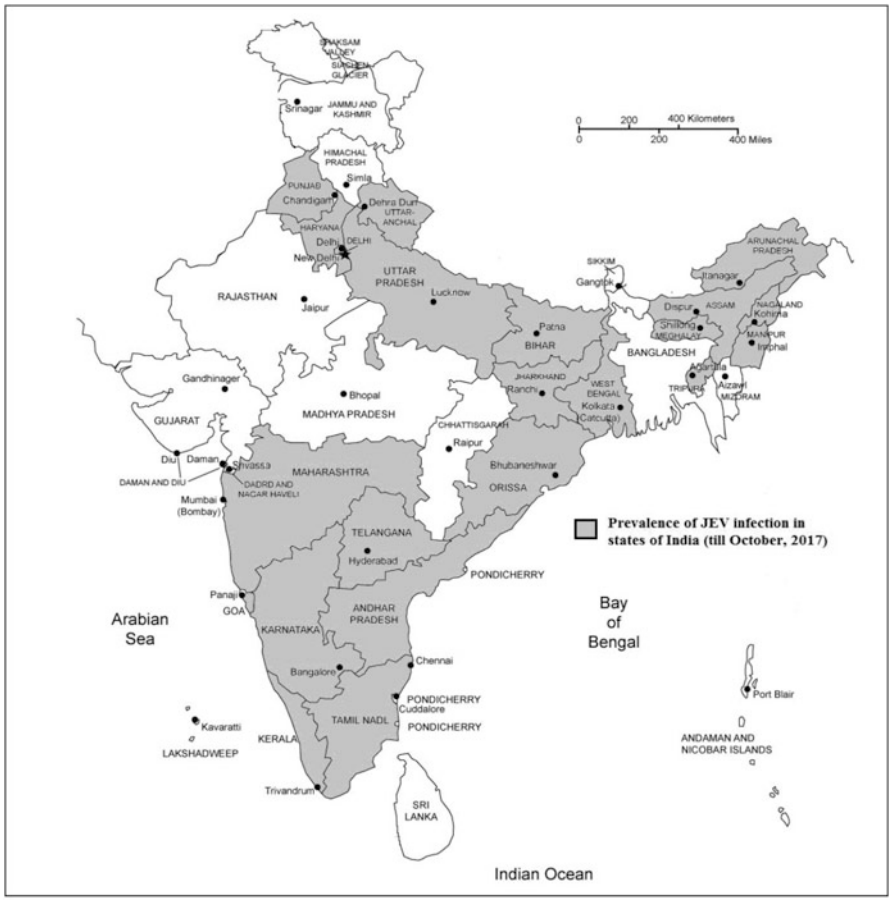

Fig. 12.3 Prevalence of Japanese encephalitis in India

reported JE (Rao et al. 2000). India is following strategies of human vaccination and vector control programme. There is no pig or equine vaccination available in India. In India, JE vaccination has been introduced in 2006 for children aged 1–15 years. This vaccine was included in the National Immunization Programme by Government of India in 2014. The districts where JE is endemic, the SA-14-14-2 JE vaccine are being used as a part of Universal Immunization Programme. More than 11 crore children's from identified JE endemic districts are immunized in India. Simultaneous detection of JEV genotype I and genotype III from cases of acute encephalitis syndrome (AES) in 2009 Gorakhpur outbreak was recorded by Fulmali et al. (2011). JE cases are mostly reported during monsoon and post-monsoon periods due to vector abundance.

Japan The name of JE is originated from Japan, and the first JEV Nakayama strain was isolated in 1935.The cases of JE have been drastically reduced in Japan in the last three decades after the implementation of National Surveillance of vaccine-preventable diseases since 1965. The figures of JE cases were above 1000 per year during the 1960s and surprisingly, during 1982–2004 only 361 cases were reported (Arai et al. 2008). The age-wise distribution revealed 78% of the JE cases were above 40 years of age (Matsunaga et al. 1999). Since 2005, inactivated Vero cell-derived JE vaccine (Beijing-1 strain) is in use. *C. tritaeniorhynchus* is the main vector in Japan and epidemics were reported from July to November. JE surveillance shows that JE is still prevalent in Japan but with combined efforts of vaccination, mechanization of rice cultivation, and mosquito control programme, the country has controlled JE to a great extent (Konishi et al. 2006; Ayukawa et al. 2004; Wang and Liang 2015; Nanishi et al. 2019). July to November is the peak season for JE occurrence in Japan, and it is predominantly observed in unvaccinated individuals only. Japan has set a very effective model of mass vaccination to control the JE. As per the South-East Asian Regional Office of the WHO, health education and training is the recommended strategy for JE prevention and control.

China China is one of the hyperendemic countries of JE. The disease is responsible for thousands of cases annually since 1943. The JEV was isolated in 1949, and until now numerous JEV strains were isolated from human, animals, mosquitoes, etc. Due to the endemic status and clinical spectrum, JE has been declared a notifiable disease in 1950. During 1950–2011 approximately two million JE cases were documented from 26 provinces of China. After the introduction of immunization the incidence has been declined significantly (Gao et al. 2014). China has developed P3 inactivated JE vaccine which was in use for the immunization. Later in 1988, the live-attenuated SA-14-14-2 vaccine was licensed and still used for JEV immunization. This vaccine is prepared by four companies in China for domestic as well as export purpose. The first report of JE from China came in the 1940s, with reporting of 10–15 cases per 100,000 in 1960–1979. Children upto15 years of age constitute the majority of JE cases in China. The peak JE season is from July to August and cases were seen even 1 month before and after the peak season. JE vaccination was introduced in China from the 1970s with mouse brain-derived JE vaccine (MBD) (P3 strain) and later with the live-attenuated SA 14-14-2 in 1989, and the same was used in National Immunization Programme since 2008 (Wang and Liang 2015). There were around 10,308 reported JE cases in 1996 which got decreased to 2541 cases in 2010 (Shi et al. 2019). JE is reported from the Tibet region also (Zhang et al. 2017).

South Korea Since 1993, JEV III has been completely replaced by genotype I in Korea. Genotype V was also isolated from *Culex* mosquito in this country. Due to vaccination policy adopted for infants, JE incidence is very low in Korea. Similarly, JE in swine is a notifiable disease of animals in Korea and sow vaccination is compulsorily done. The vaccine strain Anyang 300, G3 is being used for swine vaccination for the last 30 years (Nah et al. 2015). With the detection of the first human case of JE in 1946, the government started to include JE in national

surveillance system from 1949 and later in the year 1971 JE vaccination with the MBD was started with children with mass vaccination programme taking place since 1983 after a large outbreak in that year. These mass vaccination efforts could be seen in the decrease of JE annual cases from 100 to 1000 cases (before 1983) to 10 annual cases thereafter (Lee et al. 2012). August to October is the season of JE with *C. tritaeniorhynchus* as a principal vector (Wang and Liang 2015; Bae et al. 2018). JE sero-monitoring is mandatorily done in Korea, and since 2007, JE outbreak is not notified in pigs in Korea.

Vietnam The first isolation of JE virus was recorded in the year 1951. During the 1960s as high as 22 human cases per 100,000 were recorded, this later decreased to 1–8 per 100,000. JE surveillance is a part of their national surveillance system. In the year 1997, JE vaccination was initiated for children in the 12 high-risk districts with MBD vaccine which was later expanded to 65% of districts of Vietnam (Yen et al. 2010; Wang and Liang 2015).

Thailand In Thailand, JEV immunization began as a part of the childhood vaccination programme in the Northern provinces in 1990; this programme rapidly expanded to 36 provinces that had reported a persistent incidence of encephalitis. Study of Nitatpattana et al. (2008) conducted on the pigs and mosquitoes samples collected from the JEV confirmed human cases revealed co-circulation of genotype I and genotype III of JEV. It was further stated that genotype III is getting replaced by GI. Serological evidence was there since 1961. Encephalitis cases (JE included) were recorded in the database for routine disease surveillance in Thailand. Epidemics in Thialand were seen mainly from May to September, with record of sporadic cases (occurring throughout the year). *C. tritaeniorhynchus*, *C. gelidus*, and *C. fuscocephala* are the suspected vectors for transmission of JE in Thailand. Annually around 1500–2500 encephalitis cases were reported between 1970s and 1980s, which got decreased to 297–418 cases recorded during 2002–2008. JE was recorded more in children. The MBD JE vaccine was introduced in 1990 and at present after successful trial of chimeric live-attenuated vaccine strain (SA-14-14-2) in Thailand got recommended by WHO and had been in practice (Appaiahgari and Vrati 2010; Wang and Liang 2015).

Nepal JE has been transmitted from northern India to Nepal, and the first case was detected in the Terai region in 1978. JE is presently endemic in Nepal and outbreaks are recorded every 2–3 years span. Nepal is a hilly country, and the cases were seen mainly in low hill relatively plain areas (Bhattachan et al. 2009). July to October is the main season for JE epidemics. Between 2005 and 2010, a total of 2040 JE cases with 205 deaths were recorded in Nepal. JE mass immunization with the live-attenuated SA-14-14-2 was carried in 2006 in the epidemic area which decreases the incidence rate to 1.3 per 100,000 (Wierzba et al. 2008). Pig vaccination was initiated in the Terai districts in 2001 with the live-attenuated virus (Wang and Liang 2015). In the last 25 years, over 26,000 cases and 5000 deaths are attributed to JE in Nepal. Out of 75 districts, JE cases were recorded in 54 districts (Ghimire and Dhakal 2015). Rice cultivation, pig farming, and other climatic factors favour the existence of JEV in Nepal.

Myanmar After serological evidence in 1968, the first JE outbreak was recorded in 1974 and July to October is the season of JE in Myanmar. Majority cases were in children and teenagers. *C. tritaeniorhynchus* is the vector being suspected and the seropositivity is seen in domestic animals and human in the country (Wang and Liang 2015).

Singapore The first JE cases were reported in 1952, and after that, 100 cases were recorded during the 1970s to the early 1980s, and another 12 cases during 1985–1992. In 1992 Singapore completely closed pig farming from the state and after which the incidence of JE decreased considerably with only 06 cases are reported from 1991 to 2005. However, JEV is still being circulated in the wild boars with sero-evidence and it has been isolated from mosquitoes and human blood indicating possible future threats (Wang and Liang 2015).

Indonesia JEV circulation was first documented through serosurvey in the island of Lombok in 1960 and the later virus was isolated from vector *C. tritaeniorhynchus* in 1972. In a hospital-based survey between 2001 and 2003, the JE incidence rate was recorded to be 8.2 per 100,000 in children below 10 years. Another hospital-based survey involving 15 hospitals covering 06 provinces during 2005–2006 confirmed the presence of JE cases in all provinces and throughout the year, with majority of cases in children under 10 years. JE is endemic in Indonesia with 32 of 34 provinces reporting JE cases which are occurring throughout the year with the peak in rainy season (Garjito et al. 2019). Sero-surveillance in pigs for JE antibodies revealed higher antibody rate in Bali Island than East Java (Wang and Liang 2015). Indonesia is looked close for JE as its location is geographically close to the place where ancient JE virus originated, i.e. Malay Archipelago.

Malaysia The first human record of JE goes back to 1942 in Malaysia with first human isolation in 1951. JE major outbreaks were recorded in the year 1974 and subsequently in 1988, 1992, and 1999. During the last major JE outbreak in 1999, there were 154 JE cases with 42 being confirmed and 56 deaths. The majority of the cases were confirmed from the pig handler working at the farms. The MBD JE vaccination programme introduced in the year 2001 for children under 15 years reduced the JE incidence from 9.8 to 4.3 cases per 100,000 children under 12 years of age. *C. tritaeniorhynchus* and *C. gelidus* are the main vectors in Malaysia (Wang and Liang 2015). Sarawak state is the most affected part of the country; otherwise JE is not regarded as a major public health issue in Malaysia.

Bangladesh The first report of the JE outbreak in Bangladesh came in 1977 with 22 cases and 07 deaths mainly affecting the children. After that, low seropositivity was recorded, and majority of cases are from a rural area. May to October is the season of JE in Bangladesh (Wang and Liang 2015).

Australia JE was first reported in 1995 from human in Torres Strait inhabitant in the mainly aboriginal population (Hanna et al. 1996). After that, sero-evidence was detected in pig population with isolation from mosquito also. *C. annulirostris* is the major vector in Australia. JEV had become endemic in the Torres Strait as per survey

reports conducted between 1995 and 2005 (van den Hurk et al. 2019). The MBD vaccine is used from 1995 and now carried exclusively for Torres Strait Islands residents, and visitors (Wang and Liang 2015).

Sri Lanka JE presence was recorded since 1968 in Sri Lanka, and the first JE outbreak was recorded in 1971 with first isolation in 1974. Thereafter, three major outbreaks were recorded between 1985 and 1987 and that too in the winter months. *C. tritaeniorhynchus* and *C. gelidus* were the suspected vector. JE immunization programme was launched in 1988 using the MBD and the live-attenuated SA14-14-2 vaccine, and later they carry forward only the MBD vaccine (Wang and Liang 2015).

12.6 Transmission

JE virus is maintained in nature between culicine mosquitoes (vector), ardeid birds (reservoir), and pigs (amplifier host). Human acts as an incidental dead-end host and infection is acquired through mosquito bite harbouring JEV contracted from pigs or birds. Birds like cattle egret and heron saw in the rice field are the maintenance reservoir of the virus (Acha and Syfrez 2003). Viremia range in egret goes up to $10^{2-4.2}$ plaque-forming units (PFU)/ml which show the potential viremia in egret, whereas in chicken (*Gallus domesticus*) the viremia is $10^{1.7}$ which is considered low to infect the vector (Nemeth et al. 2012; Preziuso et al. 2018).

Swine acts as amplifier host and plays a crucial role in the transmission of JEV as they develop high viral load with long viremia after natural infection with JEV and the vector gets enough opportunity to get the JEV lading to further transmission of virus to human living in their close proximity (Diallo et al. 2018). Pigs once infected carry the viremia for 05 days and thereafter become immune lifelong. But as the herd replacement of pigs is fast and every year newborn non-immune population is being built up, so the virus always gets a naïve population who is ready to be infected. Maternal antibodies in piglet can protect for up to 4 months against JE. The 4–6-month-old pigs which have now lost the maternal antibodies and are non-vaccinated are crucial for taking up the natural infection from the mosquitoes. Vector free transmission has also been recorded in experimentally infected pigs and mice (Chai et al. 2019). Equine and human are dead-end hosts as the viremia is transient with low viral load in the peripheral blood which is not sufficient to be carried over by vector (Niazmand et al. 2019). The virus had been isolated from bats (Liu et al. 2013) and ducks (Xiao et al. 2018).

Among mosquito, *Culex* is predominantly responsible in Asia, and *C. tritaeniorhynchus* is the commonest species responsible for the transmission of the disease (Fang et al. 2019). Other species of mosquito similar to *C. tritaeniorhynchus* which lays eggs in the paddy field can also harbour JE virus. JEV is persistent and non-pathogenic to susceptible cells of mosquitoes. In that respect, one has to consider the long co-evolution of flaviviruses in mosquitoes with persistent infection. During JE epidemic season the vector (mosquito) breeding

ground is being noticed in waterlogged rice fields, irrigation channels, ponds, drains, etc. (Gao et al. 2019; Pearce et al. 2018). The active mosquito time is dusk and dawn, and hence control measures like fogging will be effective at this period effectively. The mosquitoes have preferential biting to pigs and limited in cattle (Oliveria et al. 2018). Pig sero-surveillance is used for epidemiological studies but if seropositivity is seen in cattle than it indicates active infection in that area. Mosquito life span is from 10 days to 8 weeks and can hibernate up to 6 months, so once the mosquito gets the JE virus then it can carry it for long time. Vertical transmission is being recorded for JE Virus in mosquitoes (Rosen et al. 1989). In JE transmission *C. tritaeniorhynchus* is considered the most important vector mosquito. The mosquito and even this *C.tritaeniorhynchus* can survive the winters by hibernation and it can carry over the JE virus present in it which they acquired after feeding on a viraemic host before entering hibernation. This phenomenon of carrying over the virus to next winter through infected mosquito is known as overwintering (Karna and Bowen 2019). Experimentally infected *C. tritaeniorhynchus* and *C. quinquefasciatus* have shown to transmit the JE virus to susceptible hosts by overwintering phenomena (Hurlbut 1950; Mifune 1965). A mosquito can travel 1–2 km in still air condition but can be blown through high winds or through vehicular or aircraft transport can travel long distance (Ritchie and Rochester 2001). A study in China calculated potential dispersal of *C. tritaeniorhynchus* up to 200 km per (Ming et al. 1993). The saliva of infected mosquito contains very high concentration of virus up to $10^{4.2}$ SMIC-LD50/1 mL of saliva and virus diluent is being recorded (Takahashi 1976). Birds (ardeid and heron) usually have a viremia of $10^{3.5}$ suckling SMIC LD50/0.03 mL of blood (Buescher et al. 1959; Scherer et al. 1959b) which is sufficient to infect mosquito. Even the highly competent *C. tritaeniorhychus* can be infected with low doses of virus $10^{1.0–3.5}$ suckling mouse intracerebral (SMIC) LD50 (lethal dose 50%)/0.03 mL of blood after feeding on infected birds (Hale et al. 1957; Gresser et al. 1958; Hill 1970; Takahashi 1976; Burke and Leake 1988). Pigs have even high viremias of 10^6 SMIC LD50/mL of blood which last from 24 h post-infection to 05 or more days. Furthermore, almost all domestic pigs irrespective of breed and even wild boar are capable of infecting mosquitoes (Gresser et al. 1958; Scherer et al. 1959a, b).

JEV have been isolated from around 30 species of mosquitoes, with *C. tritaeniorhynchus* being the major vector along with involvement of some other species like *C. gelidus, C. vishnui, C. pseudovishnui, C. whitmorei, C. epidesmus, C. quinquefasciatus*, *Mansonia indiana, M. uniform, Anopheles subpictus, A. peditaeniatus,* etc. (Kanojia et al. 2003; Lindahl et al. 2012).

Two basic cyclic transmission pattern of JEV exists between mosquito, pig, and human, i.e. synchronous infection in pigs and asynchronous infection in pigs. In synchronous infection of pigs few mosquitoes infects few numbers of pigs say 20% in the initial first outbreak, and then gradually number of mosquitoes get the JEV infection and it infects almost all pigs say 100% and a large population of mosquito again suck the blood from this huge pig population infected with JEV and a huge build-up of mosquito with JEV is now ready to infect human being and this is the

stage for epidemics. Another asynchronous infection in pigs is when during initial first outbreak when few mosquitoes infect few pigs say 20% and then more mosquito starts building the JEV infections and ready to infect pigs but these mosquito are not getting enough chance to bite or infect new pig population because these pigs are being protected through various means of mosquito control or vaccination which does not allow more build-up of JEV infected mosquito and here human outbreak does not occur (Impoinvil et al. 2012).

Two basic epidemiological patterns of JE, namely epidemic and endemic, are being documented. Epidemic patterns demonstrate typical seasonal characteristics mainly in the summer/monsoon season with occasional outbreaks and are seen mostly in northern areas (Northern India, China, Japan, Korea, Nepal, Bangladesh, Bhutan, Taiwan, Pakistan, Northern Vietnam, Northern Thailand, and Russia). Endemic patterns seen in tropical region and shows sporadic JE cases throughout the year and found in southern areas (Southern India, Sri Lanka, Burma, Brunei, Australia, Cambodia, Indonesia, Malaysia, Laos, Papua New Guinea, Philippines, Singapore, Southern Vietnam, Southern Thailand, and Timor-Leste) (Wang and Liang 2015). Important factors in endemic area are rice farming in larger area following traditional farming practices, vector population density, and stagnation of surface water due to improper drainage due to flood, post-rainy season, break-down of municipality services, and lack of personal care against mosquito (Witt et al. 2011). The increase in JEV activity in newer areas has been attributed to the demographic pressure of human population, intensification and expansion of rice farming, increase in pig husbandry, and introduction of vector owing to climate change, deforestation, urbanization, and increasing regional and global trade (Mackenzie et al. 2008). Other reasons for JE outbreak and spread are the lack of potential vaccination programmes and proper surveillance in these areas (Fig. 12.4).

12.7 Pathogenesis in Human

Humans are usually infected through bites of JEV infected mosquitoes. Incubation time and appearance of first symptoms take 5–15 days (Ghosh and Basu 2009). With limited details available for the early events in JE, it is anticipated that the virus infects local cell, viz. fibroblasts, endothelial cells, pericytes macrophages, and dermal dendritic cells in the skin where mosquito bite has occurred, and there the first round of virus amplification takes place. After that, the virus spreads to the brain, via newly produced virion particles, or by migratory infected immune cells, viz. dendritic cells and T-lymphocytes, which release infectious virions at their target location (Wang et al. 2017). How JEV crosses the blood–brain barrier and infects other brain cells is also not so well defined. Mouse model studies show that the virus enters the brain infect the neurons and tissue-damaging inflammation leads to breakage of the blood–brain barrier (Liu et al. 2018). Two possible mechanisms of how JEV enters the brain tissue in which first says endothelial cells of the brain capillaries may be infected with JEV, without being functionally affected, able to

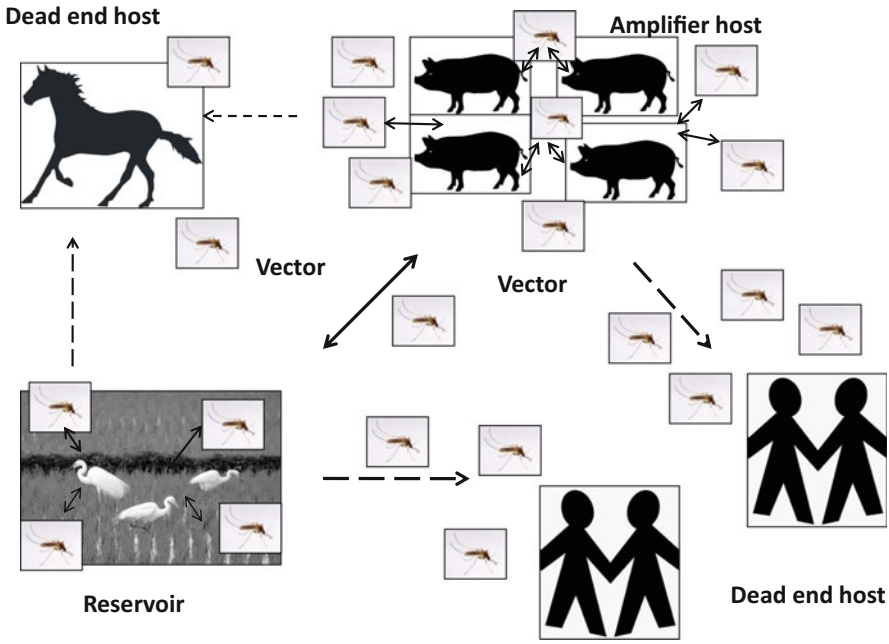

Fig. 12.4 Enzootic cycles of JEV transmission

sustain the blood–brain barrier and subsequently pass the infection to underlying microglial cells and astrocytes, which pass it further on to neurons. Secondly, JEV infected immune cells may enter through known physiological ways into the brain as in the healthy individual, that is, via the choroid plexus into the ventricular space from where they may spread the infection in the brain tissue. Thereafter, the breakdown of the blood–brain barrier may be only secondary to the infection of nerve tissue cells and after the anti-viral and inflammatory response (Filgueira and Lannes 2019). Usually, encephalitis is the most severe clinical appearance of JEV infection with a variety of first symptoms, including seizures, as well as acute sensory and neuromuscular functional deficiencies. In response to JEV infection in brain the immune cell responses may clear the infection with minimum damage in some patients but 20–40% of patients suffer from severe encephalitis and neuronal infection, and this immune response may damage key centres of the brain with long-term deficiencies or a fatal outcome. Pathological symptoms are mainly seen in brain with inflammation and congestion of grey matter showing confluent areas of haemorrhage and focal, punched out necrosis, infiltration of meninges and perivascular areas with mononuclear cells (Kumar et al. 2019). The cerebral cortex shows microglial infiltration with circumvascular necrolytic zones with total loss of neurons, whereas the white matter is fairly well preserved (Chauhan et al. 2017).

12.8 Clinical Symptom

The incubation period of 5–15 days is seen for JEV infection with the asymptomatic outcome or leads to febrile aseptic meningitis or encephalitis. The course of encephalitis and the illness can be divided into three stages—prodromal, acute encephalitic phase, and a convalescent phase. The symptoms start suddenly with a high grade of fever with a headache, which is occasionally associated with vomiting and diarrhoea. The patient and here most often children start showing seizures and that too tonic spasms, and in a matter of hours to few days the patient may go to coma and even death. Hyperventilation raised intracranial tension, shock, with death of patient is seen. Gastric haemorrhage is one of the common signs seen in seriously ill children during terminal stage. Around 33% of children affected with JE use to die during the acute stage, and many even could not reach the hospital because of very quick development of symptom and going to terminal stage in short period. Rest cases have equal fate of recovery or prolonged convalescence. These cases of prolonged convalesce show pronounced extrapyramidal signs, abnormal movements, gradually improving over weeks to months or may get lifelong implications. Clinically, JE is difficult to be distinguished from other encephalitis cases or with acute encephalitis syndrome. Therefore, laboratory testing and confirmation are advocated.

World Health Organization in 2006 defined acute encephalitis syndrome (AES) for surveillance purposes for JE endemic area which says clinically an AES patient should have at least one of the following condition (1) change in mental status (like confusion, disorientation, coma, or inability to talk); (2) seizures (not common to person or because of simple fever). Other findings include increased irritability, abnormal behaviour (WHO 2018).

WHO classified the AES cases into four categories:

1. Laboratory confirmed JE: An AES case which is confirmed to be JE based on the result of laboratory results.
2. Probable JE: An AES case from which there may be an adequate sample collection or even no sample collection but had occurred in the JE endemic geographical area during the outbreak.
3. AES—another agent: An AES case where other than JEV has been confirmed through laboratory testing.
4. AES—unknown: An AES case which came out to be negative to JE or another etiological agent through laboratory testing or that case has not been tested.

12.9 Disease in Animal

Horses manifest encephalitic disease accompanied by fever and there could be mortality also. Most often in horses, JE is seen as a sub-clinical and when clinical signs are present which is usually sporadic have three major manifestations—transitory, lethargic, or hyperexcitable. In transitory type syndrome, there is

moderate fever which lasts for a few days and can be accompanied by loss of appetite, incoordination, jaundice and the horse recovers in the next few days. Lethargic type syndrome will have a high fever for variable periods with pronounced neurological symptom, difficulty in swallowing, haemorrhagic petechiae over mucosa, and can go to even paralysis. These cases take longer time to recover which goes around 1 week or more. The third pattern is the hyperexcitable type having very high fevers, accompanied by heavy sweating, muscle tremors, pronounced neurological symptom, and loss of vision, coma, and death. Mortality in horses goes around 5% to as high as 30% with morbidity rates around 1%. Horses dying with JEV infection in post-mortem (PM) shows gross lesions in the central nervous system, viz. a diffuse non-suppurative encephalomyelitis with apparent perivascular cuffing; phagocytic destruction of nerve cells, perivascular cuffing and focal gliosis, blood vessels appear dilated with numerous mononuclear cells.

In pig herds, the disease is seen with large numbers of stillborn or weak piglets which are negative to the known abortion causes like brucellosis, swine fever, African swine fever, porcine reproductive and respiratory syndrome, etc. Reproductive disease manifestation is the most common in pigs with reproductive losses ranging between 50 and 70%. The reproductive manifestation can be abortions in sows, stillbirths or mummified foetuses, and in boars, there are sperm abnormalities. The piglets which are born with JE often display neurological symptoms and often die after birth with mortality rates as high as 100% in these piglets. It is worth to note that the adult non-immune pigs which usually do not die and after getting JE infection results in lifelong immunity. In swine, the PM sign in the mummified or stillborn foetuses shows dark appearance with neurologic damage; hydrocephalus, cerebellar hypoplasia and spinal hypomyelinogenesis, and subcutaneous oedema (Scherer et al. 1959c).

12.10 Diagnosis of JE

Virus Isolation The JEV can be isolated using a cell culture system, intracerebral inoculation of suckling mice, and mosquito inoculation. The virus isolation rate is usually less because of low circulating viral copies and fast development of neutralizing antibodies (Solomon et al. 1998a, b). Successful isolation goes with proper collection of biological sample at an appropriate time, i.e. brain tissue or biopsy sample during post-mortem/autopsy or from cerebrospinal fluid (CSF) of human within 4 days of the onset of symptom. JE had been isolated from pigs from blood, and CSF and mosquitoes also have been isolated. Isolation is usually carried out in one-day-old suckling mice or cell line like in porcine stable kidney cells, Vero cell line, mosquito cell line of *Aedes albopictus* clone C6/36 etc.

