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Infectious Diseases of Livestock

EDITED BY J A W COETZER & R C TUSTIN

VOLUME ONE

Infectious Diseases of Livestock

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Introduction

This is the second edition of the textbook *Infectious Diseases of Livestock with Special Reference to Southern Africa* which was published in 1994 and is now out of print. While the focus of the first edition was, as its title indicates, more on the diseases of farm animals in Africa south of the Sahara Desert, the book did include many important diseases not then present on the continent which it was thought did constitute a very real or potential threat to Africa. The contents of the second edition have been expanded significantly to include the majority of the important infectious diseases of livestock present in the world. Hence the shortened title, the increase in the number of pages and the set of three volumes. However, in some sections and chapters there is still emphasis on diseases, their vectors, if any, and their control pertaining to sub-Saharan Africa because of their particular socio-economic importance in this region.

The production of the first edition was inspired by the three outstanding editions of the textbook written by Dr Michiel Wilhelm Henning titled *Animal Diseases in South Africa*, the first of which appeared in 1932 and the third in 1956. These are regrettably now out of print and are virtually unobtainable, but much reference is made to them in the present work.

The demand for the first edition, of which 2 000 sets were printed, far outstripped the supply within a matter of several years, and we are still receiving enquiries about its availability almost on a daily basis. It proved, and continues to be, most popular amongst particularly veterinarians, undergraduate and postgraduate students in the fields of veterinary and animal science, and veterinary libraries.

Although fully aware of the daunting task it would take to produce the second edition, the editors decided to revise the book because of the excellent reviews the first edition had received in several well-known international journals, the two prizes that were awarded to it, and the high national and international demand for the book.

The first edition of the book won the following prizes:

- *Bill Venter Literary Award* in 1996. The purpose of this annual South African award is to recognize outstanding contributions to research by university personnel and to promote the standard of specialized works published by them.
- *Malbrant-Feunten Prize* in 1998, which is an award of the Académie Vétérinaire de France.

For the second edition we invited 197 scientists and specialists in their respective disciplines from 24 countries to contribute by writing chapters on subjects in their fields of expertise.

The definition of livestock in the book encompasses cattle, sheep, goats, pigs, horses, donkeys and mules. It will be noted that the collective noun that is frequently used for the latter three animal species and zebras is 'equid'. The book, however, not only includes diseases of livestock but also, where relevant to livestock, those of wildlife.

The country, South Africa, is mentioned relatively frequently in the book. Since publication of the first edition in 1994 there have been many political and other changes in the country including the creation of new provinces and allocation of new names, for example, to many towns and several rivers. The former Transvaal Province has been divided into four new provinces: Gauteng, North West, Limpopo and Mpumalanga; the former Cape Province now comprises the Western Cape, Eastern Cape and Northern Cape provinces; Natal has become KwaZulu-Natal; and the Orange Free State is now the Free State. We have used these names as well as the new names of the towns to which these have been allocated.

In addition to the 28 protozoal and nine rickettsial diseases in Volume 1, chapters are included on the main vectors of diseases (e.g. ticks, tsetse flies, Muscidae, Tabanidae, *Culicoides* midges and mosquitoes), the main principles/methods of control of infectious diseases, and infectious diseases of particular importance at the wildlife/livestock interface. Volume 2 contains 83 diseases caused by viruses and virus-like diseases, and Volume 3, 80 diseases caused by bacteria (including mycoplasmas), fungi and algae, as well as a small number of conditions of unknown or uncertain aetiology.

Each disease is dealt with in terms of its introduction, aetiology, epidemiology, pathogenesis, clinical signs, pathology, diagnosis, differential diagnosis and control. A comprehensive list of references is provided for each disease. To facilitate readability, the references are numbered in the text.

Each of the volumes contains a list of contents and a comprehensive index which pertain to all three volumes. Furthermore, the three volumes are so paginated that page 1 commences in Volume 1 and the last page (page 2159) of the set is in Volume 3.

We believe that, as the first edition proved to be, this textbook will be invaluable to undergraduates and postgraduates in faculties teaching veterinary medicine, and that veterinary researchers, veterinarians in farm practice, stock breeders and all those charged with the promotion of animal health and the prevention of livestock and wildlife diseases should also find it a valuable addition to their reference libraries.

Although every effort was made to complete the project in as short a time as possible, it proved to be an even more formidable and daunting task than the first edition, which took seven years from inception to publication. We sincerely hope that this book will help promote not only the health of livestock but also the control of their infectious diseases.

The editors

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Preface

The first edition of this book, now out of print, was published under the title *Infectious Diseases of Livestock with Special Reference to Southern Africa* in 1994 as a set of two volumes. It was very well received by veterinary and animal husbandry professionals, receiving glowing book reviews in several wellknown international journals as well as two prizes – the *Malbrant-Feunten Prize* in 1998 from the Académie Vétérinaire de France, and the *Bill Venter Literary Award* in 1996, which is awarded for outstanding contributions by university personnel in South Africa. This new second edition has been expanded to include the majority of infectious diseases present in farm animals across the world, this necessitating a new title and expansion to a set of three volumes.

The focus of the book is on infectious diseases of importance to livestock and wildlife. Many of these diseases have the potential of transgressing international boundaries and consequently affecting international trade in animals and their products with serious socioeconomic consequences for countries in which they occur or into which they are introduced. It is well known that several of these diseases often pose as the biggest constraint to the sustainable socioeconomic development of agricultural communities in developing countries. In addition, the highly contagious diseases such as foot-and-mouth disease, rinderpest and African swine fever, and those that have public health implications, such as Rift Valley fever and anthrax, may be used as bio-weapons or in agri-bioterrorism.

Globalization and the ever-increasing trade of animals and their products create a situation which facilitates the potential spread of many diseases to countries where they have been eradicated, often at great cost, or countries where they have not previously occurred. The risk of emergence or re-emergence of diseases in the developed world is therefore at an all time high. Good examples to illustrate this point are the recent emergence of foot-and-mouth disease in the United Kingdom and other countries, with devastating consequences. Climatic changes such as those currently occurring in many parts of the world may also be responsible for the emergence of vector-borne diseases in new geographical areas, as demonstrated by the recent outbreaks of Rift Valley fever in Saudi Arabia and Yemen, and of bluetongue in some Mediterranean countries. Bovine spongiform encephalopathy (BSE) is a good example where animal husbandry and practices played a role in the emergence of a 'new' disease. It is important to note that some of these emerging/re-emerging animal diseases may also have a significant bearing on public health, e.g. Rift Valley fever, tuberculosis and BSE.

The World Organization for Animal Health (OIE) currently classifies animal diseases as List A or List B diseases based on the nature of their spread, the socioeconomic or public health consequences they may have, and their effects on international trade in animals and animal products. The introduction (emergence) of an OIE List A disease can have a devastating effect on a country with a sizable export trade in animals and animal products. It is estimated that economic losses due to lost trade are about ten times those incurred during depopulation (stamping-out) and disinfection. Early detection and prompt regulatory measures should therefore be regarded as the most efficient means to minimize these serious losses.

The objectives of the OIE include ensuring transparency in the global animal and zoonoses situation; collecting, analysing and disseminating scientific veterinary information; providing expertise and encouraging international solidarity in the control of animal diseases; safeguarding world trade by publishing health standards for international trade in animals and animal products, providing a better guarantee of the safety of food of animal origin; and promoting animal welfare through a science-based approach. Books such as this one are of great assistance in the realization of many of these objectives.

Infectious Diseases of Livestock will remind all those concerned with livestock diseases of their national and international responsibilities. Many of the diseases, indeed, constitute a very real threat to the world, whose human population continues to expand at an exponential rate.

The editorial team should be commended for the remarkable task of collating the contributions of world-renowned scientists in their respective fields of expertise, thus adding a tremendous value to this book.

I therefore have much pleasure in recommending this book to all veterinarians and other professionals dealing with livestock and livestock diseases.

Dr B Vallat
Director General OIE

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MERIAL

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We are especially grateful to Dr B Vallat, Director General of the Office International des Epizooties (OIE), Paris, for writing the Preface.

The dimensions of this second edition of the book proved to be more formidable than were those of the first edition, and only the experience and dedication of an experienced publisher could have undertaken the task of producing it. We extend our sincere thanks to Oxford University Press Southern Africa and, in particular, its former Managing Director, Kate McCallum, for their support, advice, technical ability, patience and perseverance. The sterling and friendly assistance of Inge du Plessis, our technical and language editor, is greatly appreciated. Our thanks are also due to Marina Pearson who was responsible for the mammoth task of indexing the book and Ethné Clarke our proofreader.

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Our most heartfelt thanks are due to our families and friends for their help, forbearance and understanding.

Prof J A W Coetzer
Prof Emeritus R C Tustin
Faculty of Veterinary Science
University of Pretoria
2004

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Abridged list of abbreviations

A

AAHL	Australian Animal Health Laboratory
AAV	adeno-associated virus
ACAV	American Committee on Arthropod-borne Viruses
ADCC	antibody-dependent, cell-mediated cytotoxic
ADE	antibody-dependent enhancement
AE	attaching-and-effacing (lesions)
AEC	amino-ethyl carbozole
AEEC	attaching and effacing *E. coli*
AG	arthrogryposis
AGID	agar gel immunodiffusion
AHS	African horse sickness
AHSV	African horse sickness virus
AIDS	acquired immune deficiency syndrome
AIHV	alcelaphine herpesvirus
ALP	alkaline phosphatase
APC	antigen-presenting cells
ARDRA	amplified ribosomal DNA restriction analysis
ASF	African swine fever
ASFV	African swine fever virus
ASPV	acid-stable picornavirus
AST	aspartate aminotransferase
ATP	adenosine triphosphate

B

BACTEC	radiometric culture system
BANV	Banzi virus
BAV	bovine adenovirus
BCG	Bacillus Calmette-Guérin
BCV	bovine coronavirus
BD	Border disease; Borna disease
BDV	Border disease virus; Borna disease virus
BECV	bovine enteric calicivirus
BEF	bovine ephemeral fever
BEFV	bovine ephemeral fever virus
BHK	baby hamster kidney
BHM	bovine herpes mammillitis
BHV	bovine herpesvirus
BIV	bovine immunodeficiency virus
BLV	bovine leukaemia virus
BoTV	bovine torovirus
BPF	bovine petechial fever (Ondori disease)
BPV	bovine papillomavirus
BRSV	bovine respiratory syncytial virus
BRV	bovine rotavirus
BSE	bovine spongiform encephalopathy
BSL	biosafety level
BT	bluetongue
BTV	bluetongue virus
BVD	bovine viral diarrhoea
BVDV	bovine viral diarrhoea virus

C

CaCV	canine calicivirus
CAE	caprine arthritis-encephalitis
CAEV	caprine arthritis-encephalitis virus
CAM	chorioallantoic membrane
cAMP	cyclic adenosine monophosphate
CBC	complete blood count
CBPP	contagious bovine pleuropneumonia
CCHF	Crimean-Congo haemorrhagic fever
CCHFV	Crimean-Congo haemorrhagic fever virus
CCPP	contagious caprine pleuropneumonia
cELISA	competitive enzyme-linked immunosorbent assay
CEM	contagious equine metritis
CF	complement fixation
CFT	complement fixation test
CFU	colony forming units
cGMP	cyclic guanylate monophosphate
CHIK	Chikungunya (virus)
CID	combined immunodeficiency
CI-ELISA	competitive inhibition enzyme-linked immunosorbent assay
CIEP	counter-immuno-electrophoresis
CIN	cefsulodin, irgasan, novobiocin
CJD	Creutzfeldt-Jacob disease
CK	creatinine kinase
CLA	caseous lymphadenitis
CLO	*Cambylobacter*-like organism
CMI	cell-mediated immunity
CMV	cytomegalovirus
CNF	cytotoxic necrotizing factor
COPV	canine oral papillomavirus
CPD	cytopathic dose
CPE	cytopathic effect
CRPV	cottontail rabbit papillomavirus
CSF	classical swine fever
CSFV	classical swine fever virus
CTL	cytotoxic T-lymphocyte
CTLp	cytotoxic T-lymphocyte precursors
CTU	community tuberculin units
CWD	chronic wasting disease

D

DAEC	diffusely adherent *E. coli*
DALY	disability adjusted life year
DBILI	direct bilirubin
DFID	Department for International Development
DFMO	difluoromethylornithrine
DI	defective interfering
DISC	defective infectious single cycle
DISH	disinfection, isolation, submission, hygienic (procedures)
DIVA	differentiate infected from vaccinated animals
DMEM	Dulbecco's minimal essential medium
DMSO	dimethyl sulphoxide
DNT	dermonecrotin toxin
DPI	days post-inoculation
DTH	delayed-type hypersensitivity

E

EAEC	enteroaggregative *E. coli*
EAV	equine arteritis virus
EB	elementary body
EBHSV	European brown hare syndrome virus
EBL	enzootic bovine leukosis
ECE	equine coital exanthema
ECF	East Coast fever
ECV	equine coronavirus
ED	equine dermis
EE	equine encephalosis
EEV	equine encephalosis virus
EEE	eastern equine encephalitis
EEL	equine embryonic lung
EF	oedema factor
EFK	equine foetal kidney
EHD	epizootic haemorrhagic disease
EHDV	epizootic haemorrhagic disease virus
EHEC	enterohaemorrhagic *E. coli*
EHV	equid herpesvirus
EIA	enzyme immunoassay
EIAV	equine infectious anaemia virus
EIEC	enteroinvasive *E. coli*
ELISA	enzyme-linked immunosorbent assay
ELOSA	enzyme-linked oligonucleotide sorbent assay
EMC	encephalomyocarditis
EMCV	encephalomyocarditis virus
ENTV	enzootic nasal tumour virus
EPEC	enteropathogenic *E. coli*
EPM	equine protozoal myeloencephalitis
EqPV	equine papillomavirus
ERhV	equine rhinovirus
EspB	*E. coli* sectreted protein B
ET	electro-phoretic types
ETEC	enterotoxigenic *E. coli*
ETV	equine torovirus
EVA	equine viral arteritis

F

FA	fluorescent antibody
FAO	Food and Agricultural Organization
FBS	foetal bovine serum
FCV	feline calicivirus
FDC	follicular dendritic cells
FEK	foetal equine kidney
FFI	fatal familial insomnia
FFP	fatal fibrinous pneumonia

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FHA filamentous haemagglutinin
FIV feline immunodeficiency virus
FMD foot-and-mouth disease
FMDV foot-and-mouth disease virus
FPA fluorescent polarization assay
FPLC fast performance liquid chromotography
FPSR false positive serological reactions
FSE feline spongiform encephalopathy; focal symmetrical encephalomalacia

H

HA haemagglutinin
HADEN haemadsorbing, enteric
HAI haemagglutination inhibition
HC hog cholera
HCV hog cholera virus
HE human granulocytic ehrlichiosis; hydranencephaly
HEP high egg passage
HERV human endogenous retrovirus
HeV Hendra virus
HEV haemagglutinating encephalomyelitis virus
HEYM Herrold's egg-yolk medium
HI haemagglutination inhibition; humoral immunity
HIV human immunodeficiency virus
HN haemagglutinin-neuraminidase
HPLC high-pressure liquid chromatography
HPS Hantavirus pulmonary syndrome
HPV human papillomavirus
HRPO horseradish peroxidase
HRSV human respiratory syncytial virus
HTLV human T-cell leukaemia virus
HuCV human calicivirus

I

IAAT immunoabsorbent agglutination assay test
ICDV icosahedral cytoplasmic deoxyribovirus
ICP infected cell polypeptides
ICTV International Committee for the Taxonomy of Viruses
ID immunodiffusion
IEC intestinal cell line
iELISA indirect enzyme-linked immunoabsorbent assay
IEM immunoelectron microscopy
IETS International Embryo Transfer Society
IF immunofluorescence
IFA indirect fluorescent antibody
IFAT immunofluorescent antibody test
IFT indirect fluorescent antibody test
IHC immunohistochemical
IHT indirect haemagglutination test
IIFA indirect immunofluorescence assay
IIP indirect immunoperoxidase
ILCA International Livestock Centre for Africa
ILRI International Livestock Research Institute
INF interferon
INH isonicotinic-hydrazide
iNOS inducible nitric oxide synthase
IORP ion-regulated protein
IPB infectious pustular balanoposthitis
IPMA immunoperoxidase monolayer assay
IPV infectious pustular vulvovaginitis
IRP iron-regulated proteins
ISCOM immune-stimulating complex
ISH *in situ* hybridization
ITEME infectious thromboembolic meningoencephalitis

J

JE Japanese encephalitis
JS jaagsiekte
JSRV jaagsiekte sheep retrovirus
JV Jena virus

L

LA latex agglutination
LAM lipoarabinomannan
LAT latency-associated transcripts
LCR long control region
LCV large cell variant
LDH lactate dehydrogenase
LDV lactate dehydrogenase elevating virus
LEE locus for enterocyte effacement
LEP low egg passage
LF lethal factor
LI louping ill
LIV louping ill virus
LOS lipooligosaccharide
LPS lipopolysaccharide
LSD lumpy skin disease
LSDV lumpy skin disease virus
LT heat labile
LTR long terminal repeat

M

MAb monoclonal antibody
MAT microscopic agglutination test
MBM meat and bone meal
Mccp *Mycoplasma capricolum* subsp. *capripneumoniae*
MCF malignant catarrhal fever
MD mucosal disease
MDBK Madin-Darby bovine kidney
MDCK Madin-Darby canine kidney
MHC major histocompatibility complex
MIC minimal inhibitory concentration
MIFT micro-immunofluorescence test
MIPLD mouse intraperitoneal lethal doses
MLV modified live virus
MmmSC *Mycoplasma mycoides* subsp. *Mycoides* Small Colony
mMPSA major merozoite/piroplasm surface antigen
MMTV mouse mammary tumour virus
MOMP major outer membrane protein
MOTT mycobacteria other than tubercle bacilli
MPMV Mason-Pfizer monkey virus
MPS mononuclear phagocytic system
MPS mycoplasmal pneumonia of swine
MRLS mare reproductive loss syndrome
MRM mechanically recovered meat
MRT milk ring test
MSP major surface protein
mtDNA mitochondrial DNA
MV maedi-visna
MVV maedi-visna virus
MW molecular weight

N

NA1 Newbury agent 1
NAHMS National Animal Health Monitoring System
NCDV neonatal calf diarrhoea virus
NCR non-coding region
ND Newcastle disease
NE necrotic enteritis
NLV Norwalk-like virus
NO nitric oxide
NP nucleoprotein
NSD Nairobi sheep disease
NSDV Nairobi sheep disease virus
NSI non-structural protein
NSP non-structural viral protein
NT neutralization
NTEC necrotoxigenic *E. coli*

O

OID ovine interdigital dermatitis
OIE Office International des Epizooties
OMAGOD ovine mouth and gum obscure disease
OMP outer membrane protein
OPPV ovine progressive pneumonia virus
O-PS O-polysaccharide
ORF open reading frame
OSCPH oligosaccharide-specific capture plate hybridization
OspA outer surface protein A
OvHV ovine herpesvirus
OVI Onderstepoort Veterinary Institute

P

PA protective antigen
PAAT Programme Against African Trypanosomiasis
PACE Pan-African Programme for the Control of Epizootics

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PAM pig alveo...
PAP peroxida...
PAR progressive ...
PARC Pan-African Rinderpest Campaign
PAS periodic-acid-Schiff
PBMC peripheral blood mononuclear cells
PCMV porcine cytomegalovirus
PCR polymerase chain reaction
PCV packed cell volu... porcine circovirus; primate calicivirus
PDNS porcine dermat... and nephropathy (nephritis) syndrome
PE proliferative ... pathy
PED porcine endemic diarrhoea
PEDV porcine endemic diarrhoea virus
PEG polythylene gly...
PENV porcine enteric ...virus
PESV porcine enteric ...virus
PFGE pulsed field elect...phoresis
PFU plaque-formin...
PHE proliferative haemorrhagic enteropathy
PI parainfluenza t...
PIA porcine intestin...denomatosis
PIM polymorphic immunodominant molecule
PLD phospholipase D
PLSD pseudo-lumpy skin disease
PMWS post-weaning multi-systemic wasting syndrome
PNP proliferative and necrotizing pneumonia
PoTV porcine torovirus
PPD purified protein derivative
PPEM potentially pathogenic environmental mycobacteria
PPLO pleuropneumonia-like organism
PPR peste des petits ruminants
PPRV peste des petits ruminants virus
PR pseudorabies
PRCV porcine respiratory coronavirus
PRDC porcine respiratory disease complex
PRRS porcine reproductive and respiratory syndrome
PRRSV porcine reproductive and respiratory syndrome virus
PRV porcine rotavirus
PRV pseudorabies virus
PV parasitophorous vacuole
PVM parasitophorous vacuolar membrane

Q

QBC quantitative buffy coat

R

RA rheumatoid arthritis
RAPD random amplified polymerase DNA
RAPD-PCR random amplified polymorphic DNA-polymerase chain reaction
RB reticulate body
RBT Rose Bengal test
RDE receptor-destroying enzyme
RDP Ribosomal Database Project
RE restriction enzyme; reticulo-endothelial
REA restriction enzyme analysis
RF replicative form
RFLP restriction fragment length polymorphism
RFVL Rockefeller Foundation Virus Laboratories
RHDV rabbit haemorrhagic disease virus
RI regional ileitis
RIA radio-immuno-assay
RK rabbit kidney
R-LPS rough lipopolysaccharide
RNA ribonucleic acid
RNI reactive nitrogen intermediates
RP rinderpest
RPV rinderpest virus
RSSE Russian spring-summer encephalitis
RSV respiratory syncytial virus
RT reverse transcriptase
RT-PCR reverse transcriptase-polymerase chain reaction
RTX repeats in structural toxin
RVF Rift Valley fever
RVFV Rift Valley fever virus

S

SADC Southern Africa Development Community
SAF scrapie associated fibrils
SA-OMVV South African ovine maedi-visna virus
SAT slow (tube) agglutination test
SBE sporadic bovine encephalomyelitis
SBL sporadic bovine leukosis
SBO specified bovine offals
SCID severe combined immunodeficiency
SCV small cell variant
SD swine dysentery
SDA Sabouraud's dextrose agar
SDS sodium dodecyl sulphate
SI swine influenza
SIN Sindbis (virus)
Sip scrapie incubation period
SISS segregation, isolation, subdivision, stress reduction
SLA swine leukocyte antigens
SLE St. Louis encephalitis
S-LPS smooth lipopolysaccharide
SMEDI stillbirth, mummification, embryonic death, infertility
SMS San Miguel sea lion
SMSV San Miguel sea lion virus
S.O.S. stagnation, over-abundance and stress
SPF specific pathogen-free
SPG sucrose-potassium phosphate-glutamate medium
SPS sanitary and phyto-sanitary
SRH single radial haemolysis
SRM specified risk material
SRSV small round structured virus
SRV small round virus
SS Schweiger-Seidel
SSBP sheep scrapie brain pool
ST heat stable; swine testis
STb heat-stable enterotoxin
STEC Shiga toxin-producing *E. coli*
SUN serum urea nitrogen
(SU)-TM surface transmembrane
SVD swine vesicular disease
SVDV swine vesicular disease virus
SVN serum virus neutralization
SVS seminal vesiculitis syndrome

T

TAS trypicase-arginine-serine
TBA tracheobronchial aspirate
TBE tick-borne encephalitis
TBF tick-borne fever
TBILI total bilirubin
TC tissue culture-adapted
TCH thiophene-2-carboxylic acid hydrazide
TCID tissue culture infectious doses
TEM transmission electron microscopy
TEME thromboembolic meningo-encephalitis
TFCA transfrontier conservation area
TGE transmissible gastroenteritis
TGEV transmissible gastroenteritis virus
TME thrombotic meningoencephalitis
TME transmissible mink encephalopathy
TNF tumour necrosis factor
TPGYT trypticase-peptone-glucose-yeast extract broth with trypsin
TSE transmissible spongiform encephalopathy
TYM trypticase-yeast extract-maltose

U

UBRD undifferentiated bovine respiratory disease

V

VAT variable antigen types
VEE Venezuelan equine encephalitis
VES vesicular exanthema of swine
VESV vesicular exanthema of swine virus
VIA virus infection-associated
VIAA virion infection-associated antigen
VN virus neutralization
VNT virus neutralization test
VLP virus-like particles
VP viral protein

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VRG	vaccinia-rabies recombinant (vaccine)
VSG	variable surface glycoproteins
VS	vesicular stomatitis
VSV	vesicular stomatitis virus

W

WEE	western equine encephalitis
WHO	World Health Organization
WNV	West Nile virus
WRL	World Reference Laboratory
WSL	Wesselsbron disease
WSLV	Wesselsbron virus

Y

YARU	Yale Arbovirus Research Unit
YLD	years lived with disability
YLL	years of life lost

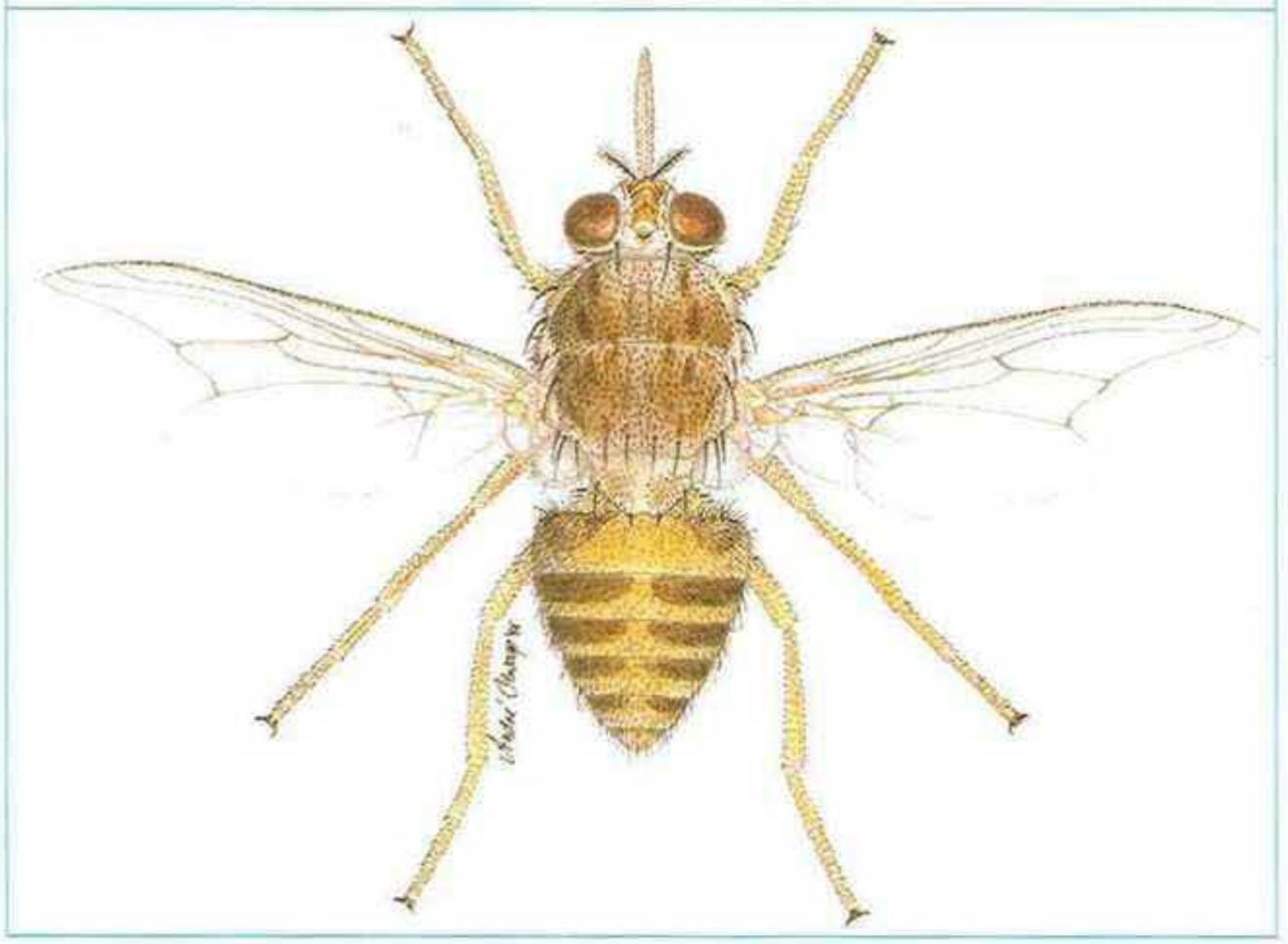
Z

ZN	Ziehl-Neelsen

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SECTION ONE

Aspects Influencing the Occurrence of Infectious Diseases



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1

Vectors: Ticks

R A I NORVAL† AND I G HORAK

Introduction

Ticks are of great economic importance as vectors of several diseases of domestic livestock and of commercially farmed wildlife in sub-Saharan Africa. Globally, 690 species and subspecies of ixodid ticks are recognized, and slightly more than 200 of these are present in the Afrotropical region.[27] In addition, 37 of the 179 species or subspecies of argasid ticks occur here.[37]

Only a small number of ticks are major vectors of diseases or causes of toxicoses in this region. These are *Amblyomma hebraeum, Amblyomma variegatum, Boophilus decoloratus, Boophilus microplus, Hyalomma dromedarii, Hyalomma truncatum, Ixodes rubicundus, Rhipicephalus appendiculatus, Rhipicephalus evertsi evertsi, Rhipicephalus zambeziensis* and argasid ticks of the *Ornithodoros moubata/porcinus* complex. However, several other species also serve as vectors, but to a lesser degree or in a more geographically localized context.

It is customary to consider domestic animals as the preferred hosts of those tick species that transmit diseases to them. However, the only ticks that nearly exclusively parasitize these animals are the introduced Asiatic blue tick, *Boophilus microplus,* of cattle; the shiny *Hyalomma, Hyalomma detritum,* of North Africa; and the cosmopolitan kennel tick, *Rhipicephalus sanguineus,* of domestic dogs. The vast majority of indigenous ticks in the sub-Saharan region are parasites of wildlife, and indeed a large number of species would be unable to complete their life cycles if there were no wild hosts available. Many of the tick species deemed to be parasites of domestic cattle, sheep, goats, horses and pigs are frequently more abundant or prevalent on equivalently sized or even smaller wild animals. Thus giraffe (*Giraffa camelopardalis*), African buffalo (*Syncerus caffer*) and eland (*Taurotragus oryx*) are excellent hosts of all stages of development of *A. hebraeum* and *A. variegatum*; impala (*Aepyceros melampus*), eland, bushbuck (*Tragelaphus scriptus*), greater kudu (*Tragelaphus strepsiceros*) and sable antelope (*Hippotragus niger*) of *B. decoloratus*; giraffe, African buffalo and eland of adult *Hyalomma marginatum rufipes* and *H. truncatum*; caracal (*Caracal caracal*) and mountain reedbuck (*Redunca fulvorufula*) of adult *I. rubicundus*; African buffalo, eland, male nyala (*Tragelaphus angasii*), greater kudu and sable antelope of all stages of development of *R. appendiculatus*; zebra (*Equus* spp.) and eland of all developmental stages of *R. evertsi evertsi*; zebra, black rhinoceros (*Diceros bicornis*), eland and gemsbok (*Oryx gazella*) of *Rhipicephalus pulchellus*; large carnivores, zebra and wild suids of adult *Rhipicephalus simus*; impala and greater kudu of all stages of *R. zambeziensis*; and warthog (*Phacochoerus africanus*) of ticks of the *O. moubata/porcinus* complex. Gerbilline rodents are hosts of the immature stages of *H. truncatum* and murid rodents the preferred hosts of those of *R. simus*. Scrub hare (*Lepus saxatilis*) are preferred hosts of all parasitic stages of *Rhipicephalus warburtoni*, as well as the immature stages of *H. marginatum rufipes* and *H. truncatum*. They are also good hosts of the immature stages of *A. hebraeum, R. appendiculatus, R. evertsi evertsi, R. pulchellus* and *R. zambeziensis*. Rock elephant shrews (*Elephantulus myurus*) are excellent hosts of the immature stages of *I. rubicundus* and of *R. warburtoni*. Helmeted guinea fowl (*Numida meleagris*) and leopard tortoise (*Geochelone pardalis*) are good hosts of the immature stages of *A. hebraeum*, and ground-frequenting birds of those of *H. marginatum rufipes*.

The diseases of major economic importance affecting cattle and transmitted by these and other ticks are heartwater, babesiosis, anaplasmosis, the theilerioses caused by various strains of *Theileria parva*, and tropical theileriosis. Of lesser importance in cattle are the generally non-pathogenic mild theilerioses, spirochaetosis, benign anaplasmosis, benign babesiosis and bovine ehrlichiosis. Sheep and goats are susceptible to the organisms causing heartwater, anaplasmosis, theileriosis, spirochaetosis, and to the virus

† Deceased, 8 April 1994

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causing Nairobi sheep disease. Horses, mules and donkeys are affected by equine piroplasmosis as well as by spirochaetosis, and pigs by porcine babesiosis and African swine fever. In addition to transmitting infectious diseases to livestock, some tick species are also associated with toxicoses such as sweating sickness, paralysis (e.g. Karoo paralysis, spring lamb paralysis) and brown ear-tick toxicosis, or with bovine dermatophilosis. Several wild ruminant species are susceptible to *Ehrlichia ruminantium*, the causal organism of heartwater or cowdriosis, or can act as carriers of this organism (**see Chapter 40: Heartwater**). Some are also susceptible to certain *Theileria* spp., while zebra are susceptible to *Babesia caballi* and *Theileria equi*, the cause of equine piroplasmosis, and wild suids to *Babesia trautmanni*, the cause of porcine babesiosis, and to infection with the virus of African swine fever.

Ixodid ticks are also important vectors of several organisms causing disease in humans in sub-Saharan Africa. These are *Rickettsia conori*, the cause of tick-bite fever or tick typhus; *Coxiella burnetii*, the cause of Q-fever; and the virus causing Crimean-Congo haemorrhagic fever. In addition, argasid ticks of the *Ornithodoros moubata* complex can transmit *Borrelia duttoni*, the cause of African relapsing fever, to humans.

The known important tick vectors of the livestock diseases and causes of toxicoses are listed in **Table 1.1**. Thereafter descriptions of the identification, hosts, life cycle, seasonal occurrence, distribution and disease transmission of each tick vector species, and the control of some, are given. A section on the control of ticks and tick-borne diseases follows: this covers the concept of endemic stability, the history of tick and tick-borne disease control in southern Africa, recent research relevant to tick control, biological control, tick and tick-borne disease models, future control methods, integrated control, and the control of ticks on wildlife.

Tick vectors

Most ticks can only be identified to species level under a stereoscopic microscope and a conventional light microscope may even be necessary for the identification of the immature stages of some. There are, however, a number of elementary observations that can be made to assist with the recognition of ticks to generic level. These are geographical region or actual locality in or at which the ticks were collected, host and site of attachment, season, and any morphological features of the ticks that are discernible with the naked eye.

Free-living adult ticks can also be characterized as 'hunters' or 'waiters'. 'Hunters' actively seek hosts by scuttling along the ground when stimulated by a host's presence or by pheromones produced by ticks already attached to a host. Ticks that follow this strategy are the *Amblyomma* and *Hyalomma* spp., and *R. warburtoni*. These ticks will very rarely be collected from the vegetation. In contrast, the 'waiters' quest for hosts from the vegetation and include *Haemaphysalis* and *Ixodes* spp., and a large number of *Rhipicephalus* spp.

Amblyomma hebraeum

South African bont tick. Suid-Afrikaanse bontbosluis (Afrik.)

Identification Adult *A. hebraeum* are large, conspicuous ticks with long mouthparts, brightly coloured patterns on the scutum of both males and females, flat eyes, and brown and white banded legs. The males have yellow-coloured festoons (**Figures 1.1 and 1.2**).

Hosts The preferred hosts of the adults are the larger domestic and wild ungulates,[104, 183, 273] to which they attach in clusters in the groin and axillae as well as on the dewlap, belly, perineum and peri-anal region.[8, 216] The larvae and nymphs feed on a wide variety of large and small mammals, birds and tortoises.[54, 110, 175, 216, 273, 293] They are very rarely found on murid rodents, even in habitats where large numbers of *A. hebraeum* larvae are present.[19, 113] On domestic livestock nymphs attach most commonly to the feet, and larvae to the face, the dewlap, neck and legs.[8] On the larger ground-frequenting birds the nymphs and larvae attach mainly on the head and neck.[110]

Life cycle *Amblyomma hebraeum* has a three-host life cycle, with larvae, nymphs and adults feeding on separate hosts. On completion of feeding, engorged ticks leave the host and seek sheltered microhabitats in which to moult or to lay eggs. The developmental periods off the host can be long. In the Eastern Cape Province of South Africa, larvae may take five months to hatch from eggs laid by female ticks that detached from hosts during March or April,[230] and nymphs may remain inactive for two to three months after moulting.[168] However, the life cycle usually lasts one year under field conditions, but may for the above reasons extend for longer. The pattern of seasonal occurrence is dependent on climate and varies considerably throughout the distribution range of the tick. In general, adults tend to be most numerous during the warm, wet summer months, larvae during the colder, dry, late autumn and winter months, and nymphs during the winter and spring months. However, varying numbers of all stages of development can often be found on hosts throughout the year.[8, 89, 168, 230] In the warmer, moist, lowveld regions of the KwaZulu-Natal, Mpumalanga and Limpopo provinces in the north-east of South Africa as well as in southern Zimbabwe, the life cycle seems to be continuous with little indication of a definite seasonal pattern of abundance for the various life stages.[94, 95, 179]

Distribution *Amblyomma hebraeum* is exclusively a southern African tick. It occurs endemically in the coastal regions of the Eastern Cape and KwaZulu-Natal provinces in

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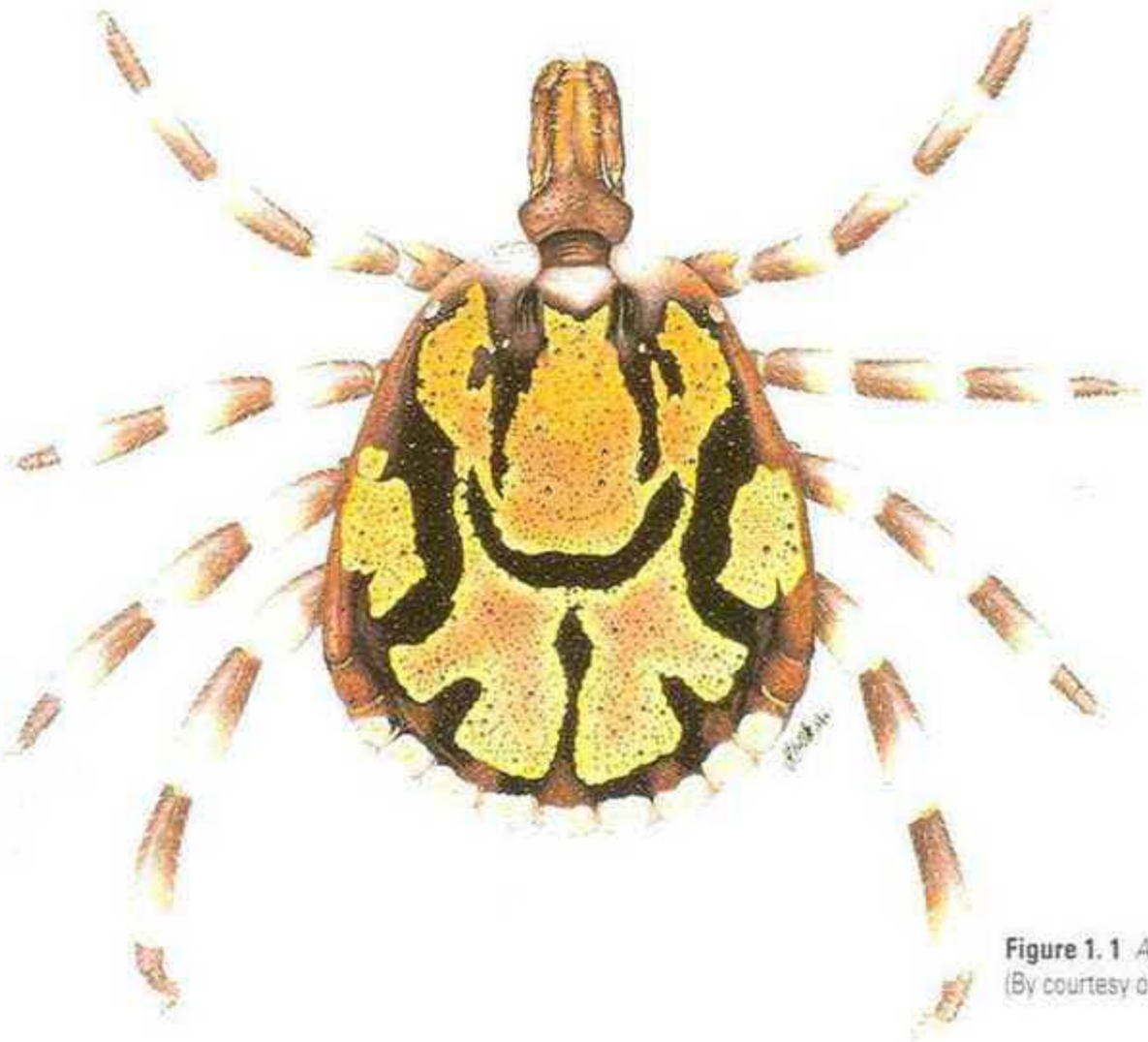


Figure 1. 1 *Amblyomma hebraeum* male
(By courtesy of J.B. Walker, OVI, Onderstepoort)



Figure 1. 2 *Amblyomma hebraeum* female
(By courtesy of J.B. Walker, OVI, Onderstepoort)

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Table 1.1 Tick vectors of diseases of domestic and wild animals in sub-Saharan Africa

DISEASE (CAUSATIVE ORGANISM)	ANIMALS AFFECTED	VECTORS	METHOD OF TRANSMISSION	REFERENCES
Heartwater or cowdriosis (*Ehrlichia (Cowdria) ruminantium*)	Cattle, sheep, goats, wild antelope	*Amblyomma hebraeum*	TS, IS, TO*	2, 4, 136
		Amblyomma variegatum	TS	34
		Amblyomma pomposum	TS	160
		Amblyomma lepidum	TS	216
		Amblyomma spp.	TS	216
Bovine ehrlichiosis (*Ehrlichia bovis*)	Cattle	*Rhipicephalus appendiculatus*	TS	148, 170
		Rhipicepalus zambeziensis	TS	257
Ovine ehrlichiosis (*Ehrlichia ovina*)	Sheep	*Rhipicephalus evertsi evertsi*	TS	162
		Rhipicephalus bursa	?	288
Anaplasmosis (*Anaplasma marginale*)	Cattle, eland	*Boophilus decoloratus*	TS, IS	221
		Boophilus microplus	TS, IS	221
		Hyalomma marginatum rufipes	IS	221
		Rhipicephalus evertsi evertsi	IS	221
		Rhipicephalus simus	TS, IS	221
Benign anaplasmosis (*Anaplasma centrale*)	Cattle	*Rhipicephalus simus*	TS	224
Ovine anaplasmosis (*Anaplasma ovis*)	Sheep, goats	*Rhipicephalus bursa*	TS	229
Bovine babesiosis or African redwater (*Babesia bigemina*)	Cattle	*Boophilus decoloratus*	TO	223
		Boophilus microplus	TO, TS?	75, 235
		Rhipicephalus evertsi evertsi	TO	25
		Rhipicephalus bursa	TS, TO	75, 288
Bovine babesiosis or Asiatic redwater (*Babesia bovis*)	Cattle	*Boophilus microplus*	TO	44, 220
Benign babesiosis (*Babesia occultans*)	Cattle	*Hyalomma marginatum rufipes*	TO	81, 274
Equine piroplasmosis (*Babesia caballi*)	Horses, donkeys, mules, zebra	*Hyalomma truncatum*	TO	49
		Rhipicephalus evertsi evertsi	TS	51, 270
		Rhipicephalus bursa	TS	60
		Rhipicephalus turanicus (as *R. sanguineus*)	TS, TO	60
Equine piroplasmosis (*Theileria equi*)**	Horses, donkeys, mules, zebra	*Rhipicephalus evertsi evertsi*	TS	270
		Rhipicephalus evertsi mimeticus	TS	222
		Rhipicephalus bursa	TS	75, 288
		Rhipicephalus turanicus (as *R. sanguineus*)		60
		Hyalomma detritum (as *H. anatolicum anatolicum*)	TS	75
Porcine babesiosis (*Babesia trautmanni*)	Pigs, warthog, bushpig	*Rhipicephalus simus*	TO	50
		Rhipicephalus turanicus	TO	134
East Coast fever (*Theileria parva* strains) (syn. *Theileria parva parva*)	Cattle	*Rhipicephalus appendiculatus*	TS	10, 45
		Rhipicephalus zambeziensis	TS	129
Zimbabwe theileriosis or January disease (*Theileria parva* strains)	Cattle	*Rhipicephalus appendiculatus*	TS	148, 181, 281
		Rhipicephalus zambeziensis	TS	129
Corridor or buffalo disease (*Theileria parva* strains)		*Rhipicephalus appendiculatus*	TS	164
		Rhipicephalus zambeziensis	TS	129, 181
		Rhipicephalus duttoni	?	31

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Table 1.1 (*continued*)

DISEASE (CAUSATIVE ORGANISM)	ANIMALS AFFECTED	VECTORS	METHOD OF TRANSMISSION	REFERENCES
Tropical theileriosis (*Theileria annulata*)	Cattle	*Hyalomma detritum* *Hyalomma dromedarii*	TS TS	18 53
Benign bovine theileriosis (*Theileria mutans, T. velifera*)	Cattle	*Amblyomma hebraeum* *Amblyomma variegatum*	TS TS	48, 127, 279 282, 283
Benign bovine theileriosis (*Theileria taurotragi*)	Cattle, eland	*Rhipicephalus appendiculatus* *Rhipicephalus zambeziensis*	TS TS	126, 281, 306 129
Ovine theileriosis (*Theileria separata*)***	Sheep	*Rhipicephalus evertsi evertsi* *Rhipicephalus evertsi mimeticus* *Rhipicephalus bursa*	TS TS TS	115 165 185
Borreliosis or spirochaetosis (*Borrelia theileri*)	Cattle, sheep, goats, horses, donkeys, mules	*Boophilus decoloratus* *Boophilus microplus* *Rhipicephalus evertsi evertsi*	TO TO TS, TO	269, 271 ? 271
African swine fever (*Asfarviridae*)	Pigs, warthog, bushpig	*Ornithodoros moubata* complex	TS, TO, S	217, 218, 219
Nairobi sheep disease (and Kisenye sheep disease?) (*Bunyaviridae*)	Sheep, goats	*Amblyomma variegatum* *Rhipicephalus appendiculatus* *Rhipicephalus pulchellus*	TS TS, TO TS, TO	36 22, 35, 39 209
Crimean-Congo haemorrhagic fever (*Bunyaviridae*)	Cattle, sheep	*Hyalomma marginatum rufipes* *Hyalomma truncatum* *Rhipicephalus evertsi evertsi* *Rhipicephalus evertsi mimeticus*	TS TS TS (?) TS (?)	267 243 267 243
Bovine dermatophilosis (*Dermatophilus congolensis*)	Cattle	*Amblyomma variegatum*		24, 145, 178
Sweating sickness (toxicosis)	Cattle	*Hyalomma truncatum*		12, 14, 161
Karoo paralysis (toxicosis)	Calves, sheep, goats, antelope	*Ixodes rubicundus*		65, 256, 285
Spring lamb paralysis (toxicosis)	Lambs	*Rhipicephalus evertsi evertsi*		80, 82
Paralysis (toxicosis)	Sheep	*Rhipicephalus bursa*		288
Paralysis (toxicosis)	Calves, lambs	*Rhipicephalus lunulatus* (as *R. tricuspis*)		128, 197, 273
Paralysis (toxicosis)	Cattle	*Rhipicephalus praetextatus* (as *R. simus*)		202
Paralysis (toxicosis)	Calves, lambs	*Rhipicephalus simus*		185
Brown tick paralysis (toxicosis)	Goat kids	*Rhipicephalus warburtoni* (as *R. pravus* group)		69
Brown-ear tick toxicosis	Cattle, antelope	*Rhipicephalus appendiculatus*		133, 274

TS = Transstadial, IS = Intrastadial, TO = Transovarial, S = Sexually transmitted between ticks

* Rarely transovarial transmission of *Ehrlichia ruminantium* by *Amblyomma hebraeum* may occur[13]

** *Babesia equi* was redescribed as *Theileria equi* by Mehlhorn and Schein in 1998.[156] It is suggested that equine piroplasmosis be used when referring to the disease caused by this organism and by *Babesia caballi*

*** The South African *Theileria ovis* described by Jansen and Neitz[115] is considered by Uilenberg[278] to be synonymous with *Theileria separata*, a parasite originally described from sheep in Tanzania.[280] *Theileria separata* does not infect goats

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South Africa, and in southern Mozambique. Its distribution also extends inland through the lowveld areas of Swaziland, the Mpumalanga, Limpopo and North West provinces of South Africa, and eastern Botswana to southern and western Zimbabwe as well as parts of the Zimbabwean highveld.[20, 175, 213, 214, 273, 290, 292] It is absent from the highveld regions of South Africa, possibly because these are too cold and treeless, but may survive locally in these regions in wooded habitats such as north-facing gullies, ravines and valleys. It was originally thought that the highveld of Zimbabwe would be unsuitable for *A. hebraeum* because of its cooler temperatures.[173] However, the Climex model, indicating ecoclimatic suitability,[254] demonstrated that conditions on the Zimbabwe highveld are favourable for its survival.[188] Recent surveys conducted there confirm this prediction.[20, 213, 214] In central Botswana its distribution is limited by increasing aridity, and in northern Zimbabwe and central Mozambique it may be restricted by interspecific competition with *Amblyomma variegatum*.[175, 260] *Amblyomma hebraeum* is associated with wooded habitats, including coastal bush, riparian woodland, thornveld and mopani woodland. It does not occur in extensive open, treeless areas.