Molecular Techniques Molecular detection of JE viral genome by reverse transcriptase-polymerase chain reaction (RT-PCR) techniques are used in blood, cerebrospinal fluids, the brain tissue of human, pigs, and experimental animals like mice. It is even used to detect viral genome from vector mosquito. The success rate of detection of viral genome from human blood is less due to short duration of virus in blood and very low-level viremia. Though it can be detected from blood and/or CSF in around 0–25% of clinically affected cases which can be improvised to some extent up to 25–30%, if sample is collected within 3 days of the onset of infection (Dubot-Peres et al. 2015; Khalakdina et al. 2010; Touch et al. 2009; Yeh et al. 2010; Swami et al. 2008). The molecular assays hold good during the early stages of the infection when seroconversion has not occurred significantly to be detected by serological assays. However, as stated above RT-PCR is not very sensitive and it most often misses to detect the viral genome in actual JE cases. Therefore, if the PCR result is positive then the case can be regarded as JE positive but negative PCR result should not be treated as JE negative and it must be complemented with serology. Other techniques like real-time PCR, loop-mediated isothermal amplification (LAMP PCR), lateral flow test (LFT) are also available (Dhanze et al. 2019a). TaqMan real-time based RT-PCR assay has been developed for the detection of JEV in swine and mosquito (Pantawane et al. 2018; Shao et al. 2018) and other real-time based also been documented (Bharucha et al. 2018). Reverse transcription LAMP coupled with a lateral flow dipstick assay for the detection of JE virus has also been developed and is claimed to be specific (Deng et al. 2015). Whole-genome sequencing confirmation has also been approved in addition to the above stated molecular tests (WHO 2018).

Serological Techniques Serological techniques are widely used for the diagnosis of JE antibodies and are regarded to be the gold standard. The detection of virus-specific antibody in the CSF is more than other clinical samples. Hence CSF based diagnosis is advocated (Ravi et al. 2006). Various serological techniques like haemagglutination inhibition test (HAI), virus neutralization test (NT) were employed for assay of antibody of JE and were recommended by WHO, OIE, and reference laboratories. However both the test requires high level of expertise and its antigen production is limited to reference laboratories only, with requirement of handling the virus, requirement of red blood cells obtained from geese in HAI and is laborious. There is a want for the development of easy to use, specific and sensitive JEV serological kit. In this search, indirect ELISA had shown some promising result. The HAI based JEV diagnosis in the paired sera is the most preferred one till the 1990s, but nowadays immunoglobulin M (IgM) capture-based enzyme-linked immunosorbent assay test as indirect ELISA or popularly known as MAC-ELISA in CSF or serum is routinely practiced and gives confirmation for recent infection in human (Cha et al. 2014). The use of MAC-ELISA as the first-line diagnostic assay in human has also been recommended by the World Health Organization for the detection of acute infections, and for best result the sample collection should be

collected within 5 days after the onset of illness. If the first initial sera turn out to be negative by MAC-ELISA, then it can be repeated after 7–10 days. There are three commercial MAC-ELISA kits available by (1) XCyton Diagnostics Limited, India, (2) the Inbios kit (InBios International Inc., United States of America), and (3) a combo kit for dengue and JE marketed by PanBio, Australia (Lewthwaite et al. 2010; Johnson et al. 2016; Sirikajornpan et al. 2018).

Indirect ELISA for pigs have been developed and used but is limited to reference laboratories only. Few indirect ELISA using whole JEV antigen for the detection of IgG JEV antibodies and had comparable sensitivity with HAI and SNT were developed and used, but none of them is available commercially for larger use (Yang et al. 2006; Hamano et al. 2007; Kolhe et al. 2015). Most of the countries still preferred whole JEV antigen harvested from cell culture for indirect ELISA and using it for pig JEV sero-surveillance. Many authors have developed JEV peptide-based and expressions based ELISAs, and few of them are in the pipeline for commercialization but at present not available commercially (Dhanze et al. 2019b; Hua et al. 2010). Many companies are claiming JEV ELISA for pigs, but their sensitivity and specificity are not fully validated and had not been recommended by OIE or reference laboratories. With less option of a better commercial JEV kit, the researcher are using and reporting the JEV seropositivity with the available kits, but these need to be further validated (Pegu et al. 2019). There is a chance of cross-reactivity with other Flaviviruses, viz. West Nile and dengue; hence, these other *flaviviruses* should also be monitored along with JE (Maeki et al. 2018; Nealon et al. 2019).

12.11 Treatment

There is no specific treatment available for JE patients, and only symptomatic treatment is the option. Most of the cases require hospitalization with supportive care under close observation. Rest, ample fluids, antipyretic, and analgesic can be used to relieve symptoms. Severe cases may require management in intensive care unit with supports to maintain clear airways, breathing, circulation, raised intracranial pressure, electrolyte balance, fever, convulsions, and parenteral antibiotics to cover for bacterial infection (Turtle and Solomon 2018). Proper nursing care is of paramount importance to prevent aspiration pneumonia, bedsores along with nutritional care of the patient (Kumar et al. 2019). The use of steroids like dexamethasone in JE patients has been tried, but its effectiveness is debatable. The tetracycline group of drug—Minocycline having antibacterial plus neuroprotective advantage has shown beneficial in animal model.

12.12 Vaccines

A vaccine against Japanese encephalitis was first introduced during the 1930s which were inactivated mouse brain-derived JEV strains of Nakayama and/or Beijing-1 made by BIKEN Company of Japan. Initially these first vaccines were used successfully in control of JE in countries like Japan, Korea, and China. Later many advance vaccines came up which were based on inactivated cell culture vaccines, chimeric virus vaccines, recombinant adenovirus-based vaccines and few of them got successfully tested in animal and human clinical trials. At present there are good vaccine options in the market and have been in practice in endemic countries (Appaiahgari and Vrati 2010; Butler et al. 2017; Hegde and Gore 2017; Li et al. 2019).

12.13 Human Vaccines

(a) *Mouse brain killed vaccine*—the inactivated vaccine derived from mouse brain was the first to be introduced by BIKEN (Japan). The Nakayama and/or Beijing strains were used initially by different companies for this mouse brain inactivated JE vaccine and had been successful in the control programme of JE in countries like China, Korea. In the initial year, this vaccine was widely used, but with reports of its neural side effect, it was phased out slowly, and BIKEN had stopped its production since 2007.

(b) *P3 strain inactivated vaccine*—The JE Beijing P-3 strain is the most virulent strain of JE known. This was converted to a cell culture-derived, formalin-inactivated JE vaccine and is widely used in China since the 1960s through Chengdu Biologicals Corporation limited. Initially, in China with the use of this vaccine in infants, they could achieve a protection level of up to 76–90%, later this vaccine was replaced by the live-attenuated vaccines because of report of low efficacy, short-lived immunity, and requirement of the booster dose.

(c) *Live-attenuated vaccine*—The field isolates of JE SA14 was a relatively weak strain in term of virulence and later converting it to a live-attenuated strain avirulent strain through serial passaging in hamster kidney cell line leads to the development of a better vaccine which is known as SA-14-14-2 strain. This single-dose vaccine produced by Chengdu Biologicals is one of the popular vaccines and is used even nowadays. This has been approved by World Health Organization. China had been using this vaccine since 1998, and country like Nepal had tried in human population with satisfactorily good efficacy since 1999 and is used in India also since 2006. The efficacy of vaccine is good in various independent studies, in Nepal an efficacy of 99.3% (same year), 98.5% (after 1 year), and 96.2% (after 5 years of vaccination) was recorded and an efficacy of 94.5% after 6 months was recorded in India. The vaccine safety profile was also recorded to be good with the development of minor post-vaccination symptom

as low-grade fever, local reactions, or irritability in 5–10% of recipients (Turtle et al. 2017; Yun et al. 2016). The environmental safety of SA-14-14-2 inactivated vaccine was checked and was found to be still safe (Liu et al. 2019).

(d) *Vero cell-derived*—inactivated JE vaccine derived from Vero cell line have also come up and had been successfully used in many countries and is available in current time also. One such is available in India as JENVAC developed by National Institute of Virology, Pune an institute of Indian Council of Medical Research using the Kolar strain (821564 XZ) which was isolated from Kolar a place in Karnataka state during the early 1980s. JENVAC is used in current practice with approval from the Drug Controller General of India and marketed by Bharat Biotech Limited.

(e) *The IC51 Vaccine*—IXIARO®—is a new generation Vero cell line derived formalin-inactivated vaccine using the SA-14-14-2 strain and is manufactured by Intercell AG (Vienna, Austria) and distributed by Novartis (Amicizia et al. 2018). This vaccine had received US Food and Drug Administration approval for use in children and adults 17 years of age or older and the vaccine was later also approved in Europe and Australia. The vaccine is available in the name of JEEV (Biological E. Ltd., Hyderabad, India) and used in current practice. This is a two-dose vaccine given on 0 and 28th day and is applicable for both children and adults.

(f) *Chimeric vaccine*—This is also a new generation vaccine and is recently cleared the human clinical trials. The vaccine was developed by Acambis, Cambridge, UK using the live-attenuated Yellow fever Virus 17 D clone with an inset of pre-membrane and envelop genes of attenuated SA-14-14-2 JE virus in between the core and nonstructural genes yellow fever virus making it a live chimeric vaccine. Its phase II trials have shown a seroconversion of 94% with single-dose, and phase III trial are also completed in Thailand and is marketed as IMOJEV and THIAJEV (Appaiahgari and Vrati 2010; Chin and Torresi 2013). The phase IV vaccine trial is also successful for this vaccine (Chotpitayasunondh et al. 2017). This vaccine is successfully used in adult and even older persons and is recommended by WHO (Table 12.2).

12.14 Animal Vaccines

(a) *Horse vaccination*—As JE affects horses as they act as a dead-end host, so there was a need to protect the racing horses and horses with high values. It also serves as a model for vaccine trial even before the use of the vaccine in human and the first horse vaccination took place in the year 1948 with mouse brain-derived JE vaccines (Nakamura 1972). Horse vaccination has reduced JE cases in horses, 337.1/100,000/year in Japan from 1948 peak outbreak to 29.74 cases/100,000/year in 1960 again further reduction to 3.33 cases/100,000/year in 1967 (Goto 1976; Nakamura 1972) along with the advancement in vaccine. Countries such

Table 12.2 JE vaccines commercialized for human use

Type of vaccine	Virus strain/type	Substrate	Manufacturer/trade name/country of origin
Inactivated	Nakayama-NIH; wild-type	Mouse brain	BIKEN, Japan
	Beijing-1 (P-1); wild-type		Japan, Korea
	Beijing-3 (P-3)	Primary hamster kidney	China
	Beijing-1	Vero cell	Japan, BIKEN
	Beijing-3 (P-3)		JEBIKV, China
	SA14-14-2		1C51—Intercell, IXIARO—Valneva, JEEV-Biological E limited, India
	Kolar-821564XY		JENVAC, India, Bharat Biotech
Live attenuated	SA14-14-2	Primary hamster kidney	China, Chengdu Biological Products
Chimeric-live-attenuated	YFV 17D containing JEV proteins	Vero cells	ChimeriVax-JE; JE-CV Acambis/ Sanofi-Pasteur (IMOJEV, THAIJEV)

as Singapore and China are also using the horse vaccine and have reported the reduction of JE cases in horses (Ellis et al. 2000). In Hong Kong, thoroughbred racing horses are vaccinated when purchased from endemic countries (Ellis et al. 2000).

(b) *Pig vaccination*—Pig vaccinated with JE does not allow high viremia and hence breaks the transmission cycle of JEV (Sasaki et al. 1982). JE vaccination in pigs is helpful in term of reduction of stillbirth in the farms. In a Taiwan study, it was proved that the JE vaccinated sows give birth to healthy piglet around 92% healthy piglet, but in the unvaccinated group, 31.6–54.1% piglets are born as stillbirths (Hsu et al. 1972; Rosen 1986). These JE vaccinated pigs are now not acting as amplifier host and did not infect mosquitoes helping in the protection of human and horses (Sasaki et al. 1982). The vaccination of pigs is an effective tool to control JE but is not widely practiced across the countries because of high turnover in pig populations, pigs with 3 month gestation period can give at least three crops per year with an average of 8–10 piglets would give a new naïve population every year and to vaccinate this huge new population is costly and require huge manpower and efforts. Moreover, the effectiveness of the live-attenuated vaccines is decreased in young pigs because of maternal antibodies (Wada 1987). There is one more hurdle where it is said that natural infection of pigs with JEV develops lifelong immunity, but with the man-made vaccine the immunity is short. The JE vaccines are available only in few countries and they have been practicing it in field like Japan, Taiwan, etc. The pig vaccine for JE is not available commercially so many endemic countries like India who contributes a high number JE cases are unable to apply this strategy (García-Nicolás et al. 2017).

12.15 Prevention

Prevention of JE in the endemic area requires a multi-approach strategy with vaccination, vector control, change of rice field irrigation system, minimizing pig-human interaction, etc. The country like Japan had been able to control JE infection with human and pig vaccination, mechanization of rice cultivation, vector control strategy, etc. Even vaccination of racehorses is also practiced in some countries. Measures to prevent JE should be targeted for vector control, in the reservoir host pig, and the protective measures in human.

1. *The Vector Mosquito control*
   Though vector control for JE is one of the practical solutions in most of the country, especially in Asian countries with huge human and animal population. It not only solves JE but many other mosquito-borne illnesses. WHO had narrated that mosquito is one of the biggest enemies to the human race, but its control is not so easy and somewhat expensive in most of the Asian countries which are either developing or underdeveloped with the huge population of both human and animals spread over a large geographical area. If properly implemented it can break the JE cycle and can control the outbreaks. Application of larvicides to rice fields, natural insecticide of *Azadirachta indica* can be applied to rice fields, placing larvivorous fish like *Gambusia affinis* in rice paddies are some of the ways. Fogging in dawn-dusk when *Culex* activity is highest should be done. Insecticide-treated mosquito nets can be used in pig sheds. Cattle are also used as a damping host for JE virus as being a dead-end host it can divert the *Culex* population from pig and human, the approach better known as zooprophylaxis. Mechanization of rice field with frequent changing of the water in rice field destroys the breeding ground for mosquito and is an effective way but limited to developed countries only, as most of the agriculture in Asian country is monsoon fed. Elevation of general hygiene practices is needed in rural as well as urban cities. The role of municipality in cleaning garbage laden waterways especially in cities is needed. The mass awareness programme from radio, television, print media, social media can make a major change and attitude change by citizen is needed to win this war against mosquito, ultimately JE and other mosquito-borne diseases. Personal protection measures against mosquito bites like use of mosquito nets during bedtime, use of mosquito repellants, and protective clothing would be useful.
2. *Preventive measures towards reservoir host pig*
   Vaccination against JEV is one of the effective strategies which has been used by some countries but is not practiced in most of the countries because of the lack of pig vaccine and high cost involved in implementation. The pig farmers should be given awareness that pig farm should be away from human houses. In most of the village setting, in developing or underdeveloped countries the pig and human house are almost common. The pig lives here side by side of the residential premises or below the same house. Government has to give incentives to these farmers of low socio-economic group to make their new pig farm away from

human house. There is need to adopt mosquito control programme in pig farms also and where it is economically not feasible especially in rural area then also the villagers should be advocated to use their indigenous low-cost knowledge for keeping mosquito away like burning of Neem leaves, etc. The government mechanism has to undertake the responsibility of fogging in this area where the farmer cannot afford it.

3. *Control strategy directed to human*
   In human, vaccination is the most helpful control measure, and earlier the vaccination that was oriented to children below 12 or 15 years of age is now applicable to adult also (Kumar 2014). Even though human vaccination will decrease human JE cases, but the virus would be maintained in the reservoir host (pig, ardeid birds) and vector mosquito and the non-vaccinated group would always be prone to the JE infection. Hence, for effective JE control and prevention programme one health approach is must with the simultaneous effort of all the departments, viz. animal husbandry, medical, municipality, irrigation, agriculture, fishery acting together to curb the menace of JEV. Countries like Japan, China, and Korea been practicing JE human vaccination since long but many countries like India, Nepal have started in the last decades only and have to go a long way to effectively vaccinate the whole population. JE vaccination is also advised for travellers who are going to endemic countries (Connor et al. 2019).

12.16 Current Scenario and Conclusions

The disease burden of Japanese encephalitis is more in Asian countries. Due to variations in the diagnostic procedures being followed globally, the true incidence of JE is not well estimated. According to the earlier estimates, approximately 68,000 JE cases occur annually, and only 10% cases are actually reported to the World Health Organization. The vast majority of people (~3 billion) from South-East Asia and Western Pacific are at the risk of JE infection. Accordingly, people from 27 countries are at the risk of JE. Depending on the annual incidence and vaccination strategies, the JE endemic countries have been grouped into high, medium, and low. For example, Korea, Taiwan, Japan, China, and India are examples of countries with a high incidence of JE. The vaccination programmes for JE are also varyingly implemented in different endemic countries. JE vaccination is being implemented since long in countries like Japan, Korea, and Taiwan. China started JE vaccination programme in 1981, but JE as a routine vaccine is implemented since 2008. In India, JE vaccination has been introduced in 2006 for children aged 1–15 years. This vaccine was included in the National Immunization Programme by Government of India in 2014. The districts where JE is endemic, the SA-14-14-2 JE vaccine are being used as a part of Universal Immunization Programme (Tandale et al. 2018). More than 11 crore children's from identified JE endemic districts are immunized in India. Climate change may pose significant impact on the JEV transmission. Identification of JEV in Tibet and Australia proves that the prediction of JEV

transmission is very difficult in the context of global warming and climate change. Although JE is considered a paediatric disease, it has also been recorded in adults with significantly high proportion. Its increasing trend in the adult further suggests for revisions in the JE prevention strategies at national and international level in the JE endemic countries. The current endemic region of JE encompasses the entire South Asia, Southeast Asia, eastern Russian Federation, Australia, Saipan, and Papua New Guinea is the globally identified endemic regions of JE.

JEV infects the CNS which causes neuroinflammation and neuronal death. Personal factors are important in the development of clinical illness in the case of humans. Age factor is very important, and neuro invasiveness is multifold in people aged above 50 years. Similarly, risk of neurological sequelae is also more in the younger age. JE infection risk also increases during pregnancy. The epidemiology of JEV is complex and unpredictable, and its transmission by non-vector route cannot be ruled out. A study has been demonstrated that JEV can be transmitted between infected and susceptible pigs even in the absence of mosquitoes. In the same study it was revealed that infectious virus dose for pigs could be as low as 10 $TCID_{50}$ per animal and mucosal virus shedding (oronasal transmission) could be the important source of virus transmission in pigs without involvement of vector.

Vaccination is the only long-term strategy for prevention and control of JE infection. At present, there are more than 15 vaccines being used for JE immunization. They are grouped into four major classes, viz. inactivated mouse brain-derived JE vaccines; inactivated Vero cell culture-derived JE vaccines; live-attenuated SA-14-14-2 JE vaccines; and live recombinant JE vaccine. WHO has recommended to gradually reduce the use of mouse brain-derived JE vaccine due to its safety concern. Virus strains used for the preparation of JE vaccines are Beijing-1, Beijing P-3, Kolar strain, SA 14-14-2 strain and recombinant vaccine using structural and non-structural genes of SA 14-14-2 virus and yellow fever 17D virus, respectively. Immunization schedule and dose regimes are also different for different group of vaccines. For example, a single dose of the live-attenuated SA-14-14-2 JE vaccine in the children aged 9 months and above will give protection for 5 years. Recombinant JE vaccine (JE-CV) is a two-dose vaccine which also gives protection up to 5 years. A two-dose inactivated Vero cell-derived JE vaccines are being used in the USA, Australia, India, and New Zealand. Use of recombinant JE-CV was licensed since 2012 in Australia and Thailand. All the JE available vaccines are based on the genotype 3 of the JEV. In India JE vaccine derived from Vero cells is manufactured by Bharat Biotech. The first JE vaccine was prepared from the Nakayama strain of JEV. It was known as mouse brain-derived inactivated JE vaccine marketed as JE-VAX. This was the only vaccine available internationally for the prevention of JE for several decades. Later it was produced in several Asian countries like India, Japan, Korea, Taiwan, Thailand, and Vietnam. In 1988, China licensed the use of the live-attenuated SA14-14-2 JE vaccine for commercial use. This vaccine is highly immunogenic, widely used and now licensed in several Asian countries like South Korea, Nepal, India, Sri Lanka, Cambodia, Laos, Myanmar, and Thailand. In 1998 China licensed another vaccine for domestic use which is a Vero cell-derived Beijing-3 JE vaccine. In Japan, similar type of vaccine prepared from Beijing-1

strain of JEV is available under trade names JEBK V and ENCEVAC licensed in 2009 and 2011, respectively. IC51 is a new type of inactivated JE vaccine derived from Vero cells using SA-14-14-2 virus strain is in use since 2009 in the USA, Europe, Canada, Australia, India, Switzerland, and Hong Kong. It is marketed under trade names IXIARO, JESPECT, and JEEV (Yun and Lee 2014). The safety and immunogenic potential of chimeric vaccine produced using Yellow Fever Virus (YFV) 17D is now well-proven. With the advent of recombinant DNA technology, precursor membrane protein (prM) and envelop (E) proteins of SA-14-14-2 strain of JEV are expressed in the YFV. This vaccine is also a type of Vero cell-derived vaccine which is commercially available under trade names IMOJEV, JE-CV, and THAIJEV. Future JE vaccine development should be focused on the circulating genotypes of the JEV. Currently, genotype I is widely circulating JEV genotype which has replaced genotype III. Unfortunately, all the available JE vaccines at present have been derived from genotype III of the JEV strains, namely Nakayama, Beijing-1, Beijing-3, and SA-14-14-2. Several approaches are being explored to develop new JE vaccines using recombinant technology and expression of immunodominant proteins in poxviruses and also plasmid DNA vaccines.

Genetic manipulation of JEV RNA is being explored to produce recombinant viruses from cloned DNA using reverse genetics. Due to the high cost of JE vaccine production and biosafety levels required for handling the JEV (BSL-3). Similarly, co-circulation of different related flaviviruses challenges vaccination and development due to cross-reactivity. In recent past research has been focused on the use of virus-like particles (VLPs). However, the VLP based vaccines are either in the pre-clinical or clinical stage of development. VLPs have great potential for future safe JE vaccine as they do not contain genetic material. Using mammalian and insect host systems; baculovirus, vaccinia virus and plasmid, retrovirus as vectors JEV VLPs are being produced to express prM and E proteins (Krol et al. 2019). Different JEV genotypes have been distributed to different geographic regions. Thus antigenic variation will exist in nature in JEV. It will pose some degree of impact on the prevention and control of this disease.

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Chapter 13
Picobirnavirus

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Abstract Picobirnaviruses (PBVs) recently occupied a place in the list of enteric viruses and are highly versatile due to their huge genetic diversity and wide host range. PBVs are small, non-enveloped viruses carrying a bi-segmented double-stranded RNA genome and have been placed within a new family '*Picobirnaviridae'*. Nevertheless, PBVs have been detected primarily from the faeces of many host species, these viruses have also been reported in the respiratory tract of pigs and plasma of horses that needs further investigation for their inhabitant behaviour. Based on the available information, PBVs may be considered as opportunistic enteric pathogens; however, prokaryotic ribosomal binding motifs have been identified in many PBV gene segments, indicating that these may be prokaryotic viruses. Though PBVs may have an ambiguous clinical implication, they might pose a potential public health concern. Because of lack of suitable animal model/cell lines, there remains an uncertainty on the pathogenesis, persistence, and evolution of PBVs. The control of PBVs largely depends on the non-vaccine approach.

Keywords Picobirnavirus · Bi-segmented · *Picobirnaviridae* · Opportunistic enteric pathogens

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13.1 Prologue

The advancement of high-throughput sequencing coupled with the human quest for exploring unknown viruses or viruses of less importance from diverse sources has led to the identification of many novel viruses and improved viral classification. Similar approaches have also been employed in several studies to probe the viral diversity in the humans and animals faecal virome, and thus, it helped in elucidating the complex aetiology of diarrhoea and identifying potential zoonotic and emerging viruses.

Hitherto, viruses belonging to at least four families, *Reoviridae* (rotavirus), *Astroviridae* (astrovirus), *Caliciviridae* (calicivirus), and *Adenoviridae* (adenovirus) have been primarily implicated as causative viral agents of diarrhoea. Additionally, *Picobirnavirus* (PBV), an emerging virus has been detected in the faecal samples of many mammalian and reptile species. There are conflicting reports on the association of PBVs with diarrhoea either as a primary or secondary causative agent. This conflict is further complicated by a recent study, which identified a typical bacterial sequence motif, the ribosomal binding site (RBS) in the 5′ untranslated regions (5′ UTRs) of many PBVs sequences suggesting that PBVs might be prokaryotic RNA viruses (Krishnamurthy and Wang 2018).

Amid these uncertainties about PBV's role in diarrhoea or their origin, this book chapter has focused on genome characteristics, prevalence, transmission, persistence, zoonotic potential, diagnosis, and treatment aspects of PBVs.

13.2 History and Virion Properties

The first-ever documented evidence of PBVs dates back to 1988 in humans and black-footed pigmy rice rats (Pereira et al. 1988a, b). At first instance, PBV was confused with *Birnaviruses* because of their bi-segmented nature of the genome. However, PBVs are quite distinct as compared to the members of *Birnaviridae* with respect to the host range (limited host range of *Birnaviruses*—fish, chicken, and turkey) and genome size *(Birnaviruses*—large segment of 3.3 kb and smaller segment of 2.8 kb), and they should be differentiated by means of molecular detection methods.

PBVs are small (35–41 nm in diameter), non-enveloped, and have double-stranded bi-segmented RNA genome (Pereira et al. 1988a; Delmas et al. 2018). The gene segment 1 (2.2–2.7 kb) encodes two or three proteins, one or two putative proteins of unknown function and a capsid protein, while segment 2 (1.2–1.9 kb) encodes the viral RNA-dependent RNA polymerase (RdRp) (Delmas et al. 2018). PBVs have been categorized into two genogroups, namely Genogroup-I (G-I) [Reference strain-1-CHN-97] and Genogroup-II (G-II) [Reference strain-4-GA-91] based on the RNA-dependent RNA polymerase (RdRp) gene (segment 2) of human PBV (Delmas et al. 2018; Malik et al. 2014a).

The nomenclature of PBVs has been derived from two words, i.e. the prefix 'pico' signifies the small diameter of the virus, and 'birna' suggests a bi-segmented nature of the genome (dsRNA). Notably, PBVs have been assigned a new taxonomic order '*Diplornavirales*', family '*Picobirnaviridae*', and genus '*Picobirnavirus*' (Delmas et al. 2018; International Committee on Taxonomy of Viruses (ICTV) 2014). The two candidate species that find a place within the genus 'Picobirnavirus' include Human Picobirnavirus (type species) and Rabbit Picobirnavirus (designated species) (Carstens and Ball 2009). Uniform nomenclature for PBVs has been identified which recommends the determination of genogroups (G-I or G-II), host, country of origin, strain, year of isolation for a specific PBV identified and should be written in described order (Fregolente and Gatti 2009). The nomenclature of a strain of PBV detected in roe deer would be as PBV/roe_deer/SLO/D38-14/2014.

13.3 Genome Organization of PBVs

About 34 complete or nearly complete genomic sequences of PBVs detected in diverse hosts are available in databases and among them, nine of them belong to novel PBVs containing mono-partite genomes (Table 13.1). Besides, the complete gene segment 1 of Lapine PBV and segment 2 of bovine, dog, and feline PBVs have also been sequenced and readily accessible from the public domain database. The striking differences do exist among these PBVs sequences and are discussed briefly below:

13.3.1 Human PBV

The segments 1 and 2 of Hy005102 strain are 2525 nt and 1745 nt in length, respectively. The GC content in the 5′-non-coding region (NCR) is low in both the segments (segment 1 = 36.5%, segment 2 = 22.6%). Notably, five-nucleotide sequence, GUAAA at the 5′-end in both the segments 1 and 2 are conserved. A polyadenylation signal (AAUAAA) in segment 1 is absent. The segment 1 of the human PBV sequence has two open-reading frames (ORF1 = 224aa and ORF2 = 552aa) (Fig. 13.1) (Wakuda et al. 2005).

13.3.2 Otarine PBV

Otarine PBV, strain PF080915 has short segments, 1 (2347 nt) and 2 (1688 nt) compared to human PBVs. Segment 1 has two ORFs that encode 163aa and 576aa proteins, respectively, while segment 2 encodes a protein of 532aa in length. The 5′-NCR (88 bases) of segment 1 has a GC content of 40.9%, but the 3′-NCR (28 bases) has GC contents of 71.4%. Similarly, the 5′-NCR (46 bases) of the

Table 13.1 Genomic characteristics of PBVs of diverse host species

S. No	Host species	Isolate name	Accession nos.	Total length (nt)	Segment-1			Segment-2 (ORF) nt
					ORF1 (nt)	ORF2 (nt)	ORF3 (nt)	
1.	Otarine PBV	HKG-PF080915	Segment 1—JQ776551; segment 2—JQ776552	Segment 1—2347; segment 2—1688	89–577	–	592–2319 (CP)	47–1645 (RdRP)
2.	Otarine PBV	PF080902	Segment 1—KU729754; segment 2—KU729755	Segment 1—2522; segment 2—1687		165–836	833–2503 (CP)	45–1643 (RdRP)
3.	Otarine PBV	PF090307	Segment 1—KU729753; segment 2—KU729767	Segment 1—2522; segment 2—1679		165–836	833–2503 (CP)	46–1635 (RdRP)
4.	Otarine PBV	PF090306	Segment 1—KU729748; segment 2—KU729766	Segment 1—2519; segment 2—1884		170–847	850–2487	46–210 (HP) 251–1846 (RdRP)
5.	Otarine PBV	PF080915	Segment 1—KU729746; segment 2—KU729760	Segment 1—2458; segment 2—1827	55–198	104–688	703–2430	39–185 (HP) 167–1783 (RdRP)
6.	Human PBV	CDC23	Segment 1—KJ663813; segment 2—KJ663814	Segment 1—2509; segment 2—1641	35–157	172–570 (a) 461–811 (b)	827–2485	1–1620[a]
7.	Human PBV	CDC16	Segment 1—KJ663815; segment 2—KJ663816	Segment 1—2056; segment 2—1717			261–> 2056[a]	35–1699
8.	Human PBV	VS6600008	Segment 1—KJ206568; segment 2—KJ206569	Segment 1—2070; segment 2—1465		115–504	529–> 2070[a]	<1–> 1465[a]
9.	Human PBV	Hy005102	Segment 1—AB186897; segment 2—AB186898	Segment 1—2525; segment 2—1745		157–831	828–2486	94–1698
10.	Chicken PBV	PBV/CHK/M3841/ HUN/2011	Segment 1—MH327933; segment 2—MH327934	Segment 1—2532; segment 2—1700	51–182	179–904	922–2502	45–1643 (RdRP)
11.	Turkey PBV	GI/PBV/Turkey/ USA/MN-1/2011	Segment 1—KJ495689; segment 2—KJ495690	Segment 1—2557; segment 2—1685		148–906	887–2539 (CP)	70–1647 (RdRP)

12.	Monkey PBV	GII/PBV/monkey/USA/196-06/2015	Segment 1—KY174982; segment 2—KY174983	Segment 1—2683; segment 2—1771		255–878	896–2566 (CP)	156–1757 (RdRP)
13.	Mouse PBV	504	Segment 1—LC110352; segment 2—LC110353	Segment 1—2490; segment 2—1409		5–730	727–2460	173–1381
14.	Porcine PBV	221/04-16/ITA/2004	Segment 1—KF861772; segment 2—KF861773	Segment 1—2524; segment 2—1730		195–794	810–2447	75–1682
15.	Porcine PBV	BEL/15V010/1966	Segment 1—KY214429; segment 2—KY214430	Segment 1—2638; segment 2—1837		159–929	926–> 2638[a] (CP)	193–1782 (RdRP)
16.	Porcine PBV	221/04-16/ITA/2004	Segment 1—KF861768; segment 2—KF861773	Segment 1—2666; segment 2—1730		185–721	734–2581 (CP)	75–1682 (RdRP)
17.	Equine PBV	Equ1	Segment 1—KR902504; segment 2—KR902503	Segment 1—2274; segment 2—1864		176–631	648–2231 (CP)	227–1813 (RdRP)
18.	Equine PBV	Equ2	Segment 1—KR902506; segment 2—KR902505	Segment 1—2734; segment 2—1730		329–997	1011–2621 (CP)	72–1700 (RdRP)
19.	Equine PBV	Equ3	Segment 1—KR902508; segment 2—KR902507	Segment 1—2494; segment 2—1704		9–764	765–2438 (CP)	71–1669 (RdRP)
20.	Fox PBV	Fox5	Segment 1—KC692367; segment 2—KC692366	Segment 1—2151; segment 2—1643		26–631	634–> 2151[a]	<1–1637[a]
21	Roe deer PBV	SLO/D38-14/2014	Segment 1-MG190028; segment 2—MG190029	Segment 1—2576; segment 2—1721	43–234	194–766	796–2511 (CP)	47–1675 (RdRP)
22.	Běihǎi picobirna-like virus 7		Segment 1—KX884063; segment 2—KX884062	Segment 1—2033; segment 2—1564		153–1976	–	64–1542 (RdRP)
23.	Běihǎi picobirna-like virus 8		Segment 1—KX884065; segment 2—KX884064	Segment 1—1924; segment 2—1534		129–1877	–	33–1526 (RdRP)
24.	Diatom colony-associated dsRNA virus 1		Segment 1—AP014890; segment 2—AP014891	Segment 1—1734; segment 2—1562		181–1704	–	26–1543 (RdRP)
Novel PBVs having mono-partite dsRNA genome								
25.	Marmot PBV[b]	HT4	KY855431	4093	139–711	726–2441 (CP)	–	2461–4053 (RdRP)

(continued)

Table 13.1 (continued)

S. No	Host species	Isolate name	Accession nos.	Total length (nt)	Segment-1			Segment-2 (ORF) nt
					ORF1 (nt)	ORF2 (nt)	ORF3 (nt)	
26.	Marmot PBV[b]	HT1	KY855428	4579	281–1192	1207–29-28 (CP)	–	2949–4544 (RdRP)
27.	Marmot PBV[b]	HT2	KY855429	4402	41–976	1351–27-96 (CP)	–	2745–4385 (RdRP)
28.	Marmot PBV[b]	HT3	KY855430	4061	149–703	726–2414	–	2427–4022
30.	Equine PBV[b]	Equ4	KR902502	4224	219–857	857–2527 (CP)	–	2600–4117 (RdRP)
31.	Beihai goldsaddle goatfish PBV[b]		MG600063	3862	29–466	522–2219 (CP)	–	2223–3830 (RdRP)
32.	Shāhé picobirna-like virus 1[b]		KX884156	3419	109–1824	–	–	1870–3399 (RdRP)
33.	Shāhé picobirna-like virus 2[b]		KX884154	3344	84–1763	–	–	1769–3331 (RdRP)
34.	Běihǎi picobirna-like virus 12[b]		KX884078	3314	62–1750	–	–	1761–3308 (RdRP)
35.	Běihǎi picobirna-like virus 13[b]		KX884081	3477	48–1970			1994–> 3477[a] (RdRP)

CP capsid protein, *RdRP* RNA-dependent RNA polymerase, *HP* hypothetical protein, *nt* nucleotide, *ORF* open reading frame
[a]Partial gene segment sequences
[b]Non-segmented genome

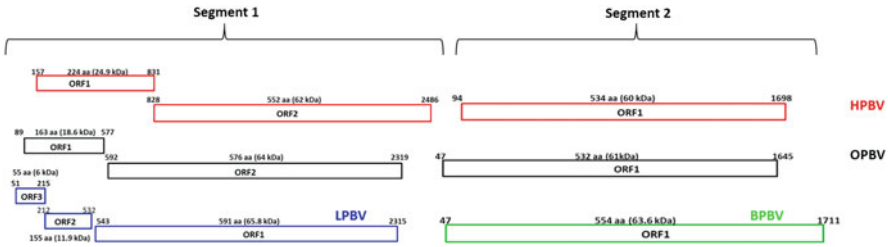

Fig. 13.1 Genomic organization of PBVs detected in the different host species (adapted from (Malik et al. 2014a)). HPBV (Human-PBV, OPBV-Otarine-PBV)

segment 2 has a GC content of 28.3%, whereas the 3′-NCR (43 bases) has GC contents of 46.5% (Woo et al. 2012).

13.3.3 Lapine PBV

Interestingly, the segment 1 of strain 35227/89 encodes three ORFs: ORF1 = 591aa, ORF2 = 155aa, and ORF3 = 55aa. The complete segment 2 has not been sequenced yet (Green et al. 1999).

13.3.4 Bovine PBV

The gene segment 2 of bovine PBV strain RUBV-P is 1758 nt in length having 41.9% GC contents. The 5′-NCR of segment 2 is AU rich (78%). It is to note that the 5′-(GUAAA) and 3′-(ACUGC) end sequences of segment 2 are conserved in this bovine PBV strain (Ghosh et al. 2009).

13.3.5 Swine PBV

The segments 1 and 2 of swine PBV strain 221/04-16/ITA/2004 are 2666 nt and 1730 nt in length, respectively. The ORF1 encodes a protein of unknown function and comprised a variable number of repetitions (Bányai et al. 2014).

13.3.6 Marmot Himalayan PBVs

A surprising huge diversity of PBVs in *M. himalayan* has been detected which indicates that it is a suitable host for PBVs. Due to huge diversity, nine PBV

assortment types have been proposed based on the phylogenetic analysis: C1:GI, C2:GIV, C4:GI, C4:GV, C5:GI, C7:GI, C8:GIV, C8:GV, and C8:GII. A model of segmentation of the PBV genome, which is mediated by a 6-bp direct repeat sequence (GAAAGG) has been proposed (Luo et al. 2018). Besides, an evolutionary model to provide an understanding of how bi-segmented PBVs could be converted into unsegmented PBVs was also proposed in this study (Luo et al. 2018).

13.3.7 Roe Deer PBV

The complete genome sequencing of PBV detected for the first time in roe deer highlighted the huge diversity and broad host range of PBVs. Segment 1 encoded three ORFs and was 2576 nt long. The ORF1 and ORF2 encode for hypothetical proteins, while the largest ORF3 encodes for a capsid protein. Interestingly, the capsid protein of the roe deer PBV strain showed only 22.3% aa identity with the related PBV aa sequences deposited in the GenBank. Segment 2 encodes for the RdRp protein and has 1721 nt with five conserved bases at the 5′ and 3′ end (Kuhar et al. 2017).

13.3.8 Horse PBV

The strain Equ1 has 2274 bp in segment 1 and 1864 bp in segment 2. Notably, PBV Equ1 belongs to members of new genogroup G-IV (Li et al. 2015).

13.3.9 Chicken PBV

The picobirnavirus strain PBV/CHK/M3841/HUN/2011 was identified through metagenomics approach. Segment 1 and 2 sequences of this chicken PBV strain were obtained by RT-PCR and have 2532 bp and 1700 bp, respectively. This PBV genome segment 1 showed low aa sequence identity to the corresponding proteins of marmot and dromedary PBVs. However, segment 2 shared a high aa sequence identity to a wolf PBV (Pankovics et al. 2018).

13.3.10 Dog and Cat PBVs

Segment 2 of strains PBV/Cat/KNA/K40/2014 and PBV/Dog/KNA/RVC7/2015 are 1784 nt and 1689 nt in length, respectively (Navarro et al. 2017).

13.3.11 Vervet Monkeys PBV

The segment 2 of strain PBV/African green monkey/KNA/016593/2015 is 1707 bp and has a high genetic diversity with other PBVs from different hosts (maximum nt and aa identities of 66.4% and 65.3%, respectively) (Gallagher et al. 2017).

13.4 Picobirnavirus in Different Animal Species

Based on the data gathered from both published articles and deposited sequences in the NCBI database, it is now possible to construct an overview of the circulation of PBVs in humans and animals such as the samples detected in 19 different groups of animals and environment, and are distributed in 28 different countries. The species detection share was of 725 monkeys (45.1%), 348 environmental sewage (21.5%), 158 humans (9.8%), 127 swine (7.9%), 46 chickens (2.9%), 45 bats (2.8%), 33 camels (2.1%), 23 bovine (1.4%), and other samples (Cervidae, fish, rabbit, reptile, caprine, feline, horse, ovine, canine, rodents, otarine, and turkey), which together corresponded to 104 (6.5%) of the samples (Fig. 13.2).

As for the distribution by country, the most dispersed PBVs are found in humans (13 countries), swine (10 countries), and cattle (6 countries). Among the analysed countries, Brazil has the highest diversity of hosts where PBVs have been detected in 10 different hosts, followed by China, India, and the USA (7 each) and the others varying from 1 to 3 different hosts (Fig. 13.3).

Regarding the published articles, the human samples have the earliest collection date (1985) until the year 2016, with 22 publications. The earliest collection date of animal samples is from 1995 and extends until the year 2016, with 57 different

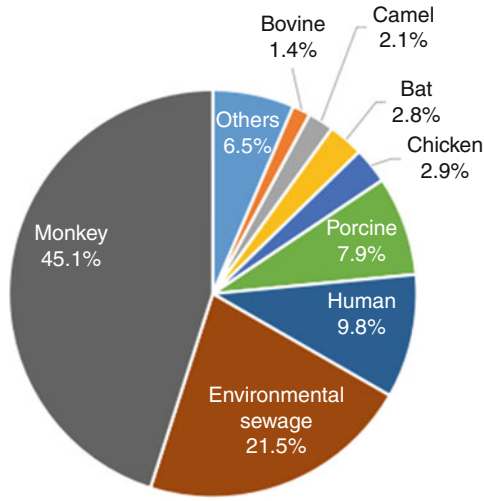

Fig. 13.2 Percentage detection of PBVs in human, animal, and environmental hosts

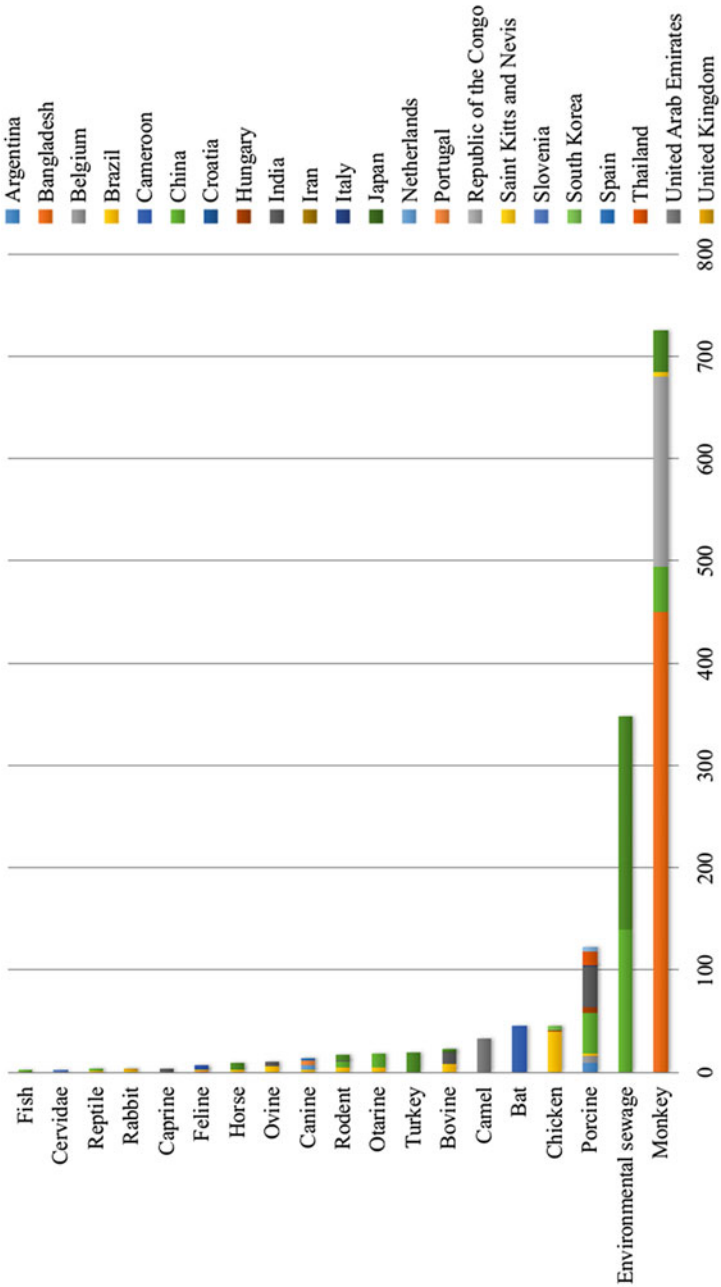

Fig. 13.3 Distribution of PBVs between hosts and countries of origin. The X-axis represents the number of PBV specific nucleotide sequences submitted to the NCBI database

publications. The number of PBV sequences submitted in the NCBI database has seen tremendous growth in recent years, especially of animal PBVs (Fig. 13.4). It is worth noting that of the 158 samples of human origin and 1451 of animal and environmental origin, 70 human samples (44.3%), and 249 animals and environmental samples (17.1%) were not published until the present date.

Epidemiological information gathered for PBVs primarily rely on three commonly used diagnostic methods such as polyacrylamide gel electrophoresis (PAGE), electron microscopy, or reverse transcription-PCR (RT-PCR) detection assays. PBVs have a wide host range as they have been detected in human, domestic, and captive animal species, including birds and reptiles in the faecal samples with or without diarrhoea and also from sewages. The prevalence studies of PBVs in different host species utilized a series of diagnostic assays worldwide suggested the presence of PBVs genome in a wide range. For example, the percentage detection of porcine PBVs by RNA-PAGE was 0.4–27.1% and RT-PCR was 9.0–65% (Ganesh et al. 2012a; Gatti et al. 1989; Bányai et al. 2008; Ludert et al. 1991; Woo et al. 2016), 0.7–8.3% by RNA-PAGE and 12% by RT-PCR in bovines (Malik et al. 2011; Buzinaro et al. 2003; Takiuchi et al. 2016), 14.3–18.0% in equines by RT-PCR (Woo et al. 2016; Ganesh et al. 2011a), 0.9–1.8% by RNA-PAGE and 0.6–4.0% by RT-PCR in canines (Smits et al. 2011; Fregolente et al. 2009; Costa et al. 2004; Ng et al. 2014), 3.4–15.3% by RNA-PAGE and 49.4% by RT-PCR in chicken (Alfieri et al. 1988; Ribeiro Silva et al. 2014), 57% by RT-PCR in turkey (Verma et al. 2015) and in other animals, it varied from 2.2–25% (RNA-PAGE) and 2.4–47.9% (RT-PCR) (Woo et al. 2016; Fregolente et al. 2009; Masachessi et al. 2007). In India, PBVs have been detected in four species so far, viz. human (1.81–2.47%), bovine calves (3.67%), porcine (18.2%), and equine (14.3%) (Ganesh et al. 2010, 2011a, b, 2012a; Malik et al. 2011; Bhattacharya et al. 2006, 2007).

13.5 Transmission

PBVs are being detected in the diarrhoeic animals either as a sole entity or frequently with enteric pathogens (Malik et al. 2014a; Ganesh et al. 2012b). Since the PBVs have been identified usually in the faeces of animals; therefore, transmission through contaminated water and the environment seems to be the major pathway (Symonds et al. 2009). Nevertheless, a few reports of isolation of PBVs from the respiratory tract of pigs and plasma of horses suggest these as secondary locations following viraemia (Li et al. 2015; Smits et al. 2011). Presently, there is no evidence of PBV transmission through the airborne droplet. The animals infected by PBV alone or mixed infection along with other enteric pathogens may have diarrhoea, nausea, and fever or none of the symptoms mentioned above, resulting in a silent and asymptomatic infection in animals.

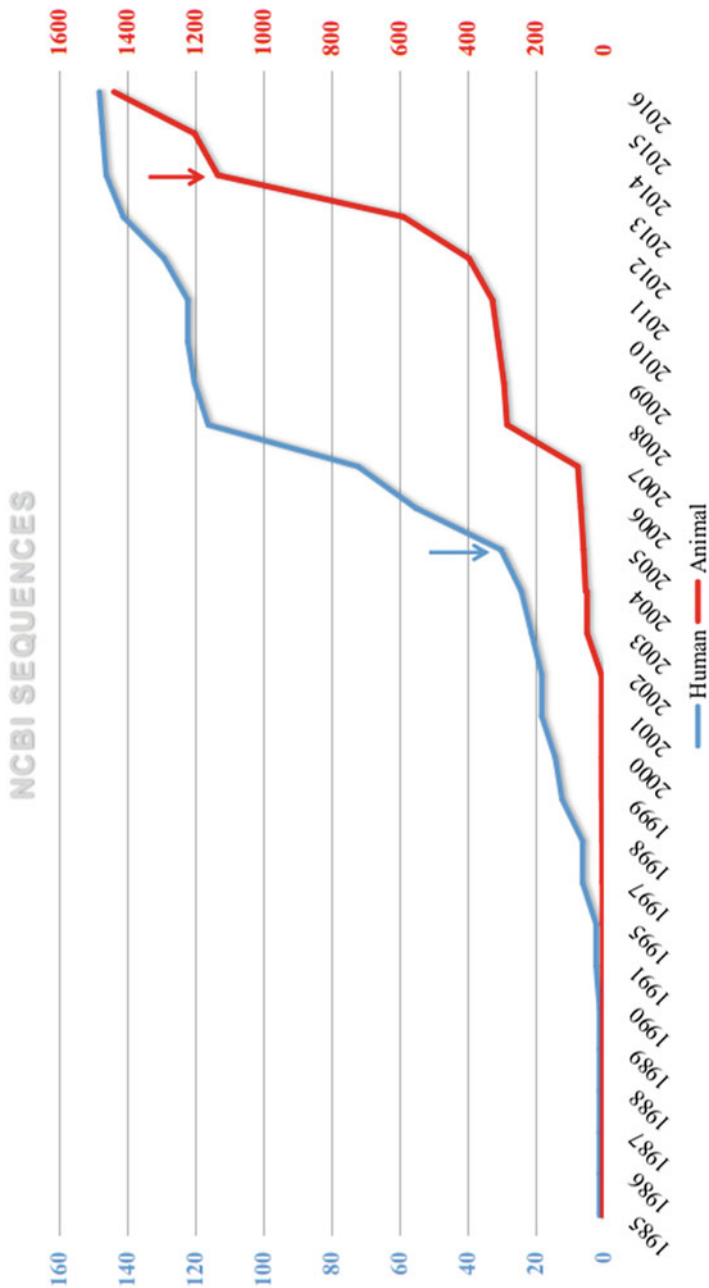

Fig. 13.4 The number of sequences deposited in the NCBI, arrows indicate the year of the first complete genome sequenced from human (blue) and animal (red—swine). Y-axis scatter values represent the human sequences and the values on the right of the X-axis represent those of animals

13.6 Pathogenesis and Persistence

Significant efforts have been undertaken to examine the role of PBVs in a wide range of associations to sporadic episodes of diarrhoea as the sole circulating pathogen or in co-infection, outbreaks of acute gastroenteritis, and in immune-compromised patients with diarrhoea, although PBVs are frequently detected in healthy non-diarrhoeal hosts, with observation of a prolonged imbalance in some individuals (Costa et al. 2004). Though PBVs were first detected in the faeces of children and animals with signs of diarrhoea, many studies failed to establish a clear association between viral excretion in faeces and disease. Thus, the concept that PBVs were of diarrhoeal aetiology remained with the perception that this virus can establish a persistent infection in the host. Numerous factors may assist the PBVs in establishing persistent infections in hosts, including age, stress syndrome, immune status, clinical disease, and environmental conditions (Bhattacharya et al. 2006; Li et al. 2015; Masachessi et al. 2012).

The role of PBVs as opportunistic pathogens associated with diarrhoea cannot be ruled out as there is evidence of persistent infections, with phases of no viral activity interspersed with periods of viral excretion, in healthy animals without a sign of disease, suggesting that this virus is well adapted to vertebrates, with peculiar host–virus interactions. In this context, pigs have been implicated by harbouring a variety of viruses that apparently establish long-term persistence beyond their emerging zoonotic potential (Fregolente and Gatti 2009; Martínez et al. 2010).

The pattern of natural PBV infection has been reported in only a few animals, highlighting the study by Haga et al. (1999) who described the weekly detection of PBV in a period of up to 4 months in giant anteaters (*Myrmecophaga tridactyla*) of a Brazilian zoo and provided evidence that infected adult animals may be persistently infected asymptomatic carriers, serving as reservoirs of infection. Masachessi et al. (2015) in a zoo in Argentina analysed armadillo and orangutan samples kept in isolated pens and also detected the viral excretion of PBV for prolonged periods for at least 6 months and 7 months, respectively. Studies conducted in the USA (Grohmann et al. 1999) and Argentina (Giordano et al. 1999) in HIV-infected patients also revealed a similar pattern of PBV excretion for periods between 45 days and 7 months. The prolonged excretion pattern intercalated for periods of 3 to 4 months without detection of PBV in faeces was described in both studies. The highest prevalence reported was in orangutans, with the virus being detected for 3 years in non-diarrhoeal hosts (Masachessi et al. 2015).

Nevertheless, several studies have revealed a continuous pattern of PBV excretion, such as in pigs and sheep during the lactogenic period in which the highest excretion rate was observed (Martínez et al. 2010; Kunz et al. 2018). Differently, in rabbits, PBV was detected more frequently in weaned animals (Ludert et al. 1995), as well as the excretion of PBV in broilers from 2 to 7 weeks (Tamehiro et al. 2003) and in asymptomatic rhea with approximately 3 weeks of age (Masachessi et al. 2012) suggesting that primary PBV infection could occur early in life in the first weeks in animals and birds establishing a persistent infection with periods of high

viral activity alternated with periods of silence. On rare occasion, PBV has also been detected in the respiratory tract of pigs (Smits et al. 2011). Though Genogroup-I PBV is frequently detected in almost all the species, simultaneous infection by both the genogroups (GG-I and GG-II) of PBVs has also been detected in pigs (Smits et al. 2011), humans (Ganesh et al. 2011b), and in bovines (Malik et al. 2014b).

Therefore, it is important to establish animal models or to adapt the virus to grow in cell culture to understand the pathobiology, the relationships between the host–virus interactions and the distinctive pattern of infection of the PBVs (Duquerroy et al. 2009).

13.7 Zoonotic Potential

PBVs are emerging and opportunistic viruses with potential zoonotic potential that have been detected in faeces of vertebrate hosts, including diarrhoeic and healthy living mammals, farm animals, companion animals, a wide variety of wild birds and zoological animals, seabirds and environmental samples around the world which has increased concern with the public health aspects of the transmission and circulation of these viruses (Delmas et al. 2018; Fregolente and Gatti 2009; Bányai et al. 2014; Luo et al. 2018; Kuhar et al. 2017; Gallagher et al. 2017; Conceição-Neto et al. 2016; Ganesh et al. 2014). In this regard, previous studies of human and animal PBVs have recorded that a specific genogroup is not restricted to a single host, contributing to its rapid spread to new geographic locations (Ghosh et al. 2009) as occurring in other enteric viruses (Cook et al. 2004). Of note, PBVs isolated from humans have been identified that are closely related to strains of genogroups I PBVs in Hungary, Venezuela, and Argentina (Giordano et al. 2011; Bányai et al. 2008; Carruyo et al. 2008) as well as new PBVs in non-human primates (NHPs) (Gallagher et al. 2017; Duraisamy et al. 2018) and roe deer (Kuhar et al. 2017). These debates have increased the interest of several worldwide scientists with a view to a better understanding of the exact role of PBVs and studies on enteric viral diversity in various host species.

It would be of interest to study the pathogenic potential of PBVs in gnotobiotic animals (Kuhar et al. 2017), in the same way that complete genomic analyses of PBVs detected from diverse hosts, and geographic locations may explain the complex dynamics of interspecies transmission and the pattern of circulating PBVs. The metagenomics approach used in the study of human and animal faeces has clarified the existence of a wide microbial diversity in the intestinal system of healthy hosts (Ganesh et al. 2012a; Tamehiro et al. 2003; Yinda et al. 2018). In this context, important studies using viral metagenomics as a tool to detect zoonoses revealed a wide diversity, as well as the emergence of new viruses recovered from a variety of animal hosts (Temmam et al. 2014; Bexfield and Kellam 2011; Barzon et al. 2011). This technique allowed expanding the evidence on the genetic diversity of PBVs by characterizing other fragments beyond the conserved region of the partial RdRp gene, increasing the number of known sequences (Fig. 13.4).

Therefore, complete genome sequencing allows inferences and evolutionary correlations that broaden the understanding of the genetic diversity of PBVs as well as their zoonotic potential.

13.8 Environment Contamination Indicator

PBVs have been commonly detected in surface water and sewage at a relatively high frequency, increasing the importance of their evaluation in environmental studies (Sassi et al. 2018; Lin and Ganesh 2013; Gibson and Borchardt 2016). Thus, the consumption of water contaminated with PBV may present a potential risk of acquiring this virus by different host species since it has been implicated as an important indicator of faecal contamination (Symonds et al. 2009; Hamza et al. 2011). Therefore, the potential health risk of the different hosts due to their presence in sewage and natural water resources used for irrigation in agriculture is cause for concern and for which they should not be neglected (Fernandez-Cassi et al. 2017). Therefore, further investigations are necessary since studies conducted in immunosuppressed hosts have indicated that this virus may be a pathogenic agent causing opportunistic diarrhoea (Giordano et al. 2011).