Disease transmission *Amblyomma hebraeum* is the main vector in southern Africa of *Ehrlichia ruminantium*, the cause of heartwater or cowdriosis in domestic and wild ruminants. Laboratory studies have demonstrated that *A. hebraeum* can carry higher infections and also a greater number of strains of *E. ruminantium* than *A. variegatum*, the other major vector of this disease.[141, 142] In addition, field observations seem to indicate that outbreaks of the disease are more commonly associated with the former than the latter tick and that these outbreaks are also more severe.[188, 213] Besides cattle, a number of wild ruminants,[210, 211, 212] as well as other animals such as scrub hare, helmeted guinea fowl and leopard tortoise can act as asymptomatic carriers of *E. ruminantium*. Other organisms transmitted by *A. hebraeum* are *Theileria mutans* and *Theileria velifera*, the cause of benign bovine theilerioses. The occurrence of foot abscesses in goats in the Eastern Cape Province, South Africa, is significantly related to the seasonal abundance of adult *A. hebraeum* and *Rhipicephalus glabroscutatum*.[138] The attachment sites of these ticks around and between the hooves afford entrance to secondary bacterial infections leading to abscessation.

Control Adult *A. hebraeum* have long feeding periods and consequently good control of the tick can be achieved by the treatment of cattle with conventional acaricides at two-weekly intervals. As the ticks attach predominantly to the undersides of cattle, control can be achieved by localized acaricide application. It should be noted that in endemic heartwater areas control of *A. hebraeum* is only necessary when large clumps of adults, forming potential sites for strike by the screw-worm fly (*Chrysomya bezziana*), occur on cattle, or when the udders or teats of heifers or cows are heavily infested. Over-rigorous control is likely to have the negative effect of disrupting the natural transmission of heartwater to young animals and thus reducing levels of naturally acquired immunity, consequently affecting endemic stability to the disease in domestic ruminant populations.[170, 172]

Regular acaricidal treatment of domestic livestock, and more particularly cattle, can result in the virtual disappearance of all developmental stages of *A. hebraeum* on sympatric wild animals up to and including the size of greater kudu as well as the disappearance of free-living larvae on the vegetation.[102, 215] Larger wild animals such as giraffe, African buffalo and eland, which are excellent hosts of all stages of development of the tick,[104, 105, 175] are probably capable of maintaining populations of *A. hebraeum* on a farm, even though the domestic livestock on the property are being treated with an acaricide. The residual population of ticks on wildlife on such mixed cattle and wildlife farms may serve as an important source of tick infestation, and consequently heartwater infection,[210, 211] for the domestic stock on the farm and thus assist in maintaining immunity.

Amblyomma variegatum

Tropical bont tick, tropiese bontbosluis (Afrik.)

Identification Adult *A. variegatum* have long mouthparts and banded legs like *A. hebraeum*, but the colour patterns on the scutum are different and they have beady eyes. In addition, the festoons of the males are dark brown in colour (**Figures 1.3 and 1.4**).

Hosts and life cycle *Amblyomma variegatum* has a host range similar to that of *A. hebraeum*.[86, 175, 216, 273] It is also a three-host tick, but differs from *A. hebraeum* in that the immature stages have a more clearly defined pattern of seasonal occurrence. In Zambia adults are most abundant in the wet season (October to February), larvae from March to July, and nymphs from May to September.[206, 308] This pattern of seasonal abundance can arise from morphogenetic diapause in the females, resulting in a delay in oviposition.[205] Similar observations of only one generation per annum have been made in other regions which have a single rainy season.[216] In Zimbabwe adults can be present throughout the year, with heavier infestations in the warmer months (September to May), and with nymphs present only from June to September.[175]

Distribution *Amblyomma variegatum* is widely distributed through West, Central and East Africa and in southern Africa extends into north-eastern Botswana, the Caprivi Strip of Namibia, and northern and central Mozambique.[273, 292] Since 1985 *A. variegatum* has spread from the north-western regions of Zimbabwe to the central and eastern highveld.[213, 214] Its spread southwards appears to be limited by interspecific competition with *A. hebraeum*,[260] with which it shares similar habitats, hosts and sites of attachment.[175, 216]

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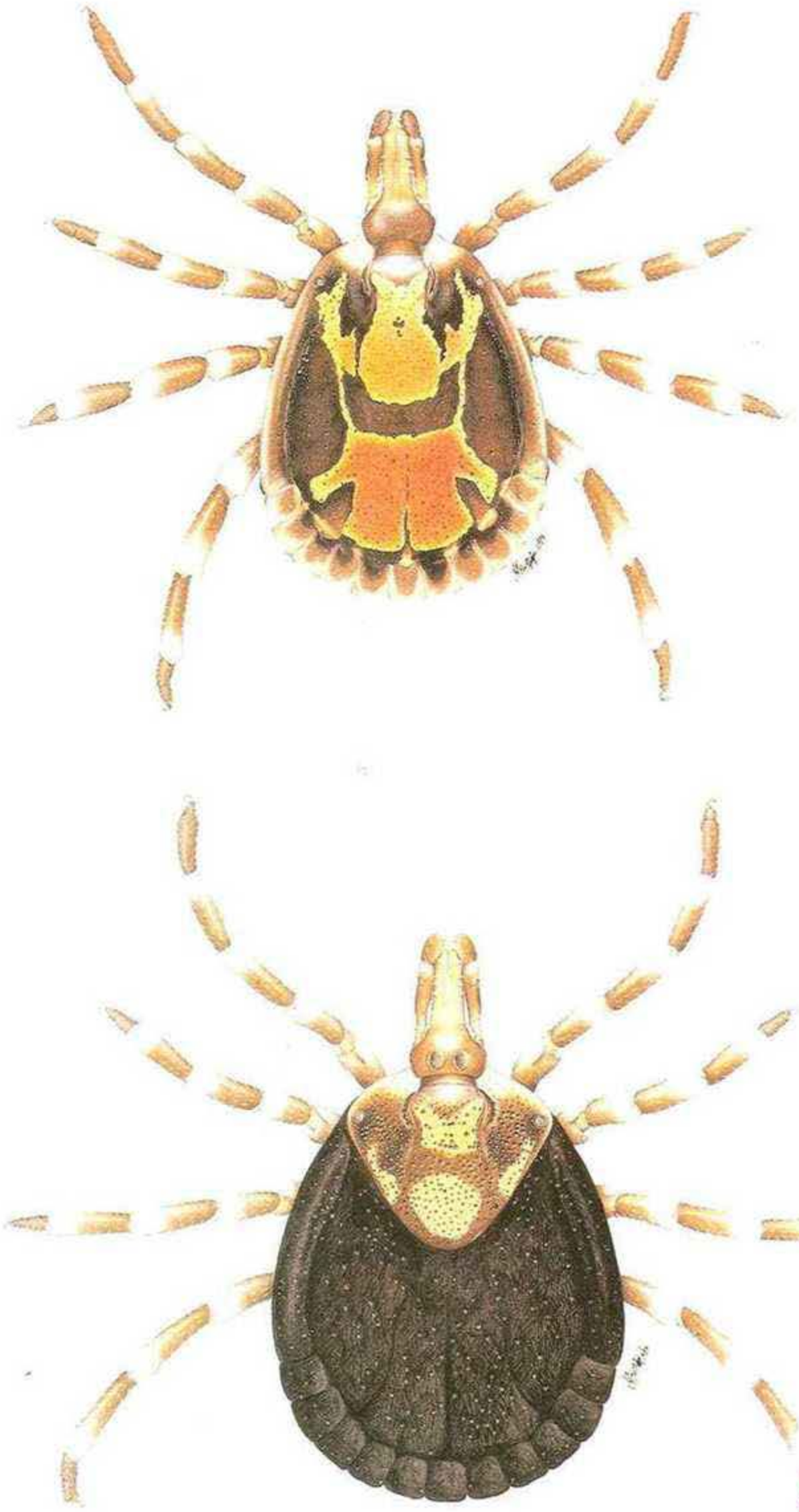


Figure 1.3 *Amblyomma variegatum* male
(By courtesy of J.B. Walker, OVI, Onderstepoort)

Figure 1.4 *Amblyomma variegatum* female
(By courtesy of J.B. Walker, OVI, Onderstepoort)

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Disease transmission *Ehrlichia ruminantium*, the cause of heartwater or cowdriosis; *Theileria mutans* and *T. velifera*, the cause of benign bovine theilerioses; *Ehrlichia bovis*, the cause of bovine ehrlichiosis; and the virus causing Nairobi sheep disease. Bovine dermatophilosis (*Dermatophilus congolensis*) is associated with the presence of the tick.

Control The control of *A. variegatum* with acaricides is similar to that described for *A. hebraeum*. However, the more clearly defined seasonality of *A. variegatum* facilitates strategic control of the adults during the wet season.

Amblyomma pomposum

Angolan bont tick

Identification *Amblyomma pomposum* closely resembles *A. variegatum* but is more heavily punctate (**Figures 1.5 and 1.6**).

Life cycle, hosts and distribution This tick has a three-host life cycle similar to those of *A. hebraeum* and *A. variegatum* and has a similar host range.[216, 242] The adults most frequently attach on the undersides of cattle.[242] It is present in Angola, parts of western Zambia, and in the southern region of the Democratic Republic of the Congo.[292] However, unlike other southern African vectors of heartwater, *A. pomposum* occurs in wet highland areas in savanna and forest.[58, 86, 242, 273] In Angola, nymphs and adults are most abundant during the rainy season, although large variations may occur depending on the region and climate.[242]

Disease transmission *Ehrlichia ruminantium*, the cause of heartwater or cowdriosis.

Amblyomma lepidum

East African bont tick

Identification *Amblyomma lepidum* closely resembles *A. hebraeum*, but has beady eyes and the males have variably coloured light and dark festoons (**Figures 1.7 and 1.8**).

Life cycle, hosts and distribution This is a three-host tick and the adults prefer cattle as hosts, although camels (*Camelus dromedarius*) may also harbour quite large infestations.[216, 287, 302] Other domestic animals can also be infested as well as a number of wild ungulates. Adults are predominantly found attached on the ventral surface of the host from the lower dewlap and axillae to the escutcheon.[302] It is widespread in eastern Sudan, Ethiopia, southern Somalia, eastern Uganda, Kenya and the northern region of central Tanzania.[292] In Tanzania, which lies south of the equator, adult ticks are most abundant between October and February, beginning either shortly before or shortly after the onset of the rainy season. However, there may be considerable variation in the timing of the peak as well as in the numbers of ticks present.[301, 302] In Ethiopia, north of the equator, adults peak during the rainy season in May to June.[202]

Disease transmission *Ehrlichia ruminantium*, the cause of heartwater or cowdriosis.

Amblyomma spp.

A number of other African *Amblyomma* spp. have also been implicated in the transmission of heartwater to domestic livestock (**see Chapter 40: Heartwater**). Except under certain unusual circumstances the ability of several of these tick species to transmit heartwater to domestic ruminants is limited because their adults, which appear to be the most efficient vectors, by preference do not feed on these animals.[216]

The males and females of all these ticks are ornate,[292] have long mouthparts, and all have three-host life cycles.[216] These ticks include:

- *A. astrion*, of which the principal hosts of the adults are cattle, sheep and African buffalo. It is distributed mainly in the Central African Republic, the northern and western regions of the Democratic Republic of the Congo, and north-western Angola;
- *A. cohaerens*, which infests African buffalo, and domestic cattle in regions in which buffalo are, or used to be, common. It has been recorded chiefly in the eastern region of the Democratic Republic of the Congo, western Uganda and central Ethiopia;
- *A. gemma*, for which domestic cattle and large, wild herbivores are the preferred hosts of the adults. It is mainly distributed in eastern Ethiopia, northern and southern Somalia, Kenya and north-eastern Tanzania;
- *A. marmoreum*, of which all stages of development, and most particularly the adults, feed on tortoises and large varanids. Adults very rarely parasitize domestic livestock but the immature stages, especially larvae, are frequently encountered on these animals. This tick is widespread in Zimbabwe and South Africa and is probably more prevalent in Mozambique, Botswana and Namibia than current records seem to indicate;
- *A. sparsum*, of which the adults prefer large reptiles, but black rhinoceros and African buffalo may also be important hosts. It rarely infests domestic hosts. Most records of this tick's occurrence are from southern and western Kenya, northern and central Tanzania and north-western Zimbabwe; and lastly
- *A. tholloni*, of which the preferred hosts of the adults are African elephant (*Loxodonta africana*) and hippopotamus (*Hippopotamus amphibius*).[175] Although larvae and nymphs have been collected from cattle, sheep and goats, domestic stock are not common hosts.[175] This tick has been recorded in the Ivory Coast and adjacent countries to the west, and throughout Central Africa to the eastern coastal regions from Kenya in the north to north-eastern South Africa in the south.

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Disease transmission *Ehrlichia ruminantium*, the cause of heartwater or cowdriosis.

Boophilus decoloratus

African blue tick, bloubosluis (Afrik.)

Identification *Boophilus decoloratus* adults are small, inconspicuous ticks with short mouthparts and slender legs. The males, which are usually considerably smaller than the females, are brownish-yellow in colour and the darker coloured intestine is visible through the lightly sclerotized scutum (**Figure 1.9**). They can usually be found paired with the females. The engorged females are blueish-brown (**Figure 1.10**) and can be seen attached particularly to the face, neck, shoulders and escutcheon of cattle.

Hosts *Boophilus decoloratus* prefers cattle as hosts. In addition to cattle it frequently parasitizes horses, donkeys, sheep and goats.[52, 111, 147, 287, 302] Contrary to earlier observations it is commonly present in large numbers on several wild ungulate species, including Burchell's zebra (*Equus burchellii*), impala, bushbuck, greater kudu, eland and sable antelope.[90, 95, 97, 106, 147, 308] Rather unusually, African buffalo do not appear to be good hosts.[106, 139, 147, 177]

Life cycle *Boophilus decoloratus* has a one-host life cycle, in which moulting from larva to nymph and nymph to adult takes place on the host.[6] The time spent on the host, from attachment of the unfed larva until detachment of the engorged female, is approximately three weeks,[6] and that spent off the host, from pre-oviposition until larval hatching and maturation, approximately five weeks. Because of this short life cycle the tick is able to pass through more than one generation annually, and is usually present in varying numbers throughout the year. In southern Africa low winter temperatures synchronize egg development and larval hatching.[236] Consequently large numbers of larvae are present on the vegetation when the weather warms in spring.[253] Waves of larvae then occur throughout the summer and into the cool months of May and June. In South Africa the largest numbers of ticks are usually present on cattle during the summer and autumn to early winter months,[8, 56, 230, 236] but a spring peak in abundance may also occur.[9, 236] In Zambia a spring peak may be followed several months later by a considerably larger late summer or winter peak.[139, 206, 207] In Nigeria, north of the equator, *B. decoloratus* is most abundant on cattle during autumn,[52] while in the Gambia peak burdens are present on these animals during the rainy season.[152] On wild herbivores in South Africa the highest burdens are usually recorded in spring and, unless these animals are stressed because of disease or drought or other conditions, their late summer and autumn burdens are low.[95, 97] Because of its one-host life cycle all stages of development occur on the host at the same time and the presence on a single host of approximately 70 engorged females can represent a total parasitic population exceeding 10 000 ticks.[95]

Distribution This tick is distributed throughout most of the moister regions of South Africa, except for areas where it has been replaced by *B. microplus*, and also occurs in cold mountainous regions such as the Drakensberg range and parts of Lesotho. It is absent from those parts of South Africa that receive an average annual rainfall of less than 380 mm, including the western Free State, and the western regions of the Northern Cape Province.[78, 112, 272] In the semi-arid to arid territory of Namibia it is present only in localized areas in the north, and in Botswana it is restricted to the higher rainfall eastern border areas and a few scattered northern localities.[290] *Boophilus decoloratus* is also present in southern Mozambique, Zimbabwe, particularly the eastern regions, Angola, much of Zambia, Malawi, south-western and northern Tanzania, Burundi, Uganda, western Kenya and in the wetter highlands and sub-highlands of Ethiopia.[147, 151, 202, 287, 302] It is also found in Central and West Africa.[52, 86, 152] It occurs in various vegetation types, including coastal mosaic, grassland, Cape shrubland, bushland, woodland and in undifferentiated montane vegetation.

Disease transmission *Babesia bigemina*, the cause of bovine babesiosis or African redwater; *Anaplasma marginale*, the cause of bovine anaplasmosis; and *Borrelia theileri*, the cause of borreliosis or spirochaetosis. Eland can be asymptomatic carriers of *A. marginale* and could thus serve as a reservoir of infection for domestic cattle.[166] *Boophilus decoloratus* is also suspected of being a vector of *Babesia trautmanni*, the cause of porcine babesiosis. However, the small numbers encountered on warthog, even in regions where large numbers of this tick are present,[90] make this seem unlikely under field conditions.

Control Because *B. decoloratus* spends approximately 21 days on its hosts to complete its parasitic life cycle it can effectively be controlled by acaricide treatment of cattle at three-weekly intervals. *Bos indicus* and Sanga-type cattle develop a considerably better resistance to this tick than *Bos taurus* cattle,[231, 232, 251] and consequently require fewer acaricide treatments.

Boophilus microplus

Asian blue tick, Asiatiese/pantropiese bloubosluis (Afrik.)

Identification Adults of *B. microplus* are slightly larger than those of *B. decoloratus*, and are slightly more red in colour, but are otherwise very similar in general appearance.

Hosts In sub-Saharan Africa domestic cattle are virtually the only hosts and other livestock species and wild ungulates are rarely parasitized, and then only if cattle infested with this tick are present.[86, 107, 147, 302]

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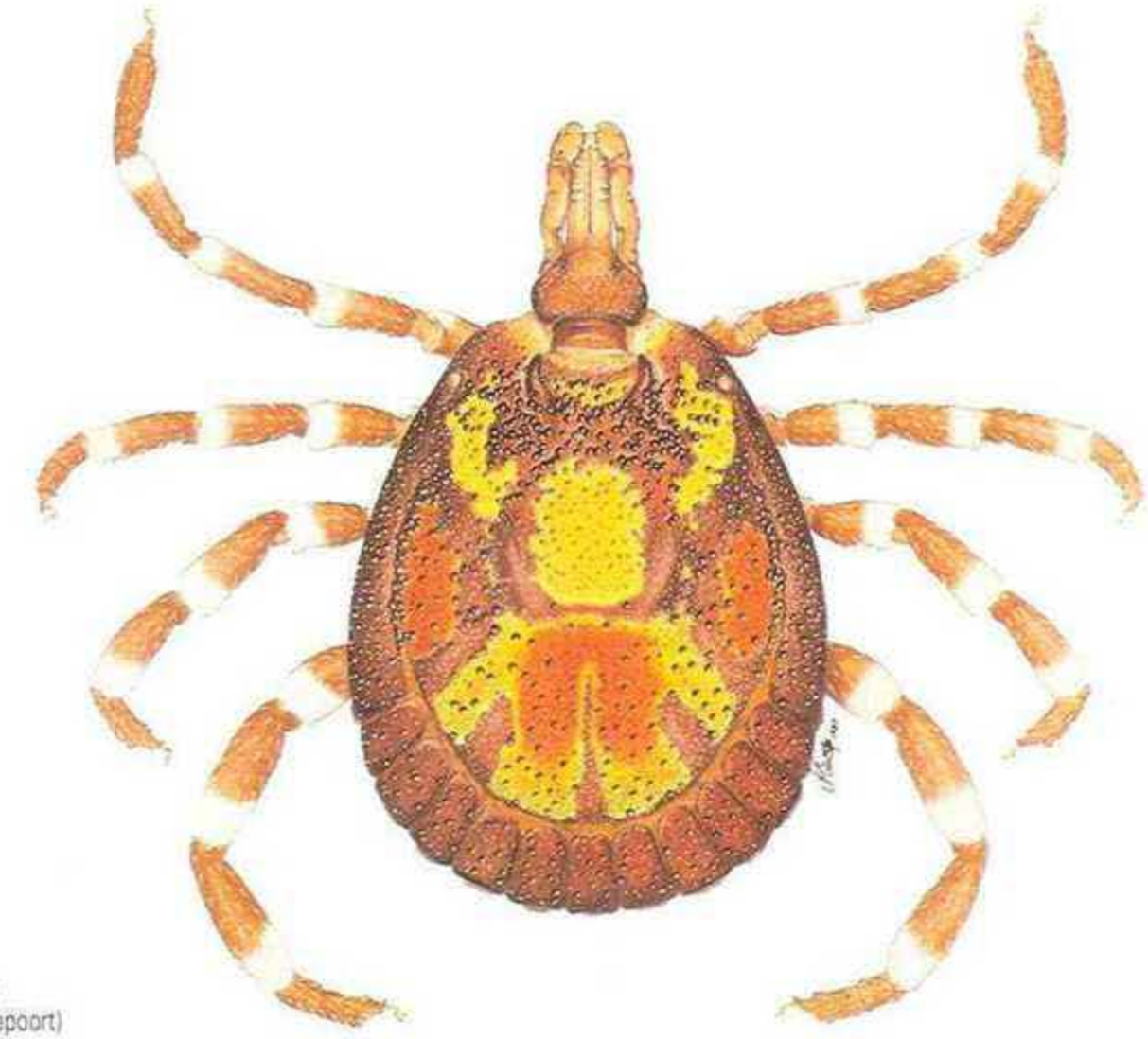


Figure 1.5 *Amblyomma pomposum* male
(By courtesy of J.B. Walker, OVI, Onderstepoort)



Figure 1.6 *Amblyomma pomposum* female
(By courtesy of J.B. Walker, OVI, Onderstepoort)

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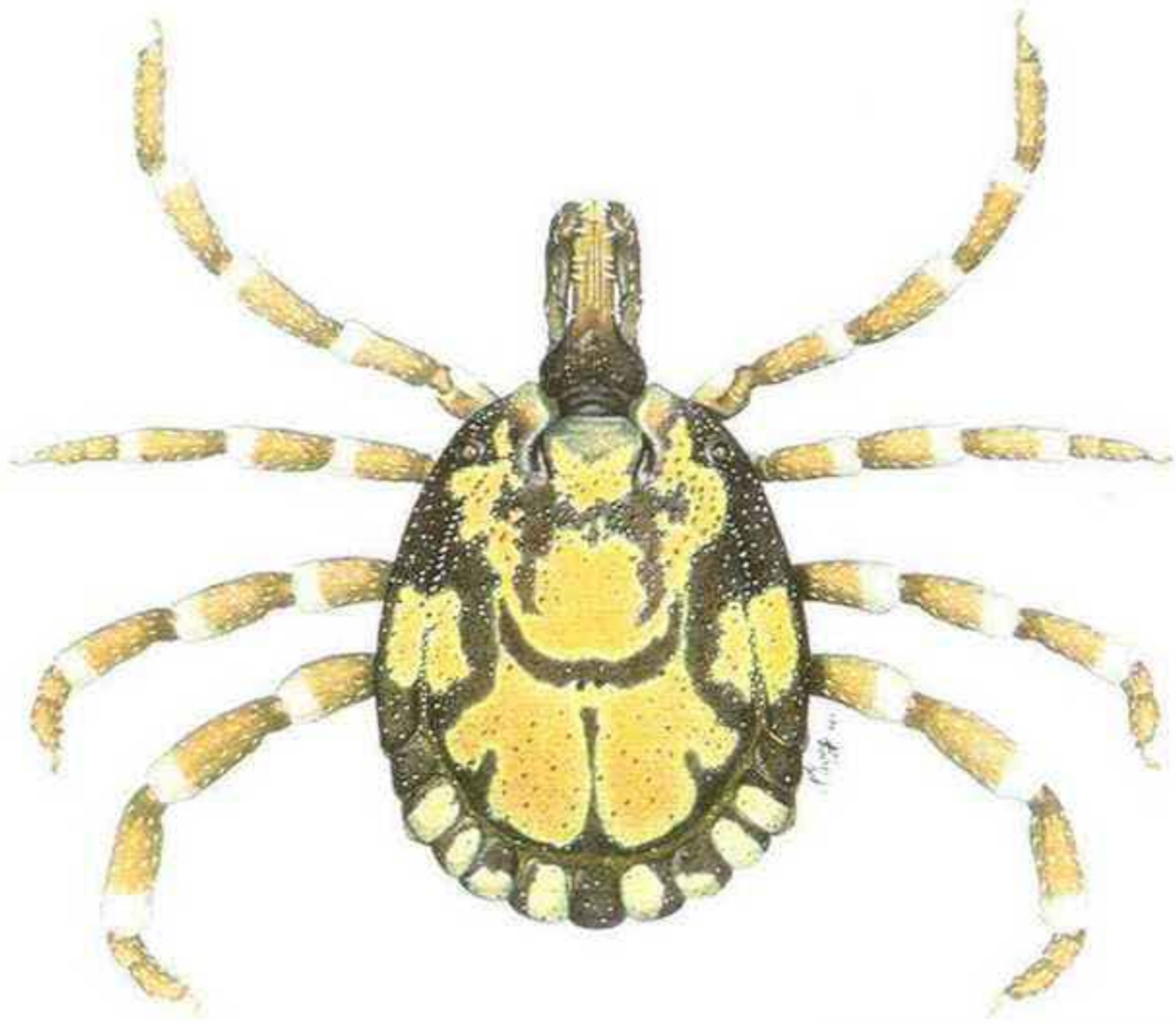


Figure 1.7 *Amblyomma lepidum* male
(By courtesy of J.B. Walker, OVI, Onderstepoort)



Figure 1.8 *Amblyomma lepidum* female
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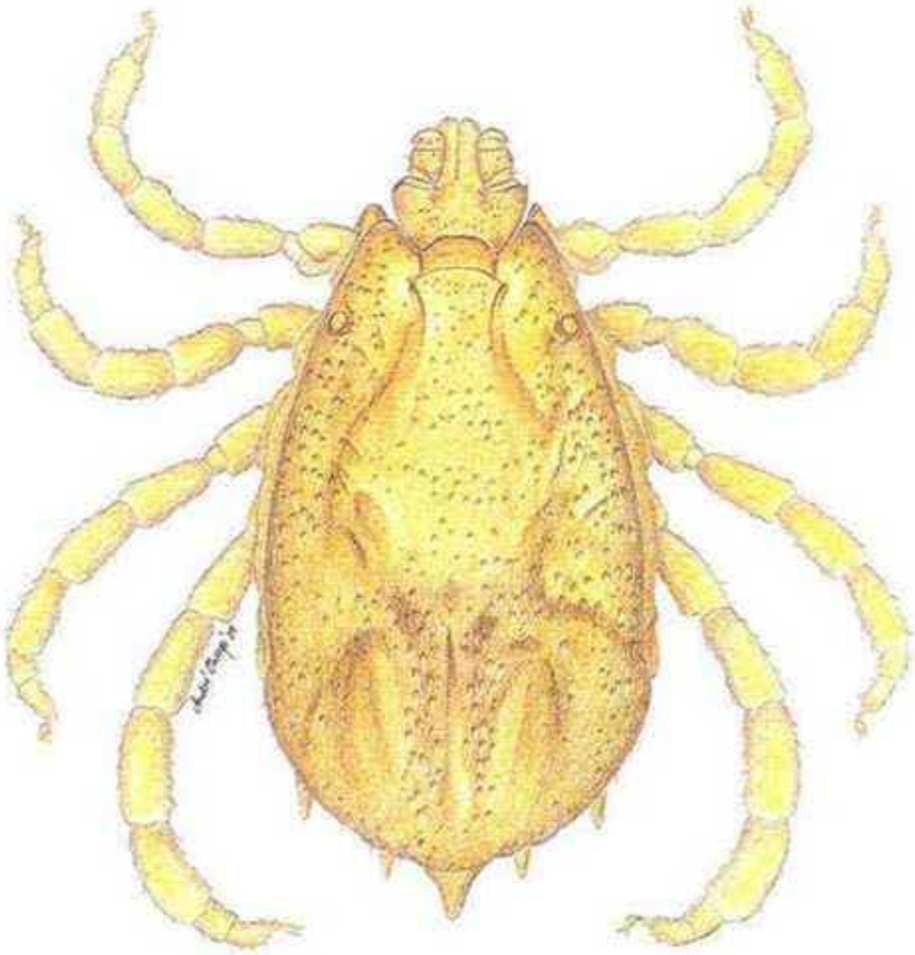


Figure 1.9 *Boophilus decoloratus* male

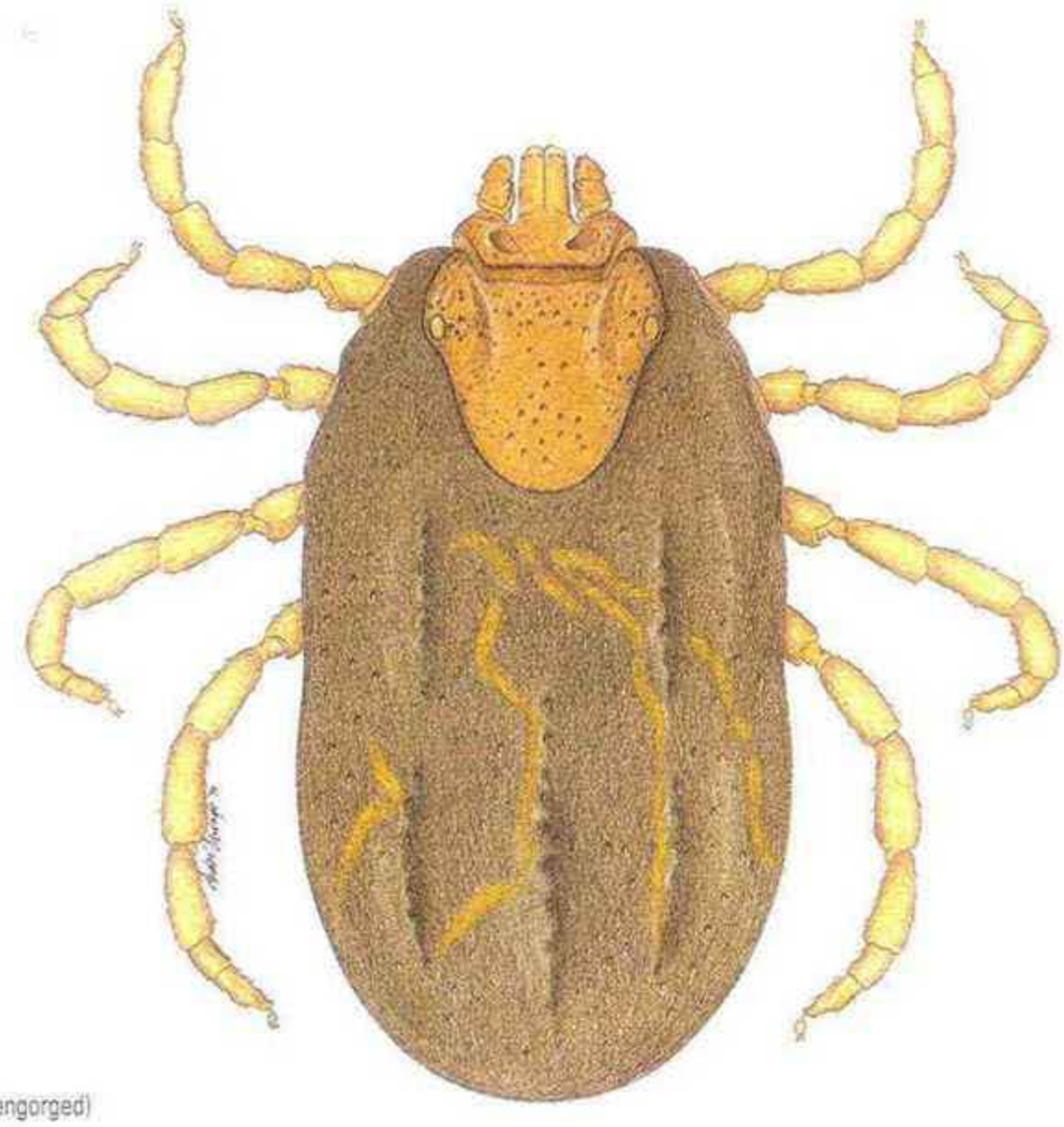


Figure 1.10 *Boophilus decoloratus* female (engorged)

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Life cycle *Boophilus microplus* has a one-host life cycle, and like *B. decoloratus* is able to complete more than one generation in a year. In Zimbabwe it appears to be present in variable numbers throughout the year.[147] In south-eastern KwaZulu-Natal, South Africa, it is most abundant on cattle from mid- to late summer.[9]

Distribution Hoogstraal[86] postulated that *B. microplus* was introduced into East and South Africa from Madagascar, where it had originally arrived with cattle from southern Asia. In South Africa it is now established in scattered areas along the southern and eastern coasts of the Western and Eastern Cape provinces and of KwaZulu-Natal. It is also present in the coastal regions of Mozambique, Tanzania and Kenya. In the interior of the subcontinent it is found in scattered localities in the Mpumalanga and Limpopo provinces of South Africa, in both the south and north of eastern Zimbabwe, in parts of the Eastern and Central provinces of Zambia, throughout Malawi and to the east and north of Lake Malawi in Tanzania.[112, 147, 287, 302] There is evidence that where favourable moist and warm climatic conditions exist it competes with and is able to replace the indigenous *B. decoloratus*.[147, 189, 254] *Boophilus microplus* spread into Zimbabwe in the 1970s, when dipping was disrupted during the pre-independence war, and replaced *B. decoloratus* in several areas.[147] Long-term surveys conducted in eastern Zambia revealed that *B. decoloratus* had almost completely been replaced by *B. microplus* in the 10 years between 1972 and 1982,[11] and its westward spread appears to be continuing.[116] The spread of *B. microplus* in Zimbabwe and Zambia has been accompanied by heavy mortality in cattle due to *B. bovis* infection, of which it is the sole vector in sub-Saharan Africa.[116, 182]

Disease transmission *Babesia bovis*, the cause of bovine babesiosis or Asiatic redwater; *Babesia bigemina*, the cause of bovine babesiosis or African redwater; *Anaplasma marginale*, the cause of bovine anaplasmosis; and *Borrelia theileri*, the cause of borreliosis or spirochaetosis. Because this tick transmits both *B. bovis* and *B. bigemina* it poses a greater potential threat to livestock production than *B. decoloratus*.

Control The control of *B. microplus* is similar to that of *B. decoloratus*. *Bos indicus*-type cattle develop a considerably better resistance to this tick than *Bos taurus*-type cattle and consequently the number of acaricide treatments required can be reduced on these animals.[259, 284] However, because *B. microplus* feeds virtually exclusively on cattle, the whole parasitic population is exposed to acaricidal intervention at any particular location as there are no alternative hosts. Because of this and its slightly shorter life cycle it would appear as if *B. microplus* is selected for resistance to acaricides more rapidly than is *B. decoloratus*.

Hyalomma truncatum

Small smooth bont-legged tick, sweetsiektebosluis (Afrik.)

Identification Adults of *H. truncatum* are medium-sized ticks with long mouthparts and dark-brown bodies, beady eyes, and long, red and white banded legs. The posterior surface of the scutum in males is characterized by a depression containing numerous large punctations, otherwise it is comparatively smooth (**Figures 1.11 and 1.12**).

Hosts The preferred hosts of the adults are large ungulates, both domestic and wild.[86, 91, 174, 287] They attach in the tail switch, around the anus, on the lower perineum, and on the legs, including around the feet.[139] The immature stages feed on hares and on certain rodents, particularly gerbils.[19, 103, 174]

Life cycle *Hyalomma truncatum* has a two-host life cycle, which normally takes a year to complete under field conditions in South Africa. Adults occur in the greatest numbers in the late wet summer months and the immature stages in the dry autumn to spring months.[89, 109, 139, 174, 234]

Distribution This tick is adapted to dry climates and is absent in Lesotho and, with the exception of the Eastern Cape, the eastern half of the Free State, south-eastern Gauteng and Mpumalanga, and southern KwaZulu-Natal, it is present throughout South Africa, Zimbabwe and much of Mozambique.[112, 174, 240] It is also present in south-eastern and north-western Botswana; central and northern Namibia; southern Angola; western, southern, central and eastern Zambia; central and southern Malawi; south-western and north-eastern Tanzania; southern, central and western Kenya; and eastern Uganda.[81, 206, 287, 302] It also occurs in many countries in north-eastern, Central and West Africa.[86, 152, 202, 273] At a local level the abundance of *H. truncatum* is influenced by the abundance of hares, which are the preferred hosts of the immature stages.

Disease transmission *Babesia caballi*, the cause of equine piroplasmosis. Because the immature stages of *H. truncatum* do not feed on horses the transmission of *B. caballi* by this tick has of necessity to take place transovarially.[49] *Hyalomma truncatum* also transmits the virus causing Crimean-Congo haemorrhagic fever in humans, and the toxin causing sweating sickness in cattle and especially in young calves. Only certain strains of *H. truncatum* produce this toxin and then it is only female ticks that do so.[14] The long mouthparts of the ticks, as well as their tendency to form clusters, can cause tissue damage resulting in secondary bacterial infections and abscessation. The attachment of adult ticks to the interdigital clefts and fetlocks of lambs almost always causes lameness.[124]

Control Hand-dressing of the preferred attachment sites with an acaricide will assist in controlling the adults. It is impractical to control the immature stages because of their preference for hares and rodents.

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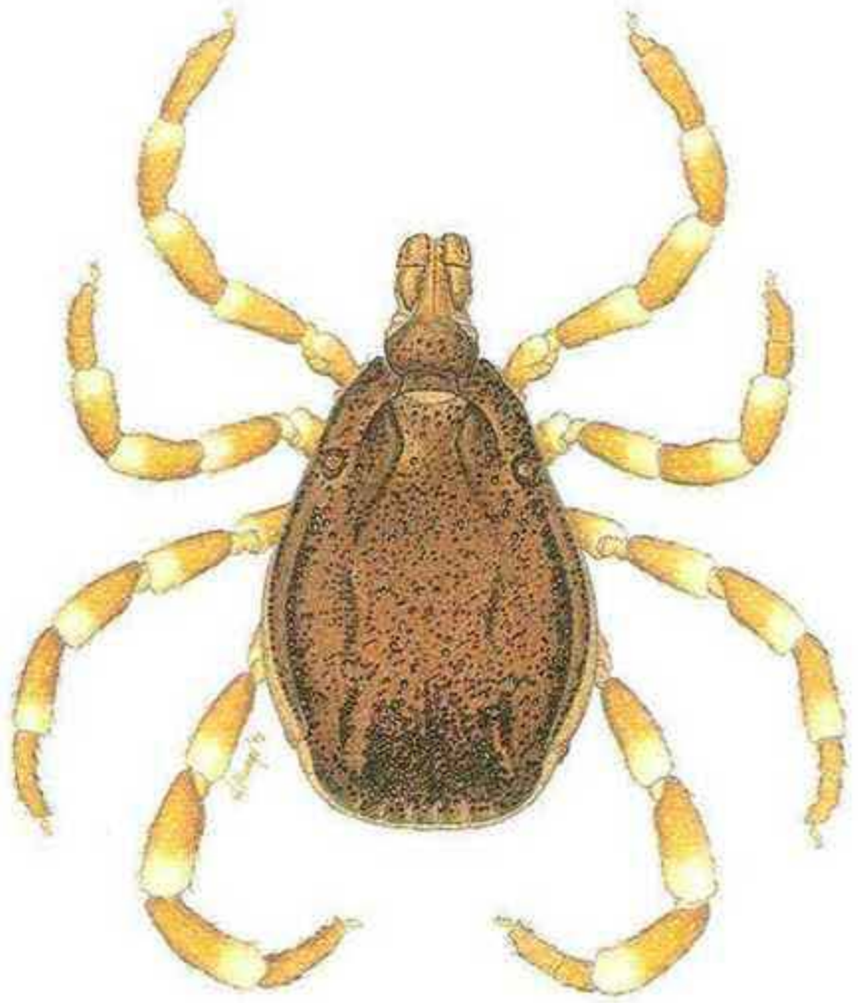


Figure 1.11 *Hyalomma truncatum* male

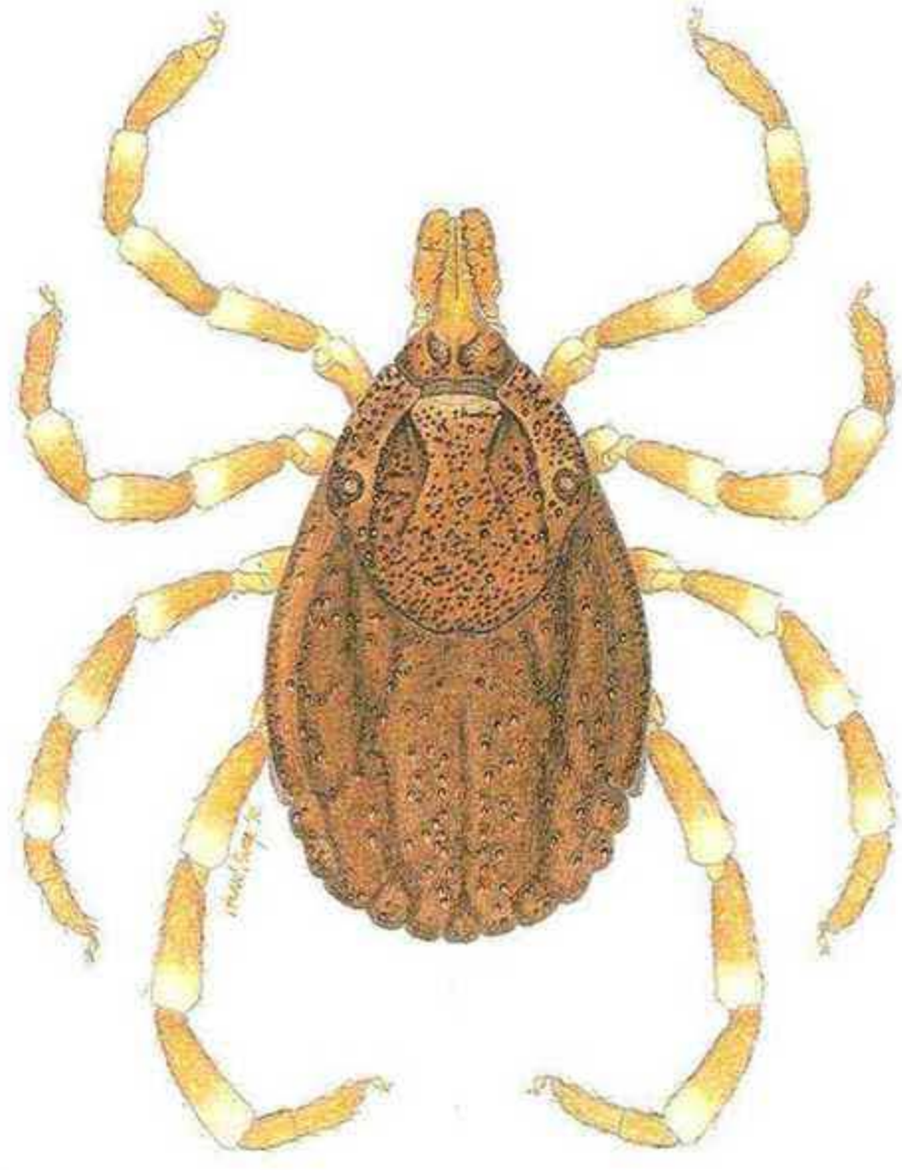


Figure 1.12 *Hyalomma truncatum* female

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Hyalomma marginatum rufipes

Coarse bont-legged tick, groot bontpootbosluis (Afrik.)

Identification Adult *H. marginatum rufipes* are large ticks with dark-brown bodies, long mouthparts, a scutum that is heavily punctate, beady eyes, and long, red and white banded legs. They differ from those of *H. truncatum* in that the whole scutum is punctate and in the males it is more circular than the rather elongate shape of the latter tick. However, it is virtually impossible to distinguish between the two species by means of the naked eye.

Hosts Adults parasitize domestic and wild ungulates, showing a preference for the larger species.[91, 174, 287] They attach mainly in the hairless peri-anal region and on the lower perineum and genitalia.[8] The immature stages are parasitic on hares, particularly scrub hares, as well as on ground-frequenting birds.[103, 174, 234]

Life cycle *Hyalomma marginatum rufipes* has a two-host life cycle, which under field conditions in South Africa takes a year to complete. The adults are most numerous in the early part of the wet season and the immature stages in the dry season.[89, 139, 174]

Distribution This tick is widely distributed in southern Africa, and appears to be absent only from the winter rainfall areas of the Western Cape Province, the mountainous areas of Lesotho and KwaZulu-Natal where snow falls in winter, and some humid, subtropical habitats along the east coast and in the north-east. It is also present in the drier regions of the southern and north-western provinces of Mozambique, south-eastern and north-western Botswana, central and northern Namibia, southern Angola, most of Zimbabwe, southern and western Zambia, central and north-eastern Tanzania, central and southern Kenya, western Burundi, eastern Uganda, and in Somalia, Sudan, Ethiopia and in West Africa.[1, 86, 112, 119, 152, 174, 202, 206, 240, 287, 302] Parasitism of birds by the immature stages undoubtedly contributes to the extensive distribution of this species.

Disease transmission *Babesia occultans*, the cause of benign bovine babesiosis; *Anaplasma marginale*, the cause of bovine anaplasmosis; and the virus causing Crimean-Congo haemorrhagic fever. Secondary bacterial infection of tick attachment sites can lead to the formation of abscesses in cattle.

Control Control can be accomplished by localized acaricide treatment. *Hyalomma marginatum rufipes* is difficult to control on cattle herds even by regular acaricide application as engorged nymphs are continuously being brought in from other areas by birds.

Hyalomma detritum

The shiny *Hyalomma*

Identification Adult *H. dentritum* are medium-sized ticks with long mouthparts, a very dark-brown, smooth, shiny scutum, and long reddish or yellowish-brown legs which may have paler bands.[86]

Hosts Domestic cattle and horses are the most common hosts, but sheep, goats and camels may also be infested.[86] All stages of development feed on the same host species. Adults attach on the inner thighs, udder, scrotum and perineum of cattle.[18]

Life cycle and distribution *Hyalomma detritum* has a two-host life cycle with the adults feeding in summer and the larvae and nymphs in autumn.[86] The detached engorged nymphs undergo a winter diapause and moult to adults the following summer.[18, 86] The life cycle is often associated with barns, stables and sheds, and livestock become infested when they are housed in these structures. This *Hyalomma* is present along the Mediterranean coast of Africa as far as Algeria and Morocco in the west.[86] It is also present in sub-Saharan Africa in north-central Sudan, which it may have invaded from the Red Sea coast or via the Nile River Valley.[86]

Disease transmission *Theileria annulata*, the cause of tropical theileriosis. In those regions in which *H. detritum* is the vector of *T. annulata*, tropical theileriosis frequently occurs on small farms on which the livestock are closely associated with barns and stables where they acquire infestations with the tick. It also transmits *Theileria equi*, the cause of equine piroplasmosis.

Hyalomma dromedarii

The camel *Hyalomma*

Identification Adult *H. dromedarii* are large ticks with long mouthparts. The scutum of the male is characterized by posterior grooves and ridges. The colour of these ticks varies from yellow-brown to nearly black. The legs are paler than the scutum and may be ringed by paler bands.[86]

Hosts The preferred hosts are camels, but cattle, sheep, goats and horses may also be infested.[87] Adults attach on the inner thighs, udder and scrotum of camels. The larvae and the nymphs feed on small burrowing animals and hares, but the nymphs may also infest camels, cattle and horses.

Life cycle and distribution *Hyalomma dromedarii* has a two- or a three-host life cycle. The larvae may feed and moult to nymphs on small mammal or leporid hosts and the adults feed on large herbivores. Or the larvae may feed on small mammal hosts, drop off and moult to nymphs, which can

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then either attach to other small mammal hosts or feed on the same large animals as the adults.[86] The life cycle appears to be continuous throughout the year. This tick is common wherever camels occur in the Far, Middle and Near East. It is also present in Mauritania in West Africa and in Morocco, Algeria, Tunisia and Libya in North Africa and is well-adapted to an arid and even desert environment.[53, 86] In north-eastern and East Africa it occurs in Sudan, Eritrea, northern, eastern and southern Ethiopia, northern Kenya and north-eastern Uganda.[86, 151, 202, 287]

Disease transmission *Theileria annulata*, the cause of tropical theileriosis.

Ixodes rubicundus

Karoo paralysis tick, Karoo-verlammingsbosluis (Afrik.)

Identification Adult *I. rubicundus* are small, reddish-brown, eyeless ticks with long mouthparts. Their legs appear to be grouped anteriorly (**Figures 1.13 and 1.14**). Males may sometimes be attached to females.

Hosts Sheep and goats are the preferred domestic hosts of the adults, on which they attach to the ventral parts of the body, neckline and upper half of the legs.[5, 255] Wild hosts include caracal and mountain reedbuck.[105] The preferred hosts of the immature stages are rock elephant shrews and red rock rabbits (*Pronolagus rupestris*).[70, 100, 105, 255]

Life cycle *Ixodes rubicundus* has a three-host life cycle that takes two years to complete. Larvae are active in autumn and winter, nymphs during winter and spring of the same year as the larvae, and the adults in late summer, autumn or early winter of the following year.[67, 105, 255]

Distribution This tick is associated with the southern slopes of hilly or mountainous terrain and occurs in Karoo and Karroid vegetation types in the semi-arid interior of South Africa. It is present in the Western and Northern Cape provinces, through much of the interior of the Eastern Cape, in the central and southern parts of the Free State, and in southern and western Gauteng in the Heidelberg and Bronkhorstspruit districts.[99, 112, 232]

Disease transmission Karoo paralysis (toxicosis) is caused by the engorging females and occurs most commonly in late summer, autumn and early winter when these ticks are present. The colder the mean minimum atmospheric temperatures during the two months preceding tick activity, the earlier this activity commences.[68] Paralysed animals will usually recover if the attached adults are removed or killed using rapid-acting acaricides, but severe losses in sheep, goats and even young calves can occur if preventive measures are not taken.[112, 252] Wild animals such as springbok (*Antidorcas marsupialis*) can also be affected.[65]

Control The control of *I. rubicundus* includes acaricidal treatment of sheep and goats prior to the onset of the cooler months of the year. These animals should also be moved from mountain camps with a southern aspect to other grazing from late summer to spring. The underbrush of trees or shrubs, which give protection not only to the free-living stages of the tick but also to the small mammal hosts of the immature stages, can be removed.[73, 252, 255] This can be accomplished by pruning, or fire, or by allowing goats to browse during the summer months. Cattle and sheep can be used before winter to keep the grass cover short, thereby creating conditions unsuitable for tick survival or for questing for hosts.[72, 73, 247] Fire can also be used for this purpose.