A study of the evaluation of environmental contamination indicators conducted at USA treatment stations using the pepper mild mottle virus (PMMoV), PBV, and Torque Teno Virus (TTV), indicated that the PMMoV was able to persist in wastewater with the higher signal of detection and correlation with enteric bacteria (Hamza et al. 2011). However, in a study that collected estuarine waters in Wales, United Kingdom, detected the presence of PBV at 22 km far from the water treatment station in all samples collected, with different detection indices involving several enteric viruses with pathogenic potential for humans (Adriaenssens et al. 2018).

Surveillance of viruses circulating in the various animal species is important since it will help in the identification of circulating viruses in a wide variety of asymptomatic or symptomatic hosts for diarrhoea and will be critical in assessing possible changes in the diversity of PBVs against changes in land use and the agricultural industry. These actions can be useful in the future and will help elucidate appropriate measures for the reduction of zoonotic transmission (Lin and Ganesh 2013; Gibson and Borchardt 2016).

It is therefore imperative to emphasize that monitoring of PBVs is essential for detecting the emergence of new zoonotic pathogens in the environment and that their potential role as an indicator of environmental quality is recognized in the future, while being incorporated into the contamination detection, that there is no universally used standard by the surveillance agencies to detect viruses contaminating water bodies.

13.9 Diagnostic Techniques

At least three diagnostic assays are largely being used for the detection of PBVs, namely direct electron microscopy, polyacrylamide gel electrophoresis, and reverse transcription-polymerase chain reaction (RT-PCR). Of these, RT-PCR is frequently used for detection as well as genotyping of PBVs. The lack of suitable animal models/cell lines for PBVs has greatly hindered in their isolation and clinic-pathological studies.

13.9.1 Electron Microscopy (EM)

PBVs appear as small (35 nm in diameter), spherical, non-enveloped entities having icosahedral symmetry. The purified viral suspensions are usually negatively stained with 2% phosphotungstic acid for 1 min. By looking at the size and morphology of the virus particles, PBVs could be easily identified.

13.9.2 Polyacrylamide Gel Electrophoresis (Page)

The PAGE is frequently used worldwide for a reliable and cost-effective diagnosis of PBV. The PBV could be visualized by its peculiar bi-segmented genome excluding *Birnaviruses* after silver staining (Herring et al. 1982). At least two genomic profiles of PBV have been identified in PAGE, i.e. large genome profile [Segment 1: 2.3–2.6 kb and Segment 2: 1.5–1.9 kb] and small genome profile [Segment 1—1.75 kb and Segment 2—1.55 kb] (Fig. 13.5). Besides, a third genome segment has also been reported in chicken (Leite et al. 1990) and dog (Volotäo et al. 2001). These viruses carrying a tri-segmented ds-RNA genome might be because of mixed infection of PBVs or with other viruses.

13.9.3 Multiplex PCR

The multiplex PCR is the ideal diagnostic test for the genomic detection and genogrouping of PBVs (Rosen et al. 2000; Bányai et al. 2003). The genogrouping of PBVs is carried out using the oligonucleotide primers targeting the RdRp gene (segment 2) of the two prototype PBV strains: GI/PBV/human/China/1-CHN-97/1997 and GII/PBV/human/USA/4-GA-91/1991. The recommended two sets of primer pairs for PBV genogrouping are given below in tabular format along with their expected amplicon sizes. The amplicon sizes of 201 bp and 369 bp are indicative of genogroup I and II, respectively.

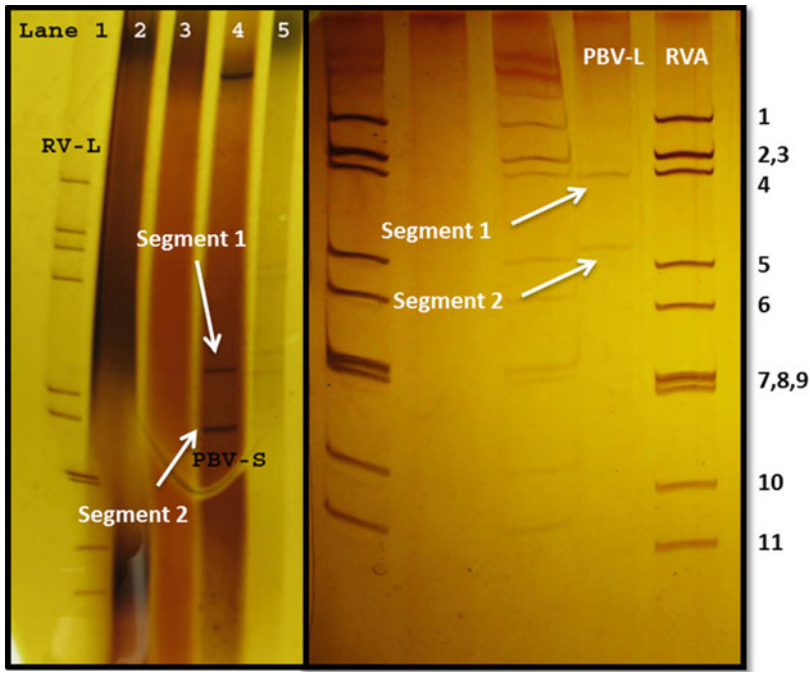

Fig. 13.5 Electrophoretic migration of PBV segments in silver-stained RNA-PAGE gel (Small genome profile-PBV-S; Large genome profile-PBV-L; RVA-Group A rotavirus) (*Source: Reprinted with permission from* (Malik et al. 2014a))

Genogroup specificity	Primers	Oligonucleotide sequences (5′-3′)	Nucleotide positions	Amplicon size (bp)	Reference strains
Genogroup I	PicoB25[+]	TGG TGT GGA TGT TTC	(665–679)	201	1-CHN-97
	PicoB43[−]	A(GA)T G (CT)T GGT CGA ACT T	(850–865)		
Genogroup II	PicoB23[+]	CGG TAT GGA TGT TTC	(685–699)	369	4-GA-91
	PicoB24[−]	AAG CGA GCC CAT GTA	(1039–1053)		

Besides, a diagnostic primer set (PBV2–19 [+] 5′-CGACGAGGTT GATAAGCGGA-3′ and PBV2–281 [−] 5′-CACAGTTCGGGCCTCC TGA-3′) targeting RdRp gene allowed detection of porcine-like PBVs in humans (Carruyo

et al. 2008). Our lab also designed and validated a set of primers targeting segment-2 for detection of diverse bovine and porcine PBVs (available on request).

13.9.4 Next Generation Sequencing

With the advancement in technologies, next-generation or high-throughput sequencing coupled with viral metagenomic analysis has come out to be a powerful tool for identifying the viruses from diverse samples. Using this technology, PBVs have recently been identified in red foxes of Croatia (Lojkić et al. 2016), feline of Portugal (Ng et al. 2014), lettuce samples of USA (Aw et al. 2016), and polluted water of Uganda (O'Brien et al. 2017).

13.10 Prevention and Control

Though PBVs have been detected in both healthy and diarrhoeic animals, at this moment, it is challenging to correlate them with clinical implications. However, they might pose a potential public health concern. The control of PBVs primarily depends on the non-vaccine approach, as no vaccine is available for them. The prophylactic measures include prevention of contamination of food, water, and the environment by applying strict hygiene, identification of transmission modes preventing the transmission of infection from animals to humans and *vice versa*. The treatment of infected cases chiefly includes supportive care in terms of preventing and treating dehydration.

13.11 Conclusion and Future Perspectives

In recent times, PBVs have been detected in several host species, including humans and have been placed in a separate new family '*Picobirnaviridae'*. The sequence and phylogenetic analysis of PBVs showed that they have vast sequence diversity and variable evolutionary dynamics in host species. Preliminary investigations have identified the reassortment events in PBVs owing to the segmented genomes, similar to other segmented viral genomes. However, novel PBVs have also been identified as possessing the monocistronic dsRNA. Furthermore, identification of prokaryotic ribosomal binding motifs in PBV gene segments indicates that these may be prokaryotic viruses. The close relationship of animal and human PBVs coupled with their detection in sewage might pose a potential public health threat. Some of the anonymities have been solved, still a long way to go to understand their replication, pathogenesis, persistence, and evolution of PBVs. Together with

ambiguous clinical implications, there is a necessity to put a lot of efforts to understand many mysteries left behind by this virus.

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Conflict of Interest There is no conflict of interest.

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Chapter 14
Drivers of Emerging Viral Zoonoses

Sandeep Ghatak, Arockisamy Arun Prince Milton, and Samir Das

Abstract Zoonotic viral diseases continue to inflict human mortality and morbidity worldwide. While efforts are on for containment of these diseases, many new viral diseases, especially those of zoonotic origin, were discovered in humans, often in newer geographical areas where diseases were not reported previously. It is now an established fact that with changes in society, demography, climatic patterns, global economy and trade, viral zoonoses have made a comeback. Many factors are responsible for the emergence of viral pathogens worldwide. These factors, known as 'drivers', are numerous and are complexly interlinked. Major drivers of the emergence include ecological perturbations caused by changes in agricultural practices, livestock husbandry, and developmental activities; global warming and changes in climatic patterns, various forces of globalization such as international travel, trade and commerce; *human demographic changes augmented by rising population and urbanization, population* mobility; human behavioural changes; microbial adaptation and evolution; changing technology and industrial practices; and deficiencies in public health infrastructure. Emergence of many new viral zoonoses, e.g. Nipah virus infection, severe acute respiratory syndrome, Middle East respiratory syndrome, highly pathogenic avian influenza, Crimean–Congo hemorrhagic fever, Alkhurma hemorrhagic fever, and others is testimony to role of complex set of drivers in predisposing emergence. Though detailed discussions of these drivers of emerging zoonotic infections are beyond the scope of the current discourse, in this chapter an attempt shall be made to discuss the most important causes of emergence of viral zoonoses so that these complex linkages are recognized and acted upon for attaining sustainable health.

Keywords Climate · Diseases · Drivers · Ecological · Emergence · Emerging · Factors · Global warming · Globalization · Infectious · Viral · Zoonoses

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14.1 Prologue

Infectious diseases are among the major causes of human mortality and morbidity all over the world. They continue to take a heavy toll on the progress made so far. However, with the advent and successful implementation of many health technologies and interventions, especially in decades around 1950s–1970s, infectious diseases were significantly under control (Fong 2013; Leibovici 2018; Rezza and Ippolito 2017; Semenza et al. 2017; van Doorn 2014; World Health Organization 2014). With changes in society, demography, climatic patterns, global economy and trade, infectious diseases made a comeback. Many new diseases that were hitherto unknown to cause human diseases were identified, new geographical areas where diseases were not reported previously were invaded and old diseases that were once thought to have been controlled reappeared in the populations (Fong 2013; Fong and Drlica 2003; Leibovici 2018; Semenza et al. 2017; van Doorn 2014). These events had a deep impact on our view of infectious diseases, and new concepts of emerging infectious diseases began to take shape. It was observed that many of the emerging infectious diseases originated from the animal world (zoonoses), bringing back emerging zoonoses in the forefront of discussions. Further examination of the newly emerging zoonoses revealed that most of them were caused by viral pathogens indicating the continual risk from the emerging zoonotic viral pathogen. A quick analysis of data obtained from 'PubMed' database of National Institute of Health, United States reveals increasing academic interest in emerging viral zoonoses (Fig. 14.1).

Many factors are responsible for such emergence of pathogens worldwide (Table 14.1). These factors (or drivers) are numerous, sometimes subtle yet complex and are often complicatedly interlinked with far-reaching consequences, predisposing emergence of new infections (Fongs 2013, 2017; Fong and Alibek 2007; Fong and Drlica 2003; Jones et al. 2008). Though a detailed discussion of these drivers of emerging zoonotic infections is beyond the scope of the current discourse, in this chapter an attempt shall be made to discuss the most important causes of the emergence of viral zoonoses.

Emerging infectious diseases are broadly described as those infections whose incidence in humans had swelled more than expected in two preceding decades or might pose a threat of increased incidence shortly (Chomel 1998). In a significant study by Jones et al. (2008), it was observed that majority (60%) of the emerging infectious diseases were of animal origin (zoonoses) and almost 72% of them were having their roots traceable to wildlife. Naturally, therefore, the term emerging zoonoses came under intensified discussion and were generally defined as zoonotic infections caused by new agents, or by already known agents, occurring in locations or in host species in which the infection was previously undocumented (Chomel 1998). While the study by Jones and colleagues (Jones et al. 2008) noted that majority of emerging infectious diseases were caused by bacteria, the contribution of emerging viral zoonoses were also significant as was evidenced by recent emergence and outbreaks of many viral infections such as Ebola hemorrhagic

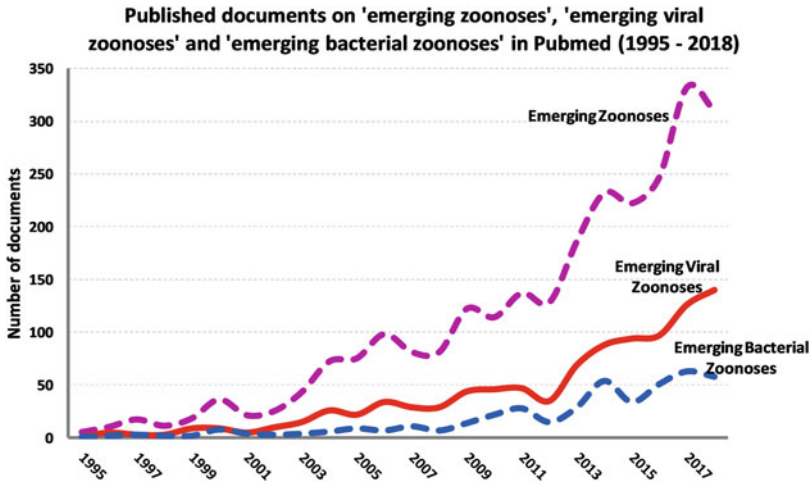

Fig. 14.1 Academic documents on 'emerging viral zoonoses' as available in the PubMed database (1995–2018) (Data source: https://www.ncbi.nlm.nih.gov/pubmed/. Accessed on 3 Aug 2019). In order to assess the academic interest in the study of emerging viral zoonoses a search was made in the 'PubMed' database using the term 'emerging viral zoonoses'. To compare the results, searches were also conducted for two comparable topics with search terms of 'emerging zoonoses' and 'emerging bacterial zoonoses'. Results revealed that search for 'emerging zoonoses' yielded greatest number of documents, followed by search with 'emerging viral zoonoses' and 'emerging bacterial zoonoses'. This was expected as the latter two terms are subsets of the former search term. However, the results also indicated greater academic interest for 'emerging viral zoonoses' compared to 'emerging bacterial zoonoses' as evidenced from available records in PubMed database

fever, Nipah virus infection, Zika virus, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), highly pathogenic avian influenza, Crimean–Congo hemorrhagic fever (CCHF), West Nile fever (WNV), Alkhurma hemorrhagic fever, Rift Valley fever (RVF), dengue hemorrhagic fever, West Nile virus fever, swine influenza, Usutu virus infection, Oropouche fever, hepatitis E infection, and many more (Al-Tayib 2019; Ambat et al. 2019; Bailey et al. 2018; Blacklaws and Daly 2018; Chu et al. 2019; Clé et al. 2019; Fong 2017; García-Bocanegra et al. 2019; Goneau et al. 2018; Jánová 2019; Sakkas et al. 2018; Sayed et al. 2019; Wang and Cowled 2015). In addition to the ever-expanding list of human viral infections, a recent study estimating the mammalian viral diversity indicated that there might be as many as 10,000 viruses with zoonotic potential (Carlson et al. 2019). This indicated that in times to come zoonotic viral infections will possibly continue to emerge and cause considerable damage to the society.

Table 14.1 Major drivers for the emergence of zoonotic viral diseases

Host related	Agent associated	Environmental
• International travel, trade and commerce	• Microbial adaptation and evolution	• Ecological changes
• International travel		• Changes in agricultural practices
• International trade and commerce		• Changes in livestock husbandry
		• Developmental activities causing ecological perturbations
• Human demographic changes		• Global warming and climate change
• Rising population density and urbanization		
• Population mobility		
• Human behavioural changes		
• Rise in tourism		
• Changing consumer behaviours		
• Changes in technology and industry		

14.2 Drivers for the Emergence of Viral Zoonotic Diseases

14.2.1 Ecological Changes

Over the long history of evolution, pathogens including viruses are often acclimatized to specific ecological settings (niches) where they perpetuate involving single or many hosts. This well-adapted lifecycle of agents is delicately balanced and depends on several interplaying factors including host abundance, patterns of vegetation, vector availability, the survival of hosts, immune status of the host population, pathogen characteristics, and climatic conditions such as precipitation, temperature, and humidity, etc. Any change(s), subtle or catastrophic, that might happen to such evenly balanced ecosystem, results in altered pattern of disease of pathogen distribution and when human hosts are involved, emergence of a zoonotic infection takes place (Dantas-Torres 2015; Fong 2013; Heffernan 2018; Karesh et al. 2012; Kilpatrick and Randolph 2012; Kock 2014; Semenza et al. 2017). In many instances, the force behind such ecological changes is anthropogenic (McMahon et al. 2018).

14.2.1.1 Changes in Agricultural Practices

Changes in agricultural practices can bring about local microecological changes prompting the emergence of zoonotic diseases. For example, when grasslands were converted into arable land, and corn cultivation began, they led to a surge in the population of a *Calomys musculinus* rodent, perhaps due to abundance in food. This, in turn, resulted in an increased number of cases of Argentinian and Bolivian hemorrhagic fevers caused by Junin and Machupo type *Coronaviruses* (Chomel 1998; Morse 1995). Increase in the incidence of Rift Valley fever in Egypt is believed to be associated with expansion of irrigated areas for agricultural development (Chomel 1998; Fawzy and Helmy 2019; Morse 1995; Morse et al. 2012).

Expansion of new agricultural activities and human habitation may also lead to the emergence of zoonotic viral infections. In the Latin American country of Peru, an outbreak of cases of vampire bat transmitted rabies occurred following sudden changes in the farming practices. It is believed that before the outbreak there was an increase in pig rearing and human settlements created near and inside forested areas. As the pig population grew vampire bats found new prey for bloodsucking. However, when the rising pig population posed threat to traditional crops in the area, many pigs were culled, and the vampire lost their usual prey for blood and turned onto human settlers with poor housing facilities. As vampire bats are among the natural reservoirs of Rabies virus, eventually an outbreak of rabies erupted among people living in the Peruvian jungles (Chomel 1998; Shipley et al. 2019; Wang and Cowled 2015).

14.2.1.2 Changes in Livestock Husbandry

Among all agricultural activities, livestock husbandry is considered to be the fastest growing sector worldwide (FAO 2011). With the burgeoning global population, increasing urbanization, and rising income level, the world today is experiencing an unprecedented demand for livestock origin foods (Mottet et al. 2018). In Asian countries, per capita intake of animal proteins per day increased from 7 gm to 25 gm between 1960 and 2013 (Van Boeckel et al. 2015). There was an estimated increase of 725% in poultry meat demand (FAO 2011). The massive rise in demand led to the intensification of livestock production systems and also expansion of extensive systems of rearing livestock, most of which took place in Asian countries (Gilbert et al. 2018). The rapid intensification of animal husbandry practices led to greater potential for spread of livestock diseases within farms due to loss of genetic diversity among farmed animals and also due to proximity of available susceptible hosts (Ostfeld 2009). Along with that with increasing global population numbers and population density, particularly in Asian countries, human–animal contact is becoming more frequent. The plausible link between intensification of livestock farming and subsequent emergence of viral zoonoses is exemplified by the emergence of Nipah virus infection in Malaysia, highly pathogenic avian influenza in Asian

countries. In late 1990s (1997–1999) with booming of piggery in Malaysia, forests were cleared to accommodate new farms. Bats which might have lost their nests started roosting in pig sheds, and pigs contracted the deadly virus (Nipah), eventually spilling over the infection to in-contact persons and then to other people (Ambat et al. 2019; Sayed et al. 2019). Similarly, Influenza A virus, which is maintained in a wild migratory birds, found readily susceptible hosts (intensively farmed chickens in various countries of Asia), when migratory birds nested around water bodies with poultry birds nearby during their annual migration resulting in the emergence of HPAI (H5N1) and subsequent epidemic spread of this viral zoonoses (Bailey et al. 2018; Blacklaws and Daly 2018; Goneau et al. 2018; Lal 2007; Morse et al. 2012; Plowright et al. 2017).

Farming of newer species of livestock and birds also provides newer opportunities for disease emergence. Crimean–Congo hemorrhagic fever is a viral hemorrhagic fever caused by *Nairovirus* of the family *Bunyaviridae* and is transmitted by ticks from animals to humans. Many wild and domestic animals including cattle, sheep, goat act as hosts for the virus. Human to human transmission occurs mostly through direct contact with body fluid (blood and secretions) of the infected person. The disease in humans is usually manifested by fever, muscular pain, body ache, stiffness in the neck, headache, diarrhoea, vomiting, neurological symptoms like the state of confusion, depression, petechial haemorrhage on the skin and mucosal surfaces. Case fatality rates hover around 30% (Bird and Mazet 2017; Chomel 1998; Möhlmann et al. 2018; Spengler et al. 2019).

While many domestic animals are hosts of the virus, birds are usually resistant with notable exceptions of ostriches which are susceptible. In South Africa, Ostriches became popular as new farm birds. An outbreak of Crimean–Congo hemorrhagic fever was reported among persons engaged in an ostrich slaughterhouse indicating the inherent risk of the emergence of viral zoonoses with new farmed animals (Chomel 1998). Similarly, an outbreak of Western equine encephalitis was reported among farmed emus in the United States (Chomel 1998).

14.2.1.3 Developmental Activities Leading to Ecological Perturbations

To meet the demand and aspirations of a surging human population, the last few decades have witnessed an unprecedented rush for harnessing various natural resources and energy. Hurried anthropogenic activities resulted in degradation of natural forests, landscape alteration through the expansion of farming and developmental activities such as dam building for hydroelectric power and irrigation, change in natural waterways, unplanned urbanization, environmental pollution, and many more. These factors, in turn, caused—loss of habitats for animals and vectors, thus destabilizing the natural foci of infections; altered the composition of various species; affected the interaction between domestic, peri-domestic, and wild animal. As a result pathogens that were once restricted to the confines of secluded ecosystems gained new opportunities to infect humans and other hosts. Expansion of human habitation into forested areas also offers scope for new infections to emerge.

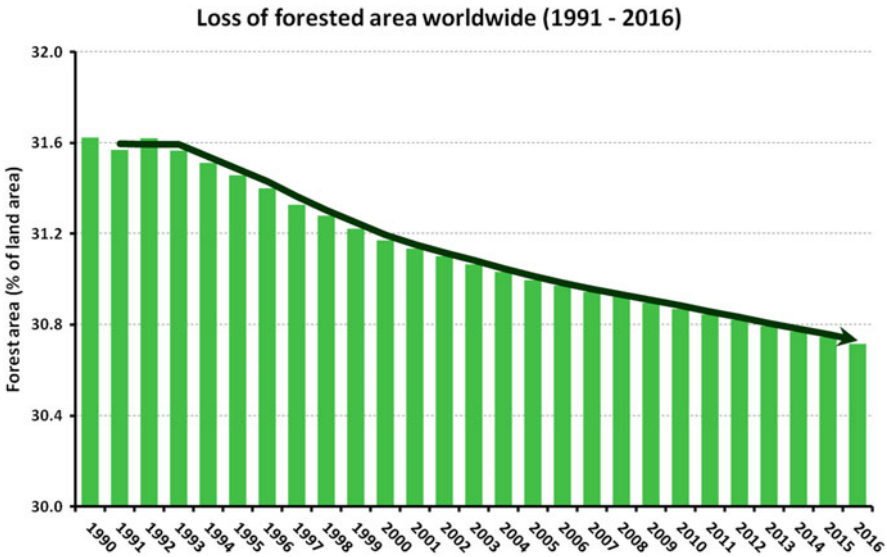

Fig. 14.2 The global trend in net deforestation (Data source: https://data.worldbank.org/indicator/ag.lnd.frst.zs. Accessed on 14 Aug 2019). During the last three decades (1990–2019) worldwide loss of forested areas amounted to approximately 1.6% of total land area of the earth. This huge loss of forest land caused loss of habitats for a multitude of species of animals and plants resulting in irreversible ecological changes. Ecological perturbations are one of the prime drivers for the emergence of viral zoonotic diseases such as Mayaro fever outbreak in Amazonian basins

There is number of instances where deforestation was linked to the emergence of zoonotic viral infections (Fig. 14.2).

It is believed that deforestation and encroachment of forest lands are key factors for the emergence of Mayaro virus infection (Acosta-Ampudia et al. 2018). Mayaro virus infections are usually restricted in the Amazonian basin area and are maintained in a sylvan cycle involving forest mosquitoes and vertebrates. However, infections were spreading in non-forested areas, possibly due to perturbations in the existing disease ecosystem (Acosta-Ampudia et al. 2018; Mackay and Arden 2016). Encroachment into the forested area has also been implicated in the emergence of Oropouche virus fever. Historically the Oropouche virus and Orthobunya virus were responsible for more than 30 epidemics and more than 500,000 cases in Latin American countries. The virus is maintained in a sylvatic cycle involving biting midges (*Culicoides paraensis*) as the arthropod vector, primates, and birds. However, human ingress into forest areas leads to infection in humans who then triggers the urban cycle of the disease as they return/travel to urban areas. Other factors that incite the disease spread include rising temperature and increased rainfall (Morse and Schluederberg 1990; Sakkas et al. 2018; Walker et al. 2018; Woolhouse et al. 2016). Thus developmental activities leading to anthropogenic influences disturbing natural ecosystem of the diseases are among the potential causes of emergence of many viral zoonoses. The emergence of Nipah virus infection could also be linked to

developmental activities (piggery development) by encroachment of forest lands (Ambat et al. 2019; Sayed et al. 2019).

14.2.2 Global Warming and Climate Change

Climate change is one of the greatest challenges faced by humanity affecting all spheres of life, including health. Current projection of climate change events predict 1–3.7 °C rise in global temperature with profound effect on biological systems (Afrough et al. 2019; Anderson et al. 2016; Dantas-Torres 2015; Glennon et al. 2018; Houghton 2019; Li and Du 2019; Mackay and Arden 2016; Rohr et al. 2019). In addition to warming events, climatic models also predict the increased frequency of extreme weather events, more precipitation in higher latitudes, desertification of lower latitude areas, among others. Effects of these weather events often lead to flooding and inundation of urban areas, untimely rainfall, higher winter temperature, etc. (Gould et al. 2017; Grubaugh et al. 2019; Jones et al. 2008; Ostfeld 2009; Petersen et al. 2018; Saker et al. 2004). In the realm of emerging viral zoonoses, all these effects indirectly help survival of viral pathogens and their arthropod vectors with increased insect vector activity and propensity (Dash et al. 2013; Fong 2017; Gould et al. 2017; Grubaugh et al. 2019; Ostfeld 2009; Petersen et al. 2018; Rezza and Ippolito 2017; Semenza et al. 2016). Some arthropod-borne zoonotic viruses have emerged as a major health problem all around the world. Expansion of vector ranges both spatially and temporally is causing the zoonotic viruses to emerge in new areas and to eventually linger in the population. For example, recent emergence of Zika virus since 2015 in American continents and Caribbean region (Blacklaws and Daly 2018; Gould et al. 2017; Heinz and Stiasny 2017; Higuera and Ramirez 2019; Manore et al. 2017). The virus is primarily spread by *Aedes aegypti* mosquitoes which appear to have expanded its range causing infections in new areas. Similar to Zika virus, other arboviruses such as Chikungunya virus and Dengue virus also emerged in recent years in many new areas or have reemerged with enhancing ferocity in endemic areas. It is estimated that in American continents infection with Chikungunya virus results in approximately 1.2 million cases (Fong 2017; Fong and Alibek 2007). Dengue virus infection is known to have caused multiple outbreaks in Asia and Africa with an urban and sylvatic cycle, which are usually separately maintained. However, recent resurgence in urban outbreaks of Dengue virus in many parts of the world is perhaps due to enhanced vector activity and failure of appropriate public health measures (Fong 2017; Fong and Alibek 2007; Fong and Drlica 2003). Apart from general warming of climate, extreme weather events also pose risks. Effects of extreme weather events may be on two fronts. First, these events may lead to earlier appearance of a seasonal infectious agent in an area or may introduce an agent in a new area as was observed for Rift Valley fever in Africa. In 1997–1998 following extremely heavy rainfall, almost 90,000 cases and 1000 deaths due to Rift Valley fever were recorded in the countries lining Horn of Africa (Saker et al. 2004). Second, existing public health services are

often overwhelmed during extreme weather events causing under-control diseases to flare up, e.g. urban settlements of developing countries usually experience outbreaks of dengue fever, chikungunya infections following extreme rainfall and waterlogging inside cities. During 1987–1992 in the USA, prolonged drought was followed by heavy rainfall in 1993, causing a sudden rise in rodent population as most of the rodent predators were killed during the preceding draught. Booming rodent population subsequently caused the emergence of Hantavirus pulmonary syndrome (Grubaugh et al. 2019; Nickerson and Schurr 2006; Saker et al. 2004).

14.2.3 International Travel, Trade and Commerce

14.2.3.1 International Travel

Dissemination of infectious diseases, including that of zoonotic diseases of viral aetiology, had historically been associated with human movements. History of zoonotic diseases reveals that many infections such as plague, yellow fever spread all over the world following human movement across countries and continents. However, in recent times the risks of spread of infections have dramatically increased in contrast to ancient times. With the advent of cheaper air travel and economic improvements, human movements across international borders have increased by an enormous scale with miraculous speed and invasive reach (Fig. 14.3).

These days, it is not impossible for any person to reach any place on earth within 24–48 h. Increased magnitude of high-speed travel to remote locations enabled many zoonotic diseases to establish a new focus of infections in distant places. There is number of instances where spread of viral zoonotic diseases has been exacerbated by air travel.

Influenza A virus is a highly infectious agent responsible for epidemics of influenza throughout the world, causing 300–600 thousand deaths globally. In addition to human infection, the virus causes infection in a wide range of hosts and is maintained in nature among wild birds, especially migratory water birds. Usually, strains of Influenza A virus infect their respective hosts, but domestic animals such as pigs may be infected by both avian and human strains of the virus. Due to segmented genomic structure of the virus, mixed infection of the pigs with avian and human type of the virus may give rise to recombinant strain with new antigenic structure which may potentially cause pandemic across the world and caused multiple pandemics in the past resulting in deaths in millions in the past. International air travel further aids in dissemination of the virus, allowing rapid global mobility of incubating hosts (humans). Recently in 2009, a novel strain of influenza virus emerged in a Mexican pig farm. With the help of international air travel the virus rapidly spreads throughout the world and caused about 123–200 thousand deaths worldwide (Findlater and Bogoch 2018; Houghton 2019; Mangili et al. 2015).

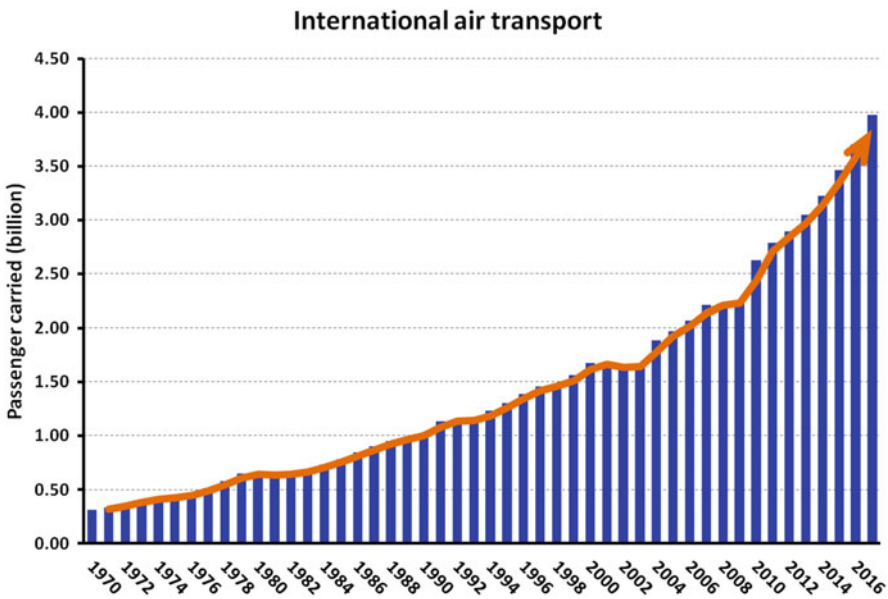

Fig. 14.3 International air travel trend (Data source: https://data.worldbank.org/indicator/IS.AIR.PSGR. Accessed on 14 Aug 2019). International air transport (passenger carried) includes both domestic and international aircraft passengers of air carriers registered in the country. From 1970 onwards there was a rapid increment in international air travel. In the year 2016, as many as nine times the people travelled through international air as compared to 1970. Such a rapid rise in air travel aggravates the transport of zoonotic viral infections throughout the world as was evidenced in case of outbreak of severe acute respiratory syndrome

Similar to Influenza A virus, in case of a global outbreak of severe acute respiratory syndrome (SARS) also, air travel played a key role in the dissemination of the infection. SARS is caused by a Coronavirus which is believed to have originated in horseshoe bats. From horseshoe bats, the virus was transmitted to civet cats from which the virus eventually was spread to humans and finally human-to-human transmission led to the epidemic spread of the disease. In February of 2003, SARS was reported for the first time from the Guangdong province in China. In about 8 months of the SARS epidemic, the disease caused more than 8000 cases and approximately 750 deaths (Findlater and Bogoch 2018; Mangili et al. 2015; Mourya et al. 2019). The disease was characterized by fever, sore throat, dry cough, respiratory difficulties, myalgia and malaise, sometimes rash and diarrhoea. Case fatality rates of SARS were about 10%. After the initial detection of these emerging viral zoonoses, the disease spread to more than 12 countries, including Taiwan, Hong Kong, Vietnam, Singapore, Philippines, and Canada mostly facilitated by international air travel and mobility of the infected incubating individuals. Rapid global spread led to international panic and loss in tourism ad trade. Estimates of economic loss due to SARS indicated a cost of USD 11 billion worldwide which included costs of various public health measures for containing the spread,

treatment, public awareness, and additional screening at airports for SARS. SARS had brought about changes in International Health Regulations practices and adoption (Findlater and Bogoch 2018; Fong and Drlica 2003; Grubaugh et al. 2019; Vonesch et al. 2019).

In 2012, the zoonotic potential of the Coronaviruses caused the emergence of another viral disease named Middle East respiratory syndrome (MERS) in Saudi Arabia. Domesticated camel is believed to be the reservoir of the MERS Coronavirus. In humans, the infection causes flu-like symptoms characterized by fever with occasional chills, cough, breathing trouble, sore throat, myalgia, and abdominal tenderness. However, vomiting and diarrhoea are also noticed. Case fatality rates may reach as high as 35%. Human-to-human transmission occurs through close contact with infected individuals. Since its first report in 2012, the disease spreads to 27 countries in four continents (Asia—Saudi Arabia, Bahrain, Iran, Jordan, Lebanon, Kuwait, Oman, Qatar, United Arab Emirates, China, Malaysia, Republic of Korea, Thailand, Philippines; Africa—Tunisia, Yemen, Algeria, Egypt; Europe—Austria, France, Germany, Greece, Italy, the Netherlands, Turkey, United Kingdom; and North America—United States) with more than 2000 cases. Since Saudi Arabia is an important tourist destination with considerable air connectivity, many more people from various countries are at risk of contracting the disease. In addition, international Hajj festival with huge inflow of tourists from all over the world over a short period of time also offers opportunity for potential spread of MERS Coronavirus (Bailey et al. 2018; Findlater and Bogoch 2018; Grubaugh et al. 2019; Li and Du 2019; World Health Organization 2014).

The global spread of other zoonotic viruses was also accelerated by international air travel. In western Africa in 2014, the epidemic of Ebola virus disease broke out. Though the virus was first discovered in 1976, the West African outbreak was perhaps the largest. Natural hosts of the virus are believed to be Fruit bats (*Pteropus*). Humans acquire Ebola virus infection from close contacts with bats and their body fluids. However, other animals and their body fluids have also been implicated in the spread of the disease to humans. Human-to-human transmission occurs similarly through close contact and their bodily secretions and fluids. Fomites and objects that had been contaminated by infected persons also play an important role in the transmission of the disease. Highly infectious nature of the disease contributes to rapid human-to-human spread, and in many cases, health workers fell victim to Ebola infection while attending to infected people. In humans, Ebola virus disease is characterized by fever, sore throat, headache, myalgia followed by vomiting, diarrhoea, and typical rashes. Eventually the disease causes multi-organ failure leading to death in many cases. Hemorrhagic signs such as gingival bleeding and blood in stool are also reported. Case fatality rates of Ebola virus disease are very high and vary between 25 and 90% with average being around 50%. The spread of the 2014 epidemic of Ebola virus disease was facilitated initially through road travel and the disease clawed out from Sierra Leone to neighbouring Guinea and Liberia. Subsequently, the disease leaped out of these countries through international air travel to several other faraway countries including the USA, Italy, United Kingdom, Spain, and Nigeria. Over 2 years the deadly epidemic caused approximately 22,000 cases

with more than 11,000 deaths. Outbreaks of the disease are continuing in various areas of African continent with huge potential for global spread (Beeching et al. 2014; Broadhurst et al. 2016; Findlater and Bogoch 2018; Malvy et al. 2019). In effect the air travel, especially the international routes, enabled many viral zoonotic diseases to leap out of its initial foci with relative ease and made the job of epidemiologists more difficult who can no longer rely on the classical parameter of geographic contiguity of disease dissemination.

14.2.3.2 International Trade and Commerce

With increasing globalization, the volume of trade within and across national borders has also increased significantly. Data from the shipping industry reveal a phenomenal increase in container cargo in various seaports during the last two decades (Fig. 14.4).

Not only did the volume of trade increased in the last decades, there was also rise in varieties of materials traded. Moreover, trading activities became more and more important in the economic output of countries, which is evident when measured against country GDPs (Fig. 14.5).

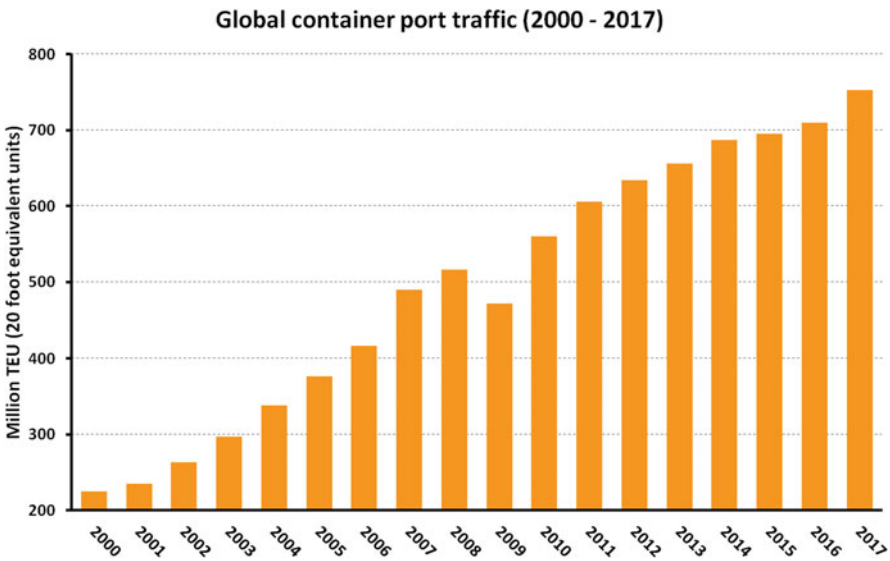

Fig. 14.4 A global upsurge in shipping activities (Data source: https://data.worldbank.org/indicator/IS.SHP.GOOD.TU. Accessed 14 Aug 2019). As per data of the World Bank, the volume of container traffic through various shipping routes increased from about 225 million TEU to more than 75 million TEU indicating a 3.5-fold increase in just 17 years. A large volume of trade in many instances facilitates spread of zoonotic infections, including viral diseases across international borders and continents

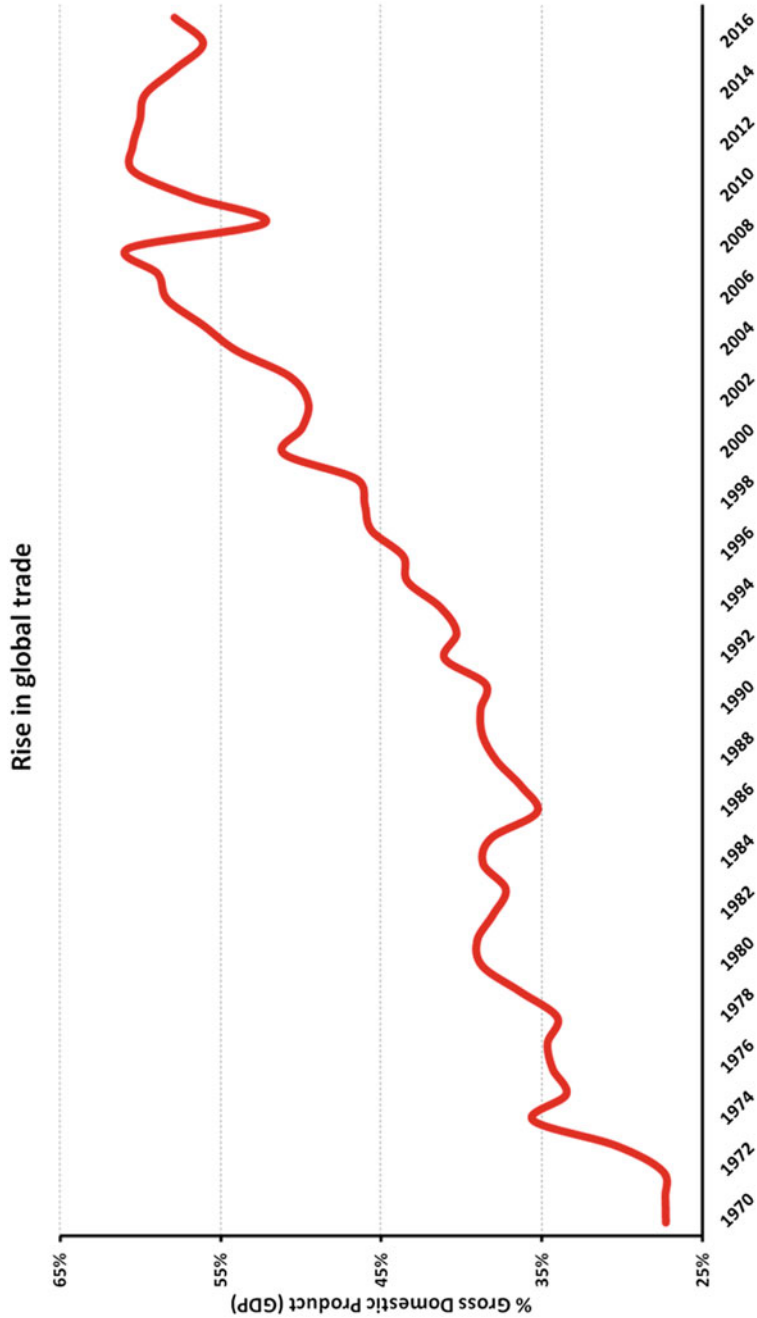

Fig. 14.5 The worldwide increase in trade volumes (Data source: https://data.worldbank.org/indicator/NE.TRD.GNFS.ZS. Accessed 14 Aug 2019). Despite occasional instability, from 1970 onwards there had been a steady rise in global trade. The rise in trade was not only in quantum of goods and services traded, the contribution of trading activities in the economies of the nations also increased. This indicated that if the current trend continues, the global economy will largely be driven by trading activities. As majority of these trading activities are across international borders, they may facilitate the emergence of viral zoonotic diseases as has been observed in the past centuries when diseases spread along the trade routes

Historically zoonotic diseases, including viral zoonosis, were disseminated along the trade routes. The plague reached the western hemisphere following the ancient trade routes. Yellow fever, an important viral zoonotic disease, is believed to have spread from Africa to America following the shipping routes (Chomel 1998; Dash et al. 2013; Esser et al. 2019; Findlater and Bogoch 2018; Higuera and Ramirez 2019; Muzemil et al. 2018; Saker et al. 2004; Tagliapietra et al. 2018).

While trades in goods and services are a general facilitator of spread of zoonotic diseases, trading in wildlife and products is another important avenue for dissemination of zoonoses. Wildlife meat is traditionally savoured by many tribal populations around the world. However, meat from wildlife has been implicated in the emergence and dissemination of Nipah virus, Ebola virus, Monkeypox virus (Ambat et al. 2019; Beeching et al. 2014; Broadhurst et al. 2016; Karesh et al. 2012; Malvy et al. 2019; Mazzola and Kelly-Cirino 2019; Muzemil et al. 2018; Petersen et al. 2018; Sayed et al. 2019). In recent years, the role of civet cats used as human food came into increasing attention following SARS epidemic. The causative agent of SARS (SARS-CoV) thrives naturally in bats, and civet cats might have picked up infection occasionally from them. However, when meats from civet cats found their way into the market, the SARS-CoV spilled over to new hosts, humans; and a new viral zoonosis, SARS emerged (de Wit et al. 2016; Parashar and Anderson 2004). Apart from meat from wildlife, various other body parts of animals are used in traditional medicines (Chomel et al. 2007). Trading of pets such as psittacine birds and rodents has become increasingly common and also might play roles in the emergence and spread of viral zoonoses (Chomel et al. 2007). The importation of Monkeypox infected pet rodents in to the USA indicated the potentials of trade in wildlife as a driver for the emergence of viral zoonoses (Chomel 1998; Petersen et al. 2019; Sklenovská and Van Ranst 2018). An increasing trend of adopting more and more exotic pets may also serve as source for spread of new viral zoonoses. In the USA and Germany, outbreak of chorio-lympho-meningitis was reported among pet owners following adoption of hamsters (Chomel 1998).

14.2.4 Human Demographic Changes

Over the last couple of decades, the world has witnessed an explosive rise in the human population with associated changes in demographic patterns. Cultural and behavioural changes were also pronounced. World population projections indicate that by the year 2050 the global population is expected to reach 9.7 billion with most of the growth in developing regions of the world (https://www.un.org/development/desa/en/about/desa-divisions/population.html). It is projected that with the rise in population, there will be even denser spatial clustering of populations in the cities of the world. Though cities usually provide better economic opportunities and better livelihood options, often the public service infrastructures including health and sanitation measures are stretched beyond limits. These situations provide fertile grounds for zoonotic diseases to spread, especially vector-borne viral zoonoses.

14.2.4.1 Rising Population Density and Urbanization

Increasing population and concentration of economic activities in selected areas have resulted in massive urbanization and increased population density all over the world including in urban areas. While the quantum of urban areas remained almost unchanged, number of people living in cities has increased steadily over the years. Almost 55% of the global population today lives in cities. At the same time, global population density rose from about 28 persons/sq. km in the 1970s to more than 59 persons/sq. km in 2019 (Fig. 14.6).

Increased population density allows more frequent contacts between infected hosts and susceptible individuals. Host population density also affects the efficiency of the vectors for transmission of the diseases. Studies have shown that increased population density does enhance the likelihood of a sustained outbreak of diseases (Tarwater and Martin 2001). Many cities in Asia have become almost endemic for several viral zoonotic diseases such as dengue, chikungunya, etc. (Dash et al. 2013; Lal 2007; Mackenzie 2005). With the rise in global temperature and the heat island effects, cities around the world provide an ambience of high host density, warmer temperature, ample breeding grounds for mosquito vectors, e.g. discarded tires accumulated water, plastic pouches with a small poodle of rainwater, clogged drainage due to choking by disposable plastic bags, etc. As a result, there is a consequent rise in the vector-borne zoonotic viruses all around the world. Recent studies on Zika and Chikungunya virus transmission also substantiate these phenomena (Chua et al. 2016; Manore et al. 2017).

14.2.4.2 Population Mobility

Movement of the population across regions had always been associated with the movement of diseases from one place to another. Migration of people occurs due to many factors and due to various reasons. The movement happens within the countries and also across international borders. In the last two decades, the number of refugee population has increased significantly after a steady fall over the preceding decades (Fig. 14.7).

Current estimates reveal that more than 25 million people are living today with refugee status. Moreover, available data from World Bank estimates that the number of international migrants has also increased significantly in 1995. Today more than 3.3% of the global population is international migrants.

While economic opportunities had traditionally been a major force behind the trans-border movement of people, other factors such as war, border conflicts, ethnic fights, and natural calamities such as drought, flood, desertification, rising sea level, etc. also contribute towards migration of people en masse. Migration of human population comes along with consequent ghettoization of the displaced population with the considerable downside of sanitation, hygiene, water quality, and overall public health infrastructure in the area. All these factors encourage diseases, including vector-borne zoonotic viral diseases to break out. The current crisis of

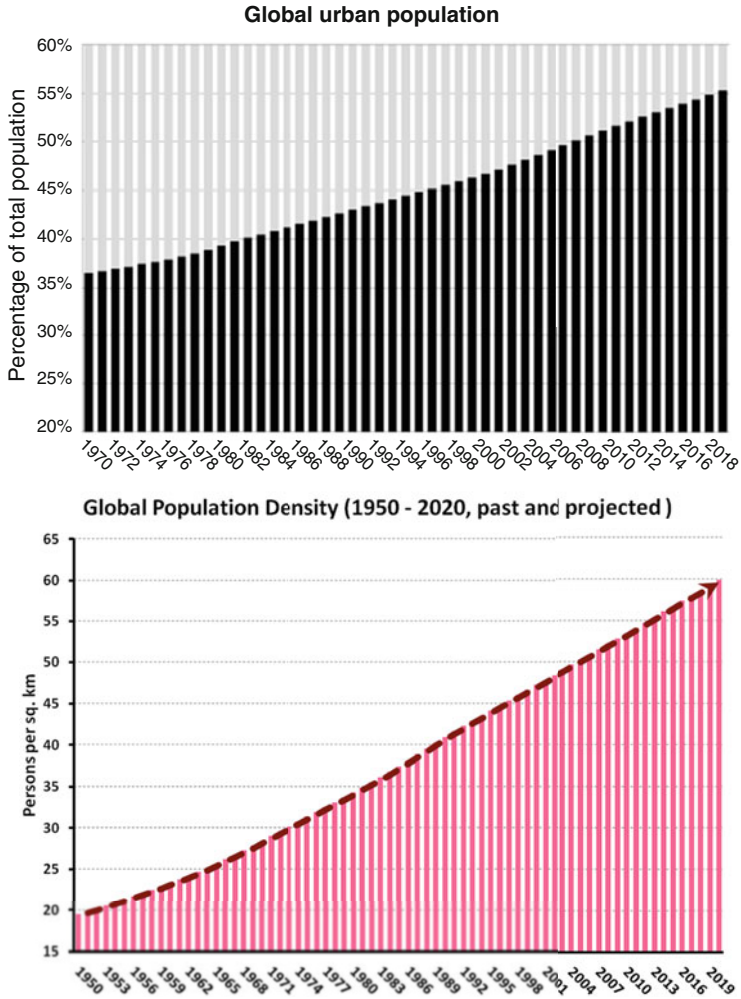

Fig. 14.6 Urbanization and population density trends (Data source: https://data.worldbank.org/indicator/sp.urb.totl.in.zs. Accessed 14 Aug 2019. https://data.worldbank.org/indicator/EN.POP.DNST. Accessed 14 Aug 2019). All over the world, there had been a steady increase in urban population. In the 1970s, about 35% of global population was urban which rose to 50% in the preceding decade. As per available data in 2018 more than half of world population lives in cities creating more stress on urban infrastructures. Global population density, on the other hand, saw meteoric surge. On every square kilometre of land, almost three times the people live today (2019) as compared to 1950. Higher population density exerts pressure on available natural resources and poses challenges to available economic opportunities

displacement of Rohingya population in Southeast Asia is an example of these. Several researchers have expressed concerns about the possibility of outbreak of diseases among these displaced peoples (Cousins 2018; Islam and Nuzhath 2018). Similar situations may also be observed in many places in Africa (Castelli and Sulis 2017; Rote and Markides 2015).

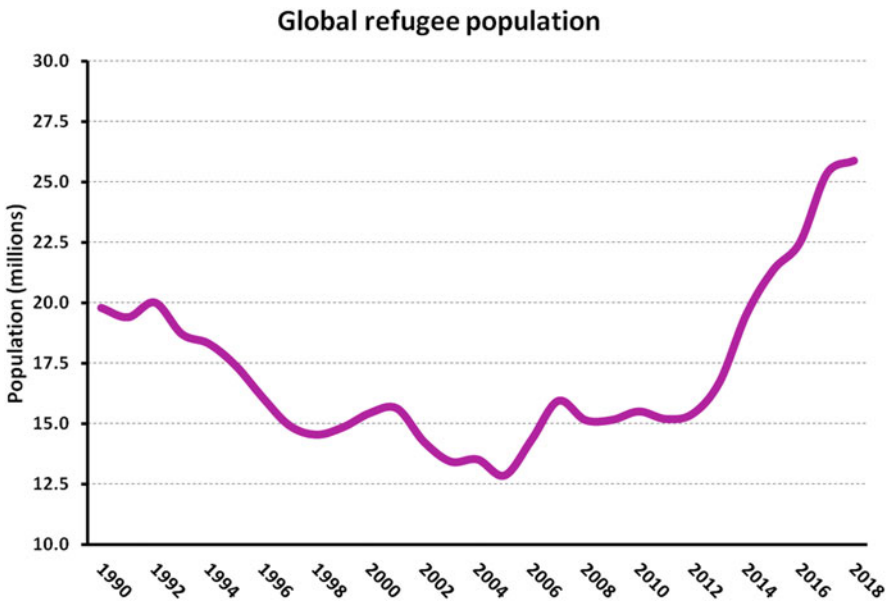

Fig. 14.7 Global refugee population (Data source: https://data.worldbank.org/indicator/sm.pop.refg. Accessed 14 Aug 2019). 'Refugees are persons who are outside their country of origin for reasons of feared persecution, conflict, generalized violence, or other circumstances that have seriously disturbed public order and, as a result, require international protection' (United Nations, 2019). From 1990 to 2004, there was a steady decline in number of refugee population worldwide. This was followed by a sharp rise in refugee population till 2018. These sections of population are considerably deprived of health care and other amenities, including sanitation and hygiene. Zoonotic infections are known to disproportionately affect such vulnerable population leading to the emergence of zoonotic infections

14.2.5 Human Behavioural Changes

With the increasing globalization of trade in goods and services accompanied by rising incomes, especially in developing countries, there had been a considerable shift in consumer behaviour and lifestyle of the people. These changes, in some cases, provide suitable stimuli for new zoonotic diseases to emerge.

14.2.5.1 Rise in Tourism

Throughout history, touring places had been an important pleasure activity of the people with surplus earnings. However, during the last two decades, there has been a phenomenal rise in international tourism throughout the world. Available data indicate that between 1995 and 2017, number of international tourists arrivals have swelled from about 524 million to more than 1.3 billion worldwide (Fig. 14.8). Not only did the quantum of tourists increased, the type of tourism also changed with more exotic destinations added to the lists of places to be visited.

Fig. 14.8 A global rise in international tourism (Data source: https://data.worldbank.org/indicator/ST.INT.ARVL. Accessed 14 Aug 2019). There has been a phenomenal rise in international tourists. According to available World Bank data the number of international tourists more than doubled during the period between 1995 and 2017

Today people are keenly interested in wildlife tourism, eco-tourism, adventure tourism, and others, which expose the non-native population to remote and/or pristine ecosystems. While the revenue earned is arguably for the preservation of these ecosystems and associated people, there are concerns for encroachment, unnecessary commercialization of forest resources, perturbation to delicate ecosystems, and potential scope for the emergence of new zoonotic diseases including viral diseases. Herpes B virus, also known as Cercopithecine Herpesvirus 1, causes zoonotic infection in humans. Natural hosts of the virus are Macaques. In humans, the disease causes fulminant encephalomyelitis with severe neurological dysfunction with high fatality rates. It has been observed that Asian macaques in Balinese temples were able to transmit Herpes B virus through direct contact. As many Asian temples are important tourist destinations and macaques thrive in the temple and adjacent territory, such contact does pose a significant threat of transmission of viral zoonoses (Chomel et al. 2007). Though, it is difficult to specifically identify the transmission of zoonotic diseases from wildlife to tourists, a recent study by Carne et al. (2017) identified possible risk factors for transmission of zoonotic infections from macaques to tourist. The authors observed that proximity between tourists and visibly diseased macaques, which were coughing and sneezing, posed particular risk and advised appropriate use of personal protective gears for preventing possible new infections.

Though most of the studies document the transmission of zoonosis from wildlife to humans, a few studies documented the occurrences of zooanthroponoses (reverse zoonoses). A review by Messenger et al. (2014) analysed cases of reverse

transmission and found that such incidents were reported from all continents except Antarctica. However, a recent report identified anthropogenic transmission of zoonotic agents to penguins of Antarctica too, thus highlighting the impacts of tourism on uninhabited natural ecosystems (Bolevich 2018).