Rhipicephalus appendiculatus

Brown ear tick, bruinoorbosluis (Afrik.)

Identification Adults of *R. appendiculatus* are medium-sized brown ticks with short mouthparts. The legs of the males increase markedly in size from the first to the fourth pair and engorged males have a slender caudal process (**Figures 1.15 and 1.16**). These ticks are frequently seen questing for hosts on blades of grass during the early part of the wet season.

Hosts This species has a wide host range. Adults parasitize medium-sized to large ruminants while the immature stages feed on most ruminant species and a variety of other mammals, including equids, carnivores and hares.[198, 288, 308] Among domestic animals, cattle are the preferred hosts and can become very heavily parasitized with all stages of development.[8, 198] Several wild ruminant species, such as impala, African buffalo, eland, male nyala, bushbuck, greater kudu and sable antelope, can also be heavily infested.[94, 133, 306] Female nyala harbour few adult ticks but are good hosts of the immature stages.[94] Adults attach in the highest numbers to the ears of their hosts but are also found on other parts of the body.[8, 139] The proportion that attaches to the ears varies between host species and is also dependent on the availability of attachment sites on the ears. Larvae and nymphs may attach to the ears as well as to other parts of the body such as the head, legs, neck and dewlap.[8]

Life cycle *Rhipicephalus appendiculatus* is a three-host tick with a strictly seasonal, single annual life cycle in southern Africa. Adults are most abundant during the rainy period in summer (December to April), larvae in the late summer and cool period after the rains (April to August), and nymphs in the winter and early spring (June to October).[8, 230, 244, 307] The pattern of seasonal occurrence is regulated by the unfed adults, which enter diapause and do not engage in host-seeking until the rains start.[245] In countries close to the equator more than one life cycle can be completed annually and no clear pattern of seasonal abundance may be evident.[119, 155]

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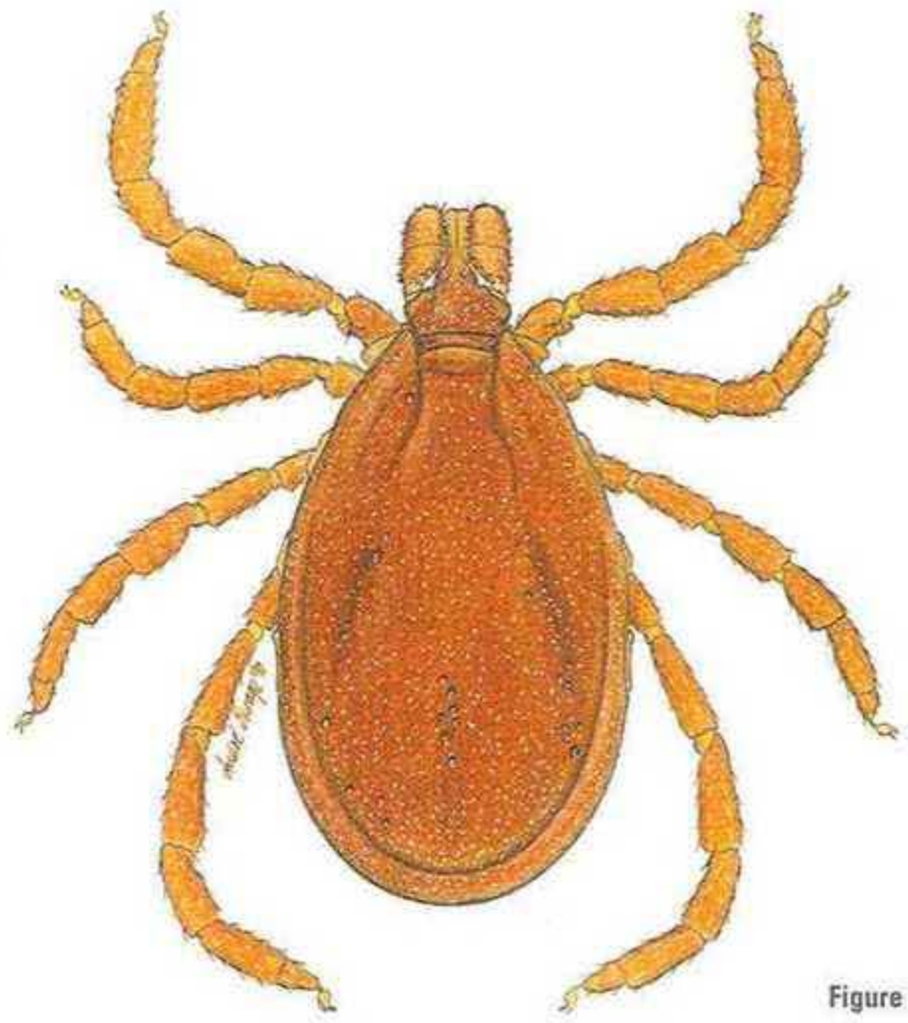


Figure 1.13 *Ixodes rubicundus* male

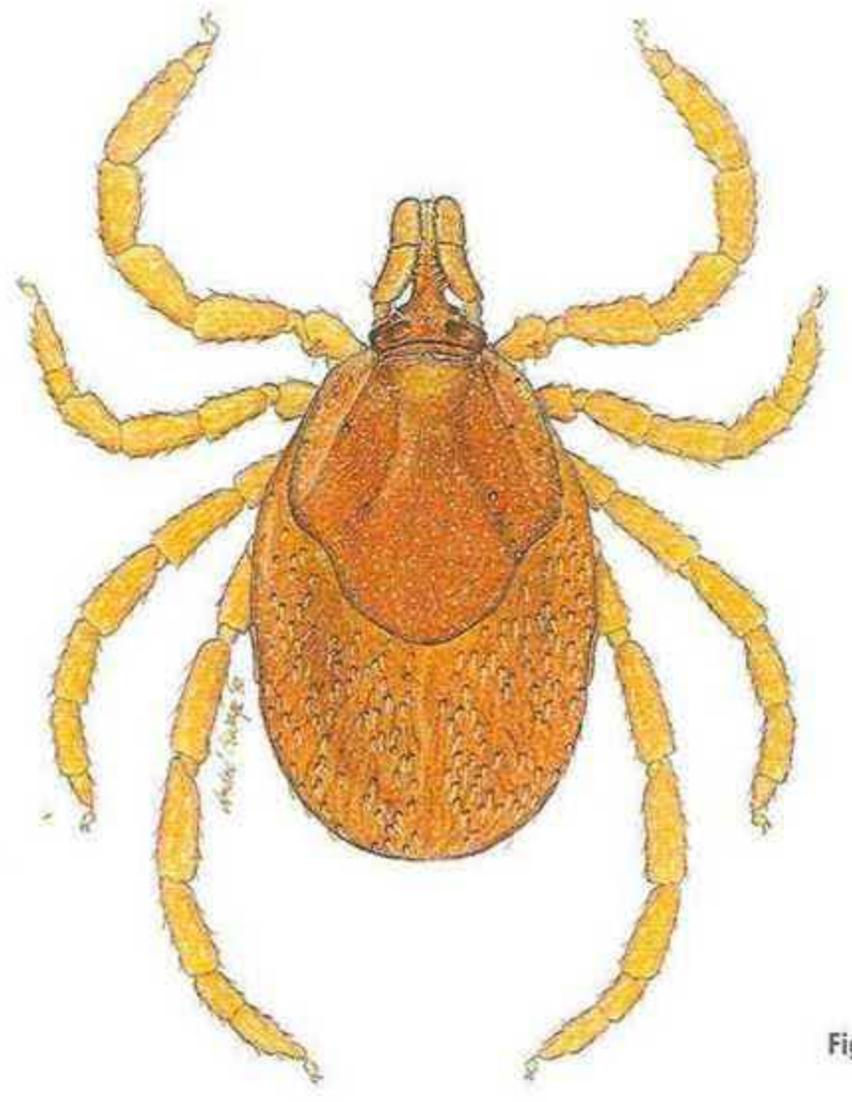


Figure 1.14 *Ixodes rubicundus* female

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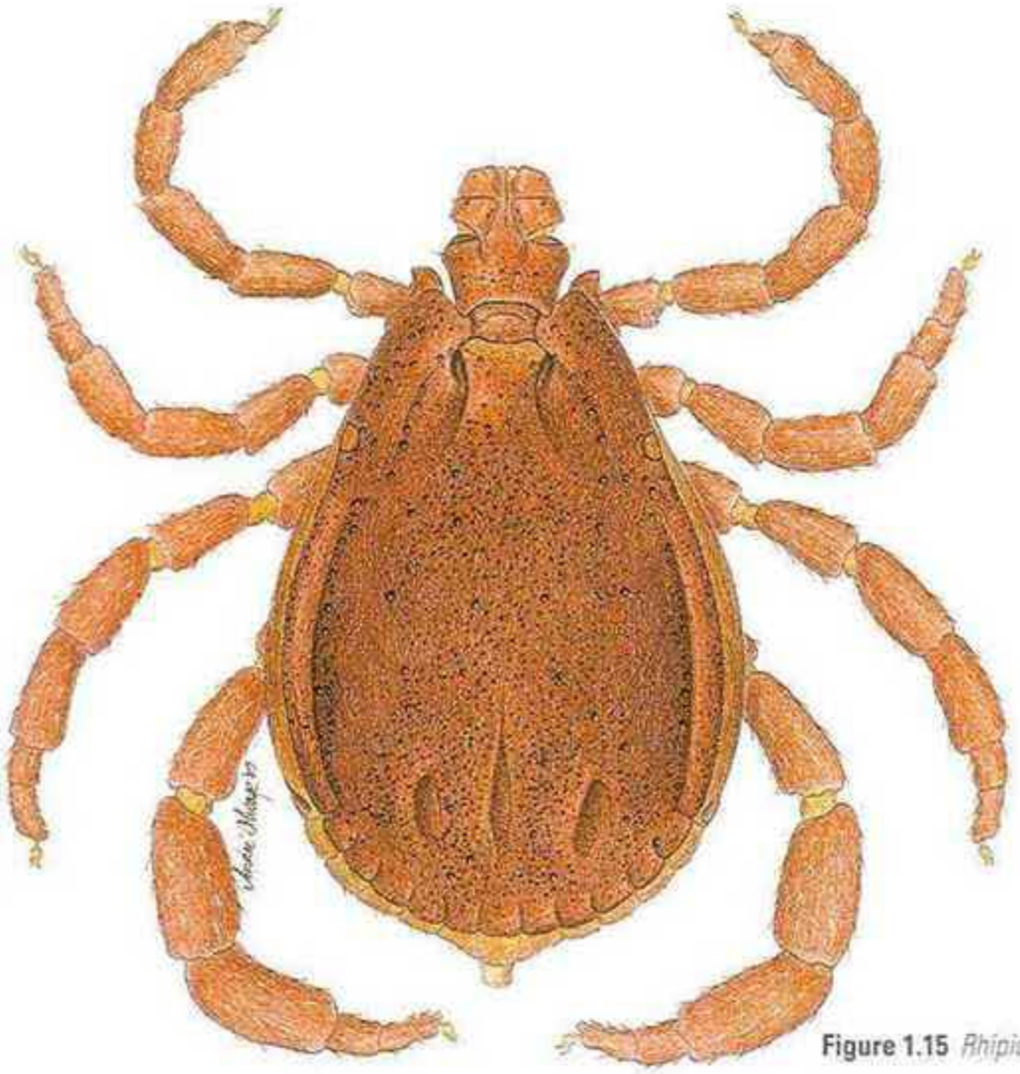


Figure 1.15 *Rhipicephalus appendiculatus* male

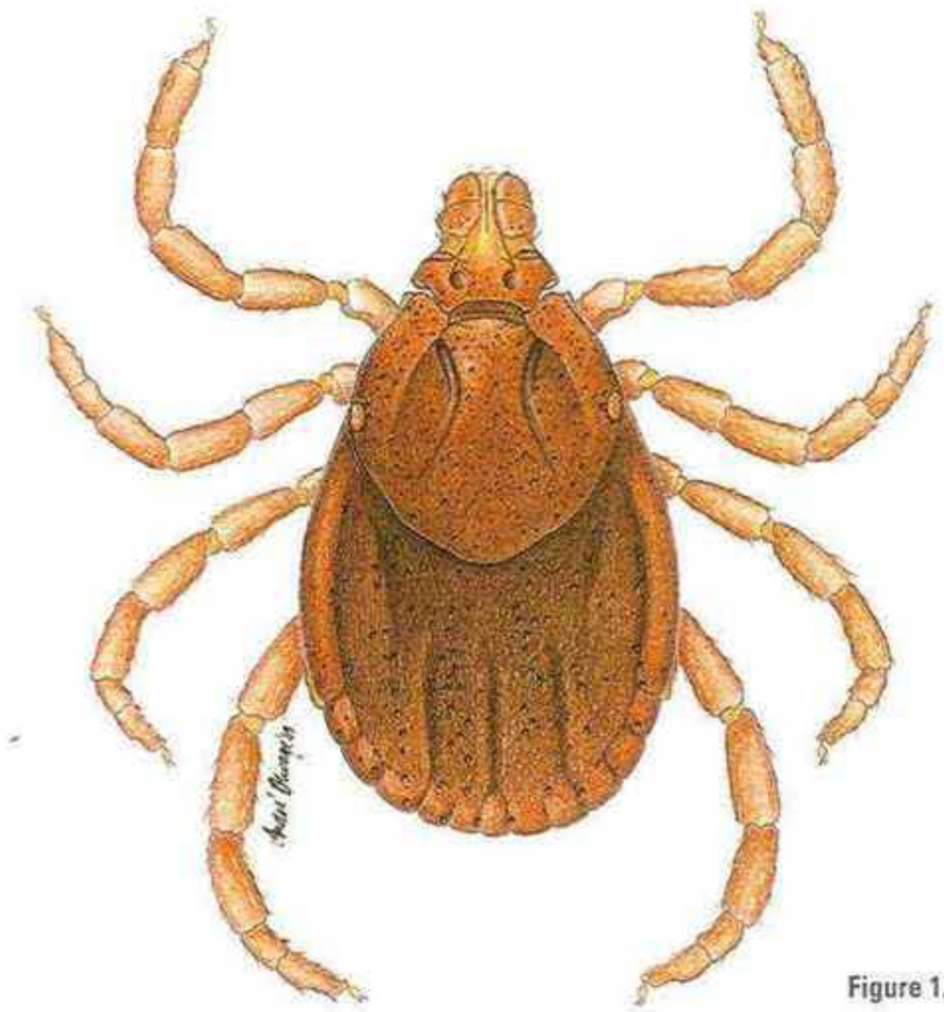


Figure 1.16 *Rhipicephalus appendiculatus* female

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Distribution *Rhipicephalus appendiculatus* is an eastern, central and southern African tick.[130, 288] Its distribution extends from southern Sudan, Uganda, south-western Kenya, eastern Democratic Republic of the Congo, Rwanda and Burundi to northern, north-eastern, central and south-western Tanzania. Further south it is confined to the wetter areas, which include the highlands of Malawi, Zambia, Zimbabwe and the Angonia and Chimoio provinces of Mozambique. The extent of its distribution in the coastal areas of Mozambique is unknown. It is also present in eastern Botswana and in Swaziland. In South Africa it occurs in the Limpopo, North West, Gauteng and Mpumalanga provinces, along the east coast of KwaZulu-Natal and nearly throughout the southern Eastern Cape Province.

This tick survives best in woodland and woodland savannah regions with good vegetation cover. It tends to disappear if overgrazing occurs and it does not survive on open plains. Its distribution may vary with rainfall. It was introduced into the south-eastern lowveld of Zimbabwe during the commencement of a wet cycle in 1973, and by 1982 it was estimated that more than 1 million ha of the lowveld was infested.[181] The tick started to disappear from this region towards the end of a dry cycle in 1983 and by 1985 it could no longer be found.[186] In eastern Zambia, *R. appendiculatus* spread southwards between 1972 and 1982 and then westwards during the 1980s and 1990s.[11]

Disease transmission *Rhipicephalus appendiculatus* is the chief vector of strains of *Theileria parva* that cause East Coast fever; other strains of *T. parva* that cause Zimbabwe theileriosis; and yet others that cause Corridor disease. This tick also transmits *Theileria taurotragi*, the cause of benign bovine theileriosis; *Ehrlichia bovis*, the cause of bovine ehrlichiosis; the virus causing Nairobi (and Kisenye?) sheep disease; and when large numbers of ticks are present they may produce sufficient toxin to cause brown ear tick toxicosis. Infection with *T. taurotragi* may cause severe or even fatal disease in eland (**see Chapter 33: *Theileria taurotragi* infection**). Exotic *Bos taurus* cattle suffer serious production losses and can become secondarily infested with the larvae of the screw-worm fly if heavily infested with adult ticks. In small wildlife parks situated in high rainfall regions large infestations with *R. appendiculatus* can constitute a serious problem in several antelope species.[133]

Control Exotic cattle should receive acaricide protection throughout the summer months. Zebu (*Bos indicus*) cattle and indigenous Sanga breeds become fairly resistant to the tick and so require fewer acaricide treatments.[195, 231, 233] In the absence of theilerioses, adequate control of adults can usually be accomplished by localized application of acaricides to the ears or head. Larvae and nymphs are considerably less damaging to cattle than adults, and do not require treatment if adequate control of the adults is achieved. Heavy infestations with adult *R. appendiculatus* can also occur on horses, and these may require treatment with acaricides during the summer. Control on other domestic livestock species is unnecessary.

Regular acaricidal treatment of cattle on a mixed cattle and game ranch in Central Province, Zambia, resulted in a significant reduction in all stages of development of *R. appendiculatus* on sympatric impala and on the vegetation of the ranch.[309] Should infestation with adult *R. appendiculatus* become a problem on wildlife in small reserves, control can be achieved by various methods based on the principle of self-application of acaricide.[57, 249]

Rhipicephalus duttoni

Angolan brown ear tick

Identification, life cycle, hosts and distribution *Rhipicephalus duttoni* is an ear tick that closely resembles *R. appendiculatus* in appearance.[250] It is a three-host species of which the adults parasitize particularly domestic cattle and African buffalo.[59, 273] It occurs in the coastal and adjacent inland regions of Angola, particularly in the south. Its southern and northern distributions extend to the north-western border of Namibia and the western coast of the Democratic Republic of the Congo respectively.[288]

Disease transmission In Angola *R. duttoni* is considered to be the vector of strains of *Theileria parva* causing Corridor disease.[31]

Rhipicephalus zambeziensis

Lowveld brown ear tick, laeveldse bruinoorbosluis (Afrik.)

Identification *Rhipicephalus zambeziensis* and *R. appendiculatus* are morphologically very similar. The major difference between the adults of the two species is that those of *R. zambeziensis* have a more conspicuously punctate scutum.[291]

Hosts and life cycle Its host range, attachment sites, life cycle and pattern of seasonal abundance are also similar to those of *R. appendiculatus*.[109, 198, 288] However, on impala the vast majority of adult ticks attach around the muzzle as opposed to the ears,[150] and a larger proportion of nymphs attach to the lower legs of greater kudu than do those of *R. appendiculatus*.[198]

Distribution The distribution of *R. zambeziensis* differs from that of *R. appendiculatus*.[130, 288] It replaces the latter tick in the hot, dry river valley systems of south-eastern Africa (e.g. Luangwa, Kafue, Zambezi, Limpopo and Sabi rivers) which separate the major highland areas. In contrast to *R. appendiculatus*, it occurs in the fairly dry environments of northern Namibia and in the lowland areas of the Mozambique interior. It is also present in southern Angola, western and south-eastern Zambia, and in Tanzania north

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of Lake Malawi.[288] The distributions of *R. zambeziensis* and *R. appendiculatus* overlap where there are gradual transitions between wet and dry areas. This occurs in parts of the Eastern and Southern provinces of Zambia bordering the Zambezi Valley, in eastern Botswana, and in the North West, Limpopo and Mpumalanga provinces of South Africa.[95, 130, 288] In these situations some interspecific hybridization may occur.[310] *Rhipicephalus zambeziensis* is absent from semi-desert and desert areas.

Disease transmission Strains of *Theileria parva* causing Corridor disease; strains of *Theileria parva* causing Zimbabwe theileriosis; *Theileria taurotragi*, the cause of benign bovine theileriosis; and *Ehrlichia bovis*, the cause of bovine ehrlichiosis. Although *R. zambeziensis* can, in the laboratory, transmit strains of *T. parva* associated with Zimbabwe theileriosis,[129] it is not associated with outbreaks of the disease in the field.[181] However, the presence of *T. parva* group antibodies in cattle in buffalo-free areas, in which the tick occurs in southern Zimbabwe, suggests that it does transmit subclinical infection.[181]

Control *Rhipicephalus zambeziensis* differs from *R. appendiculatus* in that its populations never build up to numbers that could be expected to cause significant production losses in cattle. For this reason there is little, if any, need to direct specific control measures against this tick species, other than to protect cattle from infection with the *T. parva* group of organisms where contact with buffalo occurs.

Rhipicephalus evertsi evertsi

Red-legged tick, rooipootbosluis (Afrik.)

Identification Adult *R. evertsi evertsi* are medium-sized ticks that are easily recognized by the dark-brown colour of their heavily punctate scuta, beady eyes and orange to red legs (**Figures 1.17 and 1.18**).

Hosts The preferred hosts of the adults are domestic and wild equids (horses, donkeys, mules and zebras) although cattle, sheep, goats and a number of antelope species, particularly eland, are also frequently parasitized.[86, 97, 100, 171] The adults attach in the peri-anal and inner thigh regions of their ungulate hosts.[8] Larvae and nymphs feed deep in the external ear canals,[8] and are found most commonly on the same ungulate hosts as the adults, as well as on hares.[109, 171]

Life cycle *Rhipicephalus evertsi evertsi* has a two-host life cycle in which the larvae moult to nymphs on the first host, the nymphs engorge, detach, fall off and moult, and the adults attach to the second host. With the possible exception of some of the colder regions the tick can complete more than one generation per year.[149] All stages may be present on hosts throughout the year, but their abundance can vary from season to season.

Distribution Of the 60-odd *Rhipicephalus* spp. present in sub-Saharan Africa, *R. evertsi evertsi* is the most widespread. It is most common in the eastern half of the continent, from Eritrea and Sudan in the north to South Africa in the south.[288] It tolerates a wide range of climatic conditions and in southern Africa the main factor limiting its distribution in the west is increasing aridity, with the critical rainfall level being about 250 to 280 mm per annum.[112]

Disease transmission *Anaplasma marginale*, the cause of bovine anaplasmosis; *Babesia caballi* and *Theileria equi*, the cause of equine piroplasmosis; *Theileria separata*, the cause of ovine theileriosis; *Borrelia theileri*, the cause of borreliosis or spirochaetosis; and the toxin causing spring lamb paralysis. This tick can also experimentally transmit *Babesia bigemina*, the cause of bovine babesiosis.[25] In the eastern highveld regions of the Mpumalanga and Free State provinces in South Africa, the synchronous moulting during spring of over-wintered nymphs gives rise to large numbers of adults,[111] and these may infest newly born lambs. As the females engorge they secrete a toxin which causes paralysis.[79] Locally this is known as spring lamb paralysis because of the season of its occurrence and the age of the animals affected.

Control Adult *R. evertsi evertsi* is easily controlled by localized application of acaricide to the peri-anal area. Usually no specific control measures are taken against the immature stages, but these could be controlled by the topical application of acaricides to the inner surface of the ears and to the ear canals. The danger of piroplasmosis in Thoroughbred racehorses often warrants the expense of treatment, in which case both the immature and adult ticks should be controlled.

Rhipicephalus evertsi mimeticus

Namibian red-legged tick, Namibiese rooipootbosluis (Afrik.)

Identification *Rhipicephalus evertsi mimeticus* looks like *R. evertsi evertsi*, but has red- and ivory-coloured banded legs similar to those of certain *Hyalomma* spp. However, the structure of its capitulum and its shorter mouthparts readily distinguish it from the *Hyalomma* spp.[250, 290]

Hosts, life cycle, distribution and control *Rhipicephalus evertsi mimeticus* would seem to have the same host preferences, predilection attachment sites and life cycle as *R. evertsi evertsi*.[288] The adults are most numerous from November to May, and the immature stages in February and March and from May to September.[16] This tick occurs in

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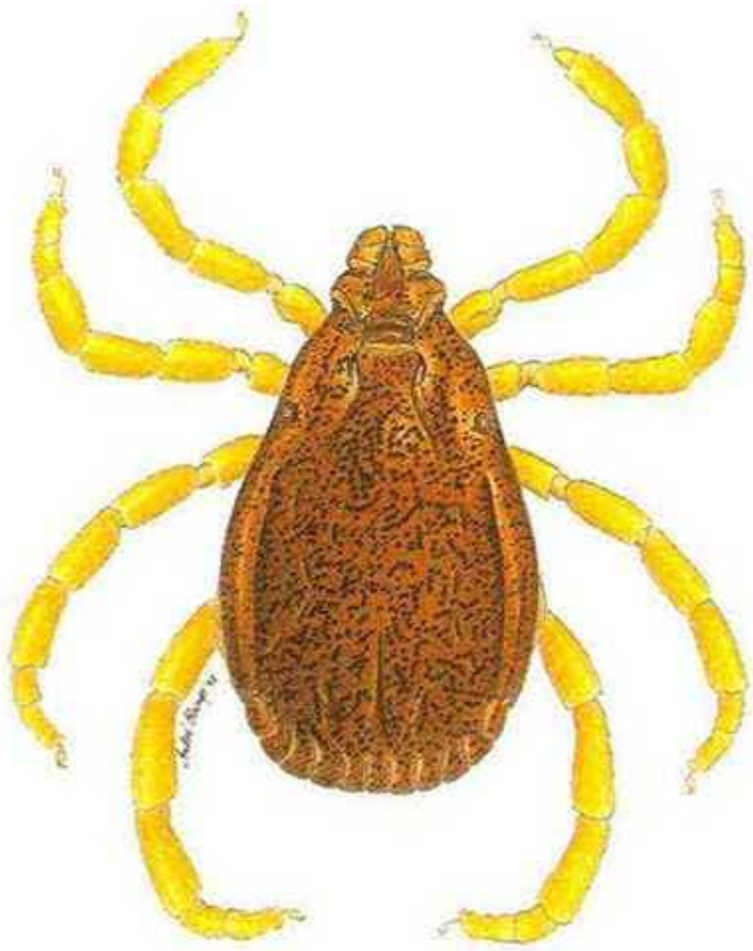


Figure 1.17 *Rhipicephalus evertsi evertsi* male

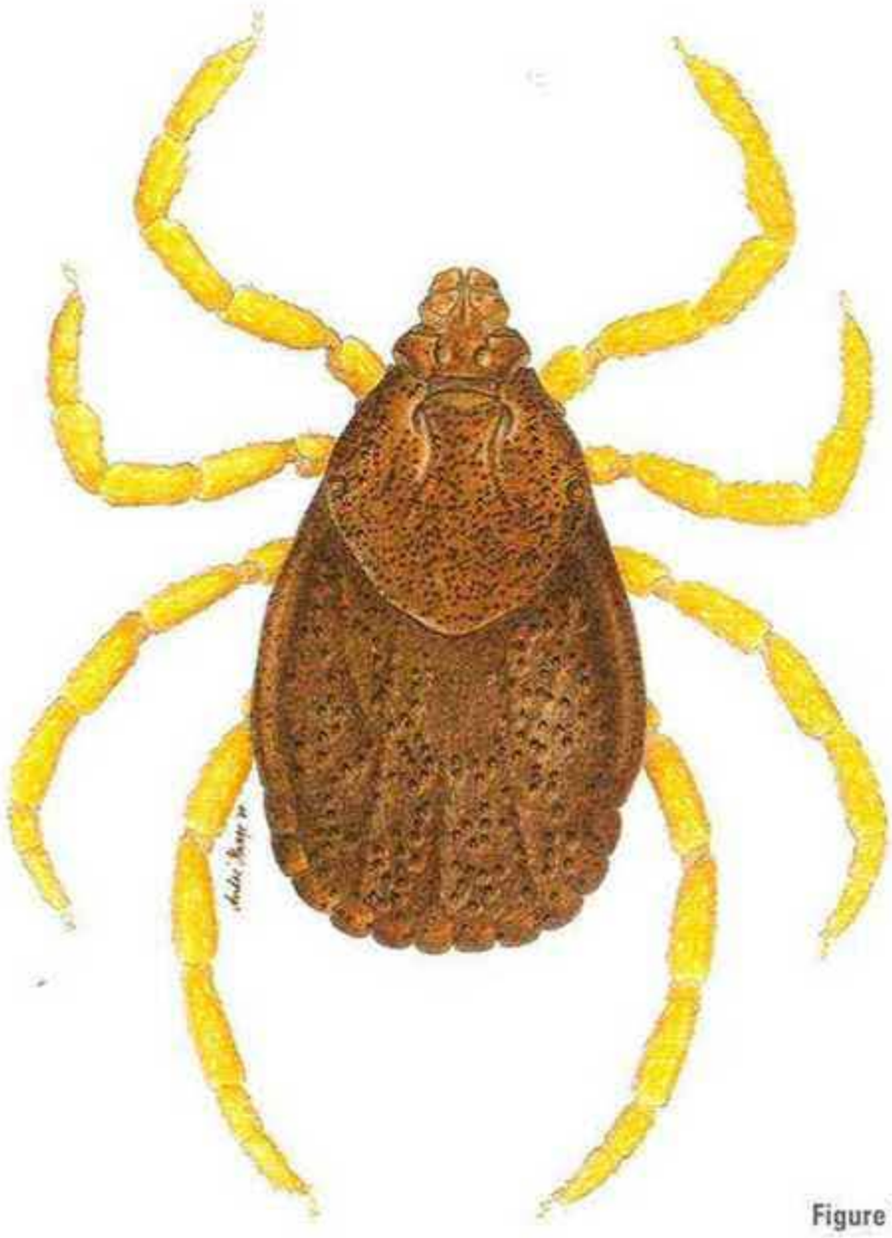


Figure 1.18 *Rhipicephalus evertsi evertsi* female

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western Botswana, central and northern Namibia and southern and western Angola.[288, 290] Its control is the same as that of *R. evertsi evertsi*.

Disease transmission *Theileria equi*, the cause of equine piroplasmosis; and *Theileria separata*, the cause of ovine theileriosis.

Rhipicephalus bursa

Identification *Rhipicephalus bursa* is a large light-brown species. The scutum is covered with numerous fine punctations. In engorged males, the light tan body wall expands laterally and posteriorly beyond the darker scutal margins.

Hosts All stages of development feed on cattle, sheep, goats and camels.[288] Adult ticks attach mainly on the inner surface of the ears of sheep but can also attach on the dorsal outer surface, as well as in the perineal and inguinal regions of these animals.[288]

Life cycle *Rhipicephalus bursa* has a two-host life cycle that takes a year to complete. Adult ticks are most abundant on sheep during summer in the northern hemisphere (June and July).[288]

Distribution The Mediterranean, Adriatic and Aegean basins, and in Libya, Tunisia, Algeria and Morocco in North Africa. *Rhipicephalus bursa* is also present in some countries in southern Europe as well as in the Near East.[288]

Disease transmission *Babesia bigemina*, the cause of bovine babesiosis or African redwater; *Babesia caballi* (experimentally) and *Theileria equi*, the cause of equine piroplasmosis; *Ehrlichia ovina* (experimentally), the cause of ovine ehrlichiosis; *Anaplasma ovis*, the cause of ovine anaplasmosis; *Theileria separata*, the cause of ovine theileriosis; and the transmission of a toxin causing paralysis in sheep.

Control Acaricide can be applied to the ears of sheep during the summer months.

Rhipicephalus lunulatus

Identification, hosts and life cycle *Rhipicephalus lunulatus* is a small dark-brown tick. Its preferred domestic hosts are cattle, sheep, pigs and dogs, and its wild hosts are warthog, bushpig (*Potamochoerus* sp.) and African buffalo.[288, 289] On cattle it attaches on the legs, including the feet, and in the tail switch.[29] The hosts of the immature stages are certain rodent species and hares.[288] It is a three-host tick, and the adults appear to be most active during the rainy season.[29, 289]

Distribution It has an extensive distribution range, from Senegal in the west to Somalia in the east and thence southwards, mainly in the eastern half of the continent, to KwaZulu-Natal, South Africa.[288]

Disease transmission *Babesia trautmanni* (suspected), the cause of porcine babesiosis. If *R. lunulatus* indeed is a vector of *B. trautmanni*, infection would have to be transmitted transovarially from the adults of one generation to the adults of the next generation because the immature stages do not feed on suids but on rodents and hares. Another possibility would be the migration of infected adult ticks from one host to another. This tick also produces a toxin causing paralysis in calves and lambs.

Rhipicephalus pulchellus

Zebra tick, yellow-backed tick

Identification Adult *R. pulchellus* are medium-sized to large ticks with a characteristic dark brown-and-ivory pattern on the scutum of the male, while the scutum of the female is predominantly ivory-coloured with anterior dark markings (**Figures 1.19 and 1.20**).

Hosts The adults prefer cattle, on which they may occur in large numbers, as well as camels, sheep and goats as hosts.[202, 287, 288] They attach primarily on the ears and the underside of the body, including the chest, belly, genital and peri-anal areas. Their preferred wild hosts are zebra, black rhinoceros, eland and gemsbok. The immature stages feed on these animals as well as on hares.[28, 287, 288]

Life cycle and distribution *Rhipicephalus pulchellus* has a three-host life cycle. The adults appear to be most active during the rainy season.[202] It is one of the most common ticks present in the Horn of Africa, as well as in and to the east of the Rift Valley from Eritrea in the north to north-eastern Tanzania in the south.[288]

Disease transmission *Theileria taurotragi*, the cause of benign bovine theileriosis; and the virus causing Nairobi sheep disease.

Control Strategic control of the adults can be achieved by acaricidal treatment during the rainy season.

Rhipicephalus simus

Glossy brown tick, blinkbruinbosluis (Afrik.)

Identification Adult *R. simus* are large, dark-brown ticks. The scutum has a glossy appearance and there are four longitudinal rows of large punctations on the scutum of the male.

Hosts The preferred domestic hosts of the adults are cattle, horses and dogs.[185, 208] The adults attach in the tail brush and around the feet of cattle and horses and around the head and

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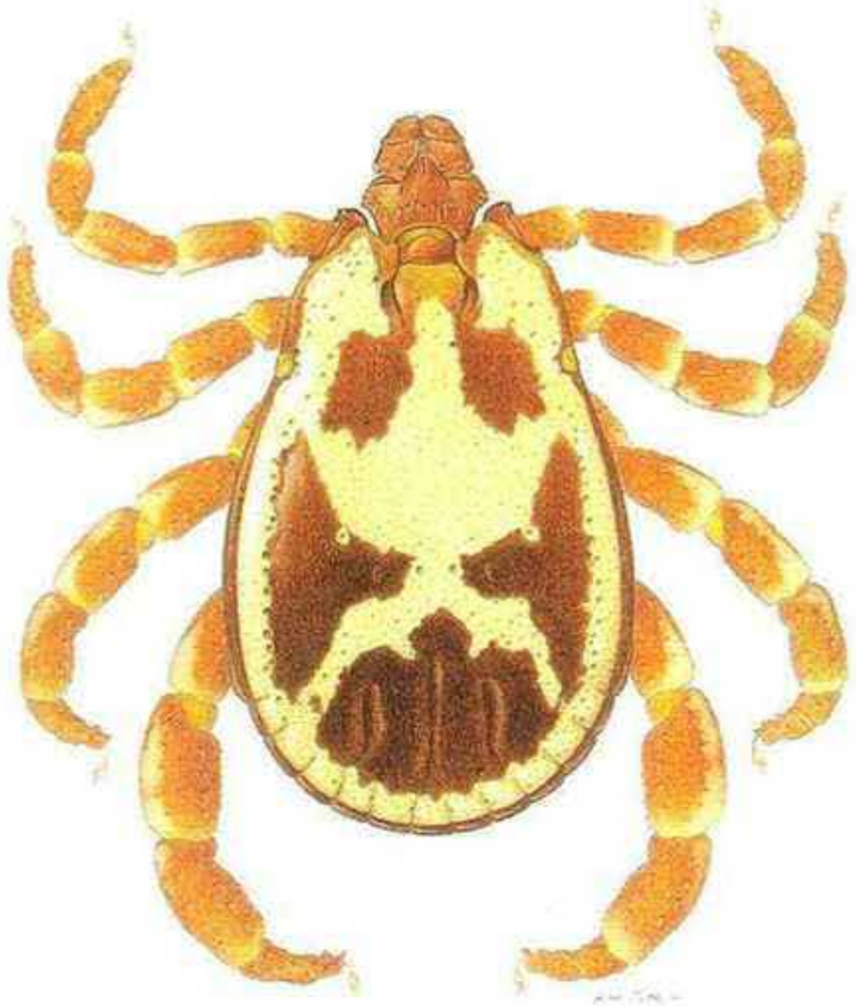


Figure 1.19 *Rhipicephalus pulchellus* male

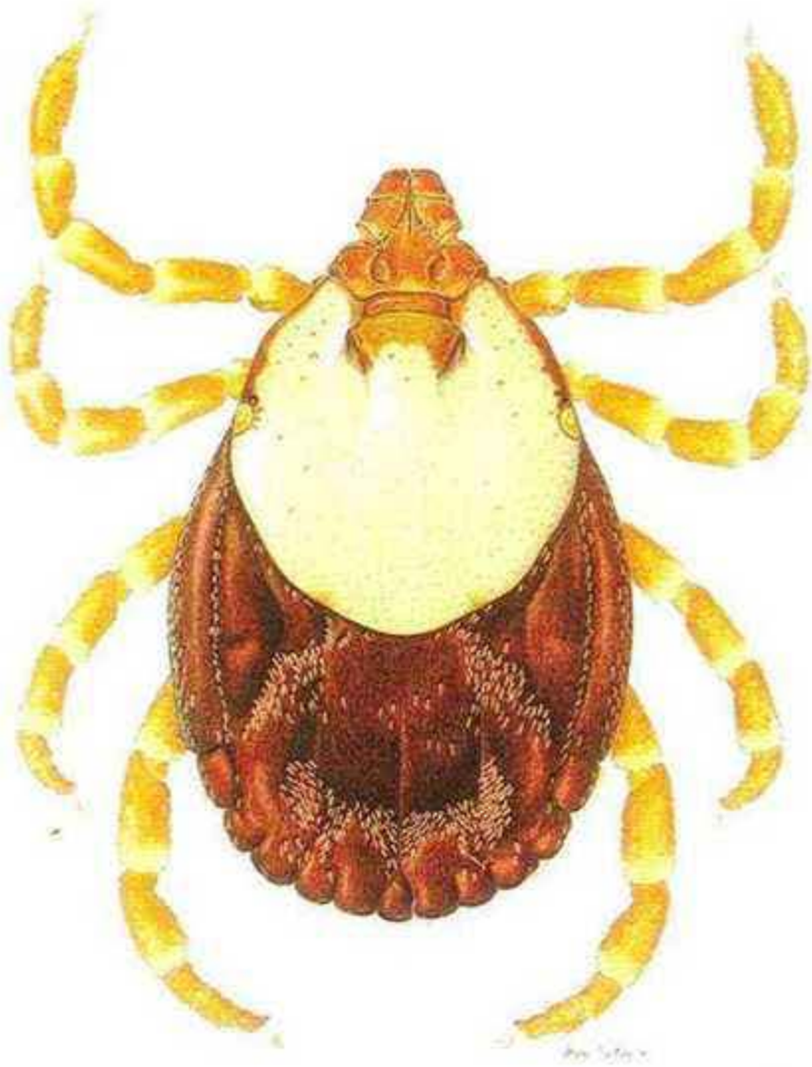


Figure 1.20 *Rhipicephalus pulchellus* female

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neck of dogs.[8, 139, 288] Monogastric wild animals, such as the larger wild carnivores, zebra, rhinoceros, warthog and bush-pig, are particularly good hosts of the adults.[93, 97, 101, 185] The immature stages are parasitic on murid rodents.[19, 185, 230] Adult abundance appears to be limited by the availability of hosts for the immature stages.[108, 169]

Life cycle and distribution *Rhipicephalus simus* is a three-host tick of which the adults are most abundant during the warm, wet summer months, and the immature stages during the cooler, dry autumn to spring seasons.[19, 93, 139, 230] It is widely distributed in the moderate to high rainfall areas of Angola, Zambia, Zimbabwe, Malawi and Mozambique in the north to South Africa in the south.[288] but rarely occurs in large numbers. In the north-east of the continent it is replaced by *Rhipicephalus praetextatus* and in the north-west by *Rhipicephalus muhsammae*, both ticks that are very similar in appearance to *R. simus* and with which it has been confused in the past.[288]

Disease transmission *Anaplasma marginale*, the cause of bovine anaplasmosis; *Anaplasma centrale*, the cause of benign anaplasmosis; *Babesia trautmanni*, the cause of porcine babesiosis; and a toxin causing paralysis in calves and lambs.

Although transstadial transmission of *A. marginale* to cattle is possible,[221] this hardly seems likely to occur in the field as the immature stages feed virtually exclusively on rodents. Intrastadial transmission by infected adult ticks, and more particularly males, wandering from one host to another seems a more likely route of infection.[221] Infection of suids with *B. trautmanni* would seem to occur by the *Babesia* being passed transovarially from one generation of adult ticks to the next generation of adult ticks, which then feed on susceptible animals.[50]

Control Except as a measure to prevent anaplasmosis in susceptible cattle and occasionally to prevent paralysis in calves and lambs, acaricidal control of this species is probably not justified.[185]

Rhipicephalus turanicus

Identification *Rhipicephalus turanicus* is a moderate-sized, reddish-brown tick, which even under a stereoscopic microscope is difficult to differentiate from the kennel tick *Rhipicephalus sanguineus*, with which it has in the past been confused.[201] The latter tick virtually exclusively parasitizes domestic dogs.[288]

Hosts The domestic animals from which adult *R. turanicus* have most frequently been collected in sub-Saharan Africa are cattle, sheep and dogs, with no collections from pigs and only a few collections from horses. Its favoured wild hosts are the larger carnivores, hares and some of the larger ground-feeding birds, with a few collections from zebra and warthog.[201, 288] The hosts of the immature stages include hedgehogs, shrews, gerbils, murid rodents and hares.[288]

Life cycle and distribution *Rhipicephalus turanicus* is a three-host tick of which the adults generally are most numerous during the late rainy to early dry seasons.[109, 201]

In sub-Saharan Africa the majority of records originate from the eastern parts of the continent from Sudan, Ethiopia and Somalia in the north to South Africa in the south. However, several collections have also been made in northern Namibia. This tick occurs in several of the Mediterranean countries as well as in their immediate neighbours and also in Russia, India and Pakistan.[288]

Disease transmission *Babesia caballi* and *Theileria equi* (both suspected), the cause of equine piroplasmosis; and *Babesia trautmanni* (experimentally), the cause of porcine babesiosis.

Because *R. turanicus* has frequently been confused with *R. sanguineus*, reports of the transmission of equine piroplasmosis by *R. sanguineus* could have referred to *R. turanicus*.[60] However, attempts to transmit *B. caballi* and *T. equi* to horses transstadially from nymphs to adults with a South African strain of *R. turanicus* have been unsuccessful.[222] Transovarial transmission of *B. trautmanni* by adult *R. turanicus* seems the most likely possibility in the field,[134] because the immature stages of this tick do not normally feed on pigs.

Rhipicephalus spp.

Hosts, life cycle, distribution and disease transmission Two *Rhipicephalus* spp., other than those already mentioned, have been implicated as the cause of livestock paralysis (toxicosis). One is *Rhipicephalus warburtoni* (previously recorded as an undescribed species of the *R. pravus* group or as *R. punctatus*). The adults of *R. warburtoni* prefer domestic goats as hosts and the immature stages rock elephant shrews.[66, 70] However, all stages of development may be encountered on scrub hares and Cape hares (*Lepus capensis*).[98] On very young Angora goat kids adult ticks attach to the head and ears, while on older goats they attach here as well as on the neck and the brisket.[71] *Rhipicephalus warburtoni* is a three-host species of which the adults are most abundant on goats from September to February, and the larvae and nymphs on elephant shrews from December to July, and from April to October respectively.[66, 70] In South Africa, it is found only in the Free State and Northern Cape provinces.[288] Acaricide can be applied to the heads and ears of very young goat kids to prevent paralysis.

The other tick is *R. praetextatus* (previously recorded as *R. simus*), whose adults and immature stages have a host preference similar to that of *R. simus*.[208] On cattle the adults attach mainly to the tail brush and the feet. *Rhipicephalus praetextatus* is a three-host tick, and its adults are most abundant during the rainy season.[208] This tick is widespread

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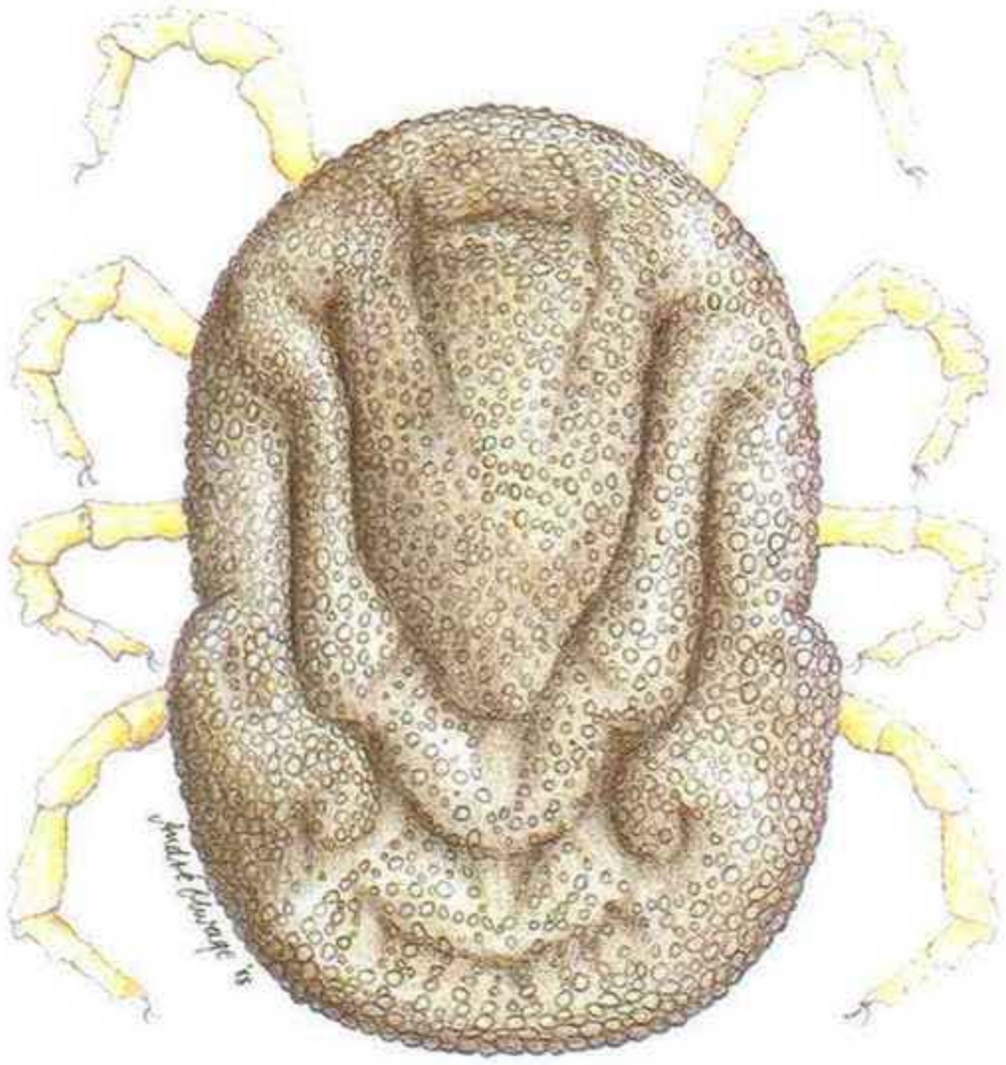


Figure 1.21 An adult tick of the *Ornithodoros moubata* complex



Figure 1.22 A typically heavy infestation of *Boophilus decoloratus*

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in north-eastern Africa from Egypt to Tanzania.[208, 288] Not only can it cause paralysis, but it can also transmit the virus causing Nairobi sheep disease.[132]

Ornithodoros moubata complex

Eyeless tampan

Identification The eyeless tampans belonging to this complex lack the sclerotized dorsal scutum that characterizes the hard ticks, and their integument has a mammilated appearance (**Figure 1.21**). Male and female ticks can only be differentiated with certainty under a stereoscopic microscope.

Hosts and life cycle Ticks of the *O. moubata* complex are parasites of warthog, and complete their life cycle in warthog burrows. The adult female tick lays a batch of eggs in a sheltered locality in the burrow after each blood meal. After hatching the larvae do not feed, and they moult to the first nymph stage after one or two days. The first nymph stage seeks a warthog host on which it feeds briefly before moulting to the second nymph stage in the burrow. This process is repeated for each of the four or five nymph stages, with the last nymph stage moulting to an adult. The feeding periods of the various nymph stages and the adults are of short duration (approximately 30 minutes), and feeding takes place when the warthog hosts are at rest in their burrows. The nymphs can survive for two years without feeding and the adults even longer.

Distribution The distribution of ticks of the *O. moubata* complex that parasitize warthog follows that of their warthog hosts, which still occur in large areas of southern Africa (**see Chapter 98: African swine fever**). However, as the density of human habitation increases, these areas are decreasing in size and in number, and wildlife reserves are becoming the foci of warthog distribution. Locally the ticks are spread via their nymphal stages, which are commonly found on warthog foraging outside their burrows.[92, 93, 277]

Disease transmission The virus causing African swine fever is spread from male to female ticks by means of the spermatophore during mating.[217] It is transmitted transstadially and transovarially by the ticks.[218, 219]

Control No control measures need be taken against ticks in warthog burrows, but to prevent their spread warthog that are to be moved should be sprayed with an acaricide prior to translocation in order to kill tampans that may be present on their hides.