14.2.5.2 Changing Consumer Behaviours

The global rise of the consumer-driven market economy led to fundamental changes in the ways goods and merchandises are traded today. The effect is more pronouncedly perceived in the food and agriculture sector than any other areas. Consumers today increasingly demand quality products with better safety. Moreover, exposure to international media and market information led to a rise in taste for exotic products resulting in trans-border movement of food and food products. As most of the consumers with disposable incomes are located in urban areas, there is increasing demand for foods, especially that of animal origins, in urban areas prompting development of peri-urban livestock farming. Peri-urban farming comes with its own set of problems, e.g. environmental pollution, creating a putative focus of zoonotic diseases that may emerge and spread to urban population nearby, providing vector breeding opportunities, etc. These factors along with others are among the inciting causes for the emergence of viral zoonoses, particularly, vector-borne zoonotic infections. In developing countries, surging demand for livestock proteins also results in cropping up of wet markets that are under-regulated. Wet markets provide ample scope for spread of new agents of viral zoonoses to new hosts (humans) resulting in the emergence of novel viral zoonoses. SARS is a prime example of such emergence as described previously (Bailey et al. 2018; de Wit et al. 2016; Lal 2007; Parashar and Anderson 2004; Wang and Anderson 2019).

14.2.6 Microbial Adaptation and Evolution

To survive in a harsh and changing world, the agents of zoonotic viral infections need to adapt themselves and evolve too with better fitness. Viruses adopt multiple strategies for their survival. The measures range from a mutation in the genome resulting in antigenic variation to evade host immune response to acquiring the ability to overwinter in hosts for a longer period. Even strategies for acquiring new host specificity (or a loss thereof) are also adopted. Thus adaptation and evolution of viral agents provide newer avenues for the emergence of new zoonotic infections. Interestingly, most of the mutational changes in viruses occur in RNA viruses, which are presumably due to lack of proofreading capacity of RNA polymerase enzyme involved in viral replications.

Influenza A virus undergoes antigenic shifts and drifts, which allows the virus to evade existing host immune response. The genome of the Influenza virus is segmented. When a particular cell is affected by two different types of virus, during

replication stage, a recombinant virus may emerge, combining genomic segments from two different strains. The resulting strain bears a new antigenic structure against which hosts do not have any immunologic memory, thus initiating a possible new pandemic (Bailey et al. 2018; Goneau et al. 2018; Lloyd-Smith et al. 2009; Morse et al. 2012). Mechanism of immune evasion through antigenic variation is employed by many flaviviruses too (Heinz and Stiasny 2017; Ye et al. 2013). Mutations in the genome of West Nile virus is known to cause variation in the expression of neutralizing epitopes (Li et al. 2005).

Similarly, antigenic variation among various genotypes of Chikungunya viruses affects cross-protection by neutralizing sera (Chua et al. 2016). Dengue virus, the causative agent of dengue hemorrhagic fever, is also known for antigenic variation for evading host immune barriers (Bell et al. 2019). In addition to short-term adaptation of viruses relatively ancient recombination events are also believed to have contributed towards the emergence of new viral zoonotic agents. It is thought that recombination events involving the Sindbis-like virus and Eastern equine encephalitis viruses led to the emergence of Western equine encephalitis virus (Chomel 1998).

14.2.7 Technology and Industry

Technology has always been crucial in shaping the history of humans. Since the time of the industrial revolution, the impact of technology on human health and infectious diseases had been immense. Technological advances affected almost all facet of human lives—including change in agricultural production systems, intensification of livestock farming, mechanization and centralization of food processing transport and marketing, organized health care and public health, enhanced communication including shortening of travel times, closed area ventilation systems, creation of new range inanimate objects and fomites harbouring infectious agents (e.g. automated teller machines, public telephones, surfaces of public transport systems, etc.), integrated water supply systems, mechanically controlled sanitation systems, and many more. One of the most current threats from evolving medical technology involves risks of acquiring zoonotic viruses following xeno-transplantation. Concerns have been raised regarding the transfer of porcine endogenous retroviruses to humans following organ transplantation (Prabha and Verghese 2012). Moreover, blood transfusion also poses risk of acquiring hidden viral infection by the recipient, and usually strict vigil is necessary.

Changes in the water supply systems worldwide definitely provided safer water to a large number of population, but at the same time centralization of the water supply system of the cities up the risks of common source epidemics due to viral hepatitis (Bloch et al. 1990; Gall et al. 2015). Similar is the risk associated with centralized processing of food products that are marketed globally (Henchion et al. 2017; Rohr et al. 2019).

14.2.8 Public Health Deficiencies

Breakdown of public health infrastructure that is supposed to prevent and preempt the spread of infections may also encourage the emergence of new zoonotic infections. Various factors contribute to deficiencies in public health infrastructures worldwide. Among the major factors include political disturbances, wars, border conflicts, ethnic struggles, insufficient investments in public health, and lack of political wills in policymakers. It has been observed that coverage of vaccinations was lower in politically troubled territories leading to an increase in the prevalence of infectious and zoonotic diseases such as yellow fever (Chomel 1998). Overpopulation, unplanned urbanization, migration, and social upheavals may also overwhelm the existing public health infrastructure leading to outbreak of diseases in peri-urban and urban areas as is witnessed by many Asian cities with almost endemic prevalence of mosquito-borne zoonotic viruses. Inadequacy of public health may also lead to spread of HIV infections, which in turn create a buildup of immuno-compromised population susceptible to many emerging and reemerging viral zoonoses, often with fatal outcomes.

14.3 Epilogue

Infectious diseases with its subset of viral zoonotic diseases had always been important in influencing the courses of human development and history. The events of biological and cultural evolutions of Homo sapiens are a history of co-evolution with infectious diseases, many of which were shared with animals. Infectious diseases and zoonoses, in particular, perpetuate in the nature within complex yet delicately balanced ecosystems which when perturbed spills out the infections to unnatural hosts, mostly humans. This understanding of zoonotic diseases ecology is central to disease emergence. With rising population, expanding globalization of trade, commerce, agricultural activities, changing the climate, declining forest covers, the root drivers for the emergence of zoonotic diseases, which are often of viral origin, sometimes become obscured. Recognizing these linkages that drive the emergence of viral diseases is crucial to our understanding of complex disease biology and attaining sustainable health for all. As highlighted in this chapter before, increasing academic interest in the study of 'emerging viral zoonoses' is a positive indication which needs to be followed by actionable information, judicious execution, and creation of a policy framework for effective handling of contingencies arising out of the emergence of viral zoonotic diseases.

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Chapter 15
Viral Zoonoses: Wildlife Perspectives

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Abstract Wildlife plays an important and complex role in the emergence of new diseases and the maintenance of endemic infectious diseases. The majority of the recent emerging diseases were caused by zoonotic viruses of wildlife origin and had significant impacts on public health and economies. Wildlife can act as a reservoir or maintenance or spill-over or amplifier hosts or simply a liaison host of diseases transmissible to human beings and farmed livestock. Anthropogenic factors like agricultural expansion, habitat destruction, urbanisation, trade of exotic or domestic animals and global travel comprise major drivers of the emergence of zoonotic disease. The viral families *Arenaviridae, Coronaviridae, Flaviviridae, Filoviridae, Hepeviridae, Hantaviridae, Herpesviridae, Nairoviridae, Orthomyxoviridae, Peribunyaviridae, Paramyxoviridae*, *Phenuiviridae, Poxviridae, Reoviridae, Rhabdoviridae,* and *Togaviridae* enclose viruses which represent most of the viral zoonoses of wildlife origin. The basic factors influencing the disease emergence from wildlife species are also the major drivers of biodiversity loss. Therefore, emerging zoonotic viruses are not only potential threats to human beings but can also be harmful to wildlife species. Thus, there is a convincing and effective chance for mutual gains for the conservation of wildlife and public health by collective and collaborative attempts.

Keywords Wildlife · Viral zoonoses · Emerging viruses · Spill-over · Spill-back · Reservoirs · Migratory birds

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15.1 Prologue

Today's world does not have any barrier between the animal and human medicine. The majority of infectious diseases affecting human are of zoonotic origin, and for several emerging diseases, wildlife serves as a reservoir (Jones et al. 2008). In the emergence of new diseases as well as maintenance of endemic infectious diseases, wildlife plays an important and complex role. The word "emerging disease" has received greater importance in the last 20 years in the popular press, owing to well-publicised disease outbreaks such as severe acute respiratory syndrome (SARS), Ebola haemorrhagic fever, Monkeypox, West Nile fever, Nipah, and Hendra viral encephalitis. These events have augmented global attention to the association of wildlife in emerging diseases (Travis et al. 2011). Over the past few decades, roughly 75% of emerging diseases including zoonoses had wildlife origin (Jones et al. 2008) and more than 70% of emerging or reemerging infectious agents are thought to have wildlife as their natural reservoirs (Taylor et al. 2001). Although wildlife has a crucial role in preserving the integrity of planet's ecosystem, it frequently embodies a significant risk of emerging zoonotic diseases (Daszak et al. 2000; Thompson et al. 2009). In significant ways, wildlife differs from other domestic animal species. They are elusive, usually have no owners or custodians, not always well recognised by zoologists and are often taken emotionally by the general public (Artois et al. 2011). Wildlife can act as a reservoir or maintenance or spill-over or amplifier hosts or simply a liaison host of diseases transmissible to human beings and farmed livestock. For example, in continental Europe, it was well-known that rabies had gone astray as a disease maintained by dogs but instead turned into disease spread by a red fox (*Vulpes vulpes*). Such situations arise where wildlife hosts are responsible for maintaining and spreading zoonotic diseases; thus, there is a rising concern in developing means to control the transmission of disease from wild animal population to humans or farm animals (Artois et al. 2011).

The majority of the recent emerging diseases were caused by zoonotic viruses of wildlife origin and had a significant influence on public health and economies (Murray et al. 2016). The part of wildlife species in diseases like severe acute respiratory syndrome (SARS), Influenza, Ebola haemorrhagic fever, Nipah viral encephalitis, and monkeypox is well-acknowledged. The emergence and rapid spread of such fatal diseases have been most important arousing public health episodes that accentuated the want for group effort between the veterinarian, wildlife professionals and public health specialists (Chomel et al. 2007). It has also increased the interests of the general public on diseases of wildlife origin, and as a result wild game managers, conservationists and government agencies have shown greater interest in surveillance and control of wildlife diseases (Gortazar et al. 2007). Pathogens of wildlife origin spill over into domestic animals, into humans and other wild animals. Zoonoses of wildlife origin have a negative bang on public health, wildlife conservation, and agricultural production (Chomel et al. 2007). It is now far and widely accepted that the complete purge of such shared pathogens is impractical if wildlife reservoirs are ignored (Gortázar et al. 2015). The viral families

Arenaviridae, Coronaviridae, Flaviviridae, Filoviridae, Hepeviridae, Hantaviridae, Herpesviridae, Nairoviridae, Orthomyxoviridae, Peribunyaviridae, Paramyxoviridae, *Phenuiviridae, Poxviridae, Reoviridae, Rhabdoviridae,* and *Togaviridae* contain viruses which represent most of the viral zoonoses of wildlife origin. The present chapter will focus on viral zoonoses involving wildlife hosts, their ecology and transmission modes, drivers of emergence, geographical distribution, and control strategies.

15.2 Spill-Over and "Spill-Back"

The spread of pathogens from domestic reservoir animals to the sympatric wild animal population, termed "spill-over", underlines the emergence of a variety of emerging infectious diseases from wildlife. Spill-over is a scrupulous threat to endangered fauna, as the existence of infected reservoir animals can reduce the infectious agent's threshold density and lead to the extinction of local populace (Daszak et al. 2000). African wild dog population *(Lycaon pictus*) has been waning since the 1960s and is now endangered and, with a patchy population of <5000, is vulnerable to stochastic events like outbreaks of disease. In 1991, synchronised with canine distemper epizootic in domestic sympatric dogs, wild dogs have become extinct in Africa.

Similarly, rabies was responsible for mortality in wild dogs, and a common viral variant has been identified in wild and sympatric dogs. In Serengeti, the emergence of rabies in wild dogs was due to the spatial expansion of human dwellings and resulting infringement of rabid domestic dogs. Spill-over outbreaks embody a stern threat to wild fauna and through "spill-back" (reverse spill-over) to the sympatric domestic animal population. Brucellosis was possibly ingrained into America through cattle. The occurrence of brucellosis in elk and bison in Yellowstone National Park (USA) is considered a probable threat to cattle grazing at the boundaries of the park. Other instances of spill-over events include bovine tuberculosis (global), sarcoptic mange in wombats (Australia) and foxes (Europe). Bovine tuberculosis also frightens to spill back to domestic livestock and eventually, to humans (Daszak et al. 2000).

15.3 Wild/Migratory Birds, Exotic Pets, Bats, and Rodents: As Reservoirs of Zoonotic Viruses

Emerging zoonotic agents have originated from numerous wildlife species like ungulates, carnivores, birds, non-human primates, bats, and rodents (Fig. 15.1) (Singh and Gajadhar 2014). Many zoonoses were originated from wildlife, and the list is expanding over time, but the relative significance and mechanism driving the

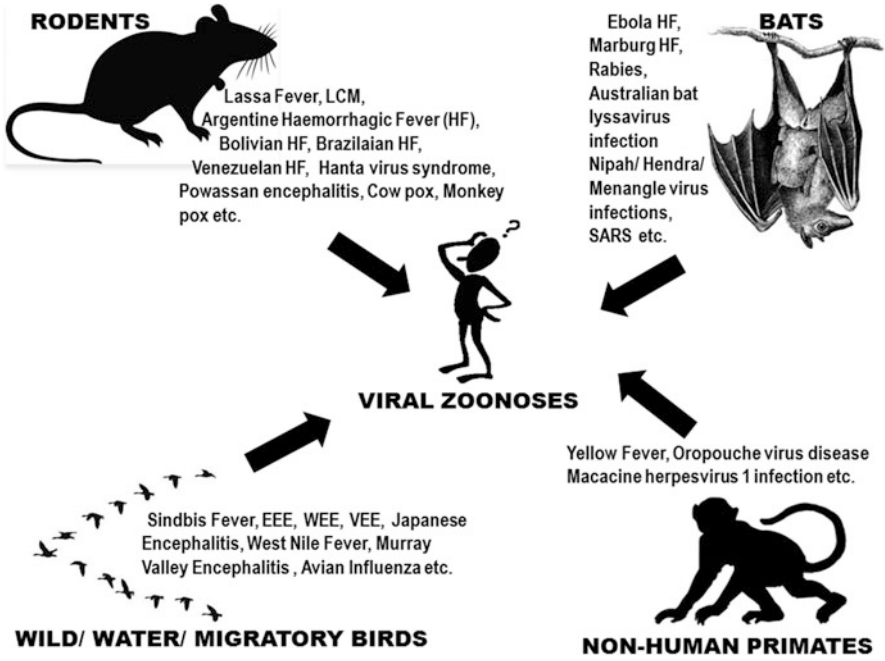

Fig. 15.1 Major wildlife reservoir group of viral zoonotic diseases

differences of various set of wild hosts in disease emergence remain unclear. With more than 1 billion cases of human zoonoses occurring each year, identifying wildlife reservoirs of the disease remains a perennial priority of public health (Han et al. 2015). Therefore identifying which species are most expected to play reservoir role of upcoming zoonotic infections and in which provinces/regions new outbreaks are expected to occur are an essential move towards a pre-emptive method to minimising zoonotic illness risk in humans.

Birds have a vital role in the transmission and spread of numerous emerging zoonotic pathogens. The West Nile virus emergence in the USA is a prominent example of how rapidly a novel zoonotic disease can become extensively dispersed. Wild birds are well-acknowledged to be reservoirs for many emerging zoonotic diseases, such as WNV, influenza A virus, Western equine encephalitis, St. Louis encephalitis, etc. Besides, wild/migratory birds are also infested with arthropod species (vector), which can disseminate zoonotic pathogens along their routes of migration, even if that particular bird species is not a capable reservoir of disease. Furthermore, avian hosts migrating over intercontinental and national borders can act as long-range competent vectors for any zoonotic pathogen. This establishes new endemic disease foci along the routes of migration (Reed et al. 2003).

Several latest epidemics have been connected with exotic pets or wildlife hosts including Ebola, SARS, and Monkeypox. For instance, the monkeypox outbreak in the USA in 2003started after importing African rodents, which infected prairie dogs

in pet shops. Many imported species of African rodents were revealed positive for the monkeypox virus related to the outbreak (Souza 2011). From 1991 to 1998, eight cases of rabies due to the new variant of rabies virus were reported in Brazil. Marmosets (*Callithrix jacchus jacchus*) reared as pets were found to be a source of transmission. In a pet care shop in France, encephalitis was detected in an Egyptian rousette bat which was recently imported from Belgium. The pet bat was found to be carrying Lagos bat lyssavirus which leads to treatment of around 120 exposed individuals (Chomel et al. 2007).

Bats (Order *Chiroptera*) offer substantial ecosystem services, like arthropod control, pollination, and seed dispersal, over a broad range of habitats. On the other hand, bats are gaining more attention as prospective reservoirs for many emerging zoonotic diseases after the recent recognition of their association with Ebola and Marburg viruses, SARS coronavirus, and Nipah and Hendra viruses. Subsequently, there has been frequent speculation that they may be exclusive in their potential to serve as a host for viruses of zoonotic nature (Calisher et al. 2006). Generally, traits of bats that may make them suitable to harbour more viruses include moderately long life spans, which helps viral persistence; flight, letting movement and spreading over long distances and extended torpor, which can diminish both immune function and viral replication (Munshi-South and Wilkinson 2010). Also, the gregarious nature of some bat species like Mexican free-tailed bat allows them to live in dense aggregations (3000 per m^2). Roosting spots can even house a diverse group of several species of bats (Luis et al. 2013). These high inter- and intraspecific contacts can favour speedy pathogen transmission, and hefty population volumes could maintain acute-immunising infections.

Furthermore, in evolutionary terms, they are ancient mammals, so it has been assumed that zoonotic viruses which evolved in them may use extremely conserved cellular receptors, hence enhancing the bat's capability to pass on viruses to other mammal species (Calisher et al. 2006). Numerous species of bats have peri-domestic behaviours, roosting in human dwellings, houses, and trees in cities, leading to repeated human contact with their excreta. In recent decades, bat–human contact is escalating due to habitat encroachment and the exploitation of bats as bushmeat (Mickleburgh et al. 2009; Luis et al. 2013).

Rodents are the diversified and superabundant living mammals on the earth. From the middle ages, it is well-known that rodents can transmit human diseases, as black rats were involved in the distribution of plague. Even today, rodents possess a significant threat to human health. Diseases distributed by rodents take two different ways. The first is a direct way, wherein rodents spread disease-causing agents to humans by biting or contaminating the food and water with their faeces or urine or through inhalation route (Hantaviruses). The second is an indirect way, wherein rodents serve as an amplifier host and transmit the pathogen to human through arthropod vectors like ticks, fleas, and mites (Crimean-Congo Haemorrhagic fever). Rodents can help to sustain the infectious agent's transmission cycles in diverse environments, varying from rural to densely populated urban areas and in the wilderness (Meerburg et al. 2009). Some rodent-borne zoonotic viral diseases are Hantavirus infections, Crimean-Congo haemorrhagic fever, Kyasanur Forest

Disease, Omsk haemorrhagic fever, Tick-borne encephalitis, Lymphocytic choriomeningitis, Lassa fever, Cowpox, etc.

15.4 Drivers of Zoonotic Disease Emergence from Wildlife

Zoonotic disease emergence is a multi-factorial event. The factors may be changes in, among others, genetics of microbes, vector distribution, human behaviour, trading, and farming practices. It is also imperative to make out that various drivers play distinctive functions in the emergence of a range of viruses, even it can be for viruses of the same family (Wang and Crameri 2014). In a joint consultation meeting of WHO/FAO/OIE held in 2004, it was ended with a conclusion that anthropogenic factors like agricultural expansion, habitat destruction, urbanisation, trade of exotic or domestic animals, and global travel comprise major drivers of zoonotic disease emergence (WHO/FAO/OIE 2004). Most of these anthropogenic factors bear negative implications for wildlife, and subsequently for human health. Nipah virus emergence demonstrated the interplay between various ecological risk factors like intensive animal agriculture, habitat destruction, and animal transport to longer distances (Greger 2007).

Agricultural drivers include intensification of farming, habitat clearing for grazing and cropping, modernisation, and newer agricultural practices. These major changes have multiple effects including pushing different wildlife species together and commingling domestic livestock and wildlife, thus facilitating spill-over and spill-back events including the transfer of novel pathogens into naive and susceptible hosts (Wang and Crameri 2014). In 1957, in India, a new flaviviral disease named after Kyasanur forest occurred owing to clearance of woods which was in turn used for grazing of cattle. Cattle are the most important host for the tick (*Haemaphysalis spinigera*) that passed the virus out from its small mammal reservoir and simian hosts. This disease now causes thousands of human cases in India each year (Greger 2007; Singh and Gajadhar 2014).

Hardwood trees cut in south-western Wisconsin, USA, form basal tree holes which collect water and increase the numbers of breeding sites for *Aedes triseriatus*, the natural mosquito vector of La Crosse virus. The reservoir of the virus is small forest mammals. The virus is also transmitted to humans, causing encephalitis, principally in pre-school age children (Williams et al. 2002).

Natural climate change is another important driver of zoonotic disease emergence from wildlife. Climate changes enhance host abundance and transmission of pathogens as in the case of the emergence of Sin Nombre Hantavirus in the USA. Higher rainfall resulted in increased grass setting and spreading out of rodent population (*Peromyscus* spp.) that are key reservoirs of the Hantavirus. Consequently, human contact with the excretions of these mice increased, which resulted in the manifestation of Hantavirus pulmonary syndrome (HPS) in humans. Even though the virus was certainly endemic in rodents for centuries, causing intermittent cases of HPS in human, until 1993, the aetiology was not discovered (Schmaljohn and Hjelle 1997).

Another important risk factor associated with zoonotic diseases emergence from wildlife is the significant increase in bushmeat consumption in several parts of the globe (Chomel et al. 2007). Although hunting of wild species for food has been in practice for millennia, a marked increase has been observed over the past few decades, and this tendency is likely to endure as one of the paramount threats to biodiversity. Bushmeat is consumed to the tune of 1–3.4 million tonnes annually in Central Africa alone (Brown 2004). The commercial trade of bushmeat in Asia, mainly in Guangdong province of China has led to the SARS epidemic (Donnelly et al. 2003) and the emergence of the H5N1 subtype of influenza virus (Chen et al. 2004). Such bushmeat consumption and illegal hunting may expose people to new or previously unknown pathogens. Increased reliance on wildlife to meet dietary protein might have increased due to land-use change, deforestation activities, and food insecurity in various parts of the world, predominantly in tropical developing countries. Change in climate is also likely to affect food security in various parts, further encouraging greater reliance on bushmeat. This is set in contradiction of increasing air travel around the globe, which already poses an important risk to public health globally employing the transportation of infectious agents (Murray et al. 2016).

Another important driver of disease emergence from wildlife is the trade of wildlife and wildlife products. Recently, globalisation has caused an unprecedented amount of such trades across the globe, both legally and illegally in the form of exotic pets, medicines, crafts, trophies, bushmeat, etc. Such trade represents a considerable risk for the public, domestic animals, and wildlife health globally (Travis et al. 2011). International legal wildlife trade is roughly US$159 billion annually (Brown 2004). Given the covert nature and large size of the business, no estimate of the volume of wildlife trafficked throughout the globe is present. The USA is involved in the maximum consumption of wildlife and wildlife products with the legal importation of live animals to the tune of 1.5 billion between 2000 and 2006 and closely 90% of which were meant for the pet industry. And as far as non-live wildlife is concerned, an average of 25 million kilograms enters the USA annually. The Monkeypox outbreak displayed that a single consignment of infected pet animals can end up in a serious impact on human health, underlining the challenges encountered by agencies trying to regulate or control the legal and illegal business of wildlife (Smith et al. 2012).

Zoological collections are also places where pathogens could spread from one species to others, to initiate a new disease. An African rodent species born and raised in an Asian zoo could be found in a South American Asian zoological collection housed adjacent to Arctic mammals from North America. Therefore the number of permutations of novel organismal biomes for pathogens or commensals to explore has increased exponentially (Brown 2004).

15.5 *Arenaviridae* Zoonotic Infections

Arenaviruses have been categorised based on their antigenic traits into two sero-complex groups, the Tacaribe group and Lassa-Lymphocytic choriomeningitis group. This has been further classified into four evolutionary lineages. All arenaviruses are more or less strongly associated with a specific mammalian host. The host distribution decides the distribution of each arenavirus (Salvato et al. 2005). The diversity of the viruses is mostly due to the long-time shared evolutionary association (co-speciation or -evolution) between the *Muridae* family of rodents and viruses of the *Arenaviridae* family (Bowen et al. 1997). In nature, the long-time persistence of arenaviruses depends on the chronic infection of the rodent host along with chronic viraemia. Out of 23 species in *Arenaviridae* family, five arenaviruses are established to cause a terrible haemorrhagic fever with a 20% case fatality rate. They are Lassa, Machupo, Junin, Guanarito, Sabia distributed in western Africa, Bolivia, Argentina, Venezuela, and Brazil, respectively (Table 15.1) (Delgado et al. 2008; Briese et al. 2009; Charrel and de Lamballerie 2010). Any manipulation of these viruses has to be done in BSL 4 facilities as they are incorporated in Category A list of pathogens designated by CDC. Lymphocytic choriomeningitis virus (LCMV) (Table 15.1) can cause congenital malformations and central nervous system infection; it has also recently been identified as a significant cause of grave infection in immunocompromised patients and organ transplantation recipients (Emonet et al. 2007; Charrel and de Lamballerie 2010). The natural hosts of the arenaviruses are rodents. Old World arenaviruses like Lassa fever virus, LCMV are allied with rodents of subfamily *Murinae* in family *Muridae*. While New World arenaviruses are related to new world rodents in the subfamily *Sigmodontinae* of family *Muridae* (Wilson and Reeder 2005). Human beings may contract arenaviruses through bites or any means of direct contact with virus-infected rodents or via inhalation of infected rodent secreta or excreta. Hence, one of the chief determinants of human infection is probably the dynamics of rodent populations. The major contributing factor aiding virus transmission to human from rodent is the peri-domestic and domestic behaviour of these rodent reservoir hosts.

Nevertheless, in majority cases, arenavirus transmission occurs after agricultural or recreational incursions into environments giving critical habitat for reservoir rodent hosts. Besides, professionals working with infected rodents in laboratory and field are at greater risk (Sewell 1995). Generally, natural ecological changes and anthropogenic modifications of the environment have been incriminated in the arenaviruses infection emergence in humans due to changes in the behaviour of the rodent population (Charrel and de Lamballerie 2010).

Table 15.1 List of *Arenaviridae*, *Peribunyaviridae*, *Phenuiviridae*, *Nairoviridae,* and *Hantaviridae* zoonotic diseases involving wildlife species

S. no	Viral zoonotic diseases	Virus aetiology genus, family (virus)	Wildlife reservoirs/ amplifiers/natural hosts/spill-over hosts	Major transmission route to humans	Geographical distribution	Human disease	BSL level	References
1	Argentine hemorrhagic fever	*Mammarenavirus*, *Arenaviridae* (Junin virus)	Reservoir—dryland vesper mouse (*Calomys musculinus*)	Direct contact with infected rodents/inhalation of rodent excreta	Argentina	Severe hemorrhagic fever	4	Charrel and de Lamballerie (2010)
2	Bolivian hemorrhagic fever	*Mammarenavirus, Arenaviridae* (Machupo virus)	Reservoir—large vesper mouse (*Calomys callosus*)	Direct contact with infected rodents/inhalation of rodent excreta; person-to-person	Bolivia	Severe hemorrhagic fever	4	Charrel and de Lamballerie (2010)
3	Brazilian hemorrhagic fever	*Mammarenavirus, Arenaviridae* (Sabia virus)	Rodents	Direct contact with infected rodents/inhalation of rodent excreta	Brazil	Severe hemorrhagic fever	4	Charrel and de Lamballerie (2010)
4	Lassa fever	*Mammarenavirus*, *Arenaviridae* (Lassa virus)	Reservoir—natal multimammate mouse (*Mastomys natalensis*)	Direct contact with infected rodents/inhalation of rodent excreta	West Africa	Severe hemorrhagic fever	4	Charrel and de Lamballerie 2010
5	Lymphocytic Choriomeningitis	*Mammarenavirus, Arenaviridae* (LCM virus)	Reservoir—house mouses (*Mus musculus, M. domesticus*)	Direct contact with infected rodents/inhalation of rodent excreta	Worldwide	Acute central nervous system disease and congenital malformations	2/3	Charrel and de Lamballerie (2010)

(continued)

Table 15.1 (continued)

S. no	Viral zoonotic diseases	Virus aetiology genus, family (virus)	Wildlife reservoirs/ amplifiers/natural hosts/spill-over hosts	Major transmission route to humans	Geographical distribution	Human disease	BSL level	References
6	Venezuelan hemorrhagic fever	*Mammarenavirus, Arenaviridae* (Guanarito virus)	Reservoir—common cane mouse *Zygodontomys brevicauda*, Alston's cotton rat (*Sigmodon alstoni*)	Direct contact with infected rodents/inhalation of rodent excreta	Venezuela	Severe hemorrhagic fever	4	Charrel and de Lamballerie (2010)
7	La Crosse encephalitis	*Orthobunyavirus, Peribunyaviridae*	Amplifying hosts—Chipmunks, squirrels	Mosquito bite (*Aedes*)	USA	Febrile encephalitis with seizures	2	Harding et al. (2018) and Maes et al. (2019)
8	Oropouche virus disease	*Orthobunyavirus, Peribunyaviridae*	Reservoir—pale-throated sloths (*Bradypus tridactylus*), non-human primates, rodents and some wild birds	Mosquito bite (*Culicoides paraensis, Culex)*	Brazil, Panama, Peru, and Trinidad and Tobago	Self-limiting dengue-like, acute febrile illness with arthralgia	3	Sakkas et al. (2018) and Maes et al. (2019)
9	Rift valley fever	*Phlebovirus, Phenuiviridae*	Wild spill-over hosts—African buffaloes, Warthog, black rhino, zebra, Thompson's gazelle, lesser kudu, impala waterbuck, lions, cheetahs, African wild dogs and jackals	Mosquito bite (*Aedes, Culex*)	Eastern and Southern Africa, Saudi Arabia	Self-limiting dengue-like, acute febrile illness with arthralgia	3	Bird et al. (2009) and Maes et al. (2019)

10	Crimean-Congo haemorrhagic fever	*Orthonairovirus*, *Nairoviridae*	Natural hosts hedgehogs, hares and ground-feeding birds	Tick bite and person-to-person	Western areas of the former Soviet Union; Southeastern and Southwestern Europe; eastern; central Asia; the Middle East and Turkey; and Africa	Mild and nonspecific febrile illness to severe hemorrhagic disease	4	Bente et al. (2013) and Maes et al. (2019)
11	Diseases caused by hantaviruses	*Orthohantavirus, Hantaviridae*	Reservoir hosts—rodents, shrews, moles, and bats	Inhalation, ingestion and transcutaneous	Worldwide	Hemorrhagic fever with renal syndrome (HFRS), Nephropathia epidemica (NE) and Hantavirus cardiopulmonary syndrome (HCPS)	3	Jiang et al. (2017)

15.6 *Peribunyaviridae* Zoonotic Infections

La Crosse Encephalitis and Oropouche Virus Disease are the two important zoonotic viral infections associated with wildlife belonging to *Peribunyaviridae* (Maes et al. 2019) (Table 15.1). La Crosse Encephalitis caused by La Crosse (LAC) virus was initially isolated in La Crosse, the USA in 1964 from the brain of a young girl diagnosed with encephalitis. In North America, LAC encephalitis is the second most frequently described mosquito-borne disease next to West Nile viral encephalitis. According to a CDC report, an inconsistent number of 30–130 human severe clinical cases has been reported in the USA annually with majority victims being children under the age of 16 (Harding et al. 2018). Unlike Yellow fever and dengue fever, LAC encephalitis infections are generally contracted in or near the wilderness. Suggested reservoir or amplifying hosts are the eastern grey squirrel and the eastern chipmunk. These animals drink from the tree holes, wherein they transmit the virus to the tree hole mosquito, *Aedes triseriatus,* which is a vector mosquito for this disease (Sutherland 2008; Harding et al. 2018). The important risk factor for LAC infection is the proximity to artificial or natural breeding sites. Humans are generally dead-end or incidental hosts and occasionally acquire an adequate dose of LAC virus from mosquito bites to build up an infection (Bewick et al. 2016; Harding et al. 2018).

Oropouche fever, similar to dengue fever is an acute febrile disease caused by Oropouche virus (OROV). OROV was initially isolated from the forest conservation worker in Trinidad. This disease is currently endemic, causing sporadic outbreaks and cases in some parts of Central and South America. OROV is an arbovirus transmitted to humans mainly by the *Culicoides paraensis* (biting midge). This virus is maintained in nature by an urban and sylvatic cycle which may comprise quite a few different vector species. In the urban cycle, the primary vector is *C. paraensis*, which has been associated with larger epidemics (Mourao et al. 2015; Sakkas et al. 2018). Wild mammals and birds are the natural reservoir hosts in the sylvatic cycle. OROV antibodies have been found in non-human primates such as black and gold howler monkeys, capuchin monkeys, black-tufted marmosets, pale-throated three-toed sloths, rodents (*Proechimys* spp.), and birds (*Thraupidae*, *Fringillidae, Columbidae*). These wild species may have some role in the transmission of OROV. Humans are most likely the link host between the two cycles of transmission because OROV is typically invading urban localities through a viraemic person who visits the forest and gets back to the urban residential area during viraemia (Cardoso et al. 2015; da Rosa et al. 2017). It is now well acknowledged that OROV is circulating in wildlife and humans at very low levels, and whenever a deviation in the natural environment (deforestation/loss of vegetation and habitat) and/or in the general population (immigration of animal and/or human) occurs, or pouches fever outbreaks are emerging (Sakkas et al. 2018).

15.7 *Phenuiviridae* Zoonotic Infections

Rift Valley Fever (RVF) is an important zoonotic viral infection caused by *Phlebovirus* of *Phenuiviridae* family (Maes et al. 2019) (Table 15.1). RVF is a mosquito-transmitted emerging zoonotic disease of animals and human beings in Africa and the Middle East region that is directly related to high rainfall conditions. This virus was first discovered from aborted sheep in 1930 in Kenya (Linthicum et al. 2016). A change from enzootic to epizootic RVF virus activity characteristically occurs following extended episodes of exceptionally plenteous rainfall and consequent inundation of dambos, which facilitates the emergence of abundant *Aedes* mosquitoes. These infected mosquitoes feed on livestock (e.g., cattle and sheep) that rapidly build up clinical disease and high-titre viraemias and in sequence; the infected animals infect bridge mosquitoes such as *Anopheline* or *Culex* spp. Humans develop disease following an infected mosquito bite or exposure to aerosols or from handling aborted materials or transcutaneous injury during necropsy or slaughtering of viraemic animals (Bird et al. 2009; Linthicum et al. 2016). The ungulate livestock, especially sheep, goats, and cattle, assume a central role in RVF epidemics and epizootics. The role of wildlife species in the maintenance of RVF virus during inter-epizootic times or as amplifier hosts has been well-studied since the discovery of the virus. Serological evidence from South Africa suggests wild rodents may play some role in the virus maintenance. A high prevalence of antibody was found in many species of wild animals, including giraffe, African buffalo, black rhino, common warthog, Thompson's gazelle, zebra, impala waterbuck, lions, African wild dogs, jackals, cheetahs, and lesser kudu during and immediately after the 2006–2007 East African epizootic (Bird et al. 2009; Linthicum et al. 2016).

15.8 *Nairoviridae* Zoonotic Infections

Crimean-Congo Haemorrhagic Fever (CCHF) is the important zoonotic viral infection caused by *Orthonairovirus* of *Nairoviridae* family (Maes et al. 2019) (Table 15.1). CCHF is the tick-borne viral zoonotic disease, causing outbreaks or sporadic human cases across a vast geographical area, from China to the Middle East and Europe (south-eastern) and many parts of Africa. It was first described in the Crimea region of the post-Soviet states in 1944 and the Congo (present DR Congo) in 1956 (Spengler et al. 2019). The CCHF virus in nature is maintained in a tick–vertebrate–tick endemic cycle, wherein the ixodid ticks serve as both vector and true reservoir of the virus as they remain infected throughout their lifetime unlike transient viraemia in mammals. *Hyalomma* ticks are the primary source of human illness, most likely because both adult and immature forms vigorously look for hosts for blood meal during every stage of maturation specifically during spring and summer. The broad distribution of *Hyalomma* ticks reveals their tolerance of varied environments, including steppe, savannah, and small forest areas, and the capability

of their vigorously questing larvae and nymphs to feed on a range of hosts, including hedgehogs hares and ground-feeding birds, whereas the adults aggressively seek out sheep, cattle, and other large ruminants (Bente et al. 2013; Spengler et al. 2019). Human beings most frequently agricultural workers, slaughterhouse workers, and medical personnel contract the infection through an infected tick bite, contact with infected blood/tissues of animals, and contact with secretions of infected patients, respectively. Climate change is frequently considered as a major factor for the virus spreading out, but evidence proposes that other factors such as agricultural abandonment, landscape fragmentation, and proliferation of wildlife hosts are also instrumental in disease emergence and outbreaks (Spengler et al. 2019).

15.9 *Hantaviridae* Zoonotic Infections

Hantaviruses (Table 15.1) of the *Hantaviridae* family are considered as emerging viruses with a rising number of clinical cases of humans worldwide. The earliest pathogenic Hantavirus was isolated in 1976, by the side of the Hantan River, in South Korea and was named as Hantaan virus. These viruses have a worldwide distribution and are major zoonotic pathogens causing severe infection in humans. More than 50 strains of Hantaviruses have been identified so far, and 24 of them have pathogenic bearing to humans (Jiang et al. 2017). The latest data states that, globally, more than 20,000 clinical cases of Hantaviruses have been estimated to occur every year, with most of the cases reported in Asia (Jiang et al. 2017). Naturally, Hantaviruses are maintained in asymptomatic specific reservoir hosts. Rodents, moles, shrews, and bats are the regular reservoir hosts of Hantaviruses. *Rattus norvegicus* and *Apodemus agrarius*, which are host species for the *Hantaan virus* and *Seoul virus*, are the principal reservoirs in the residential area and wild, respectively (Zhang et al. 2014). Though chronic and persistent infections are well established along with high-titre neutralising antibodies, these reservoirs stay as asymptomatic infected hosts (Yu and Tesh 2014). Like arenaviruses, each Hantavirus is connected with a specific rodent host, and spill-over to other species of rodents seems to provoke specific antibody production and virus clearance (Spengler et al. 2013). In general, Hantaviruses coevolve with their specific hosts (Vaheri et al. 2013). Recently a Hantavirus (Xuan Son virus) has also been found in bats in Vietnam (Arai et al. 2013). Two acute diseases are caused by hantaviruses in humans, haemorrhagic fever with renal syndrome (HFRS), and Hantavirus cardiopulmonary syndrome (HCPS). HFRS primarily came to the notice of Western physicians between 1951 and 1954, when 3200 United Nations soldiers fell ill in Korea. In Europe, more than 3000 HFRS cases occur annually (Zhang et al. 2014). HFRS outbreaks are caused by Hantaan, Dobrava, Seoul, and Puumala viruses which are prevalent mainly in Asia and Europe and are called as Old World Hantaviruses (Jiang et al. 2017). Nephropathia epidemica (NE), which is a mild type of HFRS characterised by acute kidney damage, and thrombocytopenia were first identified in Sweden (Krautkramer et al. 2013). Depending on the season, HFRS

outbreaks can vary, with most cases recorded in the winter to the early spring season in epidemic areas. Farmers are most commonly affected, especially in China (Zhang et al. 2014; Jiang et al. 2017). Lately, endemic zones have expanded beyond rural areas forming new foci of infection. Factors thought to be associated with such expansion of endemic trend are due to climate change, urbanisation, human migration, and rapid economic development (Zuo et al. 2011). HCPS, a previously unrecognised syndrome, was described first in 1993 in the USA. HCPS outbreaks are chiefly caused by Andes and Sin Nombre viruses, which are widespread in North and South America and are called as New World Hantaviruses. In contrast to HFRS, most cases of HCPS occur during early summer and late spring months (Jiang et al. 2017). The expanding geographical distribution of Hantaviruses and the variation between the "New World" and "Old World" viruses are slowly becoming less apparent.

15.10 *Togaviridae* Zoonotic Infections

Alphaviruses of *Togaviridae* family enclose zoonotic viruses which are generally transmitted by mosquitoes (Table 15.2). Alphaviruses are usually referred to as New World' and "Old World" viruses with "New World" viruses (which include Venezuelan, Eastern and Western Equine Encephalitis viruses) principally related with the serious encephalitic disease in the Americas. Old World viruses are associated with rheumatic or arthritogenic diseases in humans.

The arthritogenic alphaviruses encompass Chikungunya virus (CHIKV), the Sindbis group of viruses, Ross River virus (RRV), Barmah Forest virus (BFV), and Mayaro virus. These viruses are responsible for endemic diseases and rarely, large epidemics; for example, chikungunya epidemic in 2004–2011 resulted in 1.4–6.5 million morbidities in almost 40 countries (Suhrbier et al. 2012). Symptoms in adults due to alphaviruses infection are always associated with rheumatic ailments, principally polyarthritis and/or polyarthralgia, which can be debilitating and chronic. CHIKV was primarily isolated in Tanzania in 1952. Following the isolation, regular epidemics have been witnessed in Africa and Asia, with former outbreaks baffled with dengue fever. The largest Chikungunya epidemic was linked with the emergence of viruses that were transmitted by *Aedes albopictus* (Ng and Hapuarachchi 2010; Burt et al. 2012; Suhrbier et al. 2012). RRV and BFV were isolated in 1959 and 1974 from mosquitoes trapped in the Ross River in Queensland and Barmah Forest, Victoria, in Australia, respectively. These viruses are enzootic and endemic in Australia with RRV also identified in Papua New Guinea. Most cases occur in Northern Australia from December to February, when vector mosquitoes are at their peak. BFV and RRV infections are notifiable to Australian public health authorities (Harley et al. 2001; Jacups et al. 2008; Suhrbier et al. 2012). Sindbis virus was isolated for the first time in 1952 from mosquitoes in Egypt. Sindbis viral diseases are endemic with and restricted to Northern Europe with cases in early autumn or late summer. Sporadic cases are also reported in South Africa,

Table 15.2 List of *Togaviridae* and *Filoviridae* zoonotic diseases involving wildlife species

S. no	Viral zoonotic diseases	Virus aetiology genus, family (virus)	Wildlife reservoirs/ amplifiers/natural hosts/spill-over hosts	Major transmission route to humans	Geographical distribution	Human disease	BSL level	References
1	Chikungunya virus disease	*Alphavirus, Togaviridae*	Sylvatic natural hosts—non-human primates, bats and monkeys	Mosquito bite (*Aedes*)	South and South-east Asia, Africa, Indian Ocean Islands	Dengue-like, acute febrile illness with acute and persistent polyarthralgia	3	Burt et al. (2012)
2	Ross river fever	*Alphavirus, Togaviridae*	Reservoir—agile wallaby, and dusky rat	Mosquito bite (*Culex annulirostris, Aedes vigilax*)	Australian and South and Western Pacific regions	Febrile arthritogenic illness	2	Jacups et al. (2008)
3	Barmah forest virus	*Alphavirus, Togaviridae*	Reservoir—brushtail possums	Mosquito bite (*Culex, Aedes*)	Australia	Febrile arthritogenic illness	2	Jacups et al. (2008)
4	Sindbis fever	*Alphavirus, Togaviridae*	Amplifying hosts—migratory and water birds	Mosquito bite (*Culex, Culiseta, Aedes*)	northern Europe, South Africa, Australia	Febrile arthritogenic illness	2	Adouchief et al. (2016)
5	Mayaro fever	*Alphavirus, Togaviridae*	Wild vertebrate hosts—non-human primates, birds, marsupials and rodents	Vector transmitted—*Haemagogus*, *Culex* spp., *Aedes* spp., *Psorophora* spp., *Coquillettidia* spp.	South and Central America	Febrile arthritogenic illness	3	de Oliveira Mota et al. (2015)
6	Eastern equine encephalitis	*Alphavirus, Togaviridae*	Enzootic cycle—wild birds and mosquitoes	Mosquito bite (*Culiseta melanura*)	North and South America	Febrile disease with encephalomyelitis	3	Armstrong and Andreadis (2013)
7	Western equine encephalitis	*Alphavirus, Togaviridae*	Enzootic cycle—passerine birds (reservoir) and mosquitoes	Mosquito bite (*Ochlerotatus melanimon*, *Aedes dorsalis*, and *Ae. campestris*)	North America	Mild febrile disease with encephalomyelitis in few cases	3	Arechiga Ceballos and Aguilar Setien (2015)

8	Venezuelan equine encephalitis	*Alphavirus, Togaviridae*	Principal reservoir hosts epizootic cycles—bats, rodents, and some birds Enzootic cycles—sylvatic rodents in the genera *Sigmodon, Oryzomys, Zygodontomys, Heteromys, Peromyscus*, and *Proechimys*	Mosquito bite	Central and South America	Febrile illness; Children-fatal encephalitis and permanent neurological sequelae; pregnant women-birth defects, spontaneous abortions and stillbirths	3	Weaver et al. (2004)
9	Ebola disease	*Ebola virus, Filoviridae*	Reservoir—fruit bats	Person-to-person transmission/direct contact with non-human primates	Sub-Saharan Africa and Reston Ebola virus (REBOV), identified in the Philippines	Hemorrhagic fever and multiple organ failure	4	MacNeil and Rollin (2012)
10	Marburg virus disease	*Marburg virus, Filoviridae*	Reservoir—fruit bats	Person-to-person transmission/direct contact with non-human primates	sub-Saharan Africa	Hemorrhagic fever and multiple organ failure	4	MacNeil and Rollin (2012)

Australia, and China (Laine et al. 2004; Adouchief et al. 2016). Mayaro virus, initially isolated in 1954 is enzootic in the northern part of South America. This virus causes recurrent smaller outbreaks and sporadic cases in humans. Usually, human cases are associated with forest visits or human dwelling near the forest (de Oliveira Mota et al. 2015). All these rheumatic alphaviruses are maintained in the wilderness in the transmission cycle between mosquitoes and vertebrate hosts: non-human primates for CHIKV (Burt et al. 2012), macropods (wallabies and kangaroo) for BFV and RRV (Jacups et al. 2008), migratory and wild birds for Sindbis virus (Adouchief et al. 2016), birds, marsupials, rodents and primates for Mayaro virus (de Oliveira Mota et al. 2015). On various occasions, these reservoir hosts infect human via mosquito bite, however larger epidemics generally associated with consequent urban transmission cycles.

Alphaviral encephalomyelitis is caused by Western equine encephalomyelitis virus (WEEV), Eastern equine encephalomyelitis virus (EEEV), and Venezuelan equine encephalomyelitis virus (VEEV) (Table 15.1). All these equine alphaviruses cause a mosquito-transmitted infection that causes serious neurological disease and mortality in humans and horses in the Americas. Though related, these viruses are antigenically and genetically distinct. The first isolation of WEEV was from a horse brain in 1930 in California. WEEV is maintained in an enzootic cycle between vertebrate hosts–mosquito *(Culex tarsalis*)–passerine birds cycle. *Culex tarsalis* is associated with stream drainage and irrigated agriculture in the western United States. Bridging mosquito vectors, *Aedes dorsalis, Ochlerotatus melanimon,* and *Aedes campestris,* are implicated in the transmission of the virus to horses and humans in Utah, California, and New Mexico, respectively (Arechiga Ceballos and Aguilar Setien 2015). The first isolation of EEEV was from an infected horse brain in 1933 in New Jersey and Virginia. The EEEV is maintained in a primary transmission cycle between birds and mosquito vector, *Culex melanura.* Transmission to humans and horses is mediated by *Aedes* spp., *Culex* spp., and *Coquillettidia* spp. Transmission of virus usually occurs around the Gulf Coast of the USA and hardwood swamps in the Atlantic and the Great Lakes region (Armstrong and Andreadis 2013). During the 1930s, VEE was first identified as a disease of mules, donkeys, and horses in northern South America. In spite of wide vertebrate host range of VEE which includes humans, dogs, sheep, birds, bats, and rodents, major epidemics have not occurred in the nonexistence of equine cases. The principal reservoir hosts of VEEV are believed to be sylvatic rodent genera, *Heteromys, Oryzomys*, *Peromyscus*, *Sigmodon*, *Proechimys,* and *Zygodontomys* as they are regularly infected in nature, develop viraemia from moderate to high titre, and have high degrees of immunity (Weaver et al. 2004).

15.11 *Filoviridae* Zoonotic Infections

Under family *Filoviridae*, Ebola haemorrhagic fever and Marburg haemorrhagic fever are the two analogous diseases caused by two virus genera, Ebola virus (EBOV) and Marburg virus (MARV), respectively (Table 15.2). Despite the general rarity of their incidence, these diseases are well recognised due to the sensationalist accounts of its outbreaks. Nevertheless, EBOV and MARV are potentially pathogenic and have typically been connected with shattering outbreaks, with 25–90% case fatality rate range (Leroy et al. 2011; MacNeil and Rollin 2012). Besides, these viruses are recognised as potential bioweapons and as such are categorised as class A select agents. Present facts suggest fruit bats (*Pteropus* spp.) as the reservoir of both the viruses, and the dispersal appears to be restricted to sub-Saharan Africa (except Reston Ebola virus, spotted in the Philippines, and not documented to be related with human infection) (Taniguchi et al. 2011; MacNeil and Rollin 2012). Generally, zoonotic source of the exposure is not recognised always in outbreaks, but the introduction of these fatal viruses to have always been associated with hunting or processing bushmeat (EBOV) or persons entering mines and caves (MARV) (MacNeil and Rollin 2012). Outbreaks and clusters are principally the outcomes of person-to-person transmission. Three distinctive contact modes attribute for transmission of virus during outbreaks: (1) transmission between, close contacts, members of the family and caretakers of infected individuals; (2) direct contact with cadaver in preparation and funeral events; and (3) nosocomial transmission from infected persons to other patients or medical staff by reusing medical equipment or infringing barrier nursing (MacNeil and Rollin 2012; Cross et al. 2018).

15.12 *Flaviviridae* Zoonotic Infections

Zoonotic flaviviruses under family *Flaviviridae* are generally transmitted to humans by tick and mosquitoes. Despite being present in blood and body secretions during acute illness, flaviviruses do not get transmitted from person-to-person (contagious). Consequently, reservoirs of virus and abundance of vectors are prerequisites for epidemics. Zoonotic flaviviral diseases involving wildlife hosts can be grouped into mosquito- and tick-borne. Mosquito-borne flaviviral zoonotic diseases are Japanese encephalitis, St. Louis encephalitis Murray Valley encephalitis, Wesselsbron disease, West Nile fever, and Yellow fever. Tick-borne flaviviral zoonotic diseases are Kyasanur forest disease, Powassan encephalitis, Omsk haemorrhagic fever Tick-borne encephalitis, and Tyuleniy virus infection (Table 15.3).

Japanese encephalitis virus (JEV) causes neurological infection in humans all over Asia, affecting 70,000 people each year with nearly 10,000 fatalities. The JEV was first isolated in Japan at the beginning of the 1930s. JE is now endemic in eastern and southern Asia with more number of cases from China, Japan, India, Pakistan, and the Philippines (Erlanger et al. 2009). No cases have been reported from Europe,

Table 15.3 List of *Flaviviridae* zoonotic diseases involving wildlife species

S. no	Viral zoonotic diseases	Virus aetiology genus, family (virus)	Wildlife reservoirs/ amplifiers/natural hosts/ spill-over hosts	Major transmission route to humans	Geographical distribution	Human disease	BSL level	References
1	Japanese encephalitis	*Flavivirus, Flaviviridae*	Reservoirs—cattle egret, pond herons; other wild host species-flying foxes, snakes and frogs	Mosquito bite (*Cx. tritaeniorhynchus)*	Asia, Western Pacific countries, and Northern Australia	Febrile encephalitis and permanent neurologic or psychiatric sequelae	3	Mansfield et al. (2017)
2	Kyasanur forest disease	*Flavivirus, Flaviviridae*	Natural hosts—Blanford's rat, striped forest squirrel house shrew, ground birds; Amplifiers—black-faced langur and red-faced bonnet monkey	Nymph bite (*Haemaphysalis* spp.)	India	Haemorrhagic febrile illness and encephalitis	3	Mourya and Yadav (2016)
3	Murray valley encephalitis	*Flavivirus, Flaviviridae*	Reservoirs—water birds, such as herons and egrets	Mosquito bite (*Culex* species)	Endemic to Australia and New Guinea	Febrile encephalitis and neurological deficits	3	Floridis et al. (2018)
4	Omsk hemorrhagic fever	*Flavivirus, Flaviviridae*	Natural host—narrow-headed vole (*Microtus gregalis*); water vole (*Arvicola terrestris*) and musk rats	Tick bite (*Dermacentor*)	Western Siberia	Hemorrhagic fever	3	Dobler (2010)
5	Powassan encephalitis	*Flavivirus, Flaviviridae*	Reservoirs—woodchucks (*Marmota monax*) and white-footed mice (*Peromyscus leucopus*)	Tick bite (*Ixodes, Dermacentor*)	North America, Russian Far East	Febrile illness with neurological symptoms	3	Birge and Sonnesyn (2012)

6	Tick-borne encephalitis	*Flavivirus, Flaviviridae*	Primary reservoirs and hosts—small rodents	Hard tick bites (*Ixodes*); consuming infected milk or milk products	Europe and Asia	Febrile illness with CNS involvement (meningitis, meningoencephalitis)	3	Bogovic and Strle (2015)
7	St. Louis encephalitis	*Flavivirus, Flaviviridae*	Amplifying hosts—passerine and columbiform avian species	Mosquito bite (*Culex*)	USA, Panama, and South America	Mild febrile to severe neuroinvasive disease	3	Diaz et al. (2018)
8	Wesselsbron disease	*Flavivirus, Flaviviridae*	Cape short-eared gerbil and black rats (maintenance hosts?)	Mosquito bite (*Aedes*)	Africa	Acute, influenza-like illness with arthralgia and myalgia	3	Diagne et al. (2017)
9	West Nile fever	*Flavivirus, Flaviviridae*	Reservoir hosts—House sparrow (*Passer domesticus*), common grackles (*Quiscalus quiscula*)	Mosquito bite (*Culex* species)	Endemic in Africa, Europe, the Middle East, West and Central Asia, and most recently, North America and is spreading into Central and South America	Mild febrile to severe illness affecting the CNS (encephalitis or meningitis)	3	Chancey et al. (2015)
10	Yellow fever	*Flavivirus, Flaviviridae*	Reservoir hosts—Non-human primates	Mosquito bite (*Aedes* species)	Africa and South America	Acute haemorrhagic fever and some patients develop jaundice	3	Gardner and Ryman (2010)
11	Tyuleniy virus infection	*Flavivirus, Flaviviridae*	Natural cycle between ticks and sea birds	Tick (*Ixodes*) bite or direct contact with birds	Far Eastern Russia, France, USA	Febrile illness	2	Dobler (2010)

Africa, or the Americas. The virus is maintained in an enzootic transmission cycle between mosquitoes and wild birds, particularly large ardeid water birds such as cattle egret and pond herons. Domestic and wild pigs act as amplifier hosts. Irrigated rice fields offer a breeding ground for vector mosquitoes and also invite migratory wading birds facilitating virus maintenance in the sylvan cycle (van den Hurk et al. 2003; Miller et al. 2012; Jeffries and Walker 2015). *Culex* species of mosquitoes especially *Culex tritaeniorhynchus*, which is both ornithophilic and mammalophilic mosquito, helps in virus circulation between avian species and also acts as a bridge vector to infect livestock and humans (Guo et al. 2014; Mansfield et al. 2017). Other potential hosts in wildlife species are flying foxes, ducks, frogs, and snakes. However, these are taken as dead-end hosts as they seldom develop adequate viraemia to infect vector mosquitoes (Miller et al. 2012). JEV has not spread to Africa and Europe in spite of the presence of *Cx. tritaeniorhynchus* in these regions. This may be due to the absence of competent vectors in Europe or the non-migration of birds from tropical Asia to Africa or restricted movement of livestock from Asia to Europe (Mansfield et al. 2017).

Murray Valley encephalitis (MVE) is a mosquito-borne viral zoonotic disease endemic to Australia and New Guinea. It affects mostly children living in remote and rural areas and is potentially fatal. An enzootic cycle between *Culex annulirostris* mosquitoes and water birds maintains the virus. Apart from the primary vector, *C. annulirostris*, *Aedes normanensis* also supports the MVEV transmission to humans (Floridis et al. 2018).

St. Louis encephalitis (SLE) is again a mosquito-borne zoonotic viral disease endemic to the USA and some cases are occurring in a wide area ranging from Argentina to Canada. The virus is transmitted by several mosquito vectors in the genus *Culex*. Columbiform and passerine birds are the amplifying hosts. Most SLE cases are present with a flu-like illness and very few signs of progress to invasive encephalitis is unusual and is more common in older people (Ortiz-Martínez et al. 2017; Diaz et al. 2018).

Wesselsbron (WSL) disease is a zoonotic mosquito-borne flavivirus infection that causes teratogenic defects and abortions in sheep and cattle in Africa. These domestic animals ought to play a role in the viral life cycle, but some shreds of evidence suggest that wild animals may also be involved in virus maintenance in nature. This assumption is only supported by the isolation of the WSL virus from a black rat and Cape short-eared gerbil in Africa. The virus can also infect humans and produce dengue-like syndrome (Diagne et al. 2017).