Control of ticks and tick-borne diseases

Cattle have been present in southern Africa for more than a thousand years,[83] and until the twentieth century survived without specific control measures against ticks and tick-borne diseases. It is now known that the indigenous Sanga and Zebu breeds can develop resistance to ticks,[17, 195, 231, 232, 233, 251] and will also acquire immunity to tick-borne diseases if exposed to them at an early age.[176, 304] Situations in which cattle acquire immunity to tick-borne diseases through natural exposure as calves are called endemically stable.[26] Most losses due to tick-borne diseases occur in endemically unstable situations where there are insufficient infected ticks to ensure that all calves receive challenge. Endemic instability exists in areas that are only marginally suitable for the survival of the tick vectors or where tick populations have been suppressed but not eradicated through the use of acaricides. Tick-borne diseases also cause losses if they are introduced, together with their vectors, to new regions and spread through susceptible livestock populations, or if susceptible animals, especially exotic breeds, are moved to endemic areas.

Historical perspective

Control measures against ticks and tick-borne diseases were only instituted on a large scale in southern Africa following the introduction of East Coast fever (caused by strains of *T. parva*) from East Africa. Prior to that, tick-borne diseases had not been reported as problematic in indigenous cattle, although losses caused by babesiosis and heartwater had been experienced in imported exotic cattle.[128] One of the most serious tick-borne disease problems of the nineteenth century was the spread of heartwater through the coastal areas of the Eastern Cape Province because of the spread of the vector *A. hebraeum*, introduced by cattle which had been taken to KwaZulu-Natal for winter grazing.[135] The other was the occurrence of babesiosis in cattle imported for restocking following the rinderpest pandemic of 1896.[128]

East Coast fever, which was introduced to Mozambique, Zimbabwe and South Africa in 1901/02, caused mortality of around 95 per cent in the herds of susceptible cattle (indigenous and exotic) to which it spread.[163] It caused serious economic losses, affecting agriculture, mining and commerce, all of which still used ox-drawn transport. Considerable effort was therefore devoted to the development of control measures. Early attempts at vaccination by the eminent microbiologist Robert Koch and later by Sir Arnold Theiler were largely unsuccessful. However, once it was shown by Lounsbury that the disease was transmitted by the brown ear tick, *R. appendiculatus*,[137] more successful control measures based on tick control, quarantine procedures, pasture spelling, slaughter, and dipping in arsenic solutions were instituted. Dipping proved to be the most practical and effective measure. It was widely adopted and later made compulsory. As a result the disease was brought under control fairly rapidly and then progressively eradicated. Complete eradication of East Coast fever from southern Africa was considered to have been achieved by 1960.

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After the eradication of East Coast fever two options were open to southern African countries. They could either control the remaining major tick-borne diseases (babesiosis, anaplasmosis and heartwater) by vaccination and move towards reduced tick control and endemic stability, which had previously existed, or they could continue to control the diseases by intensive dipping. In South Africa compulsory dipping was first abolished on commercial cattle farms and the choice of control strategies was left to individual farmers. In the communal grazing regions of the Eastern Cape Province the management of the dipping service was handed over to the former homeland administrations during the 1970s. Although the service was maintained by these administrations, enforcement of the dipping programme was gradually relaxed and abolished after independence of the Transkei and Ciskei in 1975 and 1981 respectively. When these regions were reincorporated into South Africa in 1994 the Provincial Departments of Agriculture assumed responsibility for the dipping service and supplied both personnel and chemicals. Despite the relaxation of enforcement 98 per cent of livestock owners interviewed continued to partake in all dipping events, with disease control given as the main reason for participation.[146] Compulsory dipping has now been abolished throughout South Africa. However, the provinces of KwaZulu-Natal and Mpumalanga still provide personnel and chemicals in the communal grazing areas and report good attendance at each dipping event. In North West Province cattle owners utilizing a communal grazing area have been encouraged to participate in a research and extension project during which they were taught skills enabling them to make better informed decisions on the level of tick control they wished to achieve.[122]

The dipping policy in South Africa has had mixed results. Some progressive farmers took advantage of the availability of vaccines against babesiosis, anaplasmosis and heartwater; used them effectively, and were able to adopt flexible dipping regimes. Others, for reasons that were probably only partially understood, practised little or no tick control and contained tick-borne diseases through the maintenance of endemic stability. The majority, however, continued to treat cattle at regular intervals with acaricides. Few actually succeeded in eradicating ticks or tick-borne diseases,[44, 46, 47] and frequently the effect in endemic areas was to maintain instability where stability would otherwise have existed. In these circumstances losses due to tick-borne diseases commonly occurred if vaccines were not regularly used.

In Zimbabwe compulsory dipping continued to be enforced after the eradication of East Coast fever. However, it should be noted that Zimbabwe theileriosis caused by strains of *T. parva* persisted and it was partly for the control of this disease that compulsory dipping was continued. For 20 years after the eradication of East Coast fever, tick-borne diseases were effectively controlled in Zimbabwe by dipping. Control was particularly good in the communal farming areas, where dipping, together with overgrazing, which rendered the environment less suitable for tick survival, frequently resulted in the localized eradication of ticks.[167] However, this situation came to an end in the mid-1970s with the disruption of dipping in these areas during the pre-independence war. Large epidemics of tick-borne diseases occurred and losses over a five-year period amounted to more than a million head, which was one-third of the cattle owned by traditional farmers.[125, 170] These losses caused the Zimbabwe veterinary authorities to re-assess the national policy on tick and tick-borne disease control.[173] After independence in 1980, dipping was reintroduced in the traditional farming areas but was not strictly enforced. From 1985 a similar approach was adopted for commercial farmers, who were only required to have tick-free cattle if the animals were to be moved off their properties.

Intensive dipping is still practised in Swaziland, but is not as strictly enforced as in the past. In other countries in the region, including Namibia, Botswana and Angola, intensive dipping is not widely practised or enforced. It has been more extensively used in Malawi, Zambia and Mozambique for the control of theileriosis and heartwater, but not throughout each of these countries.

No movement away from intensive tick control can be made unless it is possible to control tick-borne diseases of cattle (and sometimes those of other livestock) by immunization, and then to manage the diseases to ensure continuing immunity. Management may involve regular immunization of calves or simply the maintenance of endemic stability through tick exposure. The requirements for tick-borne disease control are therefore reliable sources of vaccines, and knowledge of the epidemiology of the diseases. At present all vaccines against tick-borne diseases contain live organisms and for this reason either have a very short shelf-life (unfrozen blood vaccines) or must be stored at very low temperatures and transported in liquid nitrogen or on dry ice (frozen-blood vaccines or infected-tick stabilates). Vaccines against anaplasmosis, babesiosis and heartwater are currently available from Onderstepoort Biological Products, South Africa, and can be supplied to most parts of southern Africa.

Recent research

Debate in southern Africa on the role of acaricides for the control of tick-borne diseases was most intense in the early 1980s. By then there was considerable evidence from epidemiological studies that endemic stability was more widespread than previously believed and that intensive dipping frequently contributed to problems with tick-borne disease.[44, 46, 47, 116, 180, 181, 182] It was argued by many people that strict tick control was necessary to prevent large production losses, but no realistic information on the losses caused by African ticks was available although data existed on the effect of *B. microplus* on the productivity of cattle in Australia.[259, 265] In addition more quantitative information on the resistance of different breeds of cattle to African ticks was also required.

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It was in response to these needs that experiments have been conducted in several African and other countries.

In Zimbabwe a project to determine the effects of *A. hebraeum* infestation on live-weight gain indicated that larvae and nymphs caused no detectable losses whereas adults did, the loss per female tick completing engorgement being approximately 10 g.[192] Estimated losses in live-weight gain for *A. variegatum* appear to be considerably more severe, amounting to 46 to 61 g per engorged female,[203] while those for *A. lepidum* amounted to 6 g.[258] In the lowveld of Zimbabwe the effects of infestation with adult *A. hebraeum* on the milk production of Sanga and Sanga x Brahman cows was measured over an 11-week period. Although the relationship between tick numbers and reduction in milk yield or calf live-weight gain during the observation period was poor, provided teats had not been damaged, an average loss in live-weight gain of approximately 6 g per calf for every engorged female tick was recorded.[193]

On a mixed bushveld farm in Mpumalanga Province, South Africa, the weaning weights of calves of which the dams were predominantly infested with *B. decoloratus*, were reduced by approximately 8 g for each engorged female present on the cows.[241] In Australia mid-lactation Holstein-Friesian cows were infested at weekly intervals with gradually increasing numbers of *B. microplus* larvae for a period of 15 weeks.[117] By week 15 uninfested control cows produced 2,86 litres more milk and 0,14 kg more butterfat per day and had gained 10,6 kg more live-weight than the infested cows. It was estimated that each engorging female tick was responsible for a loss of 8,9 ml in daily milk production and 1,0 g in body-weight gain over the trial period.

In Zimbabwe larvae and nymphs of *R. appendiculatus* caused no detectable losses in live-weight gain of Sanga and European breed cattle, whereas adult ticks caused losses which amounted to approximately 4 g for every female that completed feeding.[195] Under field conditions the weight losses caused by the adults were large in *Bos taurus* cattle (about 25 kg over one summer) as the animals had low resistance to the ticks. Losses in Sanga cattle which had high resistance, however, were small and frequently insignificant.[195] Despite severe ear damage in some animals, infestations averaging 20 engorging female *R. appendiculatus* per day had no significant effect on the milk yield of *B. taurus* dairy cows nor that of crossbred *B. taurus* x Sanga cattle kept on higher and lower planes of nutrition, respectively, under commercial management on the Zimbabwe highveld.[191] Infestation with adult *R. appendiculatus* of Sanga cows receiving nutritional supplement on the Zimbabwe highveld affected their milk yield, as judged by the weights of their calves before and after suckling, but had no significant effect on the live-weight gains of the calves compared with those of tick-free cows.[196] In Zambia tick infestations, comprising mainly *A. variegatum* and *R. appendiculatus*, on Sanga cows caused a significant decrease in the amount of milk produced.[203]

Because much of the information on the effects of ticks on their cattle hosts has been obtained using experimental single species infestations, it is of limited value under field conditions in Africa since in most regions of the continent animals are infested with several species simultaneously.[42] The effects of ticks are also often linked with those of tick-borne diseases and should be considered in conjunction with the latter. Furthermore, cattle herds contain animals of different ages, sexes and types, and experiments to determine the effects of ticks should be conducted on entire herds rather than on individual or selected age groups of animals.[42]

Two projects supported by the Food and Agriculture Organization of the United Nations have investigated the economic impact of ticks on cattle in Zambia with the aim of obtaining data on the cost-effectiveness of tick control in cattle herds maintained under management and environmental conditions similar to those in the traditional sector.[42, 203] In the first the tick-free herd performed significantly better than the tick-infested herd but the value of additional production in the form of increased milk yield, calf growth rate and fertility was less than the cost of the acaricide used. In the second project greater productivity was recorded for a herd sprayed with an acaricide once a week in the wet season and at two-weekly intervals in the dry season, compared to that of a herd sprayed every two weeks in the wet season only (October to March) and a herd receiving no treatment at all. When the costs of control were taken into account, the wet season treatment, which was designed to have the greatest impact on *A. variegatum*, but also reduced the numbers of *Hyalomma* spp. and *R. appendiculatus*, gave the best result followed by treatment throughout the year. Neither of the latter acaricidal treatment regimes prevented the transmission of East Coast fever when this was introduced into the area towards the end of the trial.[42]

On three cattle ranches in Zimbabwe regular weekly treatment proved to be economically justifiable in that it reduced *A. hebraeum* populations to imperceptible levels and eliminated mortality due to heartwater.[21] On many farms this approach would not be economically viable and farmers should select the best control options for their particular circumstances.

Tick resistance in various breeds of cattle has been compared in several countries in sub-Saharan Africa. These comparisons have demonstrated that Sanga and Zebu cattle are considerably more resistant to the commonly occurring tick species than are *B. taurus* cattle, and that crossbred cattle show intermediate resistance.[194, 231, 232, 233, 248, 251] Thus, as in Australia, the potential exists for the exploitation of genetic resistance to ticks in tick control programmes in sub-Saharan Africa.

Two new chemicals for the control of ticks have recently been tested on cattle. The one is a benzoyl phenyl urea compound, known as fluazuron, and is an acarine growth-

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regulator, inhibiting the formation of chitin.[23] It is administered in a pour-on formulation, and female *B. microplus* present on animals at the time of treatment laid only a few non-viable eggs, while immature ticks died as they were unable to moult to the next stage. Protection after application lasted several weeks.[23] The efficacy of this or related compounds needs to be tested against other tick species to ensure their viability in Africa.

The second compound is a phenylpyrazone agent known as fipronil. This chemical has excellent therapeutic and persistent activity against fleas on both dogs and cats. Trials with pour-on formulations of fipronil on cattle indicate that it is effective against both *Boophilus annulatus* and *B. microplus*.[37, 38] Therapeutic and persistent efficacy are dose related and are increased by repeat treatments.

Biological control

Although several agents are effective or partially effective for the biological control of ticks it is unlikely that many of these will be commercially viable. Nevertheless, under natural conditions these agents may play a significant role in reducing tick numbers.

Domestic chickens are opportunistic predators of ticks. The indigenous varieties in particular, if allowed to scavenge amongst cattle, can consume considerable numbers of ticks, especially if the cattle are penned close to dwellings in the late afternoon and during the early morning.[55, 84, 85] Red- and yellow-billed oxpeckers (*Buphagus erythrorhynchus* and *Buphagus africanus*), which are virtually obligatory predators of ixodid ticks, take large numbers of these parasites from both domestic cattle and from several wildlife species.[7, 15, 258] The red-billed birds favour *B. decoloratus* and *R. appendiculatus* as food items,[15] whereas yellow-billed oxpeckers prefer the latter tick and *Amblyomma* spp.[286] As an aid to tick control on both wildlife and domestic cattle, red-billed oxpeckers have been reintroduced to several regions in which they originally occurred in South Africa, but from which they had in recent times disappeared.

Chalcid wasps of the genus *Ixodiphagus* are obligatory parasitoids of ixodid ticks and most species will oviposit and develop only in the nymphal stage of the ticks.[40] Several wasp larvae can successfully develop in a single engorged nymph, which is killed during this process.[40, 114] Two of the seven described species of these wasps occur in Africa, namely *Ixodiphagus hookeri* and *Ixodiphagus theilerae*.[30, 63] In Kenya adult *I. hookeri* were released over a period of one year into a field in which there were cattle naturally infested with *A. variegatum* and *R. appendiculatus*.[159] In addition the field was seeded with nymphs of both tick species. During the period of wasp release 51 per cent of *A. variegatum* nymphs but no *R. appendiculatus* nymphs collected from the cattle were infested with the parasitoid. There was also a significant decrease in the numbers of *A. variegatum* infesting the animals compared to the numbers of ticks prior to wasp release and compared to a control group of cattle.[159] In South Africa these wasps mainly parasitize the nymphs of ticks feeding on hares.[30] They can be found around the resting places of the hares and parasitize particularly the nymphs of *Hyalomma* spp.,[30] which by preference feed on these animals.[98, 109]

The infective juveniles of nematode species that are pathogenic for insects can also be pathogenic for ticks. In Guadeloupe the infective juveniles of various species of *Steinernema* and *Heterorhabditis* had no effect on any of the free-living stages of *A. variegatum* or *B. microplus* originating from the island, whereas an imported strain of *B. annulatus* was susceptible to all of them.[153] The infective juveniles of two species of *Steinernema* could kill ticks of all species tested within the genera *Amblyomma*, *Dermacentor* and *Rhipicephalus* in the USA.[123] However, it would appear that, unlike in insects, these nematodes are unable to multiply in ixodid ticks. The ticks are killed by bacteria of the genera *Xenorhabdus* and *Photorhabdus* that have a symbiotic association with the nematodes, and enter the ticks with the infective juveniles.[239]

Entomopathogenic fungi have been used in several countries for the control of agricultural pests and their use for the control of ticks is currently being investigated at various localities. In Kenya aqueous formulations of the spores of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* induced a high mortality and reduced fecundity and egg hatchability in *R. appendiculatus* feeding on cattle.[118] These formulations also caused mortalities in all life stages of *A. variegatum* and *R. appendiculatus* in the vegetation. The comparative ease with which the spores of these fungi can be produced and artificially disseminated makes them promising potential agents for the control of ticks.

Various species of tropical pasture legumes in the genus *Stylosanthes* can trap and kill larvae of *B. microplus*.[62, 263, 266] However, more ticks are likely to settle and oviposit closer to the larger tussocks of grass plants than to the stems of the legumes.[300] Similarly, questing larvae are more likely to ascend a grass tussock than the stem of a legume and thus high legume to grass ratios are needed to ensure a useful level of tick control. A number of grasses also have anti-tick effects.[41, 276] The grasses *Melinis minutiflora* and *Brachiaria brizantha* both have a lethal effect on the free-living larvae of *B. microplus*, but *M. minutiflora* also has a repellent effect, thus making it likely that larvae will avoid it.[41]

A combination of biological and acaricidal control has been successfully incorporated in pheromone-acaricide-impregnated plastic tail tag decoys.[3, 190] These tags are more specifically designed for the control of adult *A. hebraeum* and *A. variegatum*, of which the feeding males exude a pheromone that is particularly attractive to other males, females and nymphs of the same species. The incorporation into such tags of squaline, a naturally abundant mammalian skin secretion that has a long-distance tick attractant effect on *Amblyomma americanum* may provide definite advantages.[303]

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Tick and tick-borne disease models

A practical means of designing cost-effective tick control strategies is to use models.[228, 262] An ecological model was originally developed in Australia for the one-host tick, *B. microplus*.[261] It has subsequently been modified for three-host ticks and given the name T3HOST.[154] Ecological parameter values and the damage coefficient with respect to live-weight gain for *R. appendiculatus* have been fitted to T3HOST,[64] and it can now be used to simulate population dynamics and control strategies for this tick. These simulations allow the best strategies to be identified prior to the costly and time-consuming process of field-testing. For example, it has been estimated that the most cost-effective strategy for the control of *R. appendiculatus* on Zebu cattle at Lake McIlwaine in the highveld of Zimbabwe is intensive dipping for four months during the wet season (December to March).[64] The reason is that the adults, which cause losses in live-weight gain, are most abundant at this time. Extension of the dipping period could result in increased dipping costs progressively exceeding the value of increased live-weight gain. Shortening the dipping period could cause the value of decreased live-weight gain to progressively exceed the cost of dipping. Climate, geostatistics and satellite imagery have been used as predictive tools for the distribution and for the seasonal abundance of a number of economically important ticks.[61, 121, 187, 226, 227] As more data become available models will be refined and will undoubtedly become increasingly important tools for the design of tick control strategies.

Knowledge of the epidemiology of tick-borne diseases is steadily increasing and modelling is particularly useful in this respect.[32, 33, 305] Models have been developed for *E. ruminantium* transmission and its epidemiology,[43, 199] and for *B. bovis* transmission.[143, 144, 246] It should ultimately be possible to use epidemiological models to determine the best strategies for the control of tick-borne diseases in different situations.

Future control methods

It is likely that conventional live vaccines will be surpassed by a new generation of synthetic products. It is now well established that cattle and other hosts can be immunized against certain tick species by exposure to tick antigens, and considerable progress has been made in the characterization of these antigens.[294] At present the only tick against which vaccines are available commercially is *B. microplus*. This field was first pioneered by the Australians who realized that a 'concealed' antigen, which is located on the surface of the digest cells that line the gut of *B. microplus*, could be used to immunize animals against infestation with this tick.[120, 297, 299] Recombinant antigens derived from transformed cells were then incorporated in a vaccine commercially known as TickGard.[296, 298] Vaccination trials against *B. microplus* have now also been conducted on cattle in Cuba and Brazil with a commercially available recombinant antigen preparation known as Gavac.[237, 238] Both vaccines are also reported to be highly effective against *B. annulatus*.[296]

The discovery of a major gene, which infers resistance to *B. microplus*, now presents new prospects for tick control in both beef and dairy animals. This gene was found in a closed herd of the Adaptour line of cattle that results from the crossing of Hereford and Shorthorn animals, breeds that are normally highly susceptible to infestation. Homozygous carriers of this gene may acquire a stable total or nearly total resistance to *B. microplus* in early life.[76]

Many laboratories throughout the world are working on the isolation and characterization of immunizing antigens present in *Babesia*, *Anaplasma*, *Ehrlichia* and *Theileria* spp.[140, 200, 225] It seems that it is only a matter of time before some of these antigens are produced in commercial quantities, either by the application of biotechnology or by chemical synthesis. When these new generation vaccines are developed they could revolutionize our approach to the control of ticks and tick-borne diseases and have profound effects on cattle management and production throughout the tropical and subtropical regions of the world.

Integrated control

In conclusion, the control of ticks on domestic animals lies for the present in the integrated use of available technologies such as chemicals, the management of acaricide resistance, host resistance, tick vaccines, and tick-borne disease vaccines as it seems unlikely that any one of these is robust enough to stand alone.[295] The concept implies the rational use of acaricides in combination with other strategies to cost-effectively minimize production losses caused by ticks and tick-borne diseases. Such a programme could include any number of the components listed below.

Strategic control Acaricide is applied during strategic times of the year in order to control seasonal peaks in tick abundance. It is aimed mainly at adult ticks to decrease numbers to levels at which economic damage is less than the cost of control.

Opportunistic control Acaricide is applied only when tick numbers are considered to exceed the economic threshold. This strategy is effective when animals possess a high level of immunity to ticks or in regions where ticks are only sometimes a problem because of favourable climatic conditions.

Selective control Only those animals with tick burdens considered to be above the economic threshold are treated. This is more easily achieved with small herds of cattle and should reduce both treatment costs and delay the appearance of acaricide resistance. Individual animals, which continuously harbour very high tick burdens, should be culled as they are likely to pass this trait on to their offspring.

Alternation Acaricides from different chemical groups or combination formulations of acaricides are applied alternately. The inclusion of acarine growth regulators in this

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regime may further help delay the development of acaricide resistance.

Management of acaricide resistance When resistance is already present, animals must not be treated with an acaricide from the chemical group to which resistance is evident before moving them to clean or rested pasture, as this will result in the new pasture becoming contaminated with resistant ticks only. An acaricide from a completely different group should be used in such an event.

Grazing strategies Avoid camps that are heavily infested with ticks during certain seasons. Thus sheep and goats must not be grazed in camps on the southern aspect of hills or mountains in the Karoo, South Africa, during autumn and winter. The Karoo paralysis tick, *I. rubicundus*, is most abundant in this type of habitat and is present only from late summer to spring. Goats and cattle can be grazed in sheep camps during summer to denude shrubs and keep the grass short, thus eliminating favourable breeding or questing sites for the tick.

With tick species that take a year to complete their life cycles, pastures can be rested from the end of the season of adult tick abundance until the commencement of the following year's season of adult abundance. Provided no hosts suitable for the immature stages are allowed on to the pastures during the intervening months, most of these ticks should have died and the numbers of adults present when the livestock are reintroduced should be minimal.

In much of southern Africa it is common practice to burn natural pastures once every two or three years during spring to control shrub invasion and remove dead plant material. These deliberate fires coincide with the post-winter synchronous hatching of *B. decoloratus* larvae and with their questing on the vegetation and consequently many are destroyed. However, the short-term effect on tick abundance does not compensate for the damage inflicted on some vegetation types by regular burning.

A high stock density may initially favour the propagation of ticks because of the abundance of hosts. However, should stock numbers become too large the habitat may be damaged to such an extent that it becomes unfavourable for the survival of some tick species. Human perturbation of the environment by chopping down trees for firewood or removing them in order to establish pastures can have the same effect.

Host resistance Sanga or Zebu cattle can be farmed with minimal tick control, ranging from none in dry areas to strategic wet-season control in higher rainfall areas, whereas *B. taurus* cattle require intensive tick control for the greater part of the year in all but dry regions. The amount of control required for crossbred cattle will be somewhere between these two extremes.

Predators Provided non-toxic acaricides are used, oxpeckers can be reintroduced into those regions in which they originally occurred. Where cattle are penned close to rural dwellings at night indigenous chickens can be encouraged to roost close to the enclosures as this will facilitate tick predation.

Immunization against ticks Recombinant vaccines are commercially available for the control of *B. microplus* with promising results against other species. The likelihood exists that more concealed immunizing antigens will be discovered and that vaccines against a multitude of tick species will ensue.

Immunization against tick-borne diseases Immunization can currently be accomplished by using blood vaccines containing virulent or attenuated organisms or using infected tick stabilates. These rather crude vaccines will undoubtedly be surpassed by recombinant antigens produced in transformed cells, or by chemically synthesized antigens.

Tick control on wildlife

Tick control on wildlife in nature reserves encompassing more than 20 000 ha should not be necessary, particularly if a sustainable number of large carnivores are present to remove stressed, weak, sick or injured animals. Problems with ticks usually occur in small wildlife reserves or on game farms, especially those under 1 000 ha in size, on which it is not practical also to keep the larger wild carnivores. Healthy wild antelopes and equids in Africa normally harbour tick burdens in excess of several thousand.[94, 95, 97] However, with the exception of large animals such as giraffe, African buffalo and eland, which may harbour large burdens of adult ticks, the majority of ticks parasitic on wildlife are usually larvae and nymphs.[77, 90, 104] Severe adult tick infestations on wildlife are therefore frequently an indication of some underlying management problem. This might be gross overstocking,[88, 133, 184] or overstocking with particular species such as eland, which are large animals that are highly susceptible to tick infestation.[100, 131] It could also be due to the introduction of wildlife species not indigenous to a region and hence not adapted to the local tick species. In the latter event these animals may not only acquire enormous tick burdens but could also succumb to diseases such as heartwater or one of the theilerioses.

Before control measures are instituted the underlying cause of the problem should be addressed. One of the first steps in this direction would be to remove any animals that are visually very heavily tick infested as well as any diseased or weak animals. The latter animals are normally severely stressed and consequently harbour large tick burdens that can lead to contamination of their surroundings. Thereafter stocking density should be examined and corrected for each wildlife species in the reserve or on the game farm. Wildlife species that historically did not occur in the region could also be removed.

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Provided the habitat is suitable, the introduction of species such as blue wildebeest could be contemplated. These animals have an innate resistance to ticks and remarkably few adult ticks are found on them in their natural habitat.[90, 96] The introduction of oxpeckers can also be considered. This would only be feasible if the reserve or farm was located in a suitable habitat and was large enough to accommodate the birds, or if the surrounding cattle farmers use only acaricides that are not toxic to the oxpeckers. Although fire may kill large numbers of free-living ticks present on the vegetation at the time, its long-term effects seem to be limited, particularly if animals are not kept away from the burnt area once there is a flush of new vegetation.[158, 253]

Should chemical tick control be required, acaricide can be administered by means of a self-medicating applicator coupled to a bin containing licks or food concentrates to which the animals are attracted.[57, 219] In South Africa several wildlife species are more inclined to take supplementary feed in such bins during the winter months because of the seasonal shortage of grazing.[88] Consequently mainly immature ticks will be killed. Acaricide can be automatically applied to animals by constructing a single entrance to a watering point, thus forcing them to step on a pressure-plate or interrupt a light beam that will activate a jetting or pour-on device.[74, 157] Domestic cattle that are regularly treated with an acaricide, can also be used to control ticks on a small wildlife reserve. These animals can be introduced in the wet summer months when adult ticks are most abundant and can be herded during the day into those parts of the reserve where ticks are deemed to be most numerous. No new wildlife should be introduced on to a wildlife reserve or a game farm without having first been thoroughly treated with a reliable, broad-spectrum acaricide. This will reduce the risk of introducing ticks into a region in which they did not previously occur, as well as eliminating the stress of pre-existing tick burdens on the new introductions.

The regular treatment of domestic animals on mixed cattle and game farms can result in a significant reduction in tick numbers on the wildlife on the farm. Those tick species that use cattle as hosts for all their developmental stages, or that prefer cattle as hosts for their adults are particularly affected by this procedure.[102, 309]

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2

Vectors: Tsetse flies

R J PHELPS AND D F LOVEMORE

Introduction

Blood-sucking muscoid Diptera of the family Glossinidae and the genus *Glossina*, known as tsetse flies, are restricted almost entirely to Africa and some of its off-shore islands such as Zanzibar, although they are absent from others such as Madagascar. There is one old record, published in 1906, of tsetse flies from south-west Arabia,[29] and a recent one near Gizar in Saudi Arabia,[61] but they have not been reported there since then. It had long been known that domestic stock could not be kept where tsetse flies occurred and that disease was associated with the flies. However, it was not until Bruce, working in Zululand in 1895, demonstrated the transmission of pathogenic trypanosomes of livestock by tsetse flies that the reason for livestock deaths became known. (In this work, Zululand refers to the lowveld region north of the Tugela River in KwaZulu-Natal Province, South Africa.) The role of tsetse flies as vectors of human trypanosomosis, and the importance of *Glossina palpalis* in this respect, was also demonstrated by Bruce and his co-workers in 1909.[17]

The tsetse fly-infested areas of Africa could not be explored easily before the advent of mechanized transport, as animal-drawn transport was excluded by trypanosomosis. The presence of tsetse flies was thus a major obstacle to the development of much of the continent, a situation which pertains in many places even today. There are those, even now, who look upon the tsetse fly as the guardian of the natural ecosystems of Africa, and who would like this fly to remain until humans have learnt to manage the land in a sustainable manner. At present, though, the human population of Africa is expanding rapidly, and the economic situation is such that the burgeoning population can, for the most-part, be accommodated only as peasant farmers. The importance of livestock to such people is very great, not only as a source of food, draught power, and money, but also for the important role that livestock, especially cattle, play in cultural affairs. Under these circumstances it is essential to try to eliminate, or at least control, the diseases of livestock, amongst which trypanosomosis plays a major role.

Biology of tsetse flies

Anatomy and sensory systems Detailed studies have been made of the anatomy[26, 167] and the physiology[133] of tsetse flies. Leak[143] has recorded the advances in knowledge of the biology and ecology of tsetse flies over the previous 30 years.

Tsetse flies (**Figure 2.1**) have one pair of membranous, mesothoracic, functional wings, while the metathoracic wings are modified to form halteres. They can fly at about 6,3 metres per second[71] and the power of lift generated by their wings is unusually large.[90] The age of tsetse flies may be estimated from the amount of wear on the hind margin of their wings, while the length of the vein forming the blade of the 'hatchet' cell (**Figure 2.2**) can be used as an index to the size of the fly.[117] The energy substrate used in flight is proline,[22] and the stored energy is in the form of abdominal fat. The amount of proline available to the flight muscles in the thorax is limited, so the fly cannot make sustained flights. When this fuel is exhausted, the fly must rest until further proline has been synthesized in the cells of the abdominal fat body[23] and transported to the flight muscles of the thorax.

The functional mouthparts, or haustellum, of the tsetse fly consist of the labium, which is trough-like in cross section and terminates anteriorly with the denticulate labellum; the labrum, lying along the dorsal surface of the labial trough; and the hypopharynx, which lies within the tube formed between the labium and labrum and which has the salivary glands attached to its base. There are no mandibles, and the maxillae are represented by the stout maxillary palpi (**Figure 2.3**) between which the haustellum lies when at rest. The haustellum, or the haustellum plus the palpi, is often termed the proboscis. When the fly is feeding, the haustellum is lowered, the labellar teeth cut into the host's skin and the haustellum positioned in the wound. Blood is then sucked up the central lumen of the haustellum, and saliva containing an anticoagulant is released into the wound. The labium is well supplied with sensilla which monitor the blood flow and quality.

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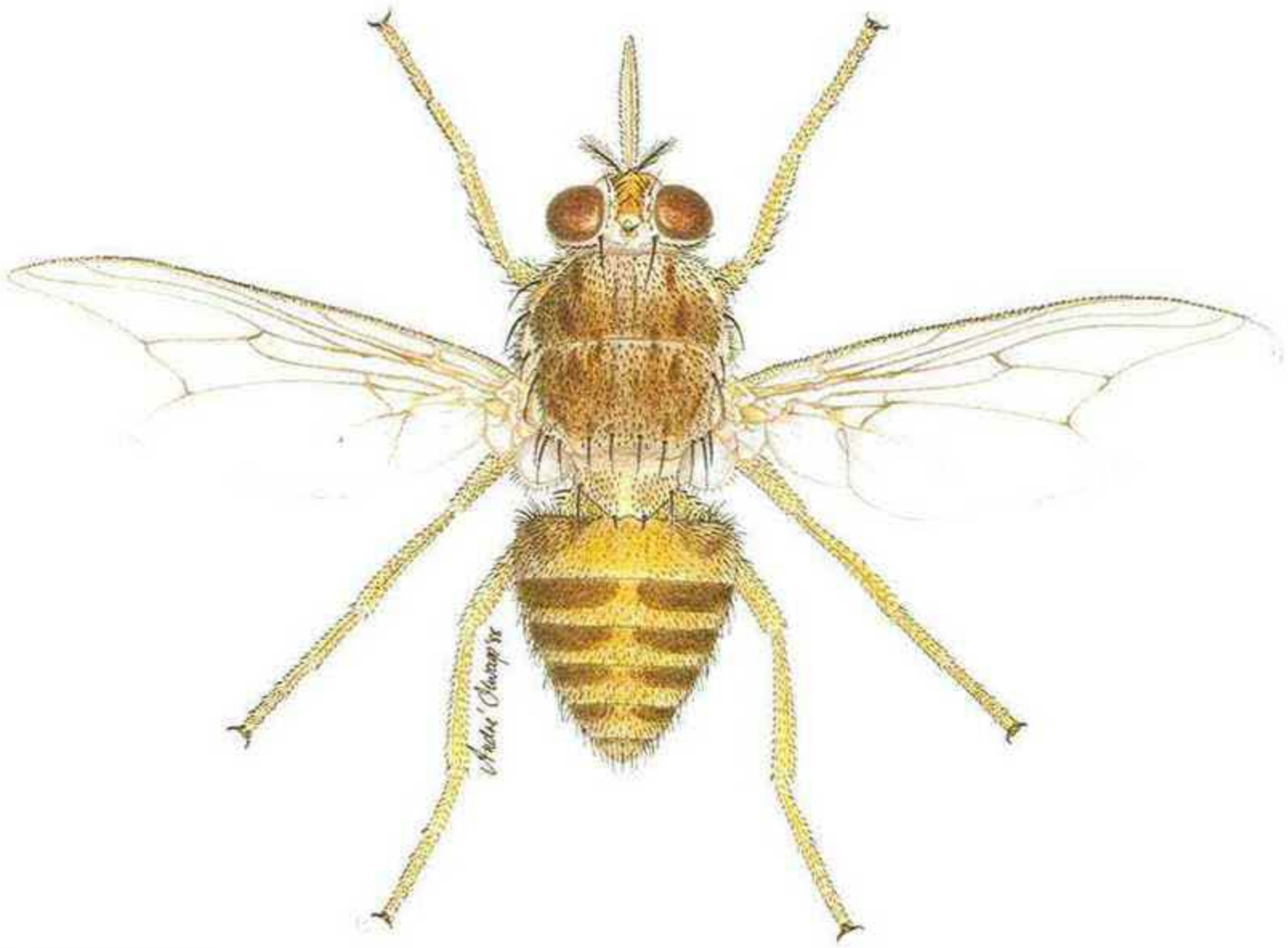


Figure 2.1 *Glossina morsitans*

Temperature of the host's skin is a primary stimulus for the fly to start probing for a feeding site, and adenosine-5-triphosphate in the host's blood stimulates the fly to engorge.[69]

Both sexes of the tsetse fly are obligate blood suckers and generally feed at three- to five-day intervals, although they may feed more frequently if the opportunity occurs. The blood meal is the source of both nutrition and water for the fly. Food reserves in the form of fat, and the amount of residual blood in a fly, give an indication of its nutritional status,[200] to which its behaviour is related.[11]

Tsetse flies can detect odours by means of sensilla situated on the antennae; if their antennae (**Figure 2.3**) are removed, flies are less able to detect their hosts than are intact flies.[236] Substances (kairomones) produced in the host's breath, such as carbon dioxide and acetone, attract tsetse flies,[235] while a further attractive component of cattle breath is 1-octen-3-ol.[244] Kairomones in the urine of African buffaloes and cattle which attract tsetse flies have been isolated and identified as phenols.[174, 245] However, some kairomones are repellent; the odour of humans repels some species of tsetse flies, notably *G. pallidipes*.[234] One of the phenols present in ox urine, 2-methoxyphenol, is repellent to *G. pallidipes* and reduces trap catches by 60 per cent.[228] Placing a source of 2-methoxyphenol next to a bait ox reduced the numbers of flies attracted to the ox, but did not affect the proportion of flies feeding on the ox after arrival. Only in areas of very light disease challenge would the repellent be of use in protecting animals in the field. Non-volatile substances may also be detected by sensilla, as is shown by the initiation of copulatory responses in the male fly by the contact of its tibial receptors with female sex pheromone, in the form of a wax on the surface of the female's thorax.[137]

Well-developed compound eyes are present in the tsetse fly and are important in host location. Hungry flies are particularly attracted to moving objects, but at shorter ranges they can also recognize stationary hosts provided these are large enough to be resolved by the eyes, which have an absolute resolution of about three degrees.[231] The eyes can also distinguish between light and dark, a useful attribute for seeking out shaded microclimates when ambient temperatures are at lethal levels.[107] The spectral sensitivity of the compound eyes of tsetse flies has been investigated[84] and it has been shown that it is the spectral reflectivity of the object which is detected by the fly. Blue-green and red covers on fly traps increase their capture rate, while green, yellow and orange covers have the opposite effect.[85] Comparison with a graded series of grey traps indicated that the flies employ some colour information and not contrast

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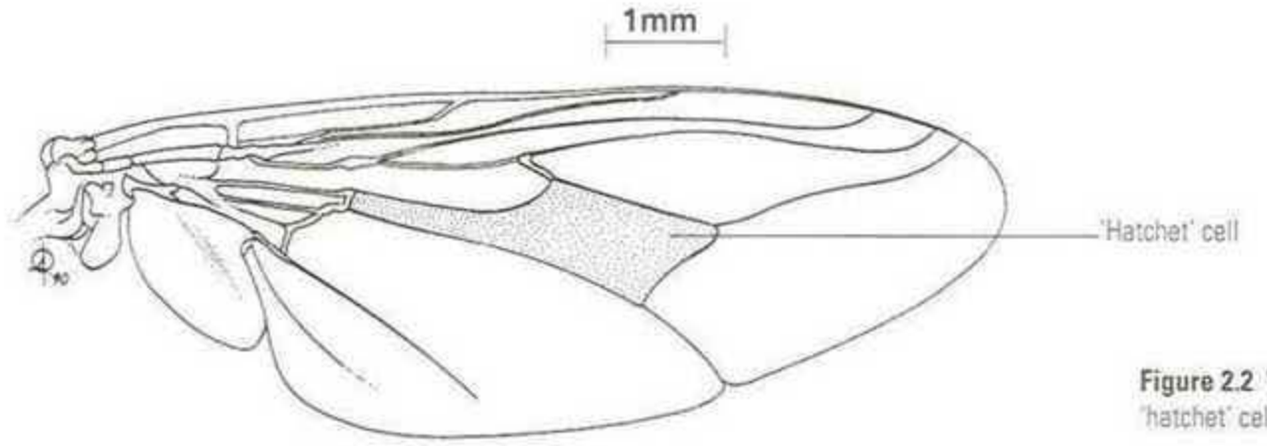


Figure 2.2 Wing of a tsetse fly, with the 'hatchet' cell indicated by stippling

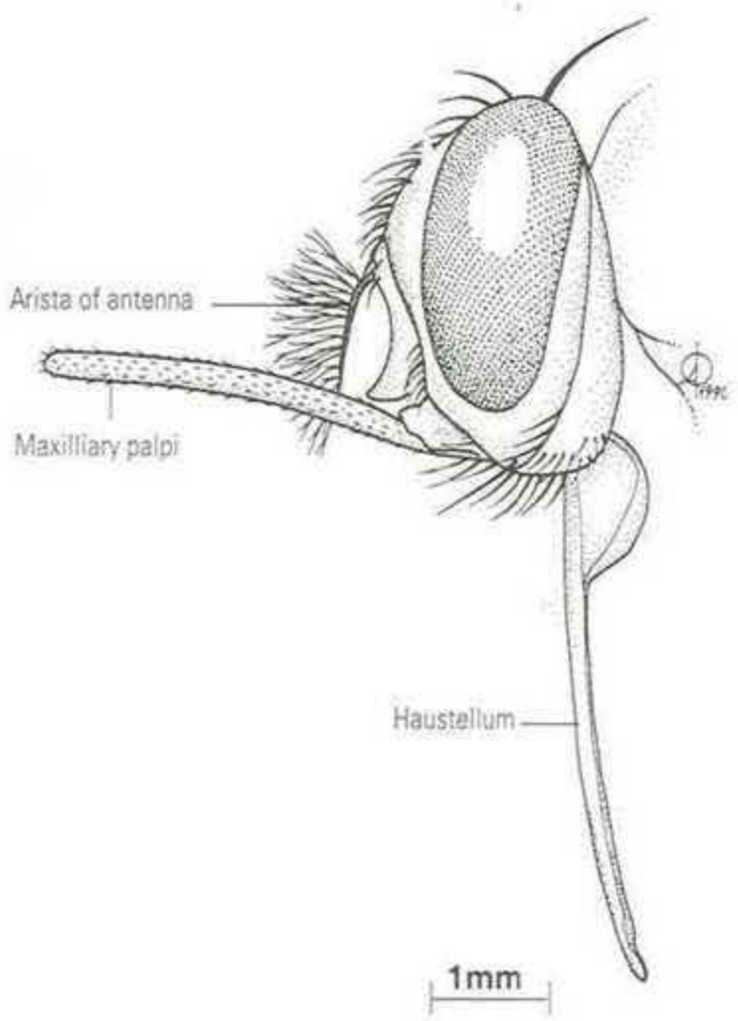


Figure 2.3 Lateral aspect of the head of a tsetse fly with the haustellum lowered to the feeding position

information alone. The same effects have been obtained with flat cloth screens of different colours;[82] black is less attractive than blue, but more flies settle on a black surface than on surfaces of other colours.

Eyes contain pteridine pigments which accumulate with age. This feature is now used to determine the age of flies of both sexes.[136]

The sexes are easily recognized in tsetse flies as the male hypopygium is well developed (**Figure 2.4**). The male claspers are concealed within the hypopygium when not in use and dissection is necessary to display these structures, which are important for specific identification. The internal genitalia of females may be used to determine the physiological age of flies, as the shedding of eggs from the four ovarioles follows a regular sequence.[30, 205] The rate at which the eggs mature is a function of temperature so, if the ambient temperatures are known, an estimate of the chronological age of the fly can be made from its physiological age.

Many aspects of tsetse fly behaviour have been investigated,[11, 12, 51, 221, 237, 251] several of which have been used to devise methods for their capture. When a full understanding of their behaviour has been attained, it may well lead to improvements in the present methods used for this purpose.

Life cycle and population dynamics Female tsetse flies emerge from their puparia about two days before males[119, 178] and, after expanding and hardening their wings, they seek a blood meal. In some species, females are receptive to males as soon as they start seeking food and often mate either when taking their first blood meal, or soon after. Females actively resist mating more than once.[120] They are viviparous; ovulation taking place about four days after the first meal with the first larva being deposited about 10 days later. At deposition, the larva is at the end of its third instar (**Figure 2.5**), having been fed on a nutritious fluid during its development within the female. Each subsequent larva follows at an interval of about 10 days. The duration of larval development in the laboratory is temperature-dependent, and may be predicted by a formula.[73] The female feeds at regular intervals throughout her life, and if not well nourished, her reproductive performance declines.[70, 160] It has been shown in the field that high host density, resulting in very well-fed flies, may cause a reduction of the interlarval period by two days, relative to that predicted on the basis of mean ambient temperature.[94] Females live longer than males in the field, and must live long enough to produce two mature larvae if the population is to remain static, and more than that to provide for mortality and population increase. The newly deposited larva moults to form the prepupa, but remains within the shed third instar cuticle, which then hardens to form the puparium (**Figure 2.6**) within an hour of larviposition.[267] Larvae are deposited in shady places with a soft substrate. Within the puparium, the prepupa moults to give the pupa, and a final moult within the puparium gives

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the pharate adult, which emerges from the puparium in due course, the duration of the puparial stages being temperature dependent. The insect does not feed from the time it leaves the female fly as a mature larva until the adult emerges from the pupa, a form of reproduction known as adenotrophic vivipary. At a constant temperature of 25 °C, males emerge after 30 days and females after 27 days, while the respective figures at a constant temperature of 16 °C are 101,8 and 100 days.[179] The integrated mean temperature from a Stevenson screen gives a good representation of an equivalent constant laboratory temperature as far as development within the puparium is concerned.[178] The life cycle of a tsetse fly is shown diagramatically in **Figure 2.7**.

Male tsetse flies emerge in equal numbers to females and, after expanding and hardening, seek a blood meal. Although the males may copulate soon after emergence, they are not fully fertile until they are a few days old. In the field, males may possibly mate more than once but, as spermatogenesis does not occur after their emergence from the puparium,[44] their sperm reserves are soon depleted and they are generally considered unlikely to mate more than once. Males feed at similar intervals to females, but have greater energy reserves for flight as they do not have to nourish larvae.[25] In general, the mean life span of males in the field is about four weeks, while that of females is at least eight weeks,[119] but these durations may vary from place to place, depending on prevailing environmental factors.

The population dynamics of tsetse flies have been investigated for many years since the development of the mark–release–recapture method by Jackson.[115] This method depends on marking flies with dots of artist's oil paint[114] and then recapturing them. Capture methods range from catching flies from a mobile or stationary bait with a hand net to the use of various types of traps, many patterns of which[85, 91, 232, 238] have been developed since that used by Harris.[98] However, no known capture method gives an unbiased sample with respect to population density, sex ratio, hunger stage, or age.[143, 183, 206, 248]

In view of the sampling biases, estimates of population density of tsetse flies vary widely. In addition, the assumptions underlying population estimates are often violated.[250] Consequently, the population ecology of tsetse flies has been reviewed,[201, 202] and a new analytical approach to population ecology has been developed.[198]

The rate at which tsetse fly populations grow is not known, although a mathematical modelling approach has been developed which indicates the limits within which the population growth rate must lie under given environmental conditions, based on current knowledge of tsetse fly biology.[92] It has been shown that no population of tsetse flies can survive if the daily mortality of females is greater than 4 per cent, and in the field possibly 2 to 3 per cent.[92] This is a very useful criterion for those concerned with tsetse fly eradication.

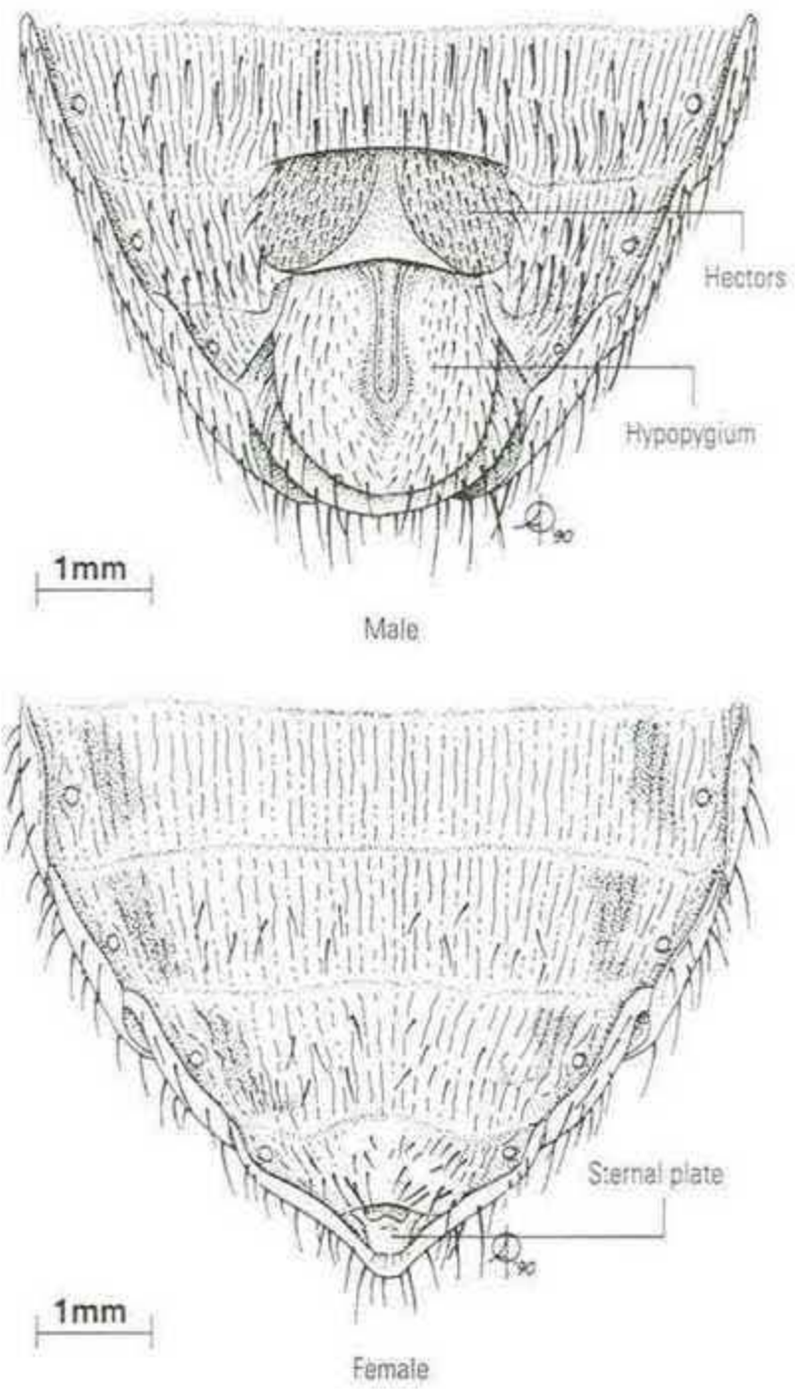


Figure 2.4 Ventral aspect of the terminal portion of the abdomen of both sexes of *Glossina morsitans*

Ecology of tsetse flies Tsetse flies are distributed over 11 million square kilometres in Africa between 14 °N and 29 °S. The northern limits of latitude are highest on the west coast, while the southern limits are greatest on the east coast, where the 17 °C effective temperature isotherm[212] gives a fair representation of the historical limits of the tsetse fly. These limits are determined by climate, often through its effect on vegetation. Although the tsetse fly and its puparium are well waterproofed,[18, 19] regions situated below the 500 mm isohyet do not support tsetse flies unless there are watercourses with some vegetation along them.

Temperature is of great importance in the ecology of the tsetse fly. Not only does it affect interlarval and puparial durations, but it also influences the fly's activity. Below 15 °C tsetse flies are inactive and above 35 °C they seek out refuge

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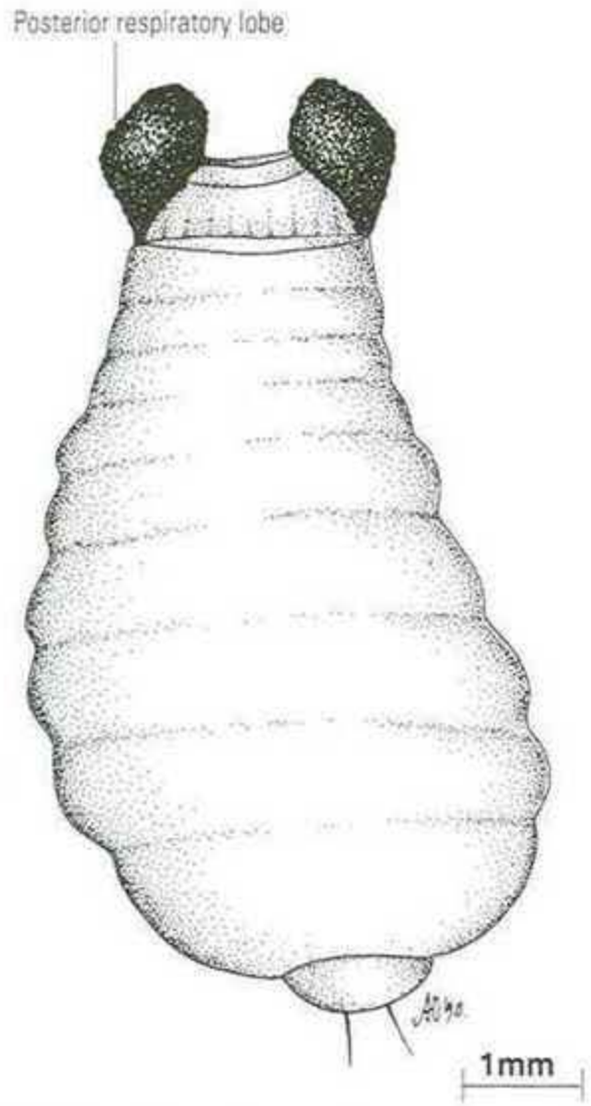


Figure 2.5 Third instar larva of a tsetse fly

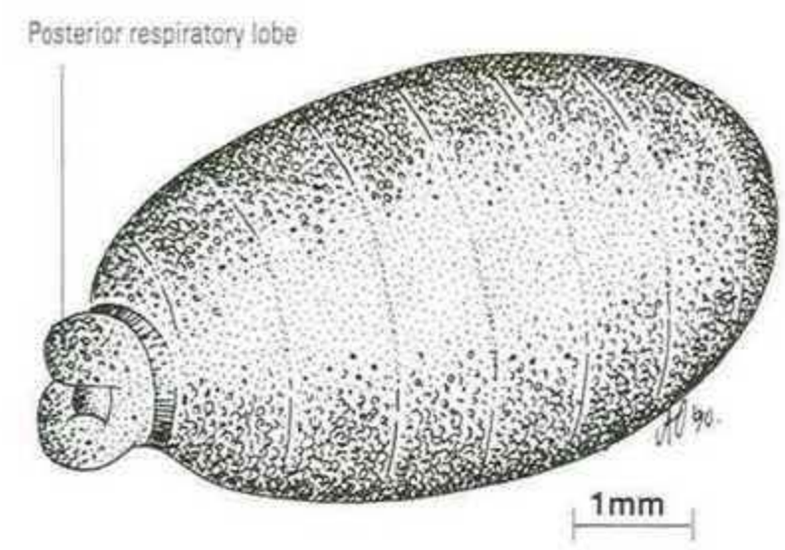


Figure 2.6 Puparium of *Glossina morsitans*

sites, such as rot-holes in trees, animal burrows and deep fissures in bark, in which they are inactive. The eyes are important in this refuge-seeking behaviour.[107] Thus the temperatures must stay within the 16 to 35 °C range for long enough during the day for tsetse flies to remain active and able to seek food. They are inactive at night. For the smaller species of tsetse flies, the puparial duration at low temperatures is so long that the insects' fat reserves become depleted prior to maturation; this is one of the ways in which tsetse fly distribution is limited.[21] The larger fly species are not subject to this limitation to the same extent. Temperatures over 40 °C are rapidly lethal to both large and small species of tsetse fly[113] and their puparia.[180] Increasing altitude moderates temperature, and this is reflected in the distribution of tsetse flies. Near the equator they may occur up to an altitude of 1 800 m, but in Zimbabwe, which lies farther south, they do not occur at altitudes above 1 200 m.[110]

The vegetation type at a given locality is greatly influenced by temperature and humidity. Apart from grasslands, which do not support tsetse flies, all forms of woodland, from savannah to rainforest, can provide a suitable habitat for some species of these flies, but no one vegetation type is suitable for all species. Artificially planted plantations can provide a suitable habitat for tsetse flies,[230] and the thickets (sometimes comprising chiefly *Lantana camara*) which develop on abandoned agricultural land are often good habitats for them.

Host preferences and location The distribution and abundance of some tsetse fly species, especially *G. morsitans* and *G. pallidipes*, which are often referred to as the game tsetse flies, are closely related to the numbers and habits of certain wild animals. The development of methods for determining the animals on which individual tsetse flies have fed, by analysis of their blood meals, has given an insight into their feeding preferences.[193, 255] Preference has been shown for the warthog (*Phacochoerus aethiopicus*) and bushpig (*Potamochoerus porcus*), as well as some Bovidae such as the kudu (*Tragelaphus strepsiceros*) and bushbuck (*Tragelaphus scriptus*). Other wild animals, including elephant (*Loxodonta africana*), black rhinoceros (*Diceros bicornis*) and African buffalo (*Syncerus caffer*), are also fed on, but less frequently. In the case of the elephant and the African buffalo, this may be due, in part, to their tendency to move over large distances. Primates are poor hosts, and some animals such as impala (*Aepyceros melampus*) and zebra (*Equus burchellii*) are rarely fed on although they may be abundant in tsetse-infested country. The blood of impala is adequate to nourish tsetse flies,[132] and the reason for flies not feeding on this animal may lie in the behaviour of these animals.[154] Behavioural reduction in the attack by tsetse flies has also been shown for juvenile warthog.[224] It has been shown that, although the blood proteins of a range of wild animals differs, tsetse flies can effectively use all the different types of blood.[164] In the absence of its preferred hosts, the tsetse fly can survive on the blood of other wild animal species. Nucleated red blood cells from birds or reptiles have also been recorded on occasions, and *G. palpalis* in particular may feed extensively on reptiles.

Domestic animals, particularly cattle[8, 184] and donkeys,[9] can be good hosts for tsetse flies, but sheep and goats are not. The analysis of tsetse fly blood meals from the northern region of the Nyanga district of Zimbabwe provides convincing evidence of *G. morsitans*' ability to survive

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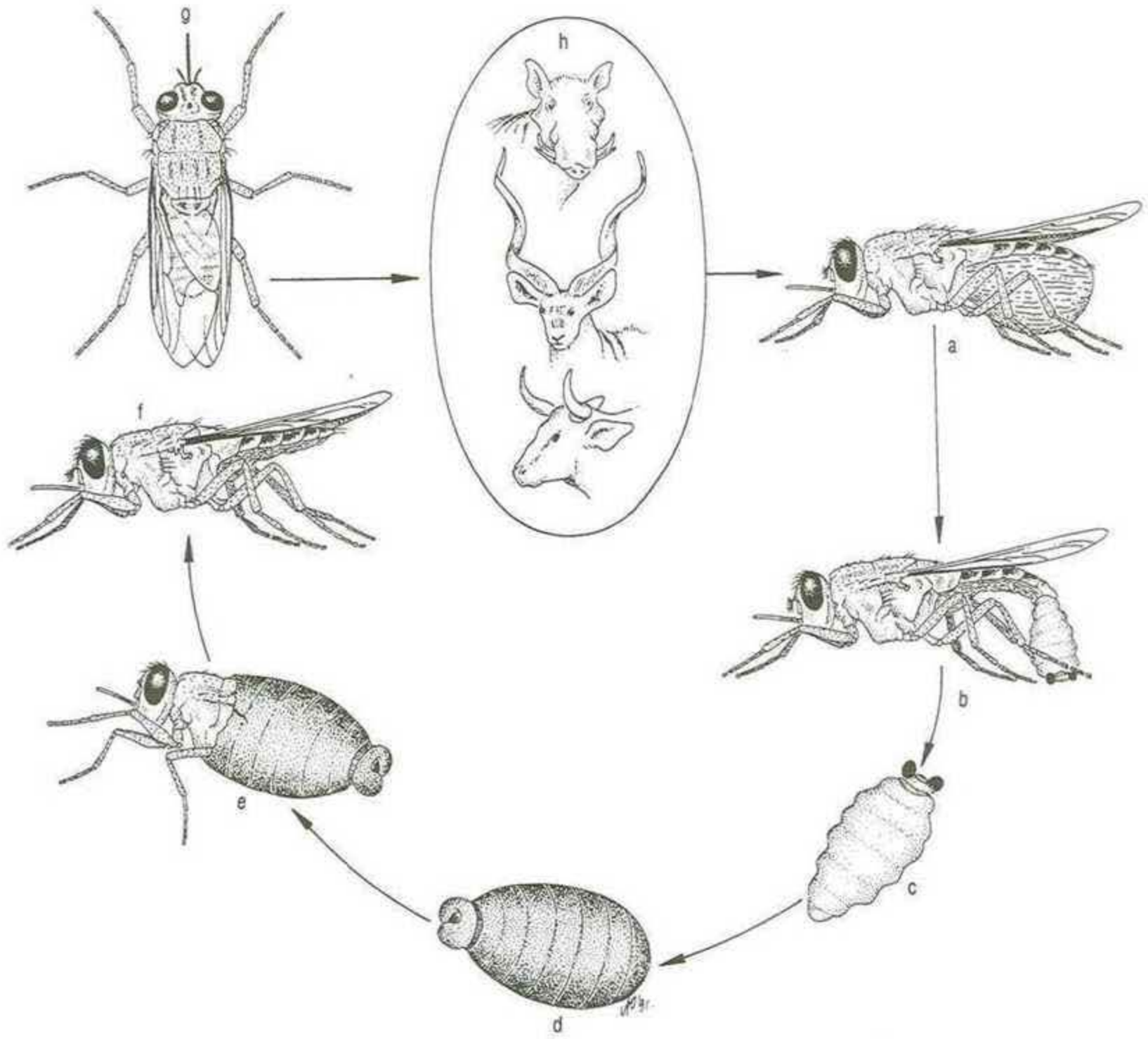


Figure 2.7 The life cycle of a tsetse fly

a = blood-engorged female
b = larvipositing female
c = third instar larva
d = puparium containing the pupa
e = fly emerging from the puparium
f = lateral aspect of a tsetse fly
g = dorsal aspect of a tsetse fly
h = some hosts of the *morsitans* group of tsetse flies

almost entirely on domestic cattle.[193] It is also known from West Africa that *G. palpalis*, which feeds on a wide range of hosts, can adapt well to feeding on peri-domestic animals (animals such as dogs and pigs which occur around the houses of people).[122]

In the laboratory, feeding defibrinated blood through a membrane is used to maintain colonies of tsetse flies.[161] For some unknown reason, pig blood is superior to cattle blood for this purpose.

Both vision and odour detection are used by the tsetse fly to locate its host.[26, 41] In the case of vision, shape, movement, colour, and light and shade differences are important aspects.[85, 99, 233] The contrast between light and shade may be important in determining alighting points on the host.[14] As the range at which a moving ox can be seen by a tsetse fly is about 140 m,[5] it seems that odours are responsible for attracting flies from greater distances.[236]

Studies have been made on the behaviour of tsetse flies in an odour plume,[24, 71, 87] but full details of how the tsetse fly navigates in the plume have not been determined. At low

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wind speeds an odour plume meanders and, even 10 m from the source, may give an incorrect indication of the direct line to the source of the odour.[13, 86] At intermediate wind speeds the odour plume straightens and gives a better indication of the direction of the odour source, but at very high wind speeds the odour plume disintegrates rapidly and is not of use in indicating the source of the odour. Williams[261] has modelled different tactics which may be used by the fly to locate an odour source, and it is likely that the fly is able to use a variety of methods, depending on the prevailing conditions. *Glossina pallidipes* has been reported to cross a clearing 923 m wide, where there was a conspicuous bush-covered cliff on the far side.[215] Possibly senses other than vision are involved in this type of movement.

Tsetse flies as vectors of trypanosomes

Tsetse flies are important mainly because they are vectors of certain species of protozoan parasites belonging to the genus *Trypanosoma*. This genus is split into four subgenera. Of these, one, *Pycnomonas*, contains only one species, *T. suis*, which is of minor importance in causing disease in Africa. The subgenera *Duttonella*, *Nannomonas* and *Trypanozoon* contain the species that cause most cases of trypanosomosis (nagana) in stock, while all the organisms causing trypanosomosis (sleeping sickness) in humans belong to the subgenus *Trypanozoon*. The individual subgenera can be recognized on morphological grounds, but the species within the subgenus *Trypanozoon* are morphologically identical and their separation is based on epidemiological or biochemical criteria. Trypanosomes belonging to the different subgenera tend to develop in different parts of the tsetse fly so, when the fly is dissected, one can often recognize the subgenus involved by its location (**see Chapter 12: African animal trypanosomoses, Figure 12.2**). The development of DNA probes for the identification of trypanosomes in tsetse flies[72] enables rapid and accurate identification of some species within the fly. Trypanosomes of major importance in southern Africa, and their usual development sites in the tsetse fly, are given in **Table 2.1**.

Transmission of the parasites after they have undergone a developmental cycle to give the metacyclic infective stage, is known as cyclical transmission. Trypanosomes can also be transmitted mechanically by syringe passage and, in the laboratory, by contamination of the mouthparts of certain other species of biting flies.[257] As trypanosomes survive for only a short time outside the mammalian or tsetse fly host, a fly with contaminated mouthparts has to feed on a susceptible host soon after the infective meal in order to transmit the parasite mechanically. *Trypanosoma evansi*, in North Africa, is thought to be transmitted entirely mechanically, and *T. vivax* in South America and Mauritius is assumed to be transmitted in this way. In southern Africa, mechanical transmission of the trypanosomes normally transmitted by tsetse flies has not been unequivocally proven in the field.[10, 257] Only some species of tsetse flies, and then only in parts of their distribution range, transmit trypanosomes which cause disease in humans (*T. brucei gambiense* in West Africa and *T. brucei rhodesiense* in central, eastern and southern Africa). In the case of livestock disease, all species of tsetse flies tested can transmit the responsible trypanosomes, although individual species may not transmit all the species of trypanosomes, and some are better vectors than others. The infection level of the tsetse fly population varies from place to place, but is generally between 5 and 15 per cent.

Table 2.1 Trypanosomes of major importance in southern Africa and their usual development sites in the tsetse fly

SUBGENUS	SPECIES	SITE OF DEVELOPMENT
Duttonella	*T. vivax*	Haustellum
Nannomonas	*T. congolense*	Haustellum and midgut
	T. simiae	Haustellum and midgut
Trypanozoon	*T. brucei brucei*	Midgut and salivary glands
	T. brucei gambiense	Midgut and salivary glands
	T. brucei rhodesiense	Midgut and salivary glands

The conditions under which tsetse flies become infected when feeding have been reported.[121, 165] Within the tsetse fly population some individuals are refractory to infection.[156] This resistance is maternally inherited and is associated with the presence of rickettsia-like organisms in the midgut,[143, 157] particularly in newly emerged flies.[159] Apparently lectins are involved in protecting the tsetse fly against infection by trypanosomes and these rickettsia-like organisms affect the level of the lectins.[158] The work on lectins and rickettsia-like organisms has so far only been done on laboratory cultures of tsetse flies and trypanosomes, and the results have not yet been confirmed in the field.

It has been suggested that the presence of trypanosomes in the haustellum of the tsetse fly interferes with the sensilla monitoring the rate of food intake and leads to infected flies probing with their mouthparts for a meal more often than uninfected flies.[166] This would be an important factor in the frequency of disease transmission, but no increase in the probing activity in infected *G. morsitans centralis* has as yet been found in the field.[163]

Many wild animal species, particularly bushbuck, kudu, warthog and bushpig, are subclinically infected with the trypanosomes that affect humans and livestock and are important reservoirs of infection.[122] Domestic animals such as cattle and pigs may be reservoir hosts of trypanosomes which affect humans.[254, 260]

Identification of *Glossina* and the species in southern Africa

Tsetse flies were formerly treated as members of the family Muscidae, or a subfamily of it. They are now generally placed in a separate family Glossinidae, which has only one genus, *Glossina*.

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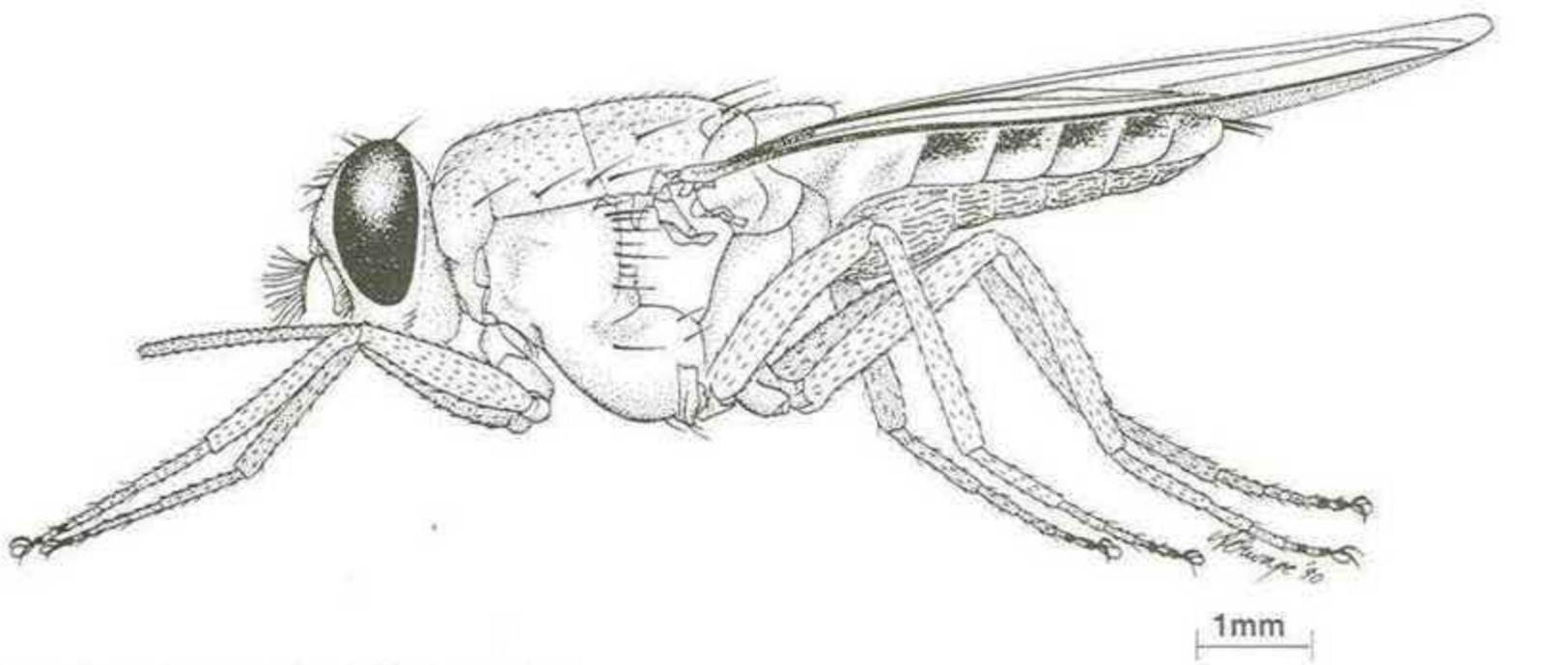


Figure 2.8 Lateral aspect of a female *Glossina morsitans*. Note forward-pointing proboscis

Flies in the genus *Glossina* can be recognized by the following features: the proboscis points forward when at rest (**Figure 2.8**); the wings have a hatchet-shaped discal cell (**Figure 2.2**), and the antennae have feathered bristles on the arista (**Figure 2.9**). When the fly is at rest, the wings lie one on top of the other (**Figure 2.10**), a feature which separates them from other common blood-sucking Diptera such as horse flies (*Tabanus* spp.) and stable flies (*Stomoxys* spp.).

There are three groups of species within the genus *Glossina*, which may be distinguished largely by their habitat preferences. However, features of their genitalia are the definitive criteria. These groups have been given subgeneric names, but these are rarely used. Species and synonyms within the subgenera have been listed by Pont.[188]

The *fusca* group (subgenus *Austenina*) inhabits rainforest or dense riparian forest. The *palpalis* group (subgenus *Nemorhina*) also inhabits rainforest, but some species extend along the riparian fringes far out into savannah woodland. The *morsitans* group (subgenus *Glossina*) is restricted mainly to savannah woodlands. In the wet season they spread throughout the woodland, but in the hot, dry season they are often associated with vegetation along the drainage lines.

The following taxa occur in southern Africa:

***fusca* group**

- *Glossina schwetzi* Newstead & Evans
- *Glossina tabaniformis* Westwood
- *Glossina nashi* Potts
- *Glossina brevipalpis* Newstead

***palpalis* group**

- *Glossina palpalis palpalis* (Robineau-Desvoidy)
- *Glossina fuscipes fuscipes* Newstead
- *Glossina fuscipes quanzensis* Pires
- *Glossina fuscipes martinii* Zumpt
- *Glossina pallicera newsteadi* (Austen)

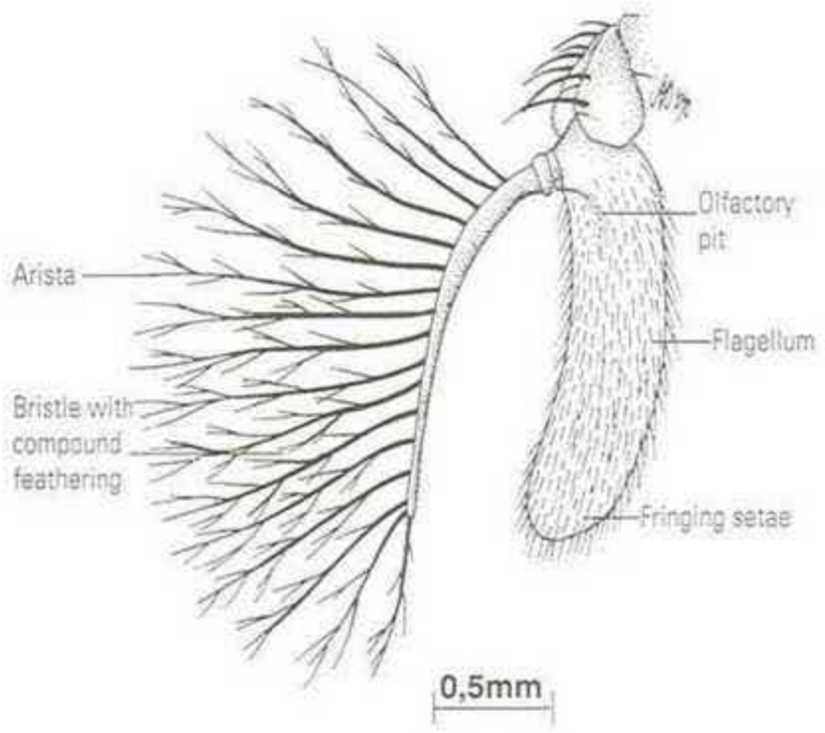


Figure 2.9 Lateral aspect of the antenna of a tsetse fly

***morsitans* group**

- *Glossina austeni austeni* Newstead
- *Glossina austeni mossurizensis* Travassos Dias
- *Glossina morsitans morsitans* Westwood
- *Glossina morsitans centralis* Machado
- *Glossina pallidipes* Austen

Apart from *G. brevipalpis*, the *fusca* group is confined to the north of Angola, and species in this group are generally of little importance in disease transmission in southern Africa. The same is true of *G. pallicera newsteadi* in the *palpalis* group.

For the non-specialist, the differentiation between *G. palpalis* and *G. fuscipes* and identification to the subspecific level of all the species is difficult, and involves mounting and

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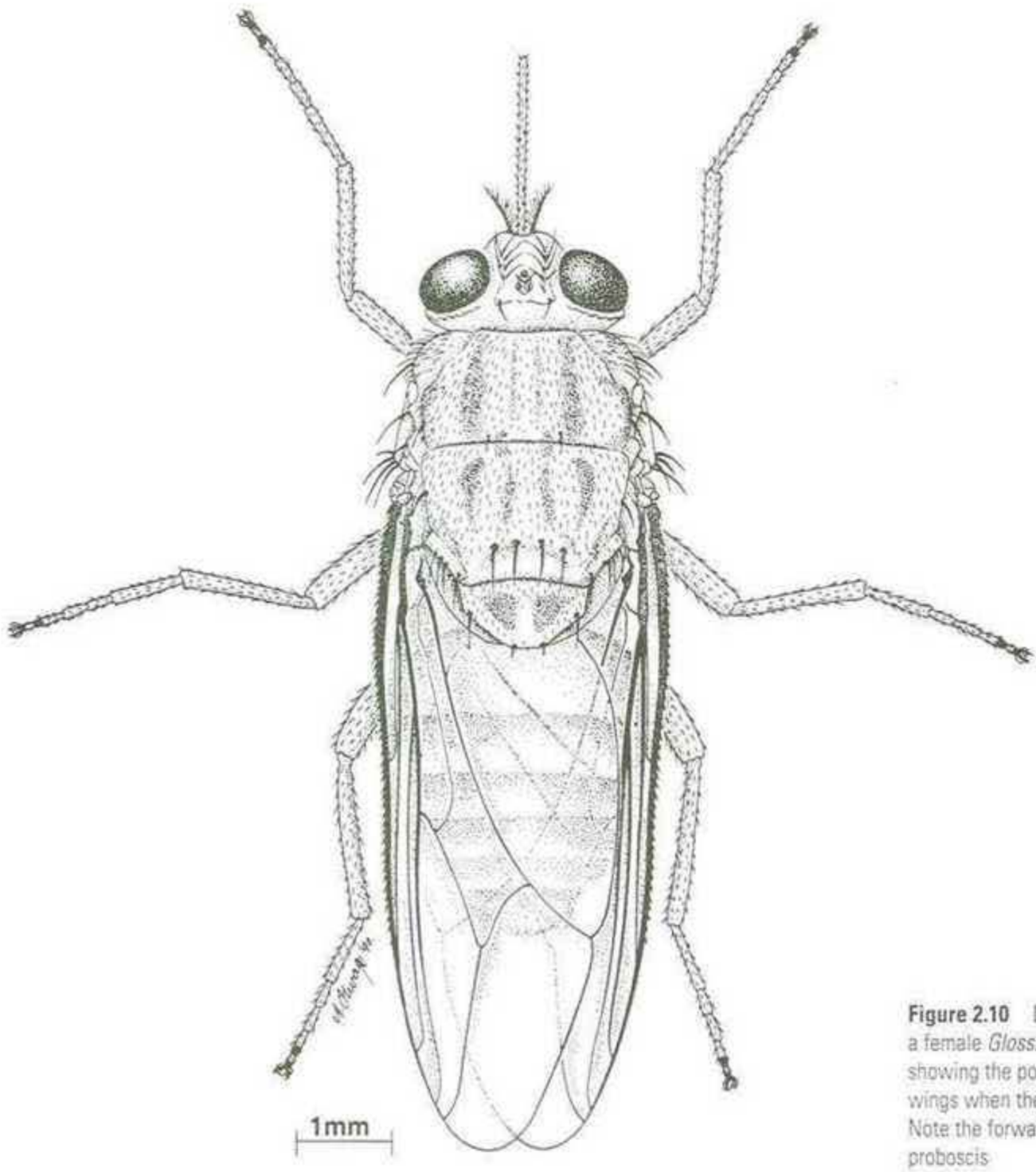


Figure 2.10 Dorsal aspect of a female *Glossina morsitans* showing the position of the wings when the fly is at rest. Note the forward-pointing proboscis

close examination of the male and female genitalia. Information given in this chapter will enable the reader to identify the subspecies of a specimen, if this is required, after it has been identified to a species level and the distribution maps given below have been consulted. Reference should be made to the keys given by Machado[148] for the subspecies of *G. morsitans*, to Machado[147] for subspecies in the *palpalis* group, and to the key to the subspecies of *G. austeni* given by Dias,[53] should they wish to confirm their identifications.

A simple key to the six species of importance in southern Africa is given below.

Key to the species of *Glossina* in southern Africa

1 Large fly, wing length 11 to 13,5 mm, medium brown in colour, anterior cross-vein thickened and darkened to appear as a spot on the wing. .....*brevipalpis*
Not as above 2

2 Upper surface of the abdomen with a dark grey background colour with black bands across it. Black banding occupies most of the surface of the abdomen. Upper surface of all segments of the hind tarsi brown or blackish .....*palpalis* group-3
Not as above 4

3 Posterior margin of the hectors straight, or in the form of a shallow curve .....*fuscipes* s.l.
Posterior margin of hectors deeply split by an anteriorly pointing triangle .....*palpalis* s.l.

4 Upper surface of abdomen bright ochreous-tawny in colour with traces of dark transverse bands which are not sharply defined .....*austeni* s.l.
Not as above 5

5 Last two joints of front and middle tarsi without contrasting brown to black tips. Abdomen brownish-yellow on the dorsal surface with distinct, contrasting, black transverse bands interrupted on the mid-line of the abdomen. Bands are square-cut at the mid-line and often appear

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as if seen through varnish. Fringing hairs of the antennal flagellum more than a third of the flagellar width ..... *pallidipes*

6 Last two joints of the front and middle tarsi with contrasting brown to black tips. Dorsal surface of abdomen brownish-yellow with contrasting bands of black interrupted at the mid-line. Bands tend to be rather indistinctly defined posteriorly, are not square-cut at the mid-line, and do not appear as if varnished. Fringing hairs of the flagellum only one seventh of the flagellar width ..... *morsitans* s.l.

The extensive studies on the genetics of the genus *Glossina* have been reviewed by Gooding.[77] The taxonomy based on morphology is generally supported by the evidence from genetic studies. In southern Africa, only *G. austeni* has a degree of uncertainty in its taxonomic affinities. Even on morphological grounds it does not fit well in the *morsitans* group, and evidence from molecular genetics and breeding data suggests that it is only distantly related to the *morsitans* group.[76] It has been proposed that *G. austeni* be split into two subspecies,[53, 55] and that the species *G. austeni* itself should be placed in a separate subgenus *Machadomyia*,[56] but it is too early to say if the latter will be widely accepted.

Differences between the Giemsa banding patterns of the chromosomes of *G. pallidipes* from Uganda and those from Zimbabwe have been demonstrated,[138] and there are also behavioural differences between these two populations. The two are, however, interfertile, so there is apparently no question of different subspecies. *Glossina pallidipes* is very variable in colour pattern, with the darker forms, in which the abdomen is almost as black as that of *G. palpalis*, coming from heavily shaded areas, while the lighter coloured forms come from less shaded areas.[20]

A new species, *G. borgesi*, was described by Pires,[187] but it is regarded by Potts[190] and Pont[188] as being a synonym of *G. pallidipes*. The male genital claspers of *G. pallidipes* in Mozambique are very variable and range from those strongly resembling the claspers of *G. longipalpis* (a West African species) to those typical of *G. pallidipes*.[47] Haeselbarth *et al.*[88] considered *G. pallidipes* to be a subspecies of *G. longipalpis*. An anatomical feature on the male claspers of *G. longipalpis* has been described[31] which may be used to differentiate between *G. longipalpis* and *G. pallidipes*. However, Thakersi[217] showed that not all *G. longipalpis* had the feature so it is not always of value in separating these two species.

Distribution of tsetse flies in southern Africa

In this section, the distribution maps and lists of species in the various countries made by previous workers[66, 162, 266] have been used as a baseline. Where possible, these data have been updated from recent publications and the

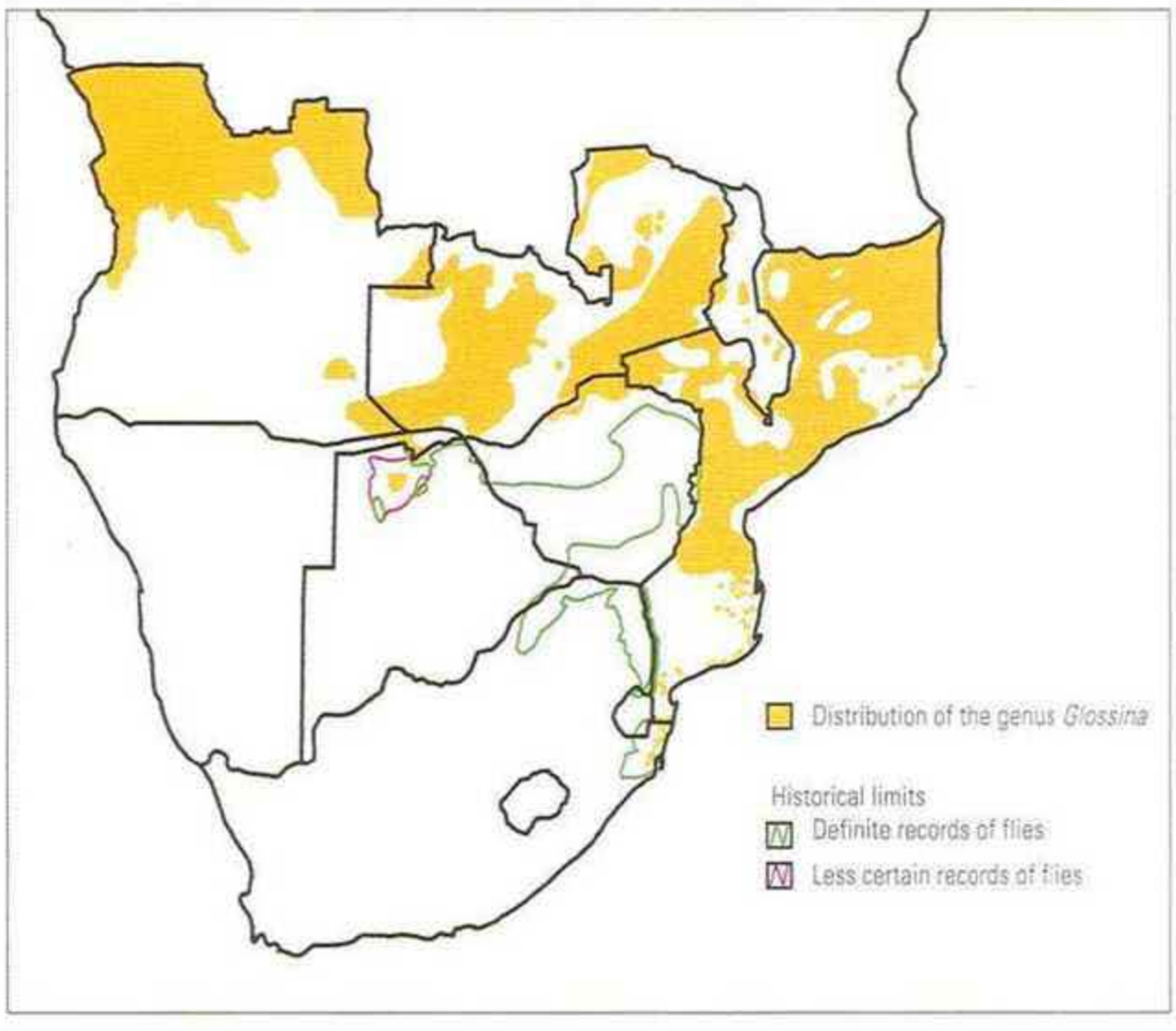


Figure 2.11 Distribution of the genus *Glossina* in southern Africa

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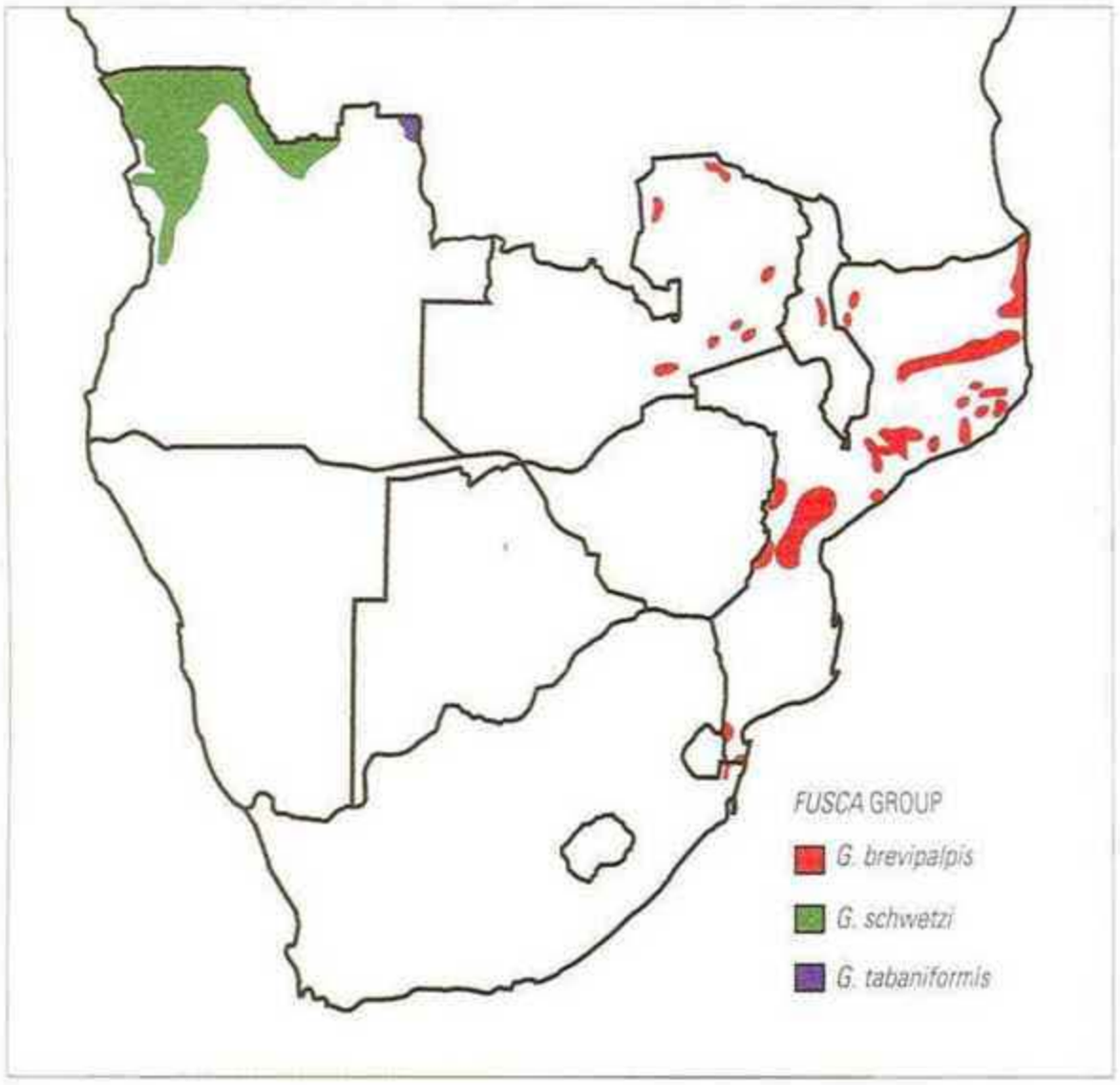


Figure 2.12 Distribution of tsetse fly species in the *fusca* group in southern Africa

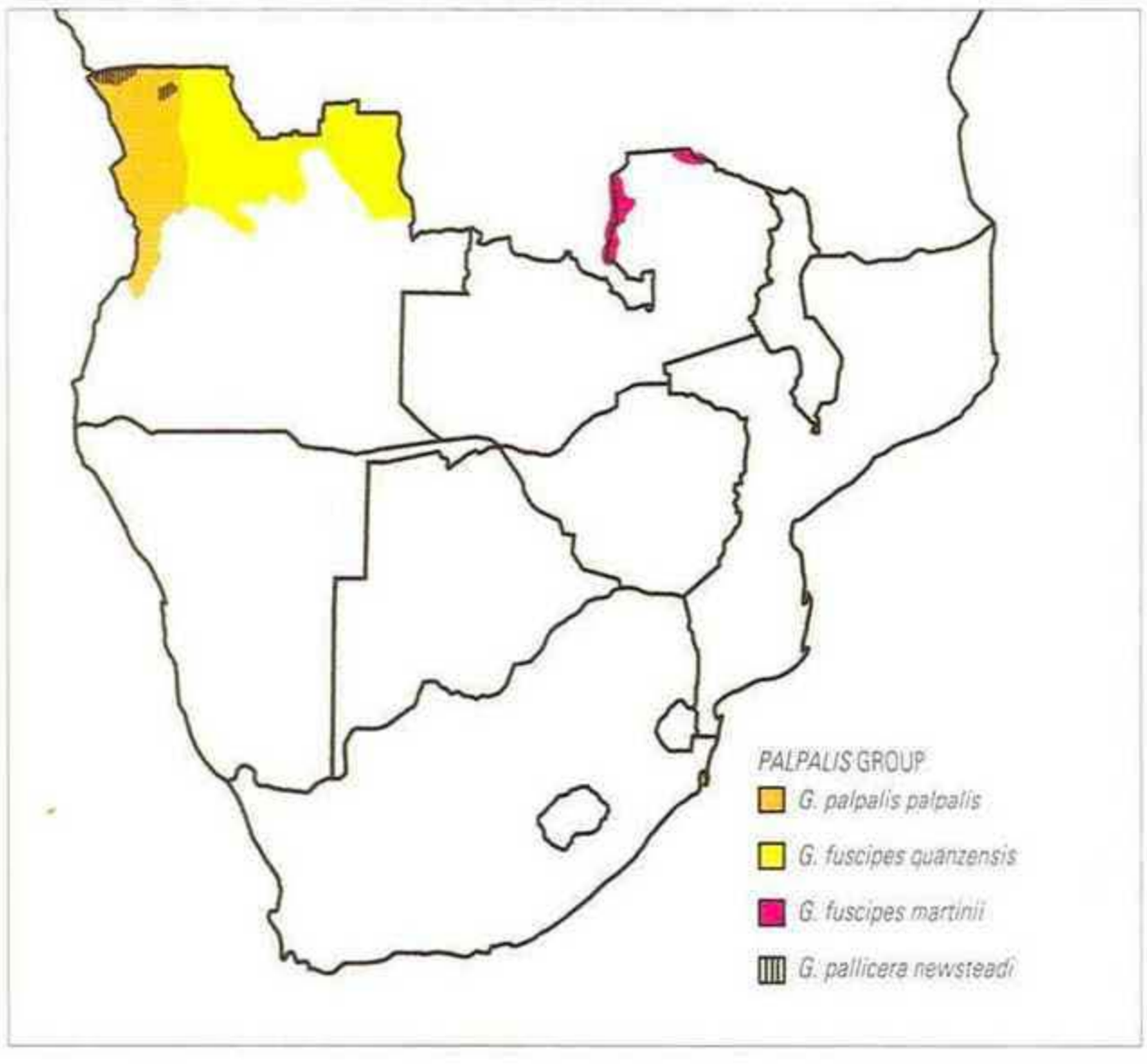


Figure 2.13 Distribution of tsetse fly species in the *palpalis* group in southern Africa

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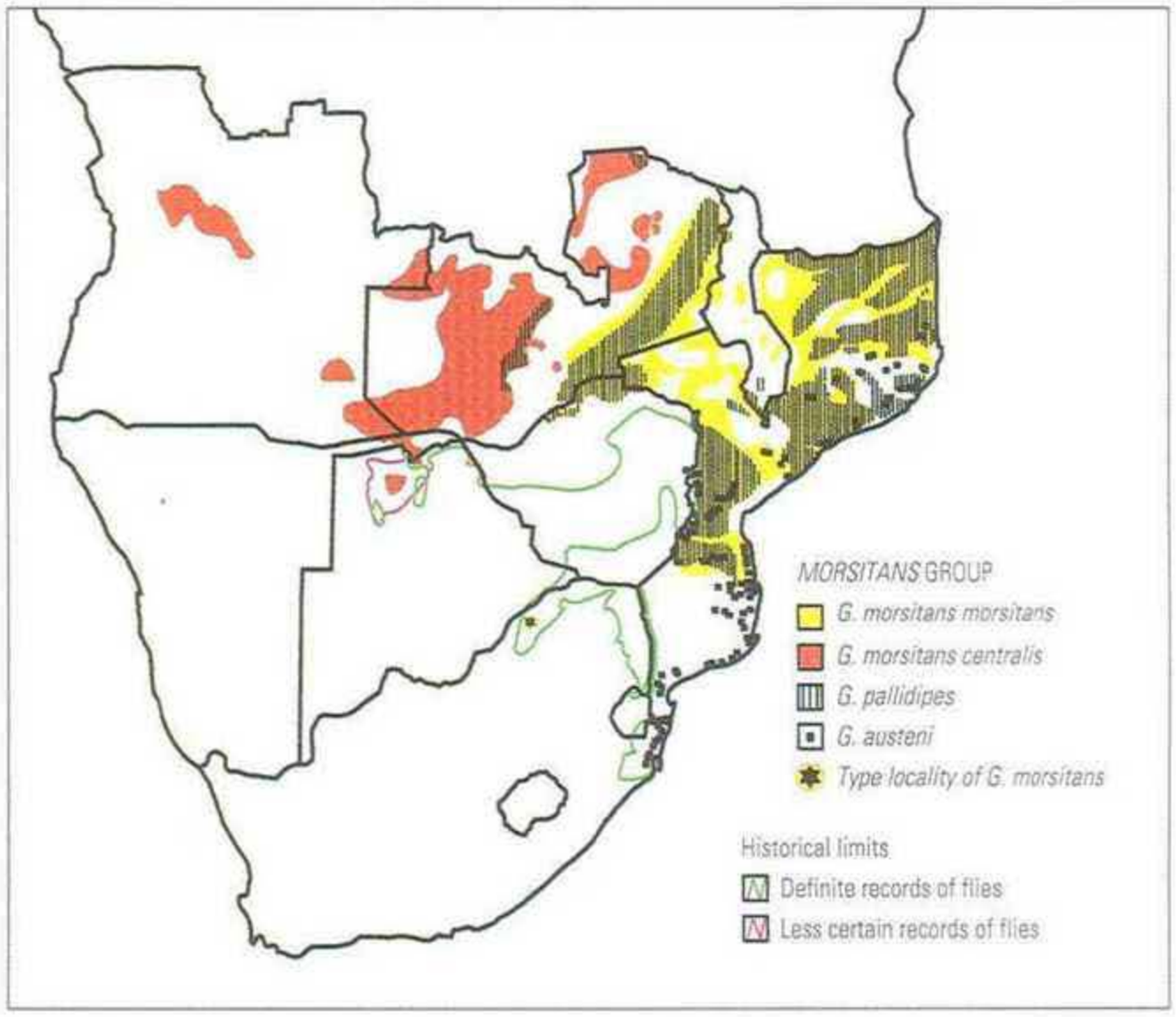


Figure 2.14 Distribution of the tsetse fly species in the *morsitans* group in southern Africa

personal knowledge of people currently working in the field, whose assistance is acknowledged with gratitude. The distribution of the genus *Glossina* in southern Africa is shown in **Figure 2.11** and that of the species *fusca, palpalis,* and *morsitans* groups within the genus is shown in **Figures 2.12, 2.13 and 2.14** respectively. Indication of the presence of flies on the distribution map (**Figure 2.11**) does not imply that they are uniformly distributed over the area, but rather that they are present where suitable habitat occurs in the region concerned. Some of the areas shown as tsetse fly-free have been cleared relatively recently and, in such localities, pockets of flies may still exist and will need to be eliminated as they are found.

The potential for use of satellite imagery in determining distribution of tsetse flies in Africa, as well as predicting mortality rates and population density, has been demonstrated.[199, 203] Predictions of the changes in tsetse distribution following global warming have also been made.[204]

Angola

The species that have been recorded from Angola, excluding Cabinda, are:

- ***fusca* group**: *G. schwetzi, G. tabaniformis*
- ***palpalis* group**: *G. fuscipes quanzensis, G. palpalis palpalis, G. pallicera newsteadi*
- ***morsitans* group**: *G. morsitans centralis*

No recent information is available from Angola, and the current boundaries of the distribution of tsetse flies there may differ considerably from those shown. This is especially true for the area infested by *G. morsitans centralis*, a species whose range has been expanding rapidly in some areas in recent years. The historical range of this species covered large areas of Angola, including the drainage basin of the Okavango River,[126] so much of southern Angola is potential tsetse fly habitat.