West Nile virus (WNV) is a mosquito-transmitted virus which causes flu-like illness to fatal neuroinvasive diseases in humans. It was first described in Uganda in 1937 from a febrile case. WNV has caused sporadic outbreaks in Israel, India, Egypt, France, and South Africa. An enzootic cycle maintains the virus between birds and mosquitoes. Birds are reservoir hosts for the WNV as they can mount high viraemia to infect mosquitoes. American crows and blue jays become commonly ill or die; however, birds like common grackles and house sparrows build up high viraemia with lesser death rates. House finches and American robins are two important

amplifiers of WNV in the USA. Additionally, 30 other vertebrate hosts such as mammals, reptiles, and amphibians are susceptible to WNV infection.

Nevertheless, only a few vertebrates including brown lemurs, eastern grey squirrels, lake frogs, hamsters, eastern chipmunks, fox squirrels, and eastern cottontail rabbits have been described to mount viraemia expected to help vector transmission. Generally, humans and horses may endure serious infection or death, but they are considered only as incidental hosts as they do not mount sufficient level of viraemia to infect vector mosquitoes. Although mosquito bite transmission is common in humans, transmission by organ transplantation, blood transfusion, transplacental route, and via breast milk is also possible. *Culex* spp. of mosquitoes that feed on both birds and mammals are considered as bridge vectors as they pass on the virus from infected birds (reservoirs) to mammalian (incidental) hosts (Van der Meulen et al. 2005; Kilpatrick et al. 2006; Chancey et al. 2015).

Yellow fever (YF) is a mosquito-transmitted flaviviral disease endemic to tropical areas of Africa and the Americas. From Africa, it was introduced into the Americas and Europe as a consequence of the slave trade. YF virus primarily affects humans and non-human primates via mosquito bite and causes devastating epidemics of grave haemorrhagic disease. The transmission to humans occurs in sylvatic (humans who enter forests), intermediate (epidemics in rural villages), and urban cycles (urban mosquito species, *Aedes aegypti*). In recent decades, the intermediate transmission cycle causing small scale epidemics in rural villages is most common in Africa, wherein the infected semi-domestic mosquitoes species feed on both humans and monkeys (Gardner and Ryman 2010). As per the World Health Organization (WHO), an outbreak involving *Ae. aegypti* is referred to as urban YF, while outbreaks associated with other species of mosquitoes are categorised as jungle YF (Bres 1986). YF is differentiated from other viral haemorrhagic fevers by the distinctive severity of liver injury and jaundice (Monath 2008; Gardner and Ryman 2010).

Kyasanur Forest disease (KFD) is a zoonotic tick-transmitted viral disease endemic in southern India. KFD virus circulates between small mammals like shrews and rodents; ground birds and a range of tick species. The natural cycle of the virus involves two monkey species, red-faced bonnet monkey, and black-faced langur and a variety of tick species, predominantly ticks of *Haemaphysalis* spp. After getting infected, monkeys amplify and broadcast the virus to a large number of ticks feed on them. In humans and monkeys, the virus causes serious haemorrhagic disease and death (Mourya and Yadav 2016).

Omsk haemorrhagic fever (OHF) is a tick-transmitted zoonotic flaviviral disease endemic in Western Siberia. The virus is naturally maintained by two independent transmission cycles (grassland cycle and wetland cycle) which are connected by migration. In the grassland cycle, the vole *Microtus gregalis* is the main maintenance host developing high viraemia levels, which infects *Dermacentor reticulates* ticks, which is the important vector of OHF virus. In the wetland cycle, *Ixodes apronophorus* appears to play a vital role as a vector, and the other water vole, *Arvicola terrestris* which migrates from grassland to wetland develops high viraemia for a longer period and seems to be an important maintenance host. In this cycle,

muskrats seem to be another key vertebrate host. Apart from humans getting OHF infection from mosquito bites, direct contact with the infected muskrat's blood and bites from infected animals is other possible routes of transmission (Dobler 2010).

Tick-borne encephalitis (TBE) is another important flaviviral infection prevalent in Europe and some regions of Asia (northern China, Japan, Siberia, and Far Eastern Russia). Three subtypes of TBE virus, namely Far-Eastern, Siberian, and European, cause the disease. Adults are often infected than children. Small rodents are the primary reservoirs which maintain the virus in nature and humans are only accidental hosts. *Apodemus flavicollis* of *Muridae* family may play a key role in the European subtype viruses transmission. TBE virus is transmitted to human mostly by tick bites. The principal mosquito vector species in Europe and Japan are *Ixodes ricinus* and *Ixodes ovatus,* respectively. While in far-east Asia, Russia, and parts of Eastern Europe, it is *Ixodes persulcatus* (Dobler 2010; Bogovic and Strle 2015). In humans, around 1% of all TBE infections are most likely acquired by consuming contaminated unpasteurised milk and milk products from farm animals, particularly goats (Mansfield et al. 2009).

Powassan virus infection is a rare tick-transmitted flaviviral infection in North America and Russia. The common reservoirs are small and medium-sized mammals like white-footed mice, woodchucks and tick species like *Ixodes* and *Dermacentor* act as vectors (Birge and Sonnesyn 2012). Tyuleniy virus infection is other rare flaviviral zoonoses at the island of Tyuleniy in Far Eastern Russia. After its first isolation from sea birds in 1969, it was also isolated from the Atlantic coast of France, Norway, and the USA. Tyuleniy virus appears to be transmitted in an enzootic cycle involving seabirds and ticks. So far three human cases have been reported with fever, pharyngitis, nausea, joint pain, and petechial exanthema. All the three cases reported were ornithologists who had direct contact with sea birds and their ticks (Hubalek and Halouzka 1996; Dobler 2010).

15.13 *Reoviridae* Zoonotic Infections

Under family *Reoviridae*, Orungo fever and Colorado tick fever are the two important viral zoonotic diseases involving wildlife species caused by orbivirus and colitivirus, respectively (Table 15.4). Orungo virus was primarily isolated in 1959 in Uganda from the blood of a human infected with the virus. Orungo virus with its four distinct serotypes is transmitted by *Aedes*, *Anopheles,* and *Culex* mosquitoes. This virus is extensively dispersed in tropical Africa. It has been isolated from humans, cattle, camels, sheep, goats, and monkeys. Antibodies against the Orungo virus have been detected in primates, cattle, and sheep. Despite high prevalence in human, only a few clinical cases and three deaths reported in Uganda. High co-infection with yellow fever has been described, revealing their analogous geographical distribution and vector mosquito (*Aedes*) species (Attoui and Jaafar 2015).

Colorado tick fever or mountain fever is a tick-borne disease prevalent in North America, particularly in the Rocky Mountain region. This virus maintains in an

Table 15.4 List of *Reoviridae, Rhabdoviridae, Paramyxoviridae, Orthomyxoviridae, Coronaviridae, Hepeviridae, and Poxviridae* zoonotic diseases involving wildlife species

S. no	Viral zoonotic diseases	Virus aetiology genus, family (virus)	Wildlife reservoirs/ amplifiers/natural hosts/spill-over hosts	Major transmission route to humans	Geographical distribution	Human disease	BSL level	References
1	Orungo fever	*Orbivirus*, *Reoviridae*	Wild host—monkeys	Culicine mosquitoes	Uganda	Febrile illness	3	Attoui and Jaafar (2015)
2	Colorado tick fever	*Coltivirus, Reoviridae*	The major naturally infected host species include the golden-mantled ground squirrel, least chipmunk, Columbian ground squirrel, yellow pine chipmunk, porcupine, deer mouse, and busy tailed wood rat	Tick-to-human transmission (*Dermacentor andersoni*)	North America	Febrile illness with neurologic Symptoms in children	2	Romero and Simonsen (2008)
3	Rabies	*Lyssavirus, Rhabdoviridae*	Wild reservoirs/ vectors—vampire bats, raccoons, skunks, wild cats, foxes jackal, wolf, badger and mongoose	Bite/wounds or cuts/inhalation/ organ transplant	Except Antarctica and Australia, distribution of the disease cover all continents	Two forms of human disease-furious, or encephalitic and paralytic or dumb	3	Singh et al. (2017)
4	Australian bat lyssavirus infection	*Lyssavirus, Rhabdoviridae*	Bats are the natural reservoir	Direct contact/bite of infected bats	Australia	Serious illness which results in paralysis and convulsions	3	Singh et al. (2017)

(continued)

Table 15.4 (continued)

S. no	Viral zoonotic diseases	Virus aetiology genus, family (virus)	Wildlife reservoirs/ amplifiers/natural hosts/spill-over hosts	Major transmission route to humans	Geographical distribution	Human disease	BSL level	References
5	Nipah virus infection	*Henipahvirus, Paramyxoviridae*	Fruit bats (flying foxes) are the natural reservoir	Direct contact (bats/pigs), food-borne, person-to-person	Southeast Asia-India, Bangladesh, Malaysia, Singapore, Philippines	Asymptomatic to acute respiratory infection, and fatal encephalitis	4	Kulkarni et al. (2013)
6	Hendra virus infection	*Henipahvirus, Paramyxoviridae*	Fruit bats (flying foxes) are the natural reservoir	Direct contact with infected horses	Australia	Mild influenza-like illness to fatal respiratory or neurological disease	4	Field (2016)
7	Menangle virus infection	*Rubulavirus, Paramyxoviridae*	Fruit bats (flying foxes) are the natural reservoir	Direct contact with infected pigs	Australia	Severe influenza-like illnesses	3	Barr et al. (2012)
8	Influenza A virus infections	*Influenza virus A, Orthomyxoviridae* (H5N1, H7N9, H9N2, H1N1, H3N2, etc.)	AI-Natural reservoirs—wild and aquatic birds SI (H1N1)—wild boars, badgers, ferrets, skunks, cheetahs, giant anteater are some spill-over hosts	Direct contact with infected birds/animals; person-to-person	worldwide	Respiratory tract infections and multiple organ failure	3	Horman et al. (2018), Schrenzel et al. (2011), and Delogu et al. (2019)
9	SARS	*Betacoronavirus, Coronaviridae* (SARS coronavirus-SARS-CoV)	Reservoir—Bats Other incidental wild hosts—palm civets, raccoon dog	Human-to-human transmission	2003 outbreak-China, Hong Kong, Vietnam, Canada and several other countries	Severe acute respiratory syndrome	3	de Wit et al. (2016)

10	Hepatitis E virus infection	*Orthohepevirus, Hepeviridae* (Hepatitis E virus HEV3, HEV4)	Wild hosts—wild boar and deer	Consumption of contaminated meat from deer and wild boar	worldwide	Self-limiting acute hepatitis and fulminant hepatic failure in patients with chronic liver disease	3	Doceul et al. (2016)
11	Macacine herpesvirus 1 (B virus or Herpesvirus simiae, or Herpes virus B) Infection	*Simplexvirus, Herpesviridae*	Enzootic natural hosts—*Macaca* spp.–*M. mulatta, M. fascicularis*	Transcutaneously (via bites) or permucosally	Asia, USA	Influenza-like illness and encephalitis	4	Lee et al. (2015)
12	Cowpox	*Orthopoxvirus, Poxviridae*	Wild rodents are reservoir	Direct contact	UK, Europe, adjacent USSR	Papular-vesicular-pustular-ulcerative skin lesions and scar formation	2	Essbauer et al. (2010)
13	Monkeypox	*Orthopoxvirus, Poxviridae*	Reservoirs—rodents, including squirrels and giant pouched rats (monkeys are considered disease hosts)	Indirect (touch, bite, or scratch) or direct contact with live or dead animals and person-to-person	Endemic in Central and West Africa, cases reported in USA, UK, and Israel	Papular-vesicular-pustular (similar to that of smallpox) skin lesions	3	Peterson et al. (2019)
14	Tanapox	*Yatapox, Poxviridae*	Monkeys (natural host)	Direct contact, arthropod vectors	Endemic in East Africa, few case reports in USA, Europe	Febrile illness with nodules on extremities	2	Dhar et al. (2004)
15	Contagious ecthyma/orf	*Parapoxvirus, Poxviridae*	Wild small ruminants (spill-over host)	Direct contact, fomites, meat	Worldwide	Solitary or multiple papules that advance through a sequence of stages, terminates in complete resolution	2	Kuhl et al. (2003)

enzootic tick–mammalian host–tick cycle involving *Dermacentor andersoni* ticks (larval and nymphal stages). The major reservoir and vector for the disease is the wood tick, *D. andersoni.* The main naturally infected vertebrate hosts include the Columbian ground squirrel, golden-mantled ground squirrel, yellow pine chipmunk, least chipmunk, porcupine, deer mouse, and bushy-tailed woodrat. Virus transmission to humans through tick bite coincides with the activity of *D. andersoni* ticks, which is generally from late March to late October (Romero and Simonsen 2008).

15.14 *Rhabdoviridae* Zoonotic Infections

Rabies and Australian lyssavirus (ALV) infection are the two important viral zoonotic diseases involving wildlife species under genus *Lyssavirus* of *Rhabdoviridae* family (Table 15.4). Rabies is a potentially zoonotic and fatal disease caused by the rabies virus. All the warm-blooded animals, including human, are affected by the virus. Rabies is distributed throughout the world and endemic in several countries except Australia and Antarctica. Every year, over 60,000 people expire due to rabies, and roughly 15 million people get the vaccine as post-exposure prophylaxis every year. Bite of infected animals and saliva of rabid hosts are mostly responsible for disease transmission. Apart from domestic dogs, wildlife like foxes, raccoons, skunks, and bats are chief reservoirs for rabies, from the enormous amount of rabies cases reported every year (Davis et al. 2013; Ellison et al. 2013; Streicker et al. 2013; Kuzmina et al. 2013). Rabies virus circulates in an urban and sylvatic cycle involving dogs, cats, and wild animals like a racoon, skunk, jackal, fox, badger, mongoose, bats, etc., as reservoirs/vectors, respectively (Condori-Condori et al. 2013; Blackwood et al. 2013; Escobar et al. 2013).

Nevertheless, both cycles may overlie in several geographical situations. Rabies was eradicated officially in the UK in 1920. But in 2002, a bat conservationist died after contracting a rabies virus (European bat lyssavirus type 2) from a bat (Fooks 2007). Presently rabies virus is absent in terrestrial animals in Australia. However Australian bat lyssavirus is present in bats. This virus is transmitted to humans and animals from bats. It was first identified in Queensland, in 1996 and so far, only three human cases have been accounted for due to bite or scratch by bats (Francis et al. 2014; Singh et al. 2017). Additionally, some rabies-related lyssaviruses have been described in Eurasia from insectivorous bats: Irkut, Aravan, Khujand, Bokeloh bat lyssavirus, West Caucasian bat viruses, and Ikoma lyssavirus (Singh et al. 2017). Till now, five human deaths have been connected to rabies-related viruses (Singh et al. 2017).

15.15 *Paramyxoviridae* Zoonotic Infections

Nipah, Hendra (genus *Henipahvirus*), and Menangle virus (genus *Rubulavirus*) infections are the three important viral zoonotic diseases involving wildlife species under the *Paramyxoviridae* family (Table 15.4). All the three viruses are transmitted

through bats. The emergence of these zoonotic viruses is alleged to be due to ecological modifications like deforestation, urbanisation, and drought that have compelled the bat populations to shift their usual habitats to agricultural areas subsequently resulting in animal and human diseases (Allocati et al. 2016; Kulkarni et al. 2013).

Nipah virus first emerged in Malaysia in 1998. It has caused an outbreak of encephalitis and respiratory illness in pigs. Nipah virus transmission from pigs to human has resulted because of direct contact with infected animals. The human-to-human transmission is also reported. In outbreaks of Bangladesh and India, an intermediary animal was not recognised, suggesting direct bat-to-human and human-to-human spread. *Pteropus* bats (*P. hypomelanus* and *P. vampyrus*) are believed to be the natural hosts. Pigs play the role of amplifying host. In human outbreaks of Malaysia and Singapore, it has been proved that infected swine was the source (Parashar et al. 2000; Kulkarni et al. 2013).

Hendra virus first emerged in 1994 in Australia has caused fatal respiratory infection in two humans and 20 horses and further several outbreaks. Pteropid bats are the reservoir of the Hendra virus. Horses infected by the secretions and excretions of infected bats are the intermediate hosts that transmit the infection to humans, who come in close contact with them. The human-to-human transmission has not been documented until now (Allocati et al. 2016). The majority of human cases have been veterinary assistants or veterinarians, underlining the prominent risk profile of this cohort (Field 2016).

Menangle virus is another zoonotic paramyxovirus able to cause disease in pigs and humans. This virus was first isolated in Australia in 1997 from stillborn piglets at a commercial pig farm. This virus was shown to infect people; 2 workers in piggery developed a serious influenza-like illness and found to have neutralising antibodies to Menangle virus. For the outbreak, bats were identified as a source, as *Pteropus poliocephalus* and *Pteropus scapulatus* bats were noticed to be roosting close to the piggery implicated in an outbreak (Barr et al. 2012).

15.16 *Orthomyxoviridae* Zoonotic Infections

Influenza A viruses under the *Orthomyxoviridae* family (Table 15.4) time and again have posed a significant threat to public health, both through pandemic outbreaks and seasonal infections. Avian influenza (AI) viruses, especially highly pathogenic (H5N1, H7N9) variants have emerged as a major zoonosis, and they circulate naturally in wild bird populations as well as in waterfowl and ducks and be able to spill over to domestic poultry birds like chickens. Aquatic birds play the role of natural reservoirs for all influenza A subtypes except some novel strains being isolated in bats (Horman et al. 2018). Many AI virus antigenic subtypes have been recovered from swine, demonstrating an ideal “mixing pot” of influenza A viruses possibly pandemic for humans. There is serological evidence of AI in one duck

hunter and two wildlife professionals with considerable exposure to wild water bird and game bird (Gill et al. 2006; Horman et al. 2018).

Swine influenza viruses (H1N1, H3N2), especially H1N1, which created a global pandemic, seem to have high infectivity for a wide array of domestic and wild animal species. The domestic animals in which the virus was detected are swine, dogs, turkeys, cats, and domestic ferrets, whereas wildlife species include skunks, cheetahs, American badger, black-footed ferret binturong, giant anteaters, and wild boar (Schrenzel et al. 2011; Delogu et al. 2019). In a few cases, animal to animal spread may have occurred, lifting apprehension about the possible development of new wild reservoirs (Schrenzel et al. 2011). The omnipresence of H1N1 pandemic strain and its capability to infect a broad range of hosts is a concern for the health of wildlife and for the likelihood of creating extra reservoirs that could change the evolution of subtype H1N1 viruses by causing diverse selection pressures and creating new ways of producing novel reassortant strains (Schrenzel et al. 2011).

15.17 *Coronaviridae* Zoonotic Infections

Coronaviruses (CoV) preceding SARS outbreak was only acknowledged to be the second reason for common cold infection next to rhinoviruses. Of late, 2 very important zoonotic-CoV were recognised: Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Table 15.4). SARS-CoV was first spotted in February 2003 in China, and soon after 4 months, 48,000 cases had been reported with about 800 fatalities in 27 countries around the world. This virus has a broad host range, and it is linked to the bushmeat industry. Bats are the principal hosts that transmit the virus to intermediate amplifier hosts such as raccoon dogs and mask palm civets that then could transmit to humans (Allocati et al. 2016). These amplifier animals are only incidental hosts as in wild or breeding facilities, no circulation of SARS-CoV-like viruses has been seen in them. Rather, bats are the natural reservoir of a broad range of coronaviruses, like SARS-CoV-like and MERS-CoV-like viruses (de Wit et al. 2016). In large scale epidemics, person-to-person transmission is considered as the major route of transmission. MERS-CoV was first recognised in 2012 in Saudi Arabia and then distributed to a few other countries causing many deaths. MERS-CoV is phylogenetically associated with SARS-CoV, and it also shares the origination that is from bats. Clinical characteristics of MERS-CoV are also similar to SARS-CoV, while this virus has also been related to some extrapulmonary manifestations, like renal complications. Experimental studies in bats prove that bats are the reservoir for MERS-CoV, as the virus replicated without manifesting any overt clinical signs (Munster et al. 2016). Animal-to human transmission is acknowledged to be important in MERS outbreak as recent studies have pointed out that dromedary camels may work as a potential source of MERS-CoV to humans (Allocati et al. 2016; de Wit et al. 2016).

15.18 *Hepeviridae* Zoonotic Infections

Under *Hepeviridae*, Hepatitis E virus (HEV) infection caused by the Hepatitis E virus is an important zoonotic viral disease from the wildlife perspective (Table 15.4). Globally, HEV is the foremost cause of hepatitis that is enterically transmitted. HEV-1 and HEV-2 genotypes infect only humans and responsible for large water transmitted epidemics. HEV-3 and HEV-4 infect both human beings and animals and are the major cause of hepatitis E cases in industrialised countries. The natural host of genotypes, HEV-3, and -4 is swine. Recently, HEV transmission from wild boar to other wild boar and domestic pigs by direct contact between animals was established (Doceul et al. 2016).

Additionally, the zoonotic spread of HEV-3 and -4 to human from wild boar, deer, and domestic pigs and human by eating contaminated meat has been confirmed (Tei et al. 2003; Doceul et al. 2016). Several reports in France, Japan, Australia, Germany, and Spain have also correlated sporadic hepatitis E cases or outbreaks with the consumption of pork or wild boar meat and offals. In France, a nationwide survey showed that consumption of pork, pork liver sausages, offal, and game meat was an important contributor for the prevalence of anti-HEV antibodies (Doceul et al. 2016).

15.19 *Herpesviridae* Zoonotic Infections

Macacine herpesvirus 1 (MaHV1 aka B virus) under the genus *Simplexvirus*, of *Herpesviridae* (Table 15.4) is a zoonotic agent enzootic among macaque (*Macaca* spp.) all over Asia. This virus is related to herpes simplex virus (HSV 1 and 2) of humans and other herpes viruses infecting non-human primates like baboons. Macaques can shed the virus without manifesting any overt clinical symptoms and also manifest vesicular lesions on the buccal cavity and genital areas. Human transmission can occur permucosally (exposure to infected macaque secretions and excretions) and transcutaneously (via bites). Among human cases, ≈40 laboratory workers have reported MaHV1 encephalitis following direct contact with the long-tailed macaques and the rhesus macaques or their infected tissues during the research (Cohen et al. 2002; Lee et al. 2015).

15.20 *Poxviridae* Zoonotic Infections

The genus *Orthopox* contains two zoonotic virus species with the involvement of wildlife species: cowpox and Monkeypox. The other two important genera that are important viral zoonotic pathogens from wildlife perspective are *Parapox* and *Yatapox,* causing contagious ecthyma/orf and Tanapox, respectively (Table 15.4).

Earlier cowpox virus was generally spotted in milking cows with the rare zoonotic transmission to milkers. Today, cowpox is renowned for infecting a wide array of hosts, including cats, zoo animals, and humans. More than 400 cases of cowpox infections have been reported in domestic cats. Human cowpox cases are mainly due to direct contact with infected cats, cows, on rare occasions with rats or zoo and circus animals (Essbauer et al. 2010). Incidental evidence of rodents being a source of infection to humans has been described in two suspected cases and one proven wild rat to women transmission (Wolfs et al. 2002).

Monkeypox was identified first in 1958 among captive monkeys imported to Denmark from Africa for research purposes. As the animal reservoir of the monkeypox is rodents, including giant pouched rats and squirrels; the given name seems to be inappropriate. From its discovery, this disease has been endemic to Central and West Africa with, sporadic and intermittent cases reported among humans transmitted from local wildlife. This virus has been identified in a range of animal species such as rats, striped mice, squirrels (rope and tree), dormice, and monkeys. Direct and indirect contact with infected live and dead animals is believed to be the driver of human cases. Monkeypox had gained international attention in 2003 when the first human cases outside Africa were reported in the USA. Several people developed a rash, fever, and respiratory symptoms, and source of exposure was investigated as pet prairie dogs (*Cynomys* sp.) (Peterson et al. 2019).

Orf, or contagious ecthyma, is a rare zoonotic disease commonly transmitted from infected sheep and goats. Humans get infection either by direct contact with infected animals or indirect contact with fomites contaminated with the virus. A case report of human Orf contracted by handling deer carcasses with bare hands is available (Kuhl et al. 2003).

Tanapox infection is extremely rare outside Africa and endemic to equatorial Africa. Non-human primate-to-human and human-to-human transmission have been described. Arthropod mediated transmission is also suggested. So far, only four human cases have been reported in the USA: where three cases were of research personnel who handled laboratory animals, and one case was a traveller recently returned from Sierra Leone. In Europe, a tanapox case was reported in a person who had recently arrived from Africa to Germany. A typical tanapox case was reported in Africa in a student working with orphaned chimpanzees (Dhar et al. 2004).

15.21 Control Strategies

Diseases shared with wildlife species are multi-host infections, which have a potential impact on public health, economy; wildlife management and conservation were wildlife itself plays a major role in the maintenance of the infection. The complete eradication of shared zoonotic pathogen is impossible ignoring its wildlife reservoir hosts. The control of such diseases needs the development of policies and strategies that will decrease the transmission of the pathogen between wildlife species and both human beings and domestic livestock. Also, a collaborative trans-disciplinary effort

in a One Health context is vital to protect the health of human, livestock, wildlife, and the environment. The following are some options that are suggested to control viral zoonotic diseases at the wildlife–livestock–human interface.

1. Establishing suitable disease surveillance and monitoring in wildlife species. Monitoring targets on the known infected population of wildlife to map temporal and spatial trends, whereas surveillance focuses on healthy wildlife to demonstrate the disease absence. After the identification of disease, descriptive studies are to be undertaken to assess whether the disease and the role of wildlife is relevant for public or animal health or for wildlife conservation and management (Artois et al. 2009; Gortázar et al. 2015).
2. Alternate options like no-action or zoning or compartmentalisation should also be given a thought, especially considering cost/benefit estimation, but monitoring of disease and population is constantly required. Compartmentalisation and zoning can be and have been employed by states or countries to define subpopulations of different health statuses for controlling the disease. Zoning is defining a particular geographical area in which a disease exists (Artois et al. 2011).
3. Translocation control ("movement control") is a well-known preventive option in controlling the disease for both livestock and wildlife. It prevents the introduction or re-introduction of infectious agents through the release of infected captive or free-living wildlife (Gilbert et al. 2005; Gortázar et al. 2015).
4. Barrier concept, which includes the use of small or large scale fencing or any other barrier, to prevent the spread of diseases by decreasing contact between animal populations. Farm biosecurity is one of the most prominent methods used to reduce wildlife–livestock–human interactions (Engeman et al. 2011; Judge et al. 2011).
5. Wildlife population control solves the problem of an increased reservoir population. Population control methods like feeding bans, increased harvesting, habitat management, random or selective culling, and reproductive control may be deployed (Gortázar et al. 2015).
6. Vaccination of wildlife emerges as a precious alternative or complementary method in disease control. As opposed to culling methods, general public easily accepts vaccination methods as it is sustainable and non-destructive (Beltrán-Beck et al. 2012).
7. Control of arthropod vectors employing insecticides, acaricides, and vaccines (tick) in the urban areas and use of protective clothing or repellents when visiting the forest areas are truly helpful methods as most of the viral zoonotic diseases are vector transmitted (Gortázar et al. 2015).
8. Proper removal of harvested wild animals (carcass, offal, and other remains) limits the potential spread of the infection mainly by mammals (Vicente et al. 2011).
9. Farming of wildlife species could diminish the risk of zoonotic infection spill over if comparable biosecurity and health measures are implemented to farmed wildlife as to domestic livestock (Murray et al. 2016).

10. Strong regulations can be instituted to prohibit and grant disincentives for illegal and legal trade of bushmeat to beat growing demand as an elegant commodity. High taxation charges may elevate the price to decrease demand and afford revenue for surveillance and enforcement efforts. Enacting high penalties may prevent participation in the illegal trade of wildlife (Murray et al. 2016).
11. Education of the general public about the risks connected with wildlife, bushmeat, and exotic pet trades (Chomel et al. 2007).
12. Future research on zoonoses involving wildlife hosts needs to embrace a collaborative trans-disciplinary approach to identify primary causes and to control their transmission (Daszak et al. 2000). Extensive studies to improve understanding of rodent–human/bat–human interactions to disrupt transmission cycles are needed to design innovative control strategies in the future.

15.22 Conclusions and Prospects

It is now well recognised in the global community that zoonotic diseases have emerged from wildlife hosts and are still emerging as a result of human and domestic livestock exposure to wildlife. The present chapter has comprehensively reviewed most of the viral zoonotic diseases from wildlife perspectives. The major pathways of disease transmission to humans from wildlife are direct exposure due to encroachment into formerly wild areas (fragmentation and degradation); growing co-mingling of domestic livestock and wildlife owing to land-use changes (habitat loss); increasing amount of international wildlife movement, overexploitation of wildlife, unsustainable practices in agriculture and other enterprises, and effect of invasive species. These basic factors influencing the disease emergence from wildlife species are also the major drivers of loss of biodiversity. Therefore, emerging zoonotic viruses are not only potential threats to humans but can also be pathogenic to wild host species. Thus, there is a convincing and effective chance for mutual gains for the conservation of wildlife and public health by collective and collaborative attempts.

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