Cabinda has all the species recorded above for Angola, apart from *G. morsitans centralis*. In addition, two *fusca* group flies, *G. nashi* and *G. haningtoni*, are present.[66]

Botswana

The only species present is *G. morsitans centralis*. Its distribution in the Okavango swamp area varies from year to year, according to the extent of seasonal flooding and the consequent movement of game animals. The historical limits to this fly's distribution in Botswana[48, 49] are shown in **Figure 2.14**. After the rinderpest pandemic of 1896 to 1897, it was reduced to a few isolated foci and, although the recovery of game and cattle numbers after the pandemic was rapid, the tsetse fly's recovery was very slow,[48] but eventually it reached its previously known limits in many places.

The pre-rinderpest tsetse fly infestation in Botswana along the north-east border with Zimbabwe, and along the

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Zambezi between Livingstone and the Chobe River, has not yet been re-established. Flies in these areas may have been *G. morsitans morsitans*, but there are no known specimens available which could be used to confirm this.

Malawi

The species which have been recorded from Malawi are:

- ***fusca* group**: *G. brevipalpis*
- ***morsitans* group**: *G. morsitans morsitans, G. pallidipes*

When describing *G. pallidipes*, Austen[3] had specimens from Malawi in the material before him. This species has not been recorded from there since then, apart from a reference to it by Moloo[162] in 1985. Recent surveys using odour-baited traps have shown this species to be distributed in Malawi as indicated in **Figure 2.14**.

The extent of the distribution of the tsetse flies in Malawi varies seasonally, with the permanently infested areas being largely in game and forest reserves. This situation is comparable to that which existed with *G. pallidipes* in Zululand, South Africa.

Mozambique

The species which have been recorded in Mozambique are:

- ***fusca* group**: *G. brevipalpis, G. fuscipleuris* Austen
- ***morsitans* group**: *G. austeni austeni, G. austeni mossurizensis, G. borgesi, G. morsitans morsitans, G. pallidipes*

In this work, *G. borgesi* is treated as a synonym of *G. pallidipes*, following the view of Potts[190] and Pont.[188] However, the variability in morphological features found in *G. pallidipes*[20, 187] and in the Giemsa banding patterns of the chromosomes of this species,[138] indicate that *G. pallidipes* may contain subspecies in a manner analogous to *G. morsitans*. It is possible that further study will show *G. borgesi* to be a valid taxon.

Glossina austeni mossurizensis is the subspecies found in miombo woodland with dense undergrowth in the high rainfall, medium to high altitude areas along the Mozambique/Zimbabwe border, whereas *G. austeni austeni* is usually found in the drier coastal thickets. The type locality of both *G. borgesi* and *G. austeni mossurizensis* is the Sitatonga hills area 50 km north-east of Espungabera.

The distribution of the species in Mozambique is based on the maps of Ford and Katondo,[66] Dias[55] and Takken.[216] However, the capture in 1990 of specimens of *G. pallidipes* in Zimbabwe along the Mozambique border, just south of the Mazoe River,[217] indicates that the distribution of this fly in Mozambique is greater than is indicated in **Figure 2.14**.

The rinderpest pandemic of 1896 to 1897 had a profound impact on the tsetse flies in Mozambique, especially south of the Save River. Before the pandemic, *G. pallidipes* and *G. morsitans* extended to about the latitude of Maputo,[54] and *G. pallidipes* presumably linked up with the fly population in Zululand, South Africa. *Glossina morsitans* apparently did not reach as far as Zululand. Since the rinderpest pandemic, *G. morsitans* and *G. pallidipes* have not crossed the arid area between the Save and Limpopo rivers in Mozambique.[103, 216] The advance of tsetse flies into the south-east of Zimbabwe[194] might well have extended to the Mwenezi (Nuanetsi) River, which is on the Limpopo drainage system, had no control measures been taken. This could possibly have enabled these two species to reinvade their former habitat in southern Mozambique and, in the case of *G. morsitans*, the north-eastern region of South Africa.

A puparial case of *G. fuscipleuris* was found in 1962 in the riverine forest along the Uanetse River (a tributary of the Incomati River) in southern Mozambique,[54] which is in an area currently free of tsetse flies, but is known to have been infested prior to the rinderpest pandemic. This species has been recorded from the Livingstonia Mountains, east of the northern end of Lake Malawi.[26] However, the climates of southern Africa are known to have changed in recent geological times, and the distribution of *G. fuscipleuris* might well have extended much further south than at present. There may have been isolated relic populations of this species in southern Mozambique up to the time of the rinderpest pandemic.

Namibia

The only species recorded from Namibia is *G. morsitans centralis*. Control operations since 1964 have removed this fly from much of the region east of the Cuando (Kwando) River and have limited its spread west of the river.

The historical limits of the fly extended to the Okavango River drainage area, so the threat of expansion of the infested area in Namibia is a very real one. A final solution to the problem in this part of Africa can only be achieved by Namibian co-operation with the authorities in Botswana, Zambia and Angola.

South Africa

The species which have been recorded from South Africa are:

- ***fusca* group**: *G. brevipalpis*
- ***morsitans* group**: *G. austeni, G. brandoni* Chubb, *G. morsitans morsitans, G. pallidipes*

The historical distribution of tsetse flies in South Africa is dealt with by Fuller.[67] The only species recorded west of the Lebombo Mountains was *G. morsitans morsitans*, whose type locality is in North West Province (**Figure 2.14**). All tsetse flies disappeared from the northern regions of South Africa after the rinderpest pandemic, although reduction in the extent of the infestation began before that,[67] possibly as a result of the activities of hunters moving up from the southern regions.

Glossina brevipalpis occurred in Zululand and probably also in the gorges of the larger rivers where they cross the

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Lebombo Mountains.[67] *Glossina fuscipleuris* may have also occurred in these gorges.[54] *Glossina brandoni*, which has its type locality in Zululand, is a synonym of *G. austeni*, which also occurs in Zululand and is probably *G. austeni austeni* as it is found in the coastal thickets. The historical records show that *G. pallidipes* was the dominant tsetse fly in Zululand, but although there have been reports of *G. morsitans* from the area, these have never been confirmed.[67] The historical distribution of tsetse flies in Zululand indicates that it must have been an extension of that in Mozambique (**Figure 2.11**). At some time, the former distribution of *G. pallidipes* in Zululand must have been linked to that in Mozambique, although in post-rinderpest times there have been no *G. pallidipes* in Mozambique closer than about 650 km to Zululand.[60]

At the time of the elimination of *Glossina pallidipes* from Zululand in 1953, it was known that both *G. brevipalpis* and *G. austeni* were still present. They were confined to riparian fringe vegetation, forests and thickets. Cattle rarely enter these densely shaded areas where the flies were located, but minor outbreaks of trypanosomosis have occurred since 1954, and were contained by using curative drugs.[125] A major outbreak of trypanosomosis occurred in communal area cattle in Zululand in 1990, and the disease was diagnosed widely in the area between the Umfolosi River and the Mozambique border[125] (**see Chapter 12: African animal trypanosomoses**).

Changes in land use, such as the establishment of game farms and forestry projects, as well as large increases in human and cattle numbers, have taken place in Zululand since the distribution of tsetse flies was last plotted in the 1950s. A survey to determine the current distribution of tsetse flies in Zululand was initiated in 1995 and is scheduled for completion in 1999.[172] The survey results to date are shown in **Figures 2.11** and **2.12**. The results show that tsetse flies now occur in some areas where they were not recorded at the time that Du Toit did his work.[60] The two species may occur together but in some localities, such as Hluhluwe and Umfolozi, only *G. brevipalpis* has been recorded, while in Tembe Elephant Reserve only *G. austeni* has been found.[125] Both species of tsetse fly were involved in the 1990 outbreak of trypanosomosis. *Glossina austeni* was found to be restricted to very densely shaded places while *G. brevipalpis* will range out from densely wooded areas into more open woodland particularly at dusk. *Glossina brevipalpis* shows some nocturnal activity, especially on moonlight nights.

Swaziland

At present, no tsetse flies exist in Swaziland.

Historically, *G. pallidipes* occurred, at least seasonally, in the south-east of the country,[60] while *G. austeni* and *G. brevipalpis* were probably present occasionally along the Swaziland/Mozambique border (**Figure 2.11**).

Zambia

The species which have been recorded from Zambia are:

- ***fusca* group**: *G. brevipalpis*
- ***palpalis* group**: *G. fuscipes martinii*
- ***morsitans* group**: *G. morsitans centralis*, *G. morsitans morsitans*, *G. pallidipes*

The map given by Ford and Katondo,[66] and the list given by Moloo,[162] indicate that *G. fuscipes fuscipes* is present in Zambia but this has not been confirmed.[181] Phelps and Lovemore[181] consider the flies recorded as *G. palpalis fuscipes*[62] to belong to the taxon *G. fuscipes martinii*, as indicated by Machado.[147]

The boundaries of tsetse fly distribution in Zambia are not monitored continuously, and advances and recessions occur naturally, so the limits shown should be regarded as approximations.

Flies from the isolated Keembe pocket of *G. morsitans centralis*, east of the main distribution zone of this species in western Zambia, have a different allele frequency for some enzyme systems from flies in the main distribution area,[78] indicating little gene flow between the two populations. The duration of this isolation is not known, and the current advances of this subspecies from the west may cause these two populations to link up.

Recent surveys, using kairomone-baited traps, have shown *G. pallidipes* to be more common in Zambia than had been previously thought.

Robinson *et al.*[196, 197] have used multivariate analysis of climatic factors and remotely sensed vegetation data to predict habitat suitable for tsetse flies in eastern Zambia. Good agreement has been found between these predictions and the known distribution of tsetse flies in the area concerned. It was also found that the separation of *G. morsitans centralis* and *G. morsitans morsitans* distribution in the northern province of Zambia (**Figure 2.14**) could be accounted for on the basis of the maximum of the minimum temperature at any given altitude. Areas with a colder minimum temperature are inhabited by *G. morsitans centralis* and those with a warmer minimum temperature by *G. morsitans morsitans*.

Zimbabwe

The species which have been recorded from Zimbabwe are:

- ***fusca* group**: *G. brevipalpis*
- ***morsitans* group**: *G. austeni*, *G. morsitans morsitans*, *G. pallidipes*

Glossina brevipalpis has been recorded along the border with Mozambique in the Chipinge district, as has *G. austeni* (probably the high-altitude, moist environment subspecies). In recent years, though, neither these species nor *G. pallidipes* have been reported from this area.

In 1992, *G. morsitans morsitans*, *G. pallidipes* and *G. austeni* were captured in odour-baited traps in the Honde Valley, on the eastern slopes of the mountainous eastern

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border area of Zimbabwe, very close to the border with Mozambique.[218] No tsetse flies had been recorded from this area previously, although relatively large numbers of cases of trypanosomosis had been recorded sporadically from cattle grazing there.

The historical distribution of tsetse flies in Zimbabwe[110, 111] is shown in **Figure 2.11**. The southern tsetse fly 'belt' had started to break up prior to the rinderpest pandemic[36] and disappeared completely after the pandemic. An advance of tsetse flies into the south-eastern corner of Zimbabwe from Mozambique in the vicinity of the Gonarezhou National Park during the late 1950s and early 1960s was controlled and this part of Zimbabwe is now free of tsetse flies.[194, 195] There have been reports from Mozambique during 1999 that tsetse flies are beginning to recover from the earlier control operations in the north-western corner of Gaza Province and could soon present a threat to south-eastern Zimbabwe again.

There are high populations of tsetse flies on the Mozambique side of parts of the eastern border of Zimbabwe (**Figure 2.11**) and occasional cases of trypanosomosis occur in cattle in adjacent areas in Zimbabwe. These cases are attributed to occasional fly movements and to cattle grazing over the border in Mozambique. Only the northern part of Zimbabwe currently has a resident population of both *G. morsitans morsitans* and *G. pallidipes*.

Although some intermediate forms, in terms of male genital structures of *G. morsitans* have been recorded, crossing experiments have confirmed that, at present, *G. morsitans morsitans* is the only subspecies present in Zimbabwe.[182] It is not known whether, in historical times, the infestation in the north-west of the country was *G. morsitans morsitans* or *G. morsitans centralis*. The latter species is expanding its range rapidly in Zambia at present, partly because the extensive control measures needed to contain the fly are beyond the resources of that country. Possibly, therefore, *G. morsitans centralis* may reach the Zambezi River in the vicinity of Victoria Falls and, should this occur, Zimbabwe may be invaded from Zambia by a subspecies of *G. morsitans* not previously recorded in Zimbabwe.

Control

Environmental modification

Effects of humans on tsetse flies in savannah and riverine areas In the past there have been many fluctuations in human populations in Africa which have been attributable to disease epidemics, tribal conflicts or the colonization of Africa.[65] The advent of western medicine has reduced the death rate in human populations in Africa dramatically and, as the birth rate has remained largely unaltered, there has been exponential growth of the human population. Where these high human populations are confined, for example by lack of additional arable land or territorial boundaries, the human density has increased greatly. Southern Africa is currently experiencing these population pressures. The situation shows no signs of stabilizing yet and, in the absence of catastrophic events, populations will continue to increase for many years. The population numbers are already in excess of any known historical levels in this part of Africa, so examples of the past interactions between humans and tsetse flies may be of academic interest only. However, some indication of the sort of interactions that may occur are given.

The effect of humans on the different subgenera of tsetse flies differs greatly, due to the differences in habitat requirements of the flies. The destruction of the forests of Africa, partly for commercial timber and partly for settlements and agricultural land, is proceeding rapidly, and this process removes the habitat of the *fusca* group of flies. It is no longer possible to leave cleared land fallow after it has become too depleted in nutrients to support crops, so clearings become permanent. The human population also destroys the food supply of the tsetse flies through hunting, so they disappear. The *fusca* group of flies are likely to survive only in forest reserves in future. In southern Africa, *G. brevipalpis*, which is the most widely distributed member of the *fusca* group, is confined to riverine forests and to the few humid forests that occur in the region. This limited distribution makes it a relatively unimportant species in the context of disease transmission in southern Africa but it is of importance in Zululand, where *G. pallidipes* has been eliminated.

The influence of humans on the *palpalis* group of flies is not always adverse, and they can often exist in close contact with people and their domestic stock. These flies, particularly *G. palpalis*, feed on a wide variety of wild and domestic animals, including reptiles, and they may be involved in disease transmission even in densely populated areas.[122] Their habitat is riparian vegetation: human activities do not always result in the elimination of this vegetation type. Agricultural activities tend to be in areas further from the streams in high rainfall areas, and the tree plantations grown there may themselves extend the habitat of the fly. Patches of natural vegetation protected by humans for cultural reasons also occur in many parts of Africa and may provide suitable habitat for *palpalis* group flies, provided domestic animals are readily available as a food source. Members of the subspecies of *G. fuscipes* can be closely associated with humans and, in western Kenya, they have been able to live in extensive thickets of *Lantana camara*, which have developed as a result of poor land management.[263] Flies in the *palpalis* group have a restricted distribution in southern Africa, but where they do occur, they may be important vectors of disease in both humans and domestic stock.

It is on the *morsitans* group of flies that humans have made their greatest impact in southern Africa. *Glossina morsitans* and *G. pallidipes* are widespread here, and are characteristic of the undisturbed savannah and wooded savannah vegetation types, where wild animals are plentiful. The agricultural activities of humans, and their use of fire, have played an important part in the development of savannah

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vegetation, so in this sense, humans have been of benefit to the tsetse fly. But humans often enter tsetse fly-infested savannah country to undertake agricultural activities. If cultivation in these areas is continued on a long-term basis, the vegetation becomes either cropland or grassland, and at the same time the wild animals are killed or move away and the flies ultimately die out. In the past, land was abandoned when its fertility had declined. Such land would, in the course of time, revert to savannah vegetation and the wild animals would return, making the area suitable again for *morsitans* group flies. Thus the situation was one of long-term rises and falls of fly populations, depending on the activity of human populations. This shifting pattern of agriculture is becoming less frequent in southern Africa, and more permanent occupation of the land by humans leads to permanent removal of the *morsitans* group of flies. The density of human settlement needed to achieve this removal of tsetse flies is not known exactly. In West African savannah, densities of 15 to 39 people per square kilometre reduce *morsitans* group flies to very low levels, and at human population levels higher than this no tsetse flies exist.[170] Some parts of southern Africa have always had a high human population density.[213] An account of the waxing and waning of the *Glossina* population in the Mossurise region of Mozambique, consequent to changes in human population densities in this heavily settled area between 1861 and 1889, was provided by Swynnerton.[213]

In the 1970s the civil war in Zimbabwe prevented control of tsetse flies along the north-east border of the country. Over a period of five years the fly (in this case *G. morsitans*) invaded the country rapidly, reached its historical limits, and even progressed slightly further in some places. However, much of the country that was reinvaded was marginal for tsetse flies and their numbers remained low, but the Umfurudzi Safari Area, lying in broken but well-watered country and with a large wild animal population, provided a good habitat and flies built up to large numbers there. This invasion gave rise to cases of trypanosomosis in cattle near Shamva, an area which had been free of tsetse flies since the rinderpest pandemic in the 1890s.

In the Sebungwe and Umniati districts of Zimbabwe, outbreaks of human trypanosomosis, in 1912 and 1934 respectively, caused the authorities of the day to eliminate the problem by moving the human population involved.[7] In the long term, this sort of action favours the vector since, in the absence of any fly control measures, the abandoned agricultural lands revert to suitable tsetse fly habitat. Similar events have occurred in East Africa.[65]

Humans also affect the distribution of tsetse flies indirectly by creating wildlife sanctuaries. If these areas are in tsetse fly-infested country, they serve as a source of the flies to domestic stock grazing on adjacent land, as has occurred in Zululand[60] and is currently the case in Malawi.[177] There is generally resistance to the use of any anti-tsetse fly measure in the sanctuaries. However, if large elephant populations are confined to the sanctuaries and are allowed to increase, they will modify the habitat. These habitat changes may make the area unsuitable for tsetse flies, and for many other animal species, as has happened in some East African parks.[64] A similar process is taking place on the escarpment of the Zambezi River Valley in Zimbabwe, where hillslope woodland is being converted to grassland by high densities of elephants.

Rinderpest This disease entered Egypt from Arabia in the late 1880s, and a pandemic of it swept down Africa in a wide variety of animals. It had reached Lake Malawi by 1892 and crossed the Zambezi in 1895 to 1896 (**see Chapter 49: Rinderpest**). There are endemic foci of infection remaining in East Africa now, but none in southern Africa.

This disease almost eliminated cattle in the infected area and severely depleted the numbers of many wild animal species in southern Africa. Not all wild animals are susceptible, but African buffalo, kudu, eland (*Taurotragus oryx*), bushbuck, bushpig and warthog died in large numbers. Other bovids, such as sable antelope (*Hippotragus niger*) and roan antelope (*Hippotragus equinus*), impala and blue wildebeest (*Connochaetes taurinus*) were less affected in the pandemic, while elephant, rhinoceros and hippopotamus (*Hippopotamus amphibius*) were unaffected.[28, 211] The susceptible animals thus include the preferred hosts of *G. morsitans* and *G. pallidipes* in particular.[193, 255] This was not, of course, known at the time of the rinderpest pandemic, but after the disease had been eradicated from southern Africa, Stevenson-Hamilton[211] pointed out that, as not all animal species were equally affected, the tsetse flies must have been associated with those that were most susceptible. The view held by some people at the time of the pandemic that rinderpest virus was itself pathogenic to tsetse flies, has been shown to be incorrect.[27] The effect of the pandemic was not uniform, and in some localities appreciable numbers of animals of the susceptible species survived.[211]

The effect on the *G. morsitans* and *G. pallidipes* populations of this severe, rapid change in the environment was dramatic. In Botswana, tsetse retracted to four small foci, while in Zambia, the Kafue tsetse fly belt shrank considerably and tsetse flies disappeared completely from the Western Province.[266] There were also changes in Namibia and southern Angola. In Zimbabwe, *G. morsitans* disappeared from much of its range,[110] and disappeared completely in South Africa.[67] In Mozambique, tsetse flies, particularly *G. morsitans* and *G. pallidipes*,[54] disappeared from the southern part of their range.[67] In parts of North Mossurise in Mozambique, *G. morsitans* numbers did not decline in spite of heavy mortality from rinderpest among wild animals.[213] As there have been similar reductions in *G. morsitans* populations after at least four outbreaks of rinderpest in East Africa, there can be little doubt that the cause was the removal of its food supply. Flies may have survived in some localities because some herds of susceptible animals remained uninfected, or because of the presence of non-susceptible

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animals such as rhinoceros and hippopotamus (often very localized in their distribution) which can provide good sources of blood for tsetse flies. Rhinoceros blood has been found in *G. morsitans* and *G. pallidipes* in Zimbabwe at some localities,[193] and *G. brevipalpis* is known to feed readily on hippopotami.[256] The exclusion of rhinoceros from the animal host list during the campaign to eliminate tsetse flies by game reduction in the 1930s may have played a considerable part in maintaining the tsetse flies[60] and suggests a possible mechanism by which flies were able to survive the rinderpest pandemic in Zululand.

Animal reduction as a tsetse fly control measure The pre-rinderpest retreat of *G. morsitans* in the Limpopo Province of South Africa[67] and in north-western Zimbabwe[36] was attributed to the activities of big game hunters and the advance of settlers from the south. The effects of rinderpest provided further evidence of the importance of wild animals to *G. morsitans* in particular. As the animal populations in southern Africa recovered after the rinderpest pandemic, so did those of the tsetse fly, and they began to spread to their former limits. The flies soon came into contact with humans and their domestic stock, and the governments of the various countries had to undertake measures to reduce the disease problems associated with tsetse flies. The most obvious way to attack the fly at that time was to remove its source of food by reducing the number of wild animals.

In Zimbabwe, the numbers of wild animals were reduced by hunting between parallel fences 16 km apart.[109] A broad spectrum of species was shot, and the method was successful in reclaiming large areas of what is now some of Zimbabwe's most productive farm land. The large-scale destruction of wildlife is abhorrent,[111] but at that time it was the only practicable method of controlling tsetse flies, which threatened disaster to the farming community in Zimbabwe. Broad spectrum game reduction campaigns against tsetse fly, either between fences or not, were undertaken in Zambia,[62, 265] Botswana[48] and Zululand (South Africa),[60] but the success achieved was generally not as good as that in Zimbabwe. As alternative methods of tsetse control became available, game reduction campaigns were abandoned in most countries.

Following the demonstration of host preferences of tsetse flies,[255] an experiment was conducted in Zimbabwe between 1962 and 1964 which showed that a spectacular decline in *G. morsitans* numbers followed the hunting out of warthog, bushpig, bushbuck and kudu.[149] Elephant and buffalo were driven out of the fenced experimental area prior to the start of the experiment, because of the damage they do to fences and also because they can be a good source of food when present in an area. No other animal species were hunted. As warthog provided about half the blood meals of *G. morsitans* at the experimental site, the effect of the removal of just warthog was tested.[243] It was shown that the flies adjusted rapidly to other preferred hosts in this area, particularly kudu. The results from these experiments led to the subsequent use in Zimbabwe of selective hunting of the four preferred species between parallel fences to prevent the advance of tsetse flies. The fence immediately adjacent to the fly area was more robust and large animals such as elephant and buffalo were kept away from it by limited hunting. This approach to animal reduction initially enabled the tsetse advances to be contained, but became ineffective during the civil war in the 1970s. Whilst hunting operations were resumed soon after the cessation of hostilities, including those conducted for the protection of fences, these activities were short-lived and by the 1990s had been abandoned completely in favour of other techniques.

Vegetation modification Studies on the biology of tsetse flies have shown that in hot weather they depend on the vegetation to shade both their refuge sites and the sites in which larvae are deposited. Vegetation patterns have been modified by fire in southern Africa, and the elimination of fire may result in the development of patterns unsuited to some tsetse species.[74, 215] The use of this approach to control tsetse flies in East Africa and Zambia has not been generally successful, as it is impossible to exclude fire from large areas of Africa completely.[74, 215] Also, this method takes effect slowly and there is usually a demand for quick results where tsetse fly problems occur. The direct effects of bush fires on adult tsetse flies and their puparia have been investigated in Zimbabwe,[35] but no evidence has been found that these fires control the flies.

As tsetse flies do not occur in grassland, the clearing of woodland to produce grassland can create a barrier to tsetse flies, but the difficulty is that, to be effective, the clearing has to be wide, although the exact width needed is not known: it apparently varies between species. Barrier clearings have been made in Zimbabwe on the eastern border with Mozambique,[111] and in Zambia,[265] Zululand,[60] Botswana[48] and Malawi, where clearing was combined with settlement.[130] Although barrier clearings have achieved moderate success, they are expensive to create and must be maintained indefinitely. They are not widely used at present. Attempts have been made to use herbicides to control regenerating vegetation,[37, 38] but the chemicals are expensive and may have deleterious effects on the environment.

Particularly in East Africa, the vegetation requirements for tsetse fly habitats can be divided into essential and non-essential components. The essential components might comprise a small proportion of the total area and, if removed, would result in the collapse of the fly population. In more arid parts of the flies' range, essential habitat is confined to water courses, but in better watered areas vegetation patterns with well-developed upper and lower canopies are thought to be essential for the flies' survival. In southern Africa the discriminative clearing approach (removal of essential habitat only) has been attempted but not widely used.

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Discriminative clearing was used between 1941 and 1942 to eliminate *G. morsitans* near Abercorn in north-eastern Zambia.[74] Vegetation was removed only in the shallow valleys at the headwaters of small streams, which formed only a small proportion of the total tsetse fly-infested area. This campaign was one of the few successful attempts at discriminative clearing. In Zimbabwe, extensive discriminative clearing in the south-east of the country was done between 1956 and 1961 in an attempt to arrest advances by both *G. morsitans* and *G. pallidipes* into Zimbabwe from Mozambique.[75] Much of this arid area is marginal country for tsetse flies,[103] but in some places riverine fringe vegetation and patches of hillslope woodland provided reasonable habitat and abundant wildlife. The clearing certainly helped to limit the flies, but it did not eliminate them. The costs were high, as much of the work was done by bulldozers, and the destruction of riverine vegetation is likely to be permanent in this very harsh part of the country. This form of tsetse fly control has not been used recently, either in Zimbabwe or elsewhere in southern Africa.

Pesticides

Application from the ground After the Second World War, the advent of synthetic organic insecticides, and particularly the long-acting organochlorines, led to new methods of tsetse control. The flies' hot, dry season habitats (i.e. their essential habitats) can be determined by sampling for adults or puparia. Within the essential habitats refuge sites of the flies can be recognized.[185, 186, 192] The determination of these sites is easier in very arid country, but even in well-watered woodland it is possible to recognize them. If these hot season resting and refuge sites are treated with suitable long-acting insecticide, applied from knapsack sprayers by teams moving through the bush, the flies will ultimately come into contact with this insecticide and be killed. This so-called ground spraying involves the recognition of essential tsetse fly habitats on aerial photographs and the subsequent development of access tracks to these places, followed by the application of insecticide to them. The insecticide is applied in the cool, dry season, so that as much country as possible can be treated before the onset of the hot weather. Where there is heavy grass cover, it may be necessary to burn this before application of the insecticide, which is decomposed by fire. The most widely used insecticides in this form of tsetse fly control have been dieldrin and DDT. However, in Botswana, Namibia and Zimbabwe they were more recently replaced by synthetic pyrethroids for environmental reasons. The substances used for this purpose have a residual life of at least four months in the dry season. Ground spraying has now been replaced almost completely in these countries by the odour-baited, insecticide-treated target technique, again for environmental reasons.

In southern Africa, an advance of tsetse flies in south-eastern Zimbabwe and south-western Mozambique was halted by ground spraying and a large area was reclaimed from the fly. This campaign was mounted jointly between 1962 and 1974 by South Africa, Mozambique and Zimbabwe.[144, 145, 146, 194, 195] Ground spraying has been the major method used to eliminate tsetse flies in Zimbabwe from 1960 to 1986, and has also been used extensively in both Nigeria[151] and Botswana.[48] In Zambia, ground spraying has been done with both knapsack[150] and motorized sprayers carried on Unimog vehicles.[176] Ground spraying has been conducted in the Caprivi strip region of Namibia and experimentally in Malawi, but large areas have not been cleared of tsetse flies by this method in either of these countries.

The use of long-acting insecticides leads to environmental contamination, and much concern has been expressed over tsetse fly control schemes based on ground spraying. Acute poisoning of birds and other wildlife by spraying with dieldrin against tsetse flies has occurred in Botswana.[80] Residues of DDT and its metabolites are accumulated by non-target organisms in sprayed areas. Following the indication of potential adverse effects on animal populations in Zimbabwe,[153] a comprehensive four-year study was undertaken in a section of the Zambezi River Valley in Zimbabwe. The study area bordered on Lake Kariba, where ground spraying with DDT and, in a small scale trial, deltamethrin against tsetse flies was being conducted. The results[57] showed little or no effect of DDT on reptile populations, some indication of effects on some ant species, and deleterious effects on populations of several species of insectivorous birds. In particular, populations of the white-headed black chat (*Thamnolaea arnoti*), which feeds extensively on ants, were severely reduced by acute DDT poisoning. Eggs of terrestrial avian raptors, such as the African goshawk (*Acipter tachiro*), showed marked thinning of the egg shell, a factor known to be associated with chronic poisoning by DDE, a metabolite of DDT. In many parts of the study area along Lake Kariba, eggs of the African fish eagle (*Haliaeetus vocifer*) showed egg shell thinning, whereas in other parts very little egg shell thinning was evident. No detectable effects of DDT were found on soil processes. Residues on sprayed tree trunks and soil were not long lasting. No severe adverse effects were demonstrated on the fish species studied. There were indications that populations of some species of bats roosting in hollow trees could be adversely affected by ground spraying with DDT.

A smaller study[57] on the environmental effects of deltamethrin used in ground spraying against tsetse flies, showed that populations of non-target invertebrate species were severely affected in both terrestrial and aquatic habitats. No major effects were seen on other animals, although the large decline in insect populations may have caused insectivorous species to move to untreated areas.

None of the species found to be adversely affected by the pesticides used in ground spraying against tsetse flies were endemic to the study area. Following elimination of the tsetse flies, it would be anticipated that invasion from surrounding unsprayed areas would occur, as well as recovery

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of the residual animal populations in the sprayed area. This would not happen if the spraying was done near settled areas, as human settlement results in major changes in the environment and many habitats are destroyed.

Another form of insecticide application from the ground is the use of pesticide dusts. This approach was used in Zululand, South Africa,[60] but not elsewhere in southern Africa.

Application from the air The first aerial spraying campaign against tsetse flies was in Zululand between 1945 and 1948.[60] Initially, a coarse spray of atomized liquid droplets was applied, but this resulted in the formation of toxic residual deposits of the chemical. This approach has not been used elsewhere in southern Africa but has been tried in West Africa, where helicopters have been used to apply the spray.[4]

The second approach to the aerial application of insecticide was the use of insecticidal smoke or thermal aerosol.[60] In this form, normally long-acting insecticides such as DDT or hexachlorocyclohexane (formerly known as benzene hexachloride or BHC) have no residual action, and the environment is simply fumigated. In Zululand, the applications were made in the early morning and late afternoon to take advantage of the temperature inversion conditions, which prevent the aerosol cloud rising from the ground. The aerosol was sprayed in the tsetse fly habitat from aircraft flying just above the tree tops. A slight wind is an advantage, as the aerosol cloud then drifts through the habitat, but strong winds disperse this cloud and its application has to be stopped until favourable conditions return.

The aerial application of insecticides initially developed by Du Toit[60] has now become more sophisticated, but the underlying principles are the same. Allsopp[1] has reviewed insecticidal methods used against tsetse flies and outlines the current approach to aerial spraying. The development of rotary atomizers which produce a uniform aerosol droplet size and, in particular, the development of night flying techniques that permit spray aircraft to operate throughout the night, have made the sequential application of insecticide from the air a more practical and economical proposition. Such applications need to be made at regular intervals, based on the time it takes for a newly emerged female tsetse fly to deposit her first mature larva, a process which is temperature-dependent and may also depend on how well fed the fly population is.[94] Applications must continue until all the puparia which were in the ground at the start of the operation have given rise to adult flies, another process which is temperature dependent. Operations are carried out in the winter months, to take advantage of the temperature inversion conditions and generally calmer weather, but this means that five cycles of spraying at 10- to 18-day intervals may be needed when the long puparial period at low temperatures is considered. Endosulfan is the most widely used insecticide in aerial spraying at present, but deltamethrin was found to be a suitable alternative in the last Regional Tsetse and Trypanosomiasis Control Programme operation in Zimbabwe. Deltamethrin and alphacypermethrin have also been used in Botswana.[266]

Large-scale aerial spraying campaigns were conducted at irregular intervals in Zimbabwe between 1953 and 1988.[33, 40, 105, 106] The more recent aerial spraying, sponsored by the Regional Tsetse and Trypanosomiasis Control Programme, has been successful against *G. morsitans* but less so against *G. pallidipes*. Aerial spraying conducted in Zambia has been largely successful.[175] As in all insecticide work against tsetse flies, the cleared areas may be reinvaded if they are not developed and settled by humans or the operations are not progressive. In Botswana, following successful preliminary aerial spraying trials in 1972, parts of the Okavango Delta were sprayed annually from 1973 to 1991 (excluding 1988). In general, although not exclusively, the annual operations up to 1979 alternated between the western and south-eastern perimeters of the Delta. Thereafter, the operations were aimed at the main body of the infestation.[266]

Although pesticide application rates are very low in the aerial spray method for tsetse fly control, many aquatic organisms are killed, especially in shallow water. The effects of aerial spraying on non-target organisms have been monitored on several occasions[39, 58, 153] and it has been found that generally, if the insecticide is applied at the correct level, and only in one year, no permanent damage to the environment occurs. These conclusions have been supported by the Scientific Environmental Monitoring Group,[207] which is part of the Regional Tsetse and Trypanosomiasis Control Programme. The same group also stated that 'if areas without open water are treated with endosulfan (6 to 24 g/ha), and areas with rivers and lakes are treated with deltamethrin (< 0.3 g/ha), then according to our present knowledge the side-effects seem to be acceptable.'[169]

Other methods for generating aerosols have been used in anti-tsetse fly work. Thermal smoke generators have been used, in which a combustible mixture of sodium chlorate, sugar and DDT are ignited to generate an insecticidal smoke.[60] Commercially available insecticidal fog generators, such as the Swingfog, have been employed in Zimbabwe and Botswana, and the vehicle-mounted TIFA has been used extensively in Zambia. The use of these methods have tended to be discontinued as aerial application techniques improved.

Ivermectin The use of ivermectin administered to animals as a means of killing tsetse flies has been investigated.[139] Although flies feeding on treated animals died, the cost of ivermectin at the dosage levels required to be effective is considered to be uneconomical. It has been suggested that formulations of ivermectins other than those used may be more suitable, but they have not yet been investigated.[258]

Insect hormones Insect growth regulators such as diflubenzuron, when applied to adult female tsetse flies, cause the female to produce larvae which do not pupariate.[123] Similar effects have been found with insect juvenile

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hormone analogues.[52, 135, 258] Field studies in Zimbabwe with the juvenile hormone mimic pyriproxyfen have demonstrated that it can be used in conjunction with traps, using the system developed by Hall and Langley,[89] to, in effect, sterilize the tsetse population.[95] The technique was developed further by applying pyriproxyfen in place of conventional insecticides in the widely used odour-baited target system for killing tsetse flies.[96] Laboratory studies on triflumuron, a chitin synthesis inhibitor,[134] showed that it induced abortion in tsetse flies at higher doses as well as preventing the emergence of adults from puparia at lower doses, thus effectively sterilizing the flies. Triflumuron has been tested on a field scale in Zimbabwe and the preliminary indications were that it would be a suitable candidate material for use in tsetse control by means of odour-baited targets. These materials are available should it become undesirable to use pyrethroids.

Traps The use of traps in attempts to control tsetse flies was first developed in Zululand, South Africa, in 1930.[98] Many *G. pallidipes* were killed in this control campaign, but the flies were not eliminated. Four per cent or more of the female flies in a population must be eliminated each day for a tsetse population to decline to extinction.[92] Despite the large numbers killed in Harris's traps, this level of elimination could not have been attained. Various traps were designed in the 1930s after the work done by Harris,[112, 214] most being based on a visual stimulus to the fly, although Fuller and Mossop[68] had shown clearly the importance of odour to *G. pallidipes*.

Since the 1970s intensive attempts have been made to improve traps for tsetse flies both in West Africa[32, 131] and in Zimbabwe.[63, 91, 238] In West Africa, after the attractiveness of blue cloth had been discovered, the effectiveness of traps for flies in the *palpalis* group was greatly increased by using traps covered with blue cloth (**Figures 2.15, 2.18 and 2.19**).

Various patterns of traps have been developed in Africa.[42] Traps may be used to monitor tsetse populations and are widely used in Africa to assess the success of control operations against tsetse flies. If the traps are treated with insecticide, or have a collector system from which the flies cannot escape, the traps can be effective in killing large numbers of flies. Such traps have been used to break transmission of trypanosomosis in livestock and humans.[15, 59, 83, 142]

In eastern and southern Africa, bright royal blue (phthalogen blue) cloth has been incorporated into traps, which originally had a white outer cover, a colour known to be attractive to some tsetse flies. Both blue and black cloth are attractive to *G. morsitans* and *G. pallidipes*,[85] but if given a choice, the flies prefer to alight on black cloth. Not all species of flies react in the same way to colour. In Zululand it has been found that *G. austeni* is strongly attracted to bright royal blue cloth but not to black cloth.[124] When given the choice, this tsetse fly alights in roughly equal numbers on black or blue cloth. Traps such as the Epsilon and F3 (**Figures 2.15 and 2.18**) used against *G. morsitans* and *G. pallidipes* have a blue outer cover to attract flies and a section of black material within the trap to induce flies to enter the traps and settle. When ready to move many of the flies on the black cloth fly upwards into the white mosquito net conical canopy, which leads to the retaining device. It is important to have the canopy well illuminated as the upward flight is in response to the light. In Zululand, both *G. brevipalpis* and *G. austeni* can be caught in a low trap with a blue outer cover and black internal crossed screens and with two conical canopies in a lateral position.[172] These two fly species tend to fly horizontally when taking off, rather than vertically as is the case with *G. pallidipes* and *G. morsitans*.

Crossed plywood panels painted blue and covered with sticky material have been used to catch *G. austeni*.[152] A much improved version of this trap has been used in surveys for both *G. austeni* and *G. brevipalpis* in Zululand.[172]

The performance of traps for *G. morsitans* and *G. pallidipes* has been greatly improved following the identification of kairomones produced by host animals. Carbon dioxide is known to attract all species of tsetse flies on which it has been tested but it is not feasible to use it on a large scale in the field. Kairomones found so far which can be used in the field are acetone, methyl ethyl ketone (butanone), 1-octen-3ol (octenol), 4-methylphenol, 3-*n*-propylphenol and 3-methylphenol. Not all of these substances are effective against all flies, and not all need be included in a bait for it to be effective. The bait used in Zimbabwe for *G. morsitans* and *G. pallidipes* consists of 3-*n*-propylphenol, octenol and 4-methylphenol in the ratio of 1:6:12 (originally 1:4:8) contained in a polythene sachet.[244, 245, 247] The sachets are made from low-density polythene lay-flat tubing with walls 0.15 mm thick and heat sealed to contain the bait. Sachets measure 5 cm by 5 cm and contain about 10 ml of bait. Release rates from the sachets are a function of the density of the polyethylene, the thickness of the sachet walls, surface area of the sachet and the temperature at which the sachet is held.[226] The relationship with temperature is exponential, and surface temperatures of sachets in the sun are up to 10 °C greater than ambient temperatures so it is difficult to give exact rates of bait release. It is often quoted as total released per day at a given ambient temperature, but the actual release rate varies greatly during the day. In addition to the bait in the sachet, acetone or butanone is released from a glass bottle with a hole in the lid.[229] Mean release rates through a 2-mm-diameter hole are about 150 mg/hour and through a 6-mm-diameter hole about 500 mg/hour.[59] Plastic bottles with appropriate thickness of the walls and surface area can be used to dispense butanone[229] and also to replace sachets for the octenol and phenol components of the bait.

The kairomones used in the field in Zimbabwe against *G. morsitans* and *G. pallidipes* are ineffective against the *palpalis* group of flies in West Africa, apart from *G. tachinoides*, which responds to the bait used in Zimbabwe, although

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Figure 2.15 Epsilon trap showing the entrance



Figure 2.16 Target with black centre and two netting panels



Figure 2.17 Black cloth target being sprayed with deltamethrin



Figure 2.18 F3 trap showing entrance and collecting apparatus



Figure 2.19 Challier/Laveissière biconical trap

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different proportions of the constituents are needed. In West Africa *G. longipalpis* (*morsitans* group) and *G. medicorum* (*fusca* group) also respond to the bait used in Zimbabwe.[210]

In Zululand, *G. austeni* responds to carbon dioxide but not to any of the other known kairomones. *Glossina brevipalpis* responds to carbon dioxide, octenol, 4-methylphenol and acetone (but not to butanone).[124] The doses of octenol and 4-methylphenol used in Zululand are higher than those used in Zimbabwe and the use of acetone is only of benefit in the dry season. The reason for this seasonal effect has not yet been determined.

Behaviour of tsetse flies in odour plumes resulting from release of kairomones has been investigated[13, 223, 225] and it has been shown that flies may react differently at different doses of odour. Comparison of synthetic odour to the odour from an ox, at the same dose, indicated that further attractant(s) exist in natural ox odour[225] for both *G. morsitans* and *G. pallidipes*. A similar conclusion was reached for *G. brevipalpis*, and possibly for *G. austeni*.[124] Comparing odour from small to large numbers of cattle with an equivalent dose of the synthetic odour used in Zimbabwe for *G. morsitans* and *G. pallidipes*[97] demonstrated a relationship between quantities of odour and fly catch, so that a tenfold increase in odour gave a 2,3- to 2,8-fold increase in catch for *G. pallidipes* and 1,4-fold increase for *G. morsitans*. It was also shown that odour from the cattle was more effective than that from the synthetic odour, again indicating that further attractant(s) remain to be identified in host odour. Skin secretions (sebum) of cattle also affect the behaviour of tsetse flies,[252] especially after alighting on a stationary cloth screen.

The way in which tsetse flies move through the habitat has an important bearing on where traps should be positioned so as to be most effective. Tsetse flies use game trails when following odour in thicket[176] and tend to fly round bushes rather than over them. Vale has developed a deterministic model to indicate how flies move through three vegetation types in Zimbabwe.[241] When placing traps in the field, they should be clearly visible to a fly but need not be in a large clearing. Provided there are breaks in the vegetation of over a metre wide, a hungry fly will move up an odour trail. There should be no distracting host-sized objects, such as rocks or small dense bushes near the trap, and if the trap is of the single entrance Epsilon or F3 type, the entrance should face downwind. It is particularly important that the canopy of the trap is well illuminated so that flies move up into the collecting device. If a trap is needed in a position that does not meet the requirements, a small amount of trimming of the vegetation can improve the quality of the position and result in better catches. In well-grassed areas, it will be necessary to make a clearing round the trap to protect the trap against fires.

Targets The objective, or target, of a hungry tsetse fly is the host animal on which it will feed. If the target is treated with a suitable residual insecticide, the flies alighting on it or simply touching it will be killed. Cattle dipped in DDT solution were used to eliminate small populations of *G. pallidipes* in Zululand in 1949.[60] The same method was investigated in East Africa,[259] but was not widely accepted. The development of synthetic pyrethroid insecticides, such as deltamethrin, which are good acaricides as well as insecticides, has revived interest in using dipped cattle to eliminate tsetse flies in lightly infested areas. In Zimbabwe, a well-developed dipping system against ticks is operated in most parts of the country. In communal, or traditional areas bordering on known tsetse fly areas, where cattle numbers are large enough to give good coverage of likely fly habitat, a dip wash containing deltamethrin is being used. Cattle are dipped every 14 days or, in the absence of dipping facilities, treated monthly with pour-on formulations of the dip. Flies alighting on the cattle are killed,[219] or knocked down so as to be available to predators, and the reduction in trypanosomosis cases in areas under treatment has been dramatic. This approach is considered a very promising form of tsetse fly control where cattle densities are high. In terms of area under treatment, deltamethrin dipping is the most widely used anti-tsetse measure in Zimbabwe at present.[208] The costs are less than those for any other method of control, provided that the infrastructure for dipping procedures is available.

Successes in using insecticide-treated cattle have been reported from southern and eastern Africa,[220] as well as from West Africa. It has been shown that pour-on applications of insecticides do not reduce the attractiveness of cattle to *G. pallidipes* and do not affect the proportion of flies that feed on the animals.[6] Disease transmission can thus still occur after the cattle have been treated, and will only cease after the tsetse population has been reduced by the insecticide, and then only if there is no invasion of flies into the area where cattle have been treated with insecticide. Various insecticides for use as pour-on, plunge dipping or spraying of cattle are now available.[2]

In comparative trials in Zimbabwe with three different pour-ons, the active ingredients of which were alphacypermethrin, deltamethrin and cyfluthrin, respectively, the dung of the animal treated with alphacypermethrin, which was dropped about seven hours after the insecticide was applied, was found to be lethal to dung beetles.[242] In the event of this phenomenon being common to other pour-ons and also dip washes, their widespread use for tsetse fly and tick control purposes could have considerable environmental implications to soil fertility.

Where tsetse flies are abundant, cattle cannot be easily maintained, but artificial targets can be used against the flies (**Figures 2.16 and 2.17**). These targets consist of a cloth screen in a frame mounted on a pole. In some versions the cloth is flanked by bands of black mosquito netting, which is not readily visible to tsetse flies. The screen and the gauze are sprayed with insecticide, often with deltamethrin suspension concentrate. In West Africa, blue screens, which are

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Figure 2.20 Blue-black odour-baited, insecticide-treated target used in Zimbabwe against *Glossina morsitans morsitans* and *G. pallidipes*. A bait sachet is shown partly withdrawn from the pocket in the blue cloth and a bottle of butanone is on the ground in front of the target

good visual attractants, are used so that flies come to the target, alight on it and receive a lethal dose of insecticide. Sufficient targets must be deployed to ensure that flies have a high probability of encountering one. Very good results have been achieved using this method in West Africa.[141]

It has been shown[239] that tsetse flies flying in an odour plume are diverted to host-like stationary objects and a variety of visual cues are involved.[222] Differences in the degree of response by different species of tsetse flies to host-derived stimuli have been demonstrated.[252] In Zimbabwe, targets are used in conjunction with the kairomones discussed above. The target first used in routine control operations consisted of a black cloth centre panel, flanked by panels of black mosquito netting, mounted on a pole and pivoted so that the target faced across the prevailing wind (**Figure 2.16**). The combination of an odour and a visual attractant has been very effective in attracting both *G. morsitans* and *G. pallidipes*, and the black cloth panel causes more flies to alight than does blue cloth. Flies colliding with the black netting also contract a lethal dose of pesticide. These targets were first shown to have potential value by their use in controlling *G. morsitans* and *G. pallidipes* on an island in Lake Kariba.[246] The first large-scale field trial (600 square kilometres) was subsequently conducted in the Zambezi River Valley, where both *G. morsitans* and *G. pallidipes* were abundant.[247] The level of success achieved in this trial, in which targets baited with acetone and octenol and sprayed with deltamethrin were deployed at three to five per square kilometre, resulted in a campaign against *G. morsitans* in the Umfurudzi area of Zimbabwe. This campaign was successful; tsetse flies were eradicated in 18 months and, complementary to this, trypanosomosis in cattle in the area disappeared completely. One of the disadvantages of the cloth and netting target was that the netting soon became torn. It was found that an all-black cloth target 1,7 m wide and 1,0 m deep was a more robust substitute. This all-black cloth target was used widely in Zimbabwe in conjunction with kairomones and with deltamethrin as the insecticide applied to the target (**Figure 2.17**).

Work in West Africa on the *palpalis* group of flies showed that bright royal blue targets were better attractants than black targets.[83] In Somalia, it was shown that a combination of blue and black cloth targets were more effective than all-black targets against *G. pallidipes*.[229] Further work in West Africa showed that the best pattern for *G. palpalis* was a bright royal blue centre panel flanked by two black panels, with both colours being attractive to the fly but the black cloth being the preferred substrate for alighting, enhancing the tendency for this fly to settle on the outer edges of any target.

A detailed study was made of colour combinations and materials that could be used to make targets for *G. morsitans* and *G. pallidipes* in Zimbabwe.[240] Although larger targets do perform better, an acceptable target had a central black panel 0,74 m wide, flanked by bright royal blue panels 0,5 m wide. All panels were 1 m high. Both *G. morsitans* and *G. pallidipes* tend to settle near the centre of a target and this trend was enhanced by the black centre panel, which is a preferred alighting stimulus for these species. An additional advantage of this pattern of target is that only the black cloth needs to be treated with insecticide. Several alternatives to cloths were identified for use in target construction, but have not yet been accepted for control operations in Zimbabwe.

In the original field trial of the odour-baited target system[247] the insecticide dosage was low and targets had to be sprayed every four months to ensure high fly mortality. A study[227] showed that a dose of 1 g, active ingredient, of deltamethrin per square metre of cloth killed almost all flies alighting on it over a period of a year. Test cloths were held in full sun and exposed to the elements in the field. Different qualities of cloth varied with respect to residue longevity and also in the extent of fading of the black dye under field conditions. Only the suspension concentrate formulation of deltamethrin gave the required longevity, although alternative insecticides may be used.[2] In Zimbabwe, the application of insecticide is now done at a central point and, to cater for possible variation in cloth quality, the insecticide is applied to give 2 g of active ingredient on the central black cloth panel (0,75 m^2). This blue and black target is the pattern used in Zimbabwe at present (**Figure 2.20**).

Investigations showed that 10 g of the phenols and octenol mixture were needed in a sachet if it was to last a year. It would also be necessary for the sachet to be in a pocket on the blue section of the target for this degree of longevity in full sun. The bait would only last about seven months if held in a pocket on the black cloth.[226] A bottle

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containing 750 ml of acetone or butanone with a 2-mm-diameter hole in the lid yields odour for a year.

The original approach to deploying odour-baited targets in Zimbabwe was to aim at a relatively uniform distribution of targets on the ground at an average of three to five per square kilometre.[247] This density gave a rapid reduction in the tsetse population, and elimination of *G. morsitans* and *G. pallidipes* could be achieved within a year. This approach has been used extensively, with the modifications in target colour and longer lasting insecticide and baits, with good results in Zimbabwe, Malawi, Namibia and Zambia, provided the guidelines for the method were followed. A modification of the pattern of deployment in Zimbabwe has been followed in the broken country near the Zambezi River Valley escarpment. As was the case at the time that ground spraying was in use, targets were deployed only in the essential hot season habitat of the flies, which was generally associated with drainage lines. These situations can be seen on aerial photographs, and careful planning to position the targets in transects along the drainage lines was done. The number of targets per square kilometre total area was still about four, but the density in essential habitat was higher. This approach has given good results in elimination of both species of flies where drainage lines are well developed. It has not always been successful where drainage is indeterminate, and in such areas a regular grid of targets may be necessary.

Following the outbreak of trypanosomosis in Zululand in 1990, the all-black odour-baited target system used in Zimbabwe was introduced in a small-scale trial. Some initial reduction in tsetse numbers was noted but the method did not eliminate the flies.[125] Research work showed that *G. brevipalpis* and *G. austeni* differed in their response to black cloth, as discussed above in the section on traps, and only *G. brevipalpis* responded to the kairomones used in Zimbabwe.[124] With the knowledge gained from experiments on the reaction of the two fly species, a target was developed which, although not the optimum for either species, was the best compromise. The target was 1.75 m long and 1 m high with a centre blue panel 75 cm wide, flanked by two 50-cm-wide black panels.[124] *Glossina brevipalpis* tends to alight towards the edges of a target and the black cloth enhances this effect so, for this species, only the black cloth would need to be treated with pesticide. *Glossina austeni* is attracted by the blue cloth but will alight on either colour of cloth so, for this species, the whole target would need to be treated. The bait used in Zimbabwe has also been modified, and is only effective for *G. brevipalpis*, while for *G. austeni* only the visual attraction to bright royal blue cloth will attract the fly. When the daily pattern of movement and dispersal of the two fly species has been determined, it should be possible to devise an odour-baited target system which will eliminate both *G. brevipalpis* and *G. austeni*.

The pattern of deployment of the odour-baited targets in the field has been modelled[241, 262] on the basis of known features of tsetse movement. The deployment needed will differ between species, and in different vegetation patterns. At the site of the target in the field, features such as good visibility, absence of distracting objects and pathways through the vegetation[241] are important, as is the case for odour-baited traps. It is necessary to have a fire break round each target, and the ground round the target should be reasonably level so the target swings easily.

There are factors that adversely affect the use of the odour-baited target system for tsetse control. The prime cause of failure is theft of targets by rural populations. Failure to make adequate fire breaks round targets is another major cause of failure of the target system. Wind damage to targets is another severe effect in many localities, and targets may become ineffective in a matter of weeks after being set up. Alternative materials to cloth may help solve this problem. In some localities, damage by wild animals such as elephants, baboons and hyaenas can reach high levels, although in much of the undeveloped country in Zimbabwe damage levels are low. Domestic cattle are also known to destroy odour-baited targets. A major difficulty in operating the system is to ensure maintenance of the targets. To relocate targets requires that their position be marked on aerial photographs or maps, and global positioning system (GPS) instruments are very useful in this respect. Access to the area being treated is essential, and the supply of the targets and baits at the correct times is a complex logistical problem in much of Africa. The capacity to operate the odour-baited target system does not exist in all the tsetse-infested countries of southern Africa, so the elimination of the tsetse fly by this means will not be a short-term operation.

Some of these difficulties might be solved if it were possible to convert trees to odour-baited targets. This can be done quite easily for tree stumps, but it has been shown that it is not easy to do for trees,[249] and was more expensive to do than using cloth targets.

Attempts have been made to encourage local communities in tsetse-infested areas to run their own tsetse control operations. A successful trial, using traps rather than targets and a simplified bait consisting of cattle urine and acetone, was run at Nguruman in Kenya, and similar systems have been operated in West Africa.[59] A community-operated odour-baited target system is under investigation in eastern Zambia. Preliminary results are encouraging, but it has not yet been demonstrated that this community will operate the system for protracted periods.

Target barriers The first large-scale trial of odour-baited targets to eliminate *G. morsitans* and *G. pallidipes* in Zimbabwe[247] demonstrated that this could be achieved in 12 months if the mean target density was three to five per square kilometre. Trap samples from a traverse across the centre of the main invasion front yielded no flies further into the treated area than 5 km. The targets also exerted a marked reduction of the tsetse population outside the treatment area. These results gave rise to the concept that targets could

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be used as a barrier to invasion by tsetse flies. Initially, it was considered that a narrow barrier with a high density of targets would give the best result. It has since been shown[168] that such barriers do not prevent some flies crossing them. Using knowledge of fly survival rates, expectation of the net daily movement of the flies and the probability of a fly being killed by an odour-baited target treated with deltamethrin, Hargrove[93] has computed the probability of flies crossing barriers of different widths and target densities. An effective barrier width of eight times the expectation of net daily movement of the flies (about a kilometre in Zimbabwe), when the target density was the same as for tsetse elimination in 12 months, was proposed. In Zimbabwe, the target density would be a mean of four per square kilometre and the width about 8 km for *G. pallidipes* and possibly less for *G. morsitans*. The number of targets involved would be less than that of a barrier of four lines of targets 0,5 km apart with a high density of targets in the lines. The probability of a fly crossing the proposed barrier would be about 0,001. As the barrier exerts an effect beyond its boundary, the fly numbers on the invasion front of the barrier decline as the barrier is approached. The predictions on barrier effectiveness do not take into account game trails, footpaths and roads that cross the barrier. Such features may reduce the effectiveness of the barrier through transport of flies on vehicles or animals.

A trial of the type of barrier proposed by Hargrove[93] was conducted on a section of the boundary between Zimbabwe and Mozambique in the Mudzi district.[253] The barrier was 40 km long, 8 km wide and target lines were 0,5 km apart with targets spaced 0,5 km apart in the line. The country is relatively flat and the target density in this regular pattern was roughly four per square kilometre. Locally owned cattle treated with deltamethrin grazed within the barrier and for a distance of about 20 km from the international boundary. The mean density of cattle in the 428 km^2 trial area was 8 to 12 per square kilometre. The distribution of cattle was not uniform and there was no control over where they grazed.

Monitoring of tsetse flies (mainly *G. morsitans* but with *G. pallidipes* also present) and trypanosomosis showed flies to occur in low numbers no further than 2 km from the invasion front and for cases of trypanosomosis to be rare. A trial was designed to check whether the insecticide-treated cattle alone would provide a barrier to flies moving into Zimbabwe. Following removal of the targets, there was a rapid increase in the distance into the barrier that flies could be caught, and also a rapid increase in the number of cases of trypanosomosis in the cattle, including some fatalities.

Following replacement of the targets, the situation soon returned to the original state. The trial demonstrated that, at this locality, the density of insecticide-treated cattle did not prevent flies from Mozambique entering Zimbabwe. How far into Zimbabwe the flies would penetrate was not determined, as it appeared that they might cross the 20 km-wide belt in which cattle had been treated with deltamethrin, so the targets were replaced. It was clearly shown that flies fed on cattle treated with insecticide and that transmission of trypanosomosis occurred.

Odour-baited target barriers to invasion by tsetse flies can be effective, but they must be carefully maintained at all times. Attempts are being made to put the maintenance of target barriers out to contract but, as the barriers will have to be maintained for the foreseeable future, it remains to be seen how effective this will be.

Other approaches

Chromosomal abnormalities Following the development of the so-called sterile male technique for insect control, the principles of which were reviewed by Knipling,[127] the method has been tried against a wide range of insects.[128] As it had been very successful against the screw-worm fly (*Cochliomyia hominivorax*) in North America, it is not surprising that it was considered for the control of *Glossina* spp. with their very low rates of reproduction. Initial theoretical considerations for using this approach against *Glossina* were reported by Simpson in 1958.[209] The irradiation of puparia as a method for rearing male tsetse flies with chromosome abnormalities (and thus effectively sterile if mated to normal females) was developed by Potts.[189]

The first field trial of the sterile male technique was conducted on an island in Lake Kariba between 1967 and 1969.[46] The test population was reduced by 98 per cent in this campaign, and eventually reached the stage where no flies could be caught by any of the available sampling methods. In this trial, chromosomal abnormalities were induced in males by chemical means as the flies emerged from field-collected puparia. This approach obviated the need for large colonies of tsetse flies to produce puparia for irradiation at a known age.[189]

A further attempt at using the sterile male method, this time based on the irradiation of puparia produced by a large colony of tsetse flies, was made some years later in East Africa.[264] The area into which the treated flies were released was isolated by a wide zone cleared of bush which, in the event, was not completely effective. The fly population in the area was reduced to a very low level, but was not eliminated. The development of membrane feeding techniques[161] has made large-scale rearing of tsetse flies more practical, and release of male flies sterilized by irradiation from such colonies has resulted in the elimination of some fly populations in West Africa.[43]

On Zanzibar island, *G. austeni* has been eliminated using the sterile male approach. Large-scale (up to one million females) rearing of flies using membrane feeding techniques was done on the mainland of Tanzania and puparia were irradiated, resulting in effectively sterile male flies. Other control measures, such as treating cattle with insecticides were also in use, but the sterile insect technique was the method which finally eliminated the fly. The last wild fly to be caught was in 1996.[191]

An alternative to rearing flies for sterilization is to

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sterilize males in a wild population by chemical means (autosterilization). The development of efficient odour-baited traps has opened the way for this approach in Zimbabwe, where a suitable sterilizing device has been developed[104] and tested successfully,[246] but the very toxic nature of the chemicals used precludes its widespread application. The juvenile hormone mimic and the chitin synthesis inhibitor mentioned above may provide a safe way to autosterilize female tsetse flies effectively. These methods eliminate the need for the costly artificially maintained tsetse fly colonies. It has been suggested that, under some circumstances, the sterilization approach to tsetse fly control may be best[140] but, until more accurate information is available on fly population dynamics, the use of population models in predicting the outcome of control measures is unlikely to be accurate.

Other approaches to control based on the genetics of tsetse flies have been investigated. The possibility of eliminating *G. morsitans* by introducing *G. swynnertoni* into the *G. morsitans* habitat, where it would not become established, was examined.[116] These two species crossmate readily in the laboratory and the interspecific cross is sterile. It was hoped that *G. morsitans* would be reduced in number, as many females would mate with male *G. swynnertoni*, however the field experiment was not successful.[118] Other suggestions for control based on genetics — such as releasing laboratory-bred strains of tsetse flies, homozygous for translocations, into natural populations[45] — have not been tried in the field.

Biological control Some animals, such as birds, wasps (*Bembex* spp.), robber flies (Asilidae) and spiders (*Hersilia* spp.), are known to prey on tsetse flies on occasion.[171] As these animals do not feed only on tsetse flies, they are not candidates for elimination of the flies.

A number of insect parasitoids which parasitize on *Glossina* puparia have been discovered.[130, 171] Recent taxonomic revisions have altered the old names of many of them.[81] One of the parasitoids was released on a large scale in an attempt to control *G. morsitans* in Malawi,[129] but it was not successful, possibly because most of the parasitoids are not host-specific.

A parasitoid wasp in the family Mutillidae (*Chrestomutilla glossinae* Turner) is well known in Zimbabwe[34, 101, 102] and has also been recorded in Zambia and Malawi. Under natural conditions it appears to parasitize only puparia of the genus *Glossina*, but in the laboratory it can, with difficulty, be reared from *Sarcophaga* sp. puparia.[34, 100] It has not yet been possible to culture the wasp in the numbers needed to attempt biological control.

A virus, widespread in Africa, that infects *G. pallidipes* and causes hypertrophy of the salivary glands of both sexes and sterility in the males has been reported.[173] If it is possible to devise methods by which the dissemination of the virus could be improved, it has potential for biological control purposes.

Strategy of trypanosomosis control

There are three main strategies which may be used, individually or in combination, to control trypanosomosis in livestock. These are the administration of curative or prophylactic drugs; the use of trypanotolerant livestock, and vector control or eradication. The choice of the tactics to be used will depend on existing circumstances. Many variables, such as the species of fly, vegetation type, topography, and both human and animal population density, must be considered. Methods which have been successful in one locality may not be effective in another. The strategy finally chosen usually depends largely on economics, and, in general, the benefits derived from controlling the disease should be greater than the cost of achieving this objective. Exceptions do occur, however, and an example is the threatened invasion of pastoral areas by vectors. Here, large expenditure may be required simply to avoid the potentially catastrophic losses that might occur if the invasion were not checked. A good example of such a situation in southern Africa was the control, between 1959 and 1969, of the advancing tsetse fly front from Mozambique into south-eastern Zimbabwe, which was threatening to reinfest the Limpopo River Valley.[193] Game parks and game or forest reserves are also special cases. Little or no benefit is gained by the park or reserve by controlling tsetse flies, but adjacent agricultural areas benefit greatly. This situation occurred in the earlier part of this century in Zululand, South Africa,[60] and is the current situation in parts of Malawi.

The economic aspects are of prime importance. Most countries in southern Africa cannot finance attempts to eliminate flies within their borders. They can only try to lessen the impact of trypanosomosis either by controlling the vectors or by using drugs to control the disease. Should it be possible for a country to eliminate the flies within its borders, as is the case in Zimbabwe, there is still the perpetual threat of reinvasion from tsetse fly-infested adjacent countries. Attempts to prevent reinvasion by the use of artificial barriers, such as zones or areas cleared of bush, generally have not proved satisfactory in the long run. The use of newer types of barriers, such as insecticide-treated, odour-baited targets, or the dipping of stock in deltamethin, is still being assessed. In any event, the maintenance of barriers is expensive and external funding agencies do not readily contribute to such ongoing control measures.

Eradication of the tsetse fly is, as of 1999, the only permanent solution to the elimination of disease caused by the trypanosomes which they transmit. Methods used to kill the tsetse flies are based on detailed knowledge of their biology and ecology.[143] To achieve eradication, the flies must be removed up to natural barriers such as lakes, open grassland, heavily settled areas or mountain ranges, not merely to territorial boundaries, unless these happen to run along natural barriers. Clearly, co-operation between neighbouring countries is imperative in order to achieve this objective

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and, as the cost is likely to be higher than most countries involved can afford, international assistance must be sought.

A proposal to provide a permanent solution to the tsetse fly problem in Malawi, much of Mozambique, eastern Zambia and Zimbabwe was prepared in 1983 by Peter Thomas Associates Consulting Services (Zimbabwe) and the Minister of Agriculture (United Kingdom).[177] In the long term, this comprised the elimination of the fly belt common to these four countries, except for the country east of Lake Malawi and north of the Zambezi. The region to be cleared of flies extended to natural barriers for almost the whole area involved and left only a small stretch along the Zambezi River in Mozambique, where an artificial barrier would be needed. The European Commission (of the European Union) agreed to finance the programme, which was known as the Regional Tsetse and Trypanosomiasis Control Programme of Malawi, Mozambique, Zambia and Zimbabwe. Implementation was divided into two phases. The first phase began in 1986 and the second in 1992. The project was closed at the end of 1998.

The first phase involved the following components: identification of the extent of the problem; the development of tsetse-control techniques; provision of training; emergency eradication operations; and environmental monitoring of control activities involving the use of insecticides. The evaluation of the first phase recommended the continuation of the project into a second phase. The prime objective of this phase was to develop a comprehensive strategic plan for an integrated strategy for the eradication of tsetse-transmitted trypanosomoses from the common fly belt. The second main objective was to complete work started in the first phase. During the second phase, because of the scale of the undertaking, the objective of the programme was redefined as integrating strategic tsetse and trypanosomosis control to support sustainable development in the countries concerned.

It is unfortunate that the project was terminated when it was, because all four countries had achieved considerable successes. In particular, Zimbabwe had cleared tsetse from 20 400 km^2 in the north-eastern part of the country, including all of the farming land which was overrun by flies during the War of Liberation.[79] This result was achieved by a combination of control methods, including aerial spraying, ground spraying, the use of targets, and the topical application of deltamethrin to cattle. The cleared area is protected by a series of target barriers located along the tsetse front and regular application of deltamethrin to all cattle depastured within 30 km of the Mozambique border.

Although the Regional Tsetse and Trypanosomiasis Control Programme has been terminated, it seems likely that collaboration in tsetse control in southern Africa will be continued through the establishment of a regional network co-ordinated by Zimbabwe as the Southern African Development Community's Centre of Excellence in tsetse control. This arrangement has still to be endorsed by the SADC Ministers of Agriculture.

In 1996, a new grouping of agencies, the Food and Agriculture Organization of the United Nations, the World Health Organization, the International Atomic Energy Agency and the Organization of African Unity/International Bureau for Animal Resources initiated a programme called Programme Against African Trypanosomiasis (PAAT). The stated objective is to promote integrated trypanosomosis control through co-ordinated international action so as to improve food security and sustainable agriculture and rural development.

In this chapter, drug treatments, trypanotolerant livestock and attempts to develop a vaccine against trypanosomes are not discussed, although they may be effective strategies for trypanosomosis control (**see Chapter 12: African animal trypanosomoses**).

Trypanosomosis will continue to be a major constraint to livestock development in much of southern Africa for some time to come. Countries such as Malawi, and to a lesser extent Zimbabwe, have a relatively high human population density, so the need to eliminate tsetse flies is more urgent. However, even in sparsely populated countries such as Zambia, the population growth rate is high and, should it remain so, it is only a matter of time before the need to eliminate trypanosomosis becomes urgent. Population growth rates may change in the future, and further improvements in measures to control trypanosomosis may be developed but, over a century after the 1895 demonstration by Bruce of the association between trypanosomes and tsetse flies,[16] there is no indication yet of any rapid or easy solution to the deleterious effects of trypanosomosis on humans and their domestic animals in southern Africa.

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3

Vectors: Muscidae

E M NEVILL

Introduction

In the Afrotropical Region (previously referred to as the Ethiopian or sub-Saharan Region) the family Muscidae embraces 870 species in 60 genera and six subfamilies.[42] However, only three subfamilies, the Stomoxyinae, Fanniinae, and Muscinae contain genera of veterinary importance, these being *Haematobia, Haematobosca, Stomoxys, Musca, Hydrotaea, Morellia, Muscina* and *Fannia*. Species in these genera are important not only because they may cause irritation to livestock, resulting in production losses,[46] but also because of their potential as mechanical vectors of various disease organisms, a fact that has been generally neglected in southern Africa. Greenberg[13] listed a multitude of organisms that have been isolated directly from various synanthropic fly species and which have the potential to be transmitted mechanically under the right circumstances. He reviewed the studies made throughout the world on fly transmission of a wide variety of diseases, such as brucellosis, anaplasmosis and summer mastitis.[14]

The literature is saturated with articles on the control of these flies. In North America much effort has gone into the chemical control of the horn fly (*Haematobia irritans irritans*) and the face fly (*Musca autumnalis*), while in Australia a biological control programme to control the buffalo fly (*Haematobia irritans exigua*) and the bush fly (*Musca vetustissima*) by the introduction of exotic dung beetles was started in the early 1970s.[3] However, the stable fly (*Stomoxys calcitrans*), the house fly (*Musca domestica*), the lesser house fly (*Fannia canicularis*) and the false stable fly (*Muscina stabulans*) continue to flourish in conditions created by humans, especially in intensive farming situations such as dairies, feedlots and chicken farms.[23] The efforts and ingenuity of humans to produce more milk, meat and eggs do not always include efforts to avoid a greater fly problem!

Members of the Muscidae are not biological transmitters of infectious diseases. Although they have been shown to be capable of mechanically transmitting numerous disease-producing organisms, they are seldom controlled for this reason. A second reason for southern African farmers' apparent apathy about these flies is that they are unaware that so many species exist, each with its own requirements for survival and its own potential for disease transmission. For example, at least 20 species of *Musca* are found on farms in various parts of South Africa, but are often confused with the house fly.[31]

The purpose of this account is to provide an insight into the life cycles, disease transmission potentials and control of the muscid flies in the subcontinent. Identification of families, sub-families, genera and certain species can be facilitated by reference to publications by Walker,[51] Greenberg[13] and Zumpt.[55] These publications also provide information on biology and distribution. For a visual comparison of six common species of Muscidae, see **Figures 3.1 to 3.6**.

Stomoxyinae

In southern Africa this subfamily is represented by eight genera, the three main livestock-associated genera being *Haematobia, Haematobosca* and *Stomoxys*.[55] The adults of these flies do not feed on nectar, but are obligatory blood-suckers. They possess a mostly slender, rigid and chitinized proboscis that enables them to puncture the skin of animals to obtain a blood meal. The proboscis cannot be retracted, projects forward and can often be seen from above (**Figures 3.1 and 3.3**).

Haematobia and *Haematobosca* spp.

Common names: Horn flies, horingvlieë (Afrik.)

These are obligatory blood-sucking muscid flies that seldom leave the host except to lay eggs in recently deposited dung. Female *Haematobia irritans irritans* flies (**Figure 3.3**) have been found to average 38.4 feeds in 24 hours and males 24 feeds.[16] This semi-permanently parasitic way of life ensures a food supply, and a larval habitat close at hand. It also leaves them very vulnerable to many insecticides applied to animals for the control of ticks.

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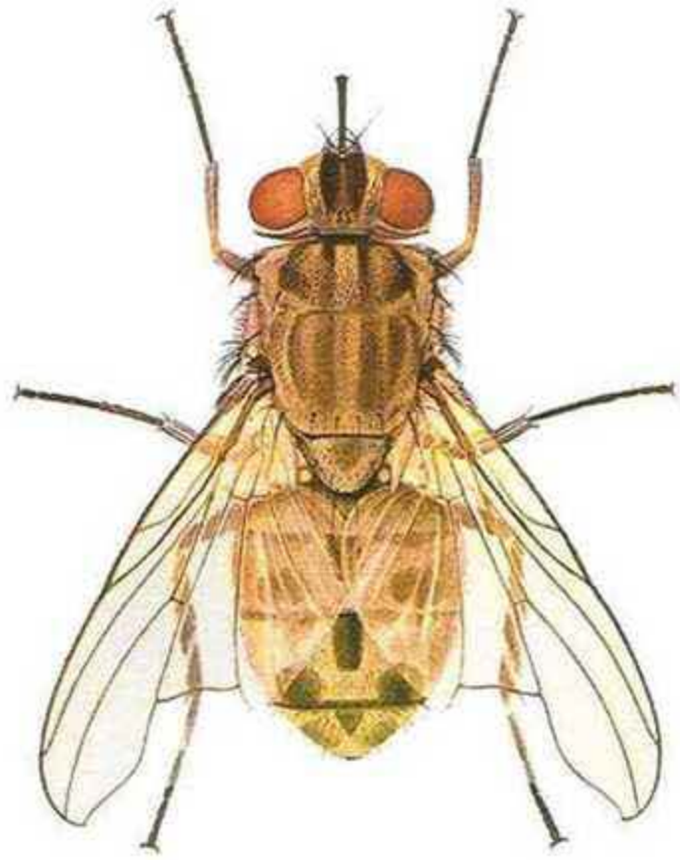


Figure 3.1

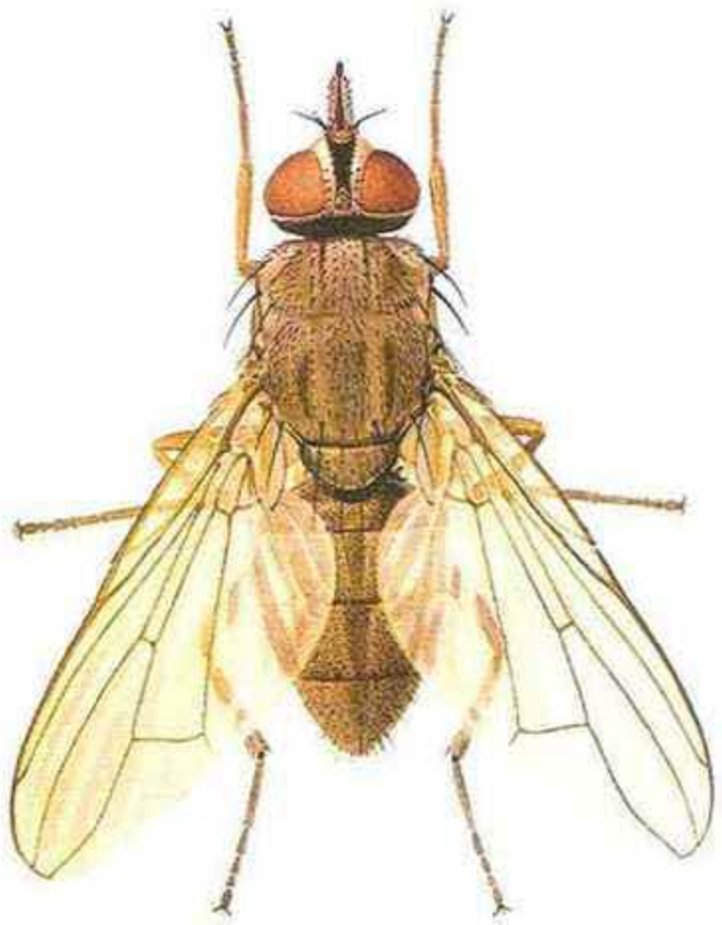


Figure 3.3

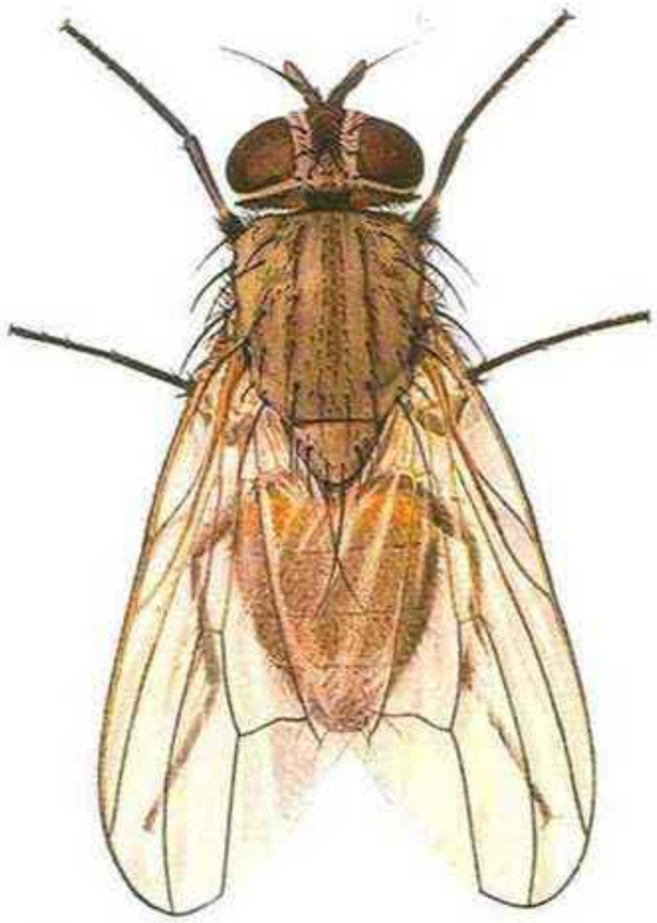


Figure 3.2

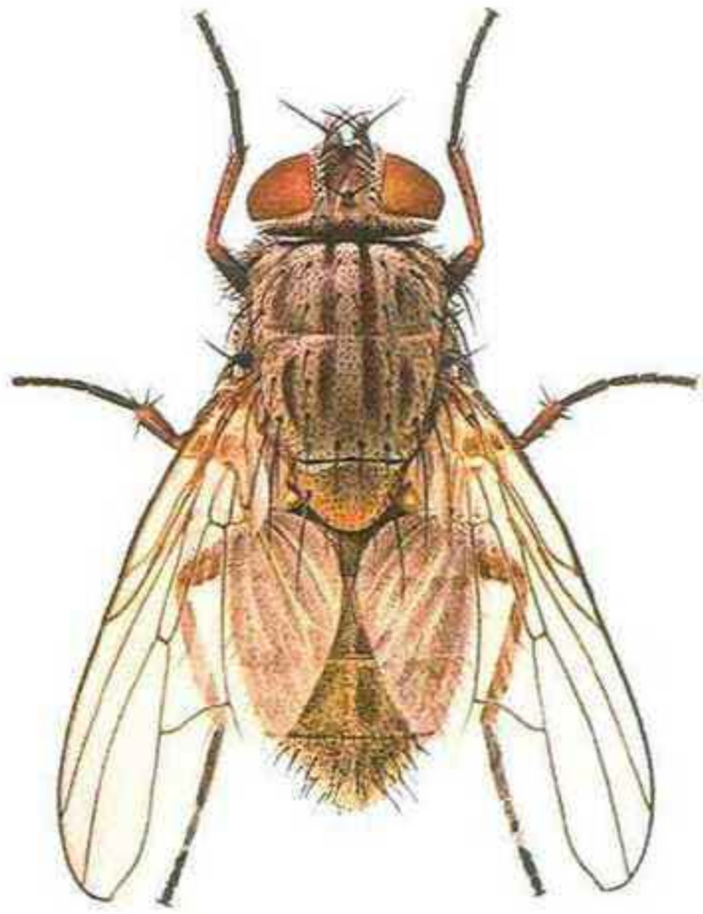


Figure 3.4

Figures 3.1 to 3.4 Common synanthropic flies, namely *Stomoxys calcitrans* (**Figure 3.1**); *Fannia canicularis* (**Figure 3.2**); *Haematobia irritans* (**Figure 3.3**); and *Muscina stabulans* (**Figure 3.4**). Their size, type of mouthparts and shape of the fourth wing vein M1+2 are important diagnostic characters. (Illustrations by F. Gregor in Greenberg, Bernard; *Flies and Disease*, Volume 1. Copyright © 1971 PUP. Reprinted by permission of Princeton University Press)

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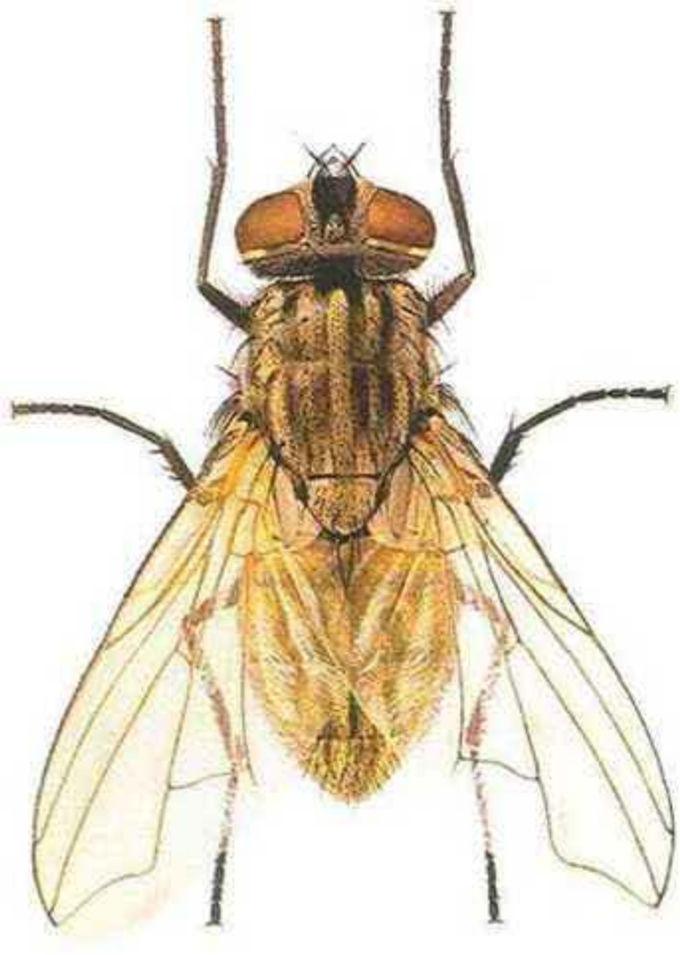


Figure 3.5

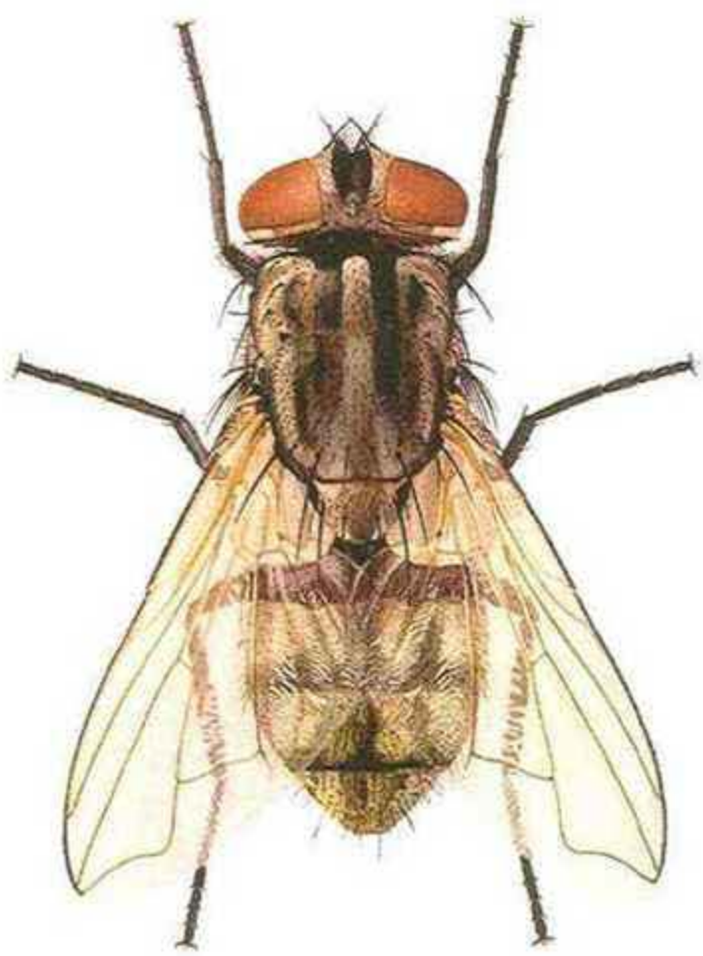


Figure 3.6

Figures 3.5 to 3.6 Two species of the genus *Musca*; namely *M. domestica* (**Figure 3.5**); and *M. sorbens* (**Figure 3.6**). Note the similar shape of the fourth wing vein M1+2, which is diagnostic for this genus. Also compare this vein with those of the four other genera of synanthropic flies depicted in Figures 3.1 to 3.4 (Illustrations by F. Gregor in Greenberg, Bernard; *Flies and Disease*, Volume 1. Copyright © 1971 PUP. Reprinted by permission of Princeton University Press)

Three species of *Haematobia* have been recorded from southern Africa, namely *H. thirouxi potans*, *H. meridiana* and *H. spinigera*, all of which are primarily associated with game, especially African buffaloes (*Syncerus caffer*).[55] In 1937/38 *H. thirouxi potans* was reported to be a problem on cattle along the coast from the Eastern Cape Province to southern KwaZulu-Natal.[9, 55] The problem disappeared after synthetic organo-chlorine insecticides were used at the end of the Second World War.[18] Today, horn flies have again become a pest on farms, either where non-insecticidal tick-detaching agents are used for tick control, or where no tick control is practised.

In southern Africa no *Haematobia* spp. have so far been proven to be vectors of any organism that causes disease in game or livestock. In many other parts of the world *Haematobia* spp. are biological vectors of *Stephanofilaria* spp.[17] The presence of stephanofilariasis in cattle has not yet been definitely confirmed in southern Africa, but in the 1980s and early 1990s stephanofilariasis-like lesions were seen in cattle in the KwaZulu-Natal Lowveld and Venda in the Limpopo Province of South Africa, and in Swaziland,[31] where they occurred in association with large numbers of *H. meridiana*.[37] *Haematobia thirouxi potans* is found in large numbers on African buffalo in South Africa. Since buffalo are carriers of the foot-and-mouth disease virus, the possibility exists that this horn fly could transmit the virus mechanically from buffalo to nearby cattle. Two attempts at the Onderstepoort Veterinary Institute to transmit the virus by this means from infected to susceptible cattle (after two hours) were unsuccessful.[48]

A second genus of horn fly, *Haematobosca*, was listed by most early authors under *Haematobia* or *Bdellolarynx*.[55] Four species of *Haematobosca* have been recorded from southern Africa, namely *H. uniseriata*, *H. latifrons*, *H. angustifrons* and *H. zuluensis*.[42] Only the first two species are common.[55] *Haematobosca uniseriata* was first recorded in the then Transvaal Province (now comprising Limpopo, Gauteng, North West and Mpumalanga provincies) in 1933, when Nieschulz and Du Toit[38] found it feeding in relatively large numbers on the belly and legs of horses near the Onderstepoort Veterinary Institute. Zumpt[55] caught specimens on a freshly killed warthog (*Phacochoerus aethiopicus*). This fly was also collected off cattle on farms in the Kuruman area in the eastern part of the Northern Cape Province during an unusually wet year (1974). In subsequent drier years it was present in much lower numbers.[29]

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Stomoxys spp.

Common names: Stable flies, stalvlieë (Afrik.)

Eight species of *Stomoxys* have been recorded in southern Africa[42] with *S. calcitrans* (**Figure 3.1**) being the major pest species. In South Africa *Stomoxys niger* (= *S. nigra*) is seldom collected, although it can, on occasion, occur in pest proportions in the wetter and hotter parts such as the Lowveld of Mpumulanga and north-eastern KwaZulu-Natal.[31] By contrast, *S. niger* was rated in Mauritius to be a serious pest of cattle and deer while *S. calcitrans* was considered to be of negligible importance.[27]

Stable flies are compulsory blood-feeders, but they differ from horn flies in that they only spend short periods feeding on their hosts, digesting their meals while resting on nearby surfaces. They feed on average twice a day,[16] usually on whichever host happens to be available at the time. If interrupted they will feed more often. This behaviour makes them ideal mechanical transmitters of any disease organisms present in the blood of their host.

Worldwide there are numerous examples where *Stomoxys* spp. (particularly *S. calcitrans*) have been shown to be either involved in, or suspected of, mechanically transmitting disease organisms such as *Brucella* spp., *Borrelia* spp. and equine infectious anaemia.[55] In Britain it has been proved experimentally that *S. calcitrans* can act as an efficient mechanical vector of capripox and African swine fever viruses.[25] It is also believed that this fly may be capable of mechanically transmitting foot-and-mouth disease virus. These findings are particularly significant for southern Africa where lumpy skin disease, African swine fever and foot-and-mouth disease are endemic. In South Africa it has been shown experimentally that *S. calcitrans* can mechanically transmit *Anaplasma marginale* within a group of cattle,[43] and that it can, in the field, act as a mechanical vector of *Besnoitia besnoiti*, the cause of bovine besnoitiosis or elephant skin disease.[2] These flies are therefore a very real means of disease transmission between animals in a herd or feedlot.

Members of the Stomoxyinae differ widely in their breeding requirements. *Haematobia* and *Haematobosca* spp. breed in freshly dropped dung from animals grazing in the veld, whereas *S. calcitrans* prefers fermenting organic material, such as rotting heaps of discarded animal feed or bedding, that may or may not contain dung. In 1997, at the Johannesburg Zoo in South Africa, the source of a severe *S. calcitrans* problem was found to be rotting kikuyu (*Pennisetum clandestinum*) lawn clippings lying in windrows on the lawns in the public area and adjacent Zoo Lake Park. For these reasons *S. calcitrans* is usually associated with intensive farming and suburban situations, while *Haematobia* and *Haematobosca* spp. are more commonly found where cattle are free-ranging.

While control of horn flies is relatively easy where facilities exist for treating livestock with insecticides, *Stomoxys* control is more difficult and must rely on a combination of farm hygiene and treatment with residual insecticides of the resting places of the adult flies and the animals themselves. A variation on some of these approaches to control utilizes the preference of adult stable flies to rest on certain surfaces. It was found that translucent white fibreglass panels, tradenamed 'Alsynite', could be used to construct an attractive sticky sampling trap for these flies.[54] If treated with a pyrethroid this device could also be used to control them,[24] but the value of this approach in their control has not yet been investigated in southern Africa. Traps baited with synthetic odour attractants, developed to lure and catch tsetse flies, also attract stable flies (and flies belonging to the Tabanidae). These traps are predominantly bright blue (pthalogen blue) and may be made of cloth, e.g. Nzi[26] and H traps,[20] or of hardboard painted with a highly sticky substance such as polybutene or 'Temoocid'.[21]

Fanniinae

The main nuisance fly in this subfamily is *Fannia canicularis* (**Figure 3.2**), the so-called lesser house fly. It is often confused with the house fly (*Musca domestica*) but is slightly smaller and more slender in build. It is a cosmopolitan fly associated primarily with intensive poultry production, where it breeds in the litter. Its sheer numbers make it a nuisance and it often infests neighbouring properties. These flies are commonly seen in daytime swarms circling in midair between cage aisles on poultry ranches, in work alleys in milking parlours, and under trees or other sheltered places. Unlike house flies, *Fannia* are less important as household pests or disease vectors.[23] In high-density poultry houses in South Africa the main control approach for it, as well as for the house fly, is the addition of a cyromazine-based additive to the feed. Cyromazine inhibits the formation of chitin so that development to the following larval stage is prevented.[31]

Muscinae

Although this subfamily contains 18 genera,[42] only species of the genus *Musca* are commonly found on livestock in southern Africa. However, low numbers of other genera such as *Muscina* and *Morellia* may sometimes be collected off livestock.

Muscina stabulans

Common name: False stable fly

The false stable fly (**Figure 3.4**) is also often mistaken for the house fly because of its similarity in colour, size, and food preference, and its habit of entering homes. In appearance it falls in between *Stomoxys* and *Musca* in that its wing venation is similar to *Stomoxys* but mouthparts resemble those of *M. domestica*. It feeds on and deposits its eggs in a wide variety of animal excrement (poultry, horse, cow, domestic

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pets) and decaying vegetable matter.[23] Its larvae have been known to prey on those of other flies.[13]

Morellia spp.

Common name: Sweat flies

These flies have been collected off cattle where they scratch the skin surface and lap up exuding blood. In South Africa they have not been found in high numbers. They are also of interest because, like *M. stabulans*, their larvae prey on other larvae in the dung in which they develop.

Hydrotaea spp.

Common names: Sheep head flies; plantation flies (*Hydrotaea irritans*)

Although species of *Hydrotaea* have not yet been recorded as pests in southern Africa, certain species elsewhere in the world have warranted serious attention. In Europe, especially the Netherlands, Denmark, Germany, Belgium and the UK, *Hydrotaea irritans* is regarded as the mechanical vector of summer mastitis.[45] In Czechoslovakia, *H. armipes* is suspected of being a mechanical vector of infectious bovine keratoconjunctivitis.[8]

Musca spp.

Common names: House flies, huisvlieë (Afrik.); face flies, gesigsvlieë (Afrik.)

Except for the cosmopolitan *M. domestica* (**Figure 3.5**), the veterinary importance of other species of the genus *Musca* has been largely overlooked in southern Africa, and there has been a tendency to attribute problems caused by them to *M. domestica*. In the 1970s and 1980s, however, various studies in South Africa on the transmission of the filarial worm, *Parafilaria bovicola*, in the Limpopo Province Bushveld,[30] a fly problem in the Northern Cape Province,[34] the transmission of bovine malignant catarrhal fever in the Limpopo Province Bushveld,[35] and in Namibia on the epidemiology of anaplasmosis in cattle,[32] have led to an increased interest in the *Musca* spp. associated with cattle and, to a lesser extent, wild-living game animals (**Table 3.1**). Six *Musca* spp. (**Table 3.1**) were common to most of the farming situations examined, namely *M. xanthomelas*, *M. lusoria*, *M. domestica*, *M. confiscata* (= *M. fasciata*), *M. nevilli* and *M. sorbens* (**Figure 3.6**). The presence of certain other species was dependent on the geographical area, the season and climatological factors. It was only near homesteads that the synanthropic *M. domestica* and *M. sorbens* were important. In the veld, away from the homesteads, there is an interaction between various symbovine *Musca* spp., and the dominant species change according to both local and the broader climatic factors. With the exception of *M. domestica* which breeds in dung and decaying organic matter and refuse, all the other *Musca* spp. mentioned above breed in fresh dung in the veld and their larvae all have to feed and survive in this medium. Over thousands of years, evolution has resulted in the development of a variety of species, each with its own preferences for food, temperature, moisture and other requirements.

Twenty-five *Musca* spp. have so far been recorded in southern Africa.[22, 42] They belong to the subgenera *Eumusca*, *Byomya*, *Musca*, *Philaematomyia*, *Viviparomusca* and *Lissosterna*.[32] The 12 *Musca* spp. listed in **Table 3.1** belong to the first four subgenera. Only five species of the last two subgenera have been recorded in southern Africa[42] and these have seldom, if ever, been collected in South Africa.[31] The observations on *Musca* spp. below are therefore restricted to 13 South African species.

Subgenus *Eumusca*

Common names: African face flies, Afrika gesigsvlieë (Afrik.)

Musca xanthomelas is often the commonest African face fly associated with cattle in the veld in South Africa. It is found throughout the year in the Limpopo Province Bushveld but is less common in the South African Highveld. It lays an average of four batches of 33 eggs each and may survive for up to 35 days at 27 °C.[33] *Musca xanthomelas* has only once been collected south of latitude 29°.[28]

Musca lusoria is the largest of the African face flies. It is common throughout the year in the Limpopo Province Bushveld, but, as is the case with *M. xanthomelas*, is also less common on the Highveld. The female is ovoviviparous, depositing one late first stage larva at a time on fresh dung, and is able to produce up to 27 larvae in her life-time of up to 75 days.[33]

Musca nevilli closely resembles *M. xanthomelas* in appearance but produces fewer eggs.[22, 33] It differs greatly, however, in its seasonal prevalence, being common only from late January to April.

Musca aethiops is rarely collected off cattle, but has been reared from the dung of both cattle and African buffalo.

Musca munroi was never collected in the surveys summarized in **Table 3.1**. On occasion it is quite common on cattle farms in parts of KwaZulu-Natal and the Eastern Cape provinces of South Africa.

Subgenus *Byomya*

Musca confiscata (= *M. fasciata*) is a very versatile species in that it may be found in reasonable numbers in both the Bushveld and Highveld areas of South Africa. It breeds in both cattle and wildebeest (*Connochaetes* spp.) dung, and feeds on cattle and presumably also on game. Its ability to utilize various situations would therefore well qualify this fly to act as a mechanical transmitter of disease organisms.

Musca sorbens (**Figure 3.6**), like *M. confiscata*, may be found throughout the year in the Limpopo Province Bushveld but, like *M. domestica*, it is associated with human habitations and does not breed in animal dung in the veld.

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Two forms of *M. sorbens* have been recognized in southern Africa.[41] Both are attracted to fresh human faeces, which appears to be their preferred breeding medium.[41]

Musca tempestatum is only encountered in the vicinity of wildebeest, in whose dung it prefers to breed.

Musca freedmani is a lesser-known fly whose preferences have not as yet been observed.

Musca lasiophthalma is occasionally collected in the Bushveld in winter, but has been collected in large numbers on the Highveld in both winter and early spring.

Musca conducens has been reared from cattle dung samples collected in the Bushveld but has seldom been collected off cattle. It is possible that the fly identified as *M. conducens* in southern Africa is different from the nominate species, which is found in Asia.[40]

Subgenus *Musca*

Musca domestica is divided into three subspecies in the Afrotropical Region.[42] Two of these subspecies are indigenous to Africa, including southern Africa. These are *M. d. calleva* (an outdoor fly) and *M. d. curviforceps* (more domestic in habits). Hybrids may also be found.[39] During the writer's studies in the Bushveld, *M. domestica* was never identified to subspecies level. It was common near homestead kraals in the first half of summer but was seldom reared from cattle dung samples collected in the veld.

Subgenus *Philaematomyia*

Musca crassirostris is a 'veld-fly', apparently common only in dry years.

Feeding habits of *Musca* spp.

All *Musca* spp. require water, sugars and protein in order to survive and reproduce. Flies that live in the veld obtain these basic requirements from fresh dung, eye and nasal secretions, wounds and sores on animals, carrion, plant and flower secretions, and the excretions of sap-sucking insects.[33, 49] Protein is essential for the development of the ovaries of the flies. Protein hunger in female face flies (*M. autumnalis*) has been directly related to the stage of oogenesis and is significantly diminished sometime before the end of each gonotrophic cycle.[50] All *Musca* spp. possess a sponge-like lapping mouth-part or labellum made up of numerous medially directed channels or pseudotracheae which radiate out from a central oral opening. Fluid is sucked up along these pseudotracheae into a central cavity and then via the labial-gutter-epipharyngeal tube into the digestive system.[10] Excess fluid is stored in a large sac-like crop from which it is either passed to the mid-gut for digestion or regurgitated by the fly if more attractive food is found. This fluid is also used to dissolve or dilute food that is too coarse or viscous to take up through the pseudotracheae.[53] The fly mixes the regurgitated fluid with the food, often using its prestomal teeth for the purpose.

All *Musca* spp. have one or more circles of prestomal teeth. They are seldom visible as they are only extruded during feeding, and are otherwise hidden by the enveloping labellar lobes of the mouth-parts. The first row of these prestomal teeth ranges in size and form according to the species. For example, short, relatively blunt teeth are present in the house fly (*M. domestica*) and an African face fly

Table 3.1 The relative abundance of various *Musca* spp. collected off cattle and reared from the dung of cattle and blue wildebeest (*Connochaetes taurinus taurinus*) in southern Africa

	COLLECTED OFF CATTLE (%)						DUNG-FEARED (%)	
MUSCA SPECIES	RSA* N. CAPE	NAMIBIA	RSA* HIGHVELD		RSA* BUSHVELD		RSA* BUSHVELD	
	KURUMAN DISTRICT 1973–1977	WINDHOEK DISTRICT 1978–1980	PRETORIA DISTRICT 1974	LYDENBURG DISTRICT 1974	VARIOUS DISTRICTS 1973–1976	THABAZIMBI DISTRICT 1987	THABAZIMBI DISTRICT	
							CATTLE 1987	WILDEBEEST 1987
M. lusoria	11,4	25,3	6,6	4,1	45,7	12,6	14,4	1,6
M. xanthomelas	28,2	52,8	0,7	2,1	9,3	27,8	44,9	0,4
M. nevilli	3,3	1,4	0,2		10,3	15,9	4,9	1,8
M. domestica	18,1	5,8	23,4	26,2	18,8	32,5	0,6	0,1
*M. confiscata***	6,2	12,0	7,5	36,4	7,3	7,1	12,0	41,9
M. sorbens	2,3	0,1	1,6	14,4	7,1	3,1		
M. crassirostris	20,4	0,7			1,0	0,2	9,2	
M. tempestatum						0,4	0,6	51,6
M. freedmani	8,8	0,5			0,1	0,2	0,3	0,1
M. lasiophthalma	1,2	1,3	60,0	16,9	0,3			
M. aethiops						0,1	1,6	
M. conducens					0,2	0,2	11,6	2,5
Fly totals	3 262	18 000	889	195	20 045	2 380	2 631	1 140

* Republic of South Africa

** According to Pont[42] *M. confiscata* is the replacement name for *M. fasciata*

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(*M. xanthomelas*);[4] longer sharper teeth in the face fly *M. autumnalis*, which occurs in Europe and North America and two African face flies (*M. nevilli* and *M. lusoria*); and large coarse teeth in *M. crassirostris*[10] (**Figures 3.7 to 3.12**).

The feeding habits of flies differ according to the type of prestomal teeth present. *Musca domestica* and *M. xanthomelas* (**Figures 3.7 and 3.8**), feed mainly on the lachrymal secretions and wounds already present on an animal; *M. autumnalis* (**Figure 3.9**) feeds directly on conjunctival tissue[5, 44] and *M. lusoria* (the southern African counterpart of *M. autumnalis*) (**Figure 3.10**)[10] may be expected to feed in the same manner. It has also been shown that *M. nevilli* (**Figure 3.11**) has prestomal teeth almost identical to those of *M. autumnalis* (**Figure 3.9**).[6] *Musca crassirostris* seldom visits the eyes or wounds on cattle, but crawls under the hair of animals to rasp away the skin surface with its large, coarse teeth until blood is drawn, on which it will engorge (**Figure 3.12**). As an obligatory blood-feeder, *M. crassirostris* is therefore the *Musca* equivalent of *Stomoxys*. *Musca conducens* has a similar feeding mechanism, although the mouth-parts are not as heavily developed as are those in *M. crassirostris*.

Disease transmission potential of the Muscinae

Very little is known about the transmission of infectious agents by *Musca* spp. in southern Africa; that which has been recorded is based largely on circumstantial evidence. *Musca confiscata* (= *M. fasciata*) has been suspected of mechanically transmitting lumpy skin disease virus.[52] Also, during an outbreak of enzootic abortion in sheep in the Northern Cape Province in 1975, a *Chlamydophila* sp. was isolated from *Musca* spp. collected around sheep.[19]

In the search for the vectors of *Parafilaria bovicola*,[30] 20 000 specimens of 11 *Musca* spp. were collected in the Bushveld of South Africa and identified (**Table 3.1**). Of these, *M. lusoria*, *M. xanthomelas* and *M. nevilli*, all belonging to the subgenus *Eumusca*, were shown to be vectors. These flies accounted for 65,3 per cent of all the specimens collected. As African face flies breed in fresh dung deposited in the veld, it is only in these situations that they occur in large enough numbers to act as vectors of the larvae of this worm. Face flies are unable to develop in the dung of cattle which have been fed a supplementary grain diet, as this dung ferments; it is, however, highly suitable for this purpose for house flies. This previously unsuspected predominance of African face flies in the veld has stimulated thinking about the possible involvement of these and other *Musca* spp. in the transmission of infectious agents, not only between cattle but also between carrier game animals and cattle in southern Africa.

One of the diseases most likely to be transmitted mechanically by flies in the veld is infectious keratoconjunctivitis ('pinkeye'), which may be caused by a number of different organisms, including *Moraxella bovis*, *Mycoplasma* spp., *Chlamydophila* sp. or rickettsiae (**see Chapter 140: *Moraxella* spp. infections**). No work has yet been done in southern Africa on the transmission of any of these organisms, but detailed studies have been conducted in the USA on the transmission of *M. bovis* by the face fly, *M. autumnalis*.[15] The North American studies proved conclusively that the damage done to the eye surface by the prestomal teeth of *M. autumnalis* while feeding predisposes the eyes of cattle to infection.[44] In addition, experimental studies have shown that *M. bovis* may be present in material regurgitated from the crops of face flies, and that infection of the eyes of cattle may follow exposure to infected flies.[1, 12] Furthermore, a higher prevalence of 'pinkeye' in the field has been related to face fly numbers.[11] The African face flies fall into the same subgenus as *M. autumnalis*, and have similar feeding habits and prestomal teeth (especially *M. lusoria* and *M. nevilli*). They may therefore transmit *M. bovis* in the same manner.

In South Africa investigations have been conducted into the possible role of *Musca* spp. in the field transmission of anaplasmosis, foot-and-mouth disease and malignant catarrhal fever. Unfortunately none of these studies has been sufficiently intensive to prove or disprove the vectorial role of these flies.

The control of flies and disease

Under intensive conditions A multi-faceted approach is necessary for the control of so-called filth flies, the most common of which is the house fly (*M. domestica*). This should include good management, the judicious use of pesticides, and the encouragement of parasites and predators.

Good management practices are essential to reduce and eliminate conditions that favour fly development.[23] These should include:

- a system of manure management focusing on its collection, processing, storage and utilization as a fertilizer or compost,
- proper water management, including supply, drainage, and disposal systems, taking into account above-normal rainfall in the area concerned, and
- general farm sanitation involving the routine disposal of other animal wastes, spilt feed, organic debris, and even household garbage.[23]

Although certain registered insecticides may be applied directly to livestock, insecticides are best used as residual sprays on resting surfaces and incorporated in attractive baits in feedlots and dairies. In this way only resting and feeding adult flies will be killed and not the wasp parasites searching the breeding medium for fly pupae to parasitize nor other arthropod predators in search of eggs and larvae on which to feed. Certain poultry feed additives such as cyromazine, which inhibits the moulting of fly larvae, are harmless to non-dipteran parasites and predators.

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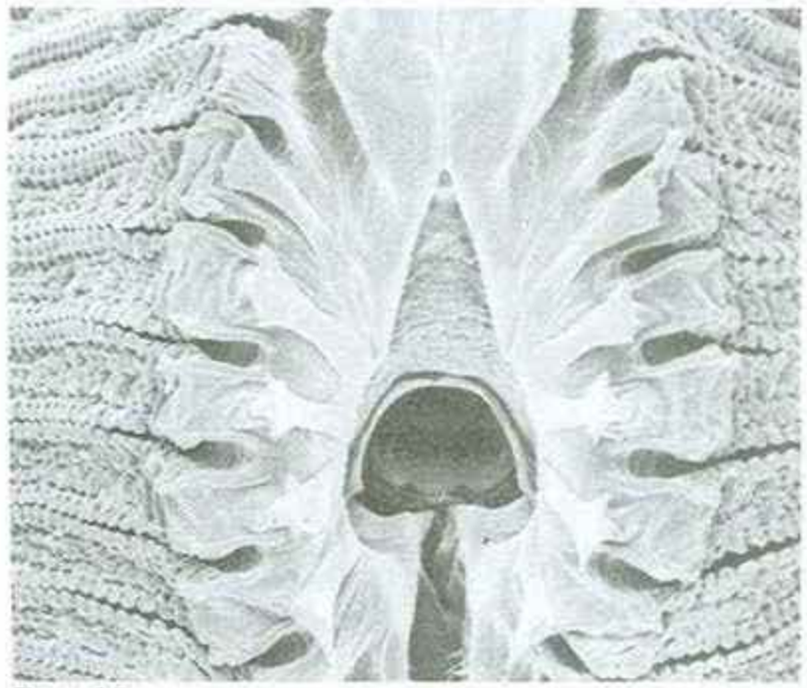


Figure 3.7

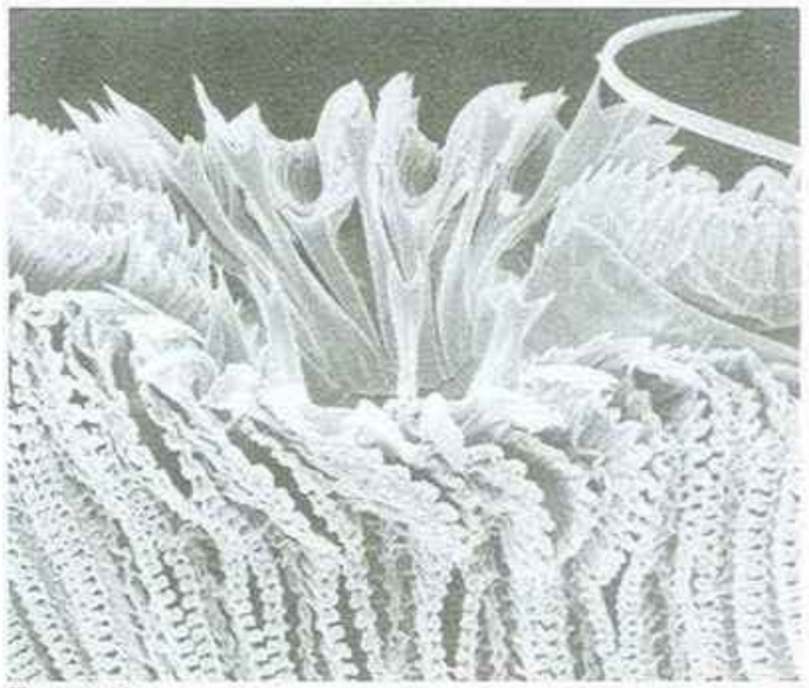


Figure 3.10



Figure 3.8



Figure 3.11

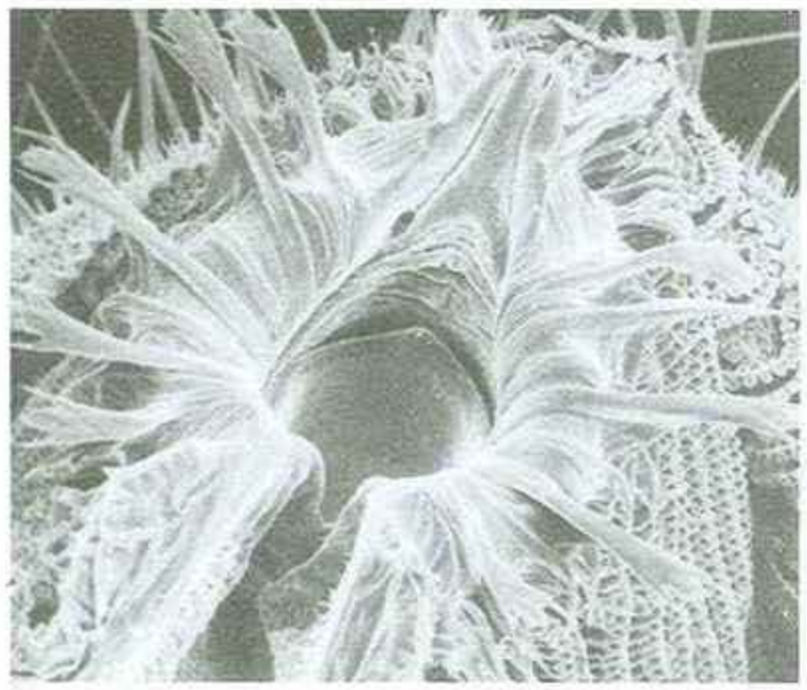


Figure 3.9

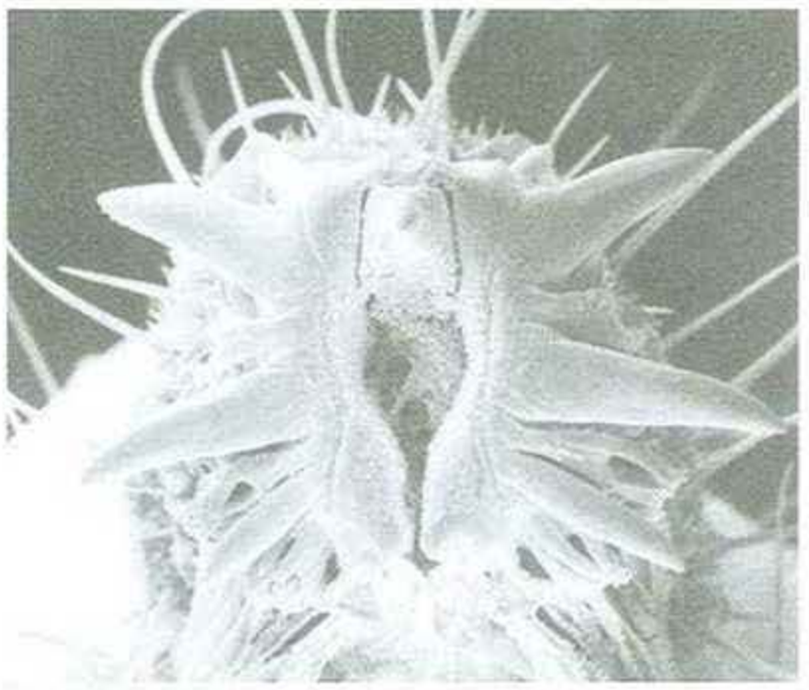


Figure 3.12

Figures 3.7 to 3.12 Scanning electron micrographs of the prestomal teeth of six *Musca* spp. namely *M. domestica* (**Figure 3.7**), *M. xanthomelas* (**Figure 3.8**), *M. autumnalis* (**Figure 3.9**), *M. lusoria* (**Figure 3.10**), *M. nevilli* (**Figure 3.11**) and *M. crassirostris* (**Figure 3.12**). (By courtesy of Drs A.B. Broce and R.J. Elzinga, Kansas State University, USA)

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Cyromazine is also available in a formulation that can be applied directly to fly-breeding material.[31]

Under extensive conditions Field studies indicate that most fly species which breed in the veld seek out the nearest host animals and do not fly far, unless the animals are removed from the area. Preliminary proof of this has come from insecticidal trials against African face flies in the Limpopo Province Bushveld of South Africa.[7] A marked fly reduction could be maintained in an insecticide-treated herd of cattle while the control herd, only 3 km away, had a high incidence of flies, indicating that these flies had not come into contact with the treated animals.

Reduction of fly numbers on cattle in the veld should therefore lead to a reduction in the transmission of certain infective organisms. This has been clearly demonstrated by Nevill *et al.*,[36] who drastically reduced the prevalence of *P. bovicola* infestation in cattle by weekly to fortnightly dipping with a pyrethroid insecticide. An easier method of face fly control is to attach pyrethroid-impregnated tags to both ears of an animal. In this way, in one British study, a reduction of up to 90 per cent in face fly numbers was achieved during a two- to three-month period, and infectious keratoconjunctivitis was almost completely eliminated.[47] This principle of fly reduction should be applied in situations where losses due to fly-transmitted diseases warrant the effort and expenditure. In South Africa no impregnated ear-tags are registered for fly control in livestock. This is because treatment is aimed primarily at ticks, for which ear-tags are unsuitable. However, pour-on pyrethroids, which are often effective for fly control, have become popular.[31]

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4

Vectors: Tabanidae

E M NEVILL, R J PHELPS AND B R STUCKENBERG

Introduction

The reason for the inclusion of a chapter on flies of the family Tabanidae in this book is their great potential as mechanical transmitters of infectious disease agents. Tabanidae are commonly known as horse flies, deer flies, clegs, and, in South Africa, as blindevlieë (Afrik.) (blind flies). The large size and variety of genera and species of Tabanidae in southern Africa should have made them an obvious choice for study by biologists, but studies on them have been mostly restricted to their taxonomy while those on their biology, role in disease transmission and control are limited.

Biosystematics

The taxonomy of the Tabanidae of the Afrotropical Region embraces three subfamilies (Pangoniinae, Chrysopsinae and Tabaninae) containing 31 genera and 727 species.[9, 10, 37–39] Four hundred and ten of these species occur in southern Africa, and of them about 80 per cent belong to only five genera, namely *Haematopota*, *Tabanus*, *Philoliche*, *Rhigioglossa* (= *Mesomyia*), and *Chrysops*.

Flies belonging to the Pangoniinae are usually of little known economic importance.[30] Various species of *Philoliche* of the subfamily Pangoniinae are, however, persistent pursuers of blood meals from cattle and horses in localized situations.[46] Of the Tabanidae recorded from southern Africa, three genera of the Chrysopsinae, namely *Rhigioglossa* (39 species), *Chrysops* (23 species) and *Tabanocella* (10 species); and five genera of the subfamily Tabaninae, namely *Haematopota* (137 species), *Tabanus* (68 species), *Hybomitra* (nine species), *Atylotus* (six species) and *Ancala* (four species) appear in a list of tabanid-transmitted disease agents.[32]

The taxonomy of tabanids of Angola,[14, 16] Mozambique,[15] Zimbabwe[24] and South Africa[48] has been reviewed.

In general, the biology of the Tabanidae has been poorly studied throughout the world, but this is even more the case in southern Africa. Chainey and Oldroyd[10] summarized the larval biology of these flies very succinctly as follows:

> Tabanid larvae live in wet places but need to reach the atmosphere periodically as they breathe air through a posterior siphon. The usual habitat is the wet mud at the margins of ponds, lakes and streams, but those of some species live in sand on the seashore. Some *Tabanus* larvae inhabit pockets of damp soil in generally dry areas, and *Haematopota* larvae occur mainly in areas of wet soil. Tree-hole breeding occurs in a few Tabanidae; larvae of *Thaumastocera* for example living in the accumulated debris in rot-holes of palms and forest trees . . . larvae of *Haematopota* and *Tabanus* feed on other insects, crustacea, worms or their own kind, but many tabanid larvae, such as those of *Chrysops*, feed on vegetable debris.

Adult tabanid flies are generally large, usually with well-developed eyes. Their mouthparts are adapted for both sucking and lapping, with both sexes feeding on nectar. The females of most species also feed on blood, which is needed for the development of the ovaries. The mandibles and maxilla are serrated and are used to cut into the skin and lacerate the dermal tissues, and thus induce the formation of a pool of blood which is then lapped up. A small amount of blood is trapped within the component parts of the mouthparts when they are withdrawn. Mechanical transmission of disease organisms is thus facilitated by this action. The coarse structure of the mouthparts and the mode of feeding results in painful bites causing cattle to try to dislodge or kill the flies by striking them with their tongues.[5, 30] Further details on the life cycles and bionomics of Tabanidae can be obtained from a chapter in the book by Kettle.[30]

The density of tabanid populations is closely associated with breeding sites. Populations around dams, vleis and natural wetlands may be high and composed of only a few species or perhaps even a single dominant one, which in South Africa is often a species of *Haematopota*.

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In southern Africa, the breeding sites of many tabanids in Malawi[33-35] and the larval feeding habits of *Tabanus biguttatus* Wied. and *Amanella emergens* Oldroyd in South Africa[8] have been recorded, but published information on the biology of adults of species of the Afrotropical Region is rather sparse and deals mainly with flight seasons, abundance and nocturnal activity.[10, 48] It is considered that certain physical adaptations aimed at distracting the attentions of visually orientated biting flies have evolved in some African wildlife species.[45] It has been demonstrated in the north-eastern KwaZulu-Natal Province of South Africa that the striping of zebra makes them virtually undetectable to tsetse flies (*Glossina* spp.) and tabanids.[25] Field observations also indicate that antelope species with a countershaded haircoat pattern, characterized particularly by a pale belly that eliminates the rounded appearance of the abdomen which is enhanced by shadowing in sunlight, are evidently unattractive to tabanids.[45] In contrast, mammals with uniform colouring, such as elephant, rhinoceros and hippopotamus, which present a strongly rounded appearance in sunlight, are highly attractive to large tabanid species such as *Tabanus taeniola* P. de B. and *Philoliche aethiopica* Thunberg. Experimental evidence has shown that a dark, single colour, horizontal cylinder is a most attractive shape for tabanids, and indeed the Harris Fly Trap represents essentially such a form.[26]

Tabanidae are unequally distributed in South Africa.[48] In general, many species of the rich savannah and tropical woodland tabanid fauna of Africa occur in the eastern coastal lowlands as far south as the Eastern Cape Province, and also range to a much more limited extent into the subarid Limpopo Valley and onto the Highveld. In the southern and south-western parts of the Western Cape Province a taxonomically very distinct tabanid fauna is characteristic of the macchia (fynbos) and the fold mountains, as well as the adjacent Karroid areas. These two major faunal units have few species in common.[48]

Regions with a high rainfall have the highest number of species. Topographically diversified areas also have a more diverse tabanid fauna. Consequently the plateau slopes, the Mpumalanga–Limpopo Lowveld, the coastal plain of KwaZulu-Natal, the Eastern Cape Province, and the southern and south-western part of the Western Cape Province have a much richer tabanid fauna than do the interior plateau areas and the arid western and northern regions of South Africa.[48]

A large proportion of species occurring in the northern and eastern lowlands of South Africa is shared with Mozambique, Zimbabwe, Zambia and Angola.

Certain species dominate in particular areas in southern Africa. In South African sand-dune areas along the southern and western coast of the Western Cape Province, a distinctive tabanid fauna is evident, with *Rhigioglossa edentula* Wied. being particularly evident.[46] The grassland plains of the Highveld support a limited fauna, though some species (e.g. *Haematopota* spp.) may achieve high densities where there are suitable breeding sites.[46] Wooded areas, or patches of indigenous forest, favour a greater diversity of tabanid species and may harbour dense populations of these flies. A common assemblage of flies may consist, for example, of species of the genera *Philoliche*, *Haematopota*, *Tabanocella* and perhaps *Amanella*, and the component species will vary from locality to locality. Members of such a guild of species aggregate around hosts that are potential sources of blood meals: such hosts may range in size from small antelope to cattle, and the flies will follow them with a marked degree of persistence. Movement of the host outside the forest or woodland results in selective withdrawal of those tabanid species that are unwilling to remain in open areas in full sunlight.

Many tabanids have preferred feeding sites on animals; the smaller tabanid species tend to settle on the legs, whereas the large species usually seek blood meals on the sides of the body or on the flanks. This behaviour has been interpreted as a form of resource partitioning through which interspecific competition for feeding sites may be reduced. This aspect has not been studied extensively in Africa, apart from observations made in Uganda[29] and Zimbabwe.[41]

Most tabanid species are active as adult flies only during the rainy season. Mass emergence of the flies may occur with the advent of the first rains, or in meteorological conditions in which such rain is possible. Consequently, in the winter-rainfall area of South Africa tabanids occur commonly from late winter to early summer, whereas in summer-rainfall regions their occurrence may be highly correlated with the rainy season of November to March. Some species may persist in low numbers throughout the year in lowland areas without frost, and a few fly only in the dry season. In hot climates, some species can be seen drinking at water sources such as the edges of natural pools: it has been observed in the Kruger National Park in South Africa that cemented water troughs provided for game are constantly visited by species of Tabanidae in drought years when natural water sources are scarce. Rearing records show that males and females occur in equal numbers, but the males may seldom be seen as they do not take blood meals and so are not attracted to mammals and only rarely to traps.[11, 43, 46]

Large tabanids mainly attack large mammals (cattle, horses, large antelope, elephant) and their presence may affect or modify behavioural patterns of animals. The blood-sucking activity of three common large species of Tabanidae (*Tabanus biguttatus*, *T. taeniola* and *Ancala africana* Gray) (**Figure 4.1**) have an important influence on the summer diurnal activity of hippopotami in north-eastern KwaZulu-Natal.[47] Because of the presence of large numbers of these flies in conjunction with high solar radiation, hippopotami are obliged to remain more or less submerged in the water during daylight hours.

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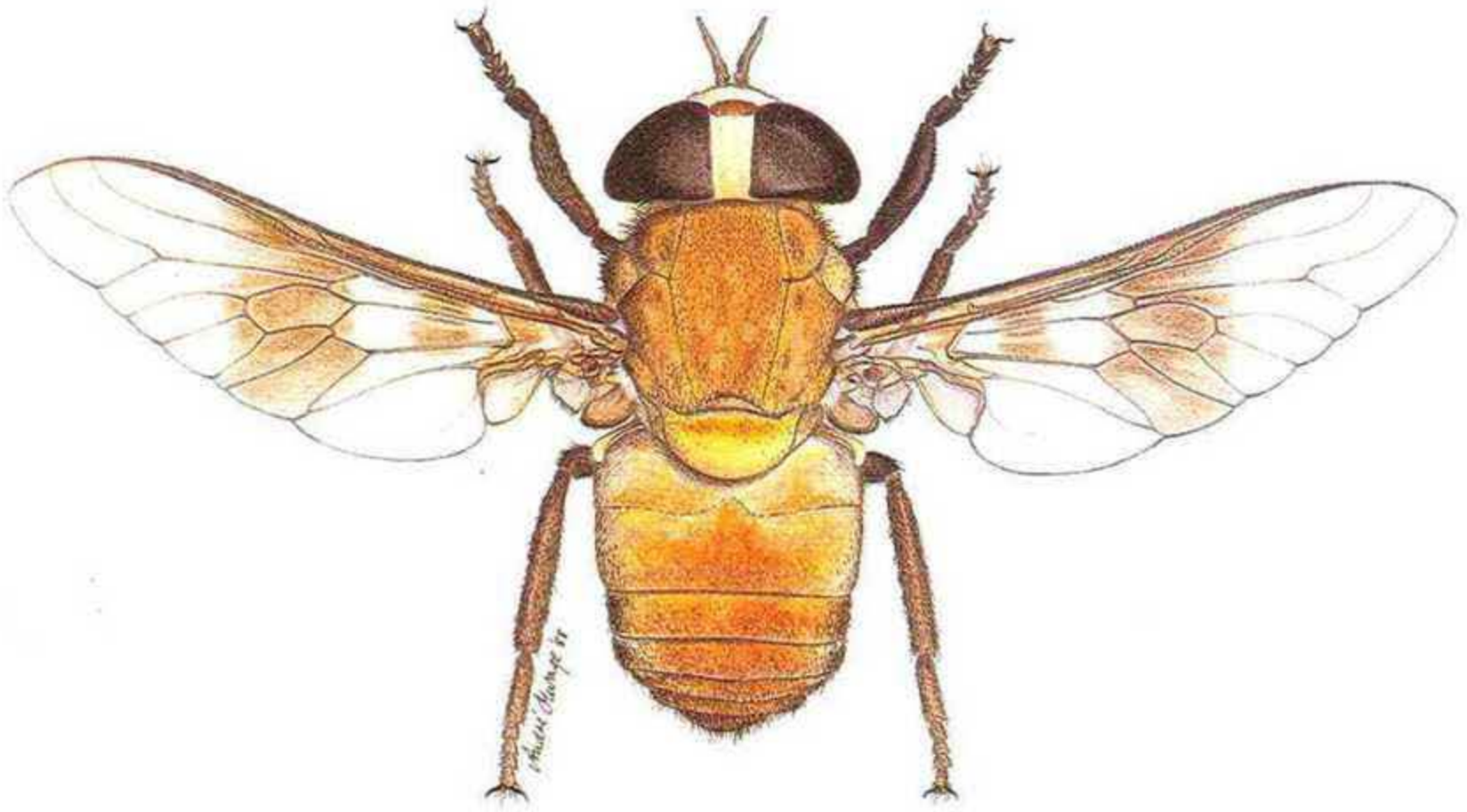


Figure 4.1 *Ancala africana*

Disease transmission

The infectious diseases or agents with which horseflies have been implicated as playing a role in their transmission are summarized in **Table 4.1**. The method of transmission may be either biological, which is uncommon, or mechanical.

Only two disease-producing micro-organisms are known to be transmitted biologically by tabanid flies (**Table 4.1**). Salivary gland infections of *Haemoproteus metchnikovi* are known to occur in the deerfly (*Chrysops callidus* O.S.) after feeding on turtles.[17] The other is *Trypanosoma theileri* which causes a benign infection in cattle. The results of carefully conducted trials in Germany suggest that transmission in the field takes place by contamination of the oral mucosa of cattle with metacyclid stages from gut contents or faeces of tabanids, when the cattle defend themselves and kill feeding tabanids with their tongues.[5]

Of interest is that although both salivarian and stercorarian trypanosomes undergo development in certain insects, infection rates of salivarian trypanosomes in the vector are often low, whereas stercorarian trypanosome infection rates may approach 100 per cent.[30]

In addition to infectious agents, various filarial worms develop in, and are transmitted by, species of tabanids throughout the world (**Table 4.1**).

Tabanids are the insect group most frequently associated with the mechanical transmission of pathogens — a result of a fly feeding successively on an infective host and then on a susceptible animal.[17–22, 28, 53–56] Transmission is facilitated by the fact that not only are they generally large insects and their presence worrying to animals, but also that their bites are painful. Feeding is therefore often interrupted and dispersal to alternate hosts frequent. The factors which influence mechanical transmission are:[17]

- the survival time of the pathogen outside the host;
- the proximity of infected and susceptible hosts;
- vector mobility;
- persistence of feeding (the less persistent feeders are probably most important in mechanical transmission);
- vector size (the larger tabanids would appear to be better transmitters of pathogens);
- the total tabanid population (this appears to be the most consistent phenomenon associated with peaks of infection);
- painful bites (this can be directly correlated with disease transmission);
- the host response to the bite of tabanids may be diminished during the acute stage of some infectious diseases and therefore the early febrile periods of chronic and acute infections may be the critical periods for transmission as pathogen titres in the blood of the host are often highest then;
- the quantity of infectious material transported by individual vectors between hosts;
- the amount of infectious material deposited at a wound/portal of entry;

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Table 4.1 Disease agents associated with tabanids[17, 32]

AGENT	ASSOCIATION
VIRUSES	
Bovine leukaemia	ET
California encephalitis	
Jamestown Canyon serotype	I
LaCrosse serotype	I
Equine infectious anaemia	ET, NT
Hog cholera	I, ET, NT
Influenza	EE
Rinderpest	ET, NT
Tick-borne encephalitis	I
Vesicular stomatitis	ET
Western equine encephalitis	I
BACTERIA AND RICKETTSIAS	
Anaplasma marginale	ET, NT
Bacillus anthracis	I, ET, NT
Brucella abortus	ET
Brucella melitensis	ET
Brucella suis	ET
Clostridium chauvoei	ET
Clostridium perfringens	EE
Coxiella burnetii	I
Erysipelothrix rhusiopathiae	ET
Francisella tularensis	I, ET, NT
Fusobacterium necrophorum	EE
Listeria monocytogenes	ET
Borrelia burgdorferi	I
Pasteurella multocida	ET
Ehrlichia risticii	EE
PROTOZOA	
Babesia ovata	EE
Besnoitia besnoiti	ET, NT
Haemoproteus metchnikovi	D, I, ET, B
Trypanosoma theileri	D, I, ET, NT, B
Trypanosoma evansi	ET, NT
Trypanosoma equiperdum	ET
Tsetse-borne trypanosomes	
Trypanosoma vivax	EE
Trypanosoma congolense	ET
Trypanosoma simiae	EE
Trypanosoma brucei brucei	ET
Trypanosoma brucei gambiense (*rhodesiense* strain)	ET
HELMINTHS	
Loa loa	I, D, ET, NT, B
Dirofilaria repens	D (exp.), B
Dirofilaria roemeri	I, D, ET, B
Elaeophora schneideri	I, D, ET, B

B = biological transmission
D = development of organism observed in tabanid
EE = epidemiological evidence
ET = experimental transmission in unnatural manner
NT = natural mechanical transmission
(exp.) = in experimentally infected flies
I = isolation from wild tabanids

- the titre of the pathogen in the blood of the infected host;
- seasonal changes and the persistence of infectious agents in the circulation of the mammalian host (e.g. under constant vector pressure an animal with a 1 per cent parasitaemia of *Anaplasma marginale* for 50 days may be equivalent, as a source of infection, to an animal with a parasitaemia of 50 per cent for one day);
- the density and age of the host; and
- prevalence of infection and degree of immunity in a host population.

Tabanids possibly played a role in the mechanical transmission of nagana (trypanosomosis) during the major epidemics in north-eastern KwaZulu-Natal.[3, 56] Sporadic outbreaks of the disease still occur in this part of KwaZulu-Natal,[6] and biting flies such as tabanids and horn flies (*Haematobia* spp.) may well play a role in its transmission.[36]

In southern Africa the only recorded experimental work on the mechanical transmission by tabanids of a pathogen is that done on *Besnoitia besnoiti*.[4] The flies that successfully transmitted the parasite by bite from cattle to rabbit and from cattle to cattle were *Atylotus nigromaculatus* Ricardo, *Tabanocella denticornis* Wied. and *Haematopota albihirta* Karsch, the only tabanid species which could be captured in sufficient numbers at the Onderstepoort Veterinary Institute. In three instances only a single fly was necessary to transmit *B. besnoiti*. Flies remained infective for up to 24 hours.[4]

Certain large species, notably *Tabanus taeniola* and *Philoliche aethiopica*, are uninhibited by large expanses of open terrain and are capable of sustained, powerful flight. Their often noisy flight, persistent attendance in large numbers and painful bites may induce restlessness in domestic stock.[46]

In southern Africa no work has been done on the evaluation of economic losses due to tabanid bites. This would be difficult as the appearance of a large number of these flies is often localized, sporadic and can occur simultaneously with other fly genera. In 1965 the bites of horse flies led to cattle losses in the USA amounting to 30 million dollars from reduced weight gain and 10 million dollars from reduced milk production.[44] Cows treated with synergized pyrethrins showed a significant increase in butter fat production, and similarly treated beef cattle gained 9 to 14 kg more than untreated cattle, over a 38-day period.[7]

Control

Methods of control generally encompass either the use of baited or unbaited traps or insecticide-treated animals. However, no concerted effort is made to apply these methods in southern Africa and there is also no published work on the control of tabanids in the subcontinent.

Studies in Zimbabwe on the reactions of tabanids to mobile and stationary baits, with and without human and ox odour, showed that, although odour did entice more flies to stationary baits, the main attraction was visual.[51] It has been shown in Zimbabwe that 1-octen-3-ol and 4-methylphenol, which are known attractants for some *Glossina* spp.[49, 50] are

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strongly attractive to *Tabanus pullulus* Austen and *T. copemani* Austen, and possibly attractive to several other species.[40] The indications are that it may be possible to develop odour-baited traps to control some tabanids.

Control of adult tabanids in other parts of the world is not commonly practised. Various approaches have, however, been tested. Because of their visual attraction to baits, use has been made of Manning or box traps, Manitoba or canopy traps, and sticky traps,[1, 42, 52] with or without the addition of carbon dioxide. In Georgia, USA, the effectiveness of the canopy trap in capturing female Tabanidae increased significantly when 1-octen-3-ol was used as an attractant.[23] The success of these traps often depends on their being placed in unobstructed flyways such as footpaths or the edge of a forest.[42]

Another approach to tabanid control is the use of residual insecticides on animals. Permethrin is lethal to horse flies and persists on treated animals for about two weeks.[2, 27] It has been found that 10 ppm permethrin is needed to kill tabanids compared with only 2 ppm for stable flies.[27]

Use of trapping or insecticides for the control of tabanids may be warranted under fairly intensive farming situations but, under extensive conditions, their control may not be successful due to large and mobile populations of one or more host species. In Oklahoma, USA, it has been shown that *Tabanus abactor* Philip is capable of flying at least 2,4 km and that the estimated number of host-seeking females per hectare can exceed 2 000 flies per day.[12, 13] However, a noticeable decrease in horse fly numbers, mostly *Tabanus nigrovittatus* Macq., resulted when 300 to 730 box traps were placed around the perimeters of Cape Cod salt marshes in the USA.[52]

Since both male and female tabanids visit flowers for nectar, they will pick up pollen and so may be pollinators for some plants. A potential side-effect of the widespread use of odour-baited, insecticide-treated targets and traps to control tsetse flies might be to reduce tabanid populations, as it has been shown that at least some species of tabanids are strongly attracted to such targets and traps.[40] This topic was investigated at Rekomitjie Research Station in Zimbabwe.[31] By comparing pollen taken from tabanids with that from plants available at the same time, it was demonstrated that, at this locality, tabanids were not specializing on any particular plant species. In addition, direct observation revealed that plants visited by tabanids attracted many other insect species not known to be attracted to anti-tsetse devices. At Rekomitjie, any reduction in tabanid numbers would not have an impact on pollination.

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5

Vectors: *Culicoides* spp.

Synonyms: Biting midges; brandassies (Afrik.)

R MEISWINKEL, G J VENTER AND E M NEVILL

Introduction

Culicoides biting midges are mosquito-like in their behaviour with the females of nearly all species being obligate blood-suckers (**Figures 5.1 and 5.2**) and being active only at night, especially when environmental conditions are calm and warm. The males feed on plant sap. *Culicoides* are 1 to 3 mm in size and are thus considerably smaller than mosquitoes but are more abundant and, on occasion, are found in enormous numbers in climates ranging from tropical to temperate. Most species possess grey- and white-patterned wings; these patterns being a very useful aid in their identification. To date 1 210 species of *Culicoides* have been described worldwide[38] but it is certain that many more await discovery; for example, one third of the 112 species known to occur in South Africa are new to Science.[216]

Culicoides are well represented in the fossil record (39 species) with the lineage of the family Ceratopogonidae going back at least 130 million years.[38] Midges clearly recognizable as *Culicoides* have been described from upper Cretaceous amber (88 to 93,5 million years ago), and it is therefore probable that *Culicoides* once fed on dinosaurs. A modern equivalent is the exclusive association between some *Culicoides* spp. and the African elephant (*Loxodonta africana*).[223] At present 38 subgenera comprise the genus *Culicoides* but many more await delineation. This taxonomic diversity is testimony to a long and complex evolutionary history reflected in the fact that *Culicoides* today feed on a broad spectrum of hosts including reptiles, mammals, birds and humans. It has been shown that in south-east Asia some *Culicoides* spp. will even feed on blood-engorged mosquitoes. Their blood-thirsty attacks on humans in some parts of the world are legion, and have earned them scientific names such as *damnosus, irritans, vexans* and *diabolicus*. These attacks can be so intense as to retard development and production in industries such as forestry and tourism.[155, 202] In Africa animals appear to be the hosts most intensively bitten. Under exceptional circumstances, such as prevail during overly wet periods, more than one million *Culicoides* can be captured in a single blacklight trap set at a horse stable on a warm summer night. Remarkably, such numbers probably represent only 1 per cent of the *Culicoides* population actively seeking blood, and illustrates the nightly levels of irritation, and possible risk of infection, that livestock may seasonally have to endure.[219]

Certain species have a proven involvement in the transmission of at least two of the 15 OIE list 'A' diseases, namely bluetongue (BT) and African horse sickness (AHS). They also transmit epizootic haemorrhagic disease (EHD) of deer, and equine encephalosis (EE).[116, 328] Thus, in sub-Saharan Africa, *Culicoides* are of great economic and veterinary significance. Indeed, it has been suggested that *Culicoides* midges were involved in two of the ten biblical plagues of ancient Egypt.[210] This is not improbable, as the suppressant effect of *Culicoides*-transmitted disease must have manifested soon after the domestication of livestock some ten millennia ago. In support of this contention is that epidemics of devastating extent have occurred in the modern era, the most notable being when AHS swept through the Near and Middle East in 1959/60 causing an estimated 300 000 deaths.[164] Another example is the AHS epidemic that decimated 70 000 equids in the then Cape of Good Hope Colony in southern Africa during the 1854/55 'season'.[19] The unprecedented scale of the latter outbreak, which claimed 40 per cent of the colony's total horse population in a mere eight months, attests not only to the efficiency of *Culicoides* as disseminators of infection but also to the modern-day necessity of protective annual vaccination. In eastern and southern Africa specifically this prophylactic regimen cannot be abandoned as it has long been known that there is a clear link between above average rainfall and outbreaks of *Culicoides*-borne orbiviral diseases.

The first studies on sub-Saharan *Culicoides* date back to 1908 when two species were described from Namibia[122] but, whilst of a high standard, the subsequent continental research effort has been mostly short-term and fragmentary. The last decade, however, has brought significant advances

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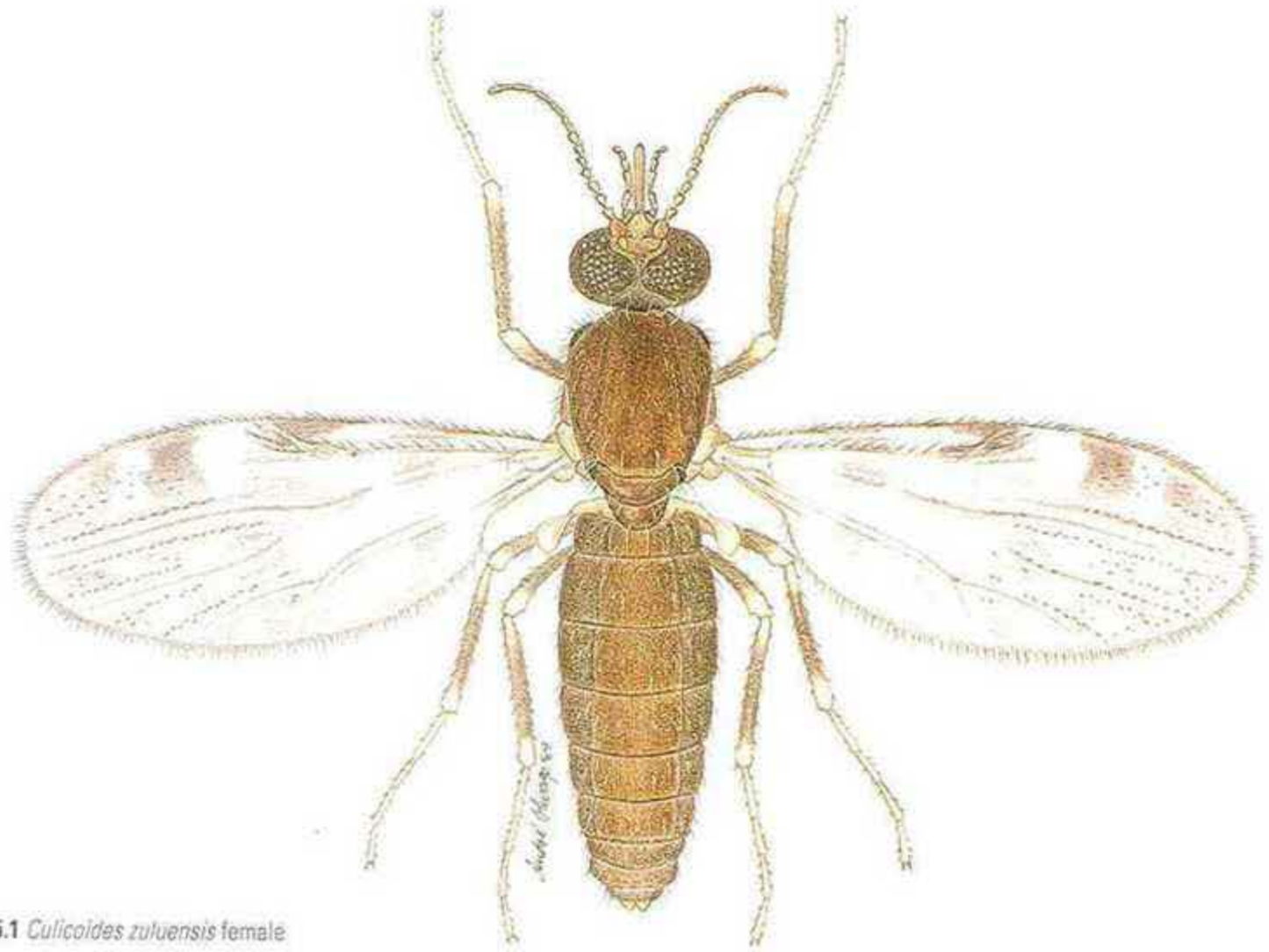


Figure 5.1 *Culicoides zuluensis* female

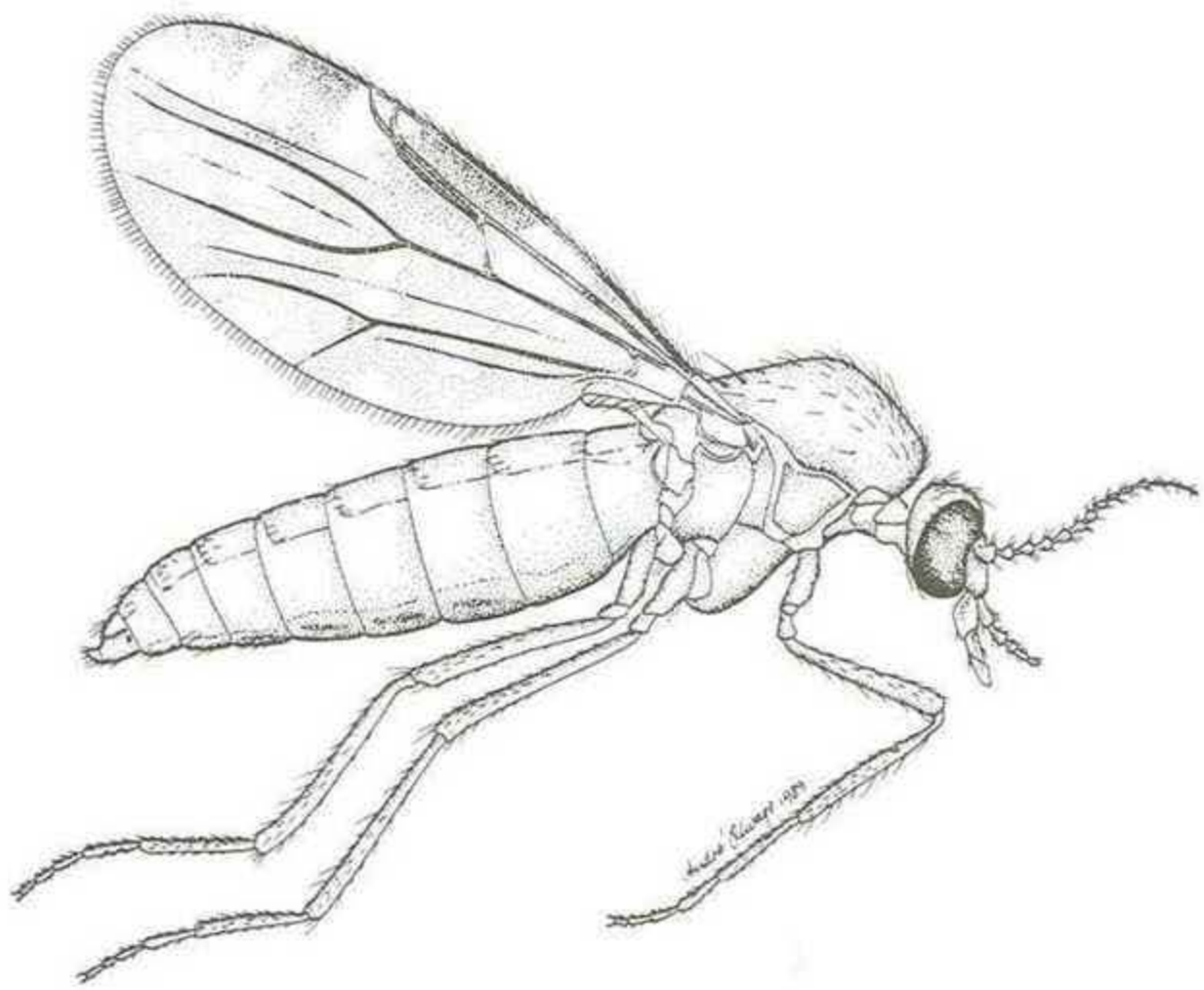


Figure 5.2 *Culicoides* spp.: lateral view

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especially in our basic understanding of *Culicoides* systematics, which includes the discovery that the most important Old World vector of BT and AHS, *C. imicola*, is but one member of a complex that comprises at least 10 species. Not only does each species have a unique biology, but laboratory infection studies have shown that a recently described species within the complex, *C. bolitinos*, may in certain instances be a more efficient field vector of bluetongue virus (BTV) than *C. imicola*. Further insights gained from field outbreaks strongly support the laboratory findings around *C. bolitinos*, and thus the 'single-vector status quo' that has stood since the time of du Toit's seminal studies[115] on *C. imicola* more than 50 years ago, is gradually being eroded. In addition, the long-neglected study of the interaction between *Culicoides* and African game animals (which naturally cycle the above-mentioned viruses) has finally received some attention, and yielded much that is of interest. This growth in basic knowledge was not only multi-disciplinary in its embrace but also formed part of an attempt to 'modernize' the study of *Culicoides* in Africa. Another consequence was the production of three satellite-based predictive risk maps for *C. imicola*, the first for any species of *Culicoides* in the world. Also harnessed for the first time were molecular techniques such as the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and mitochondrial DNA (mtDNA) extraction. Not only have these studies resulted in a partial phylogeny of the Imicola complex but, importantly, have also reaffirmed the stability of 'old-fashioned' morphological identifications, and so reassure us that the steady advances being made on the unique life cycle of each species do indeed reflect 'realities' in nature. These advances are reviewed here, and because the research has focused principally on vectors of orbiviruses that affect livestock, this chapter concentrates on assembling much of the knowledge accumulated thus far on the two proven vectors *C. imicola* and *C. bolitinos*. Included is an introductory preamble of the standardized protocols used to collect, subsample and age-grade *Culicoides*, which were developed principally in South Africa over the last 30 years, and are now used in various parts of Africa and beyond. It is essential that data collected over a wide geographic area and during a number of seasons and years be comparable so that accurate distribution databases can be created for the subsequent development of disease risk-maps. At various junctures in this review an indication is given of the research still needed to refine our understanding of African *Culicoides* and the diseases they transmit.

Collecting adult *Culicoides*

The majority of investigations conducted on *Culicoides* worldwide deal primarily with the monitoring of disease vectors as their acknowledged role in epidemics of disease impacts directly on management practices, such as vaccination, and quarantine and export of live animals to international destinations. The primary, almost sole, monitoring tool used for the capture of *Culicoides* is the light trap. However, other trapping methods of some variety have been developed over many years for the capture of adult *Culicoides*. Those most commonly used in Africa include various models of white- or blacklight traps, emergence traps (for larval habitat studies), aspirators (for the collection of live midges off human and animal hosts), and truck traps (which involve the use of a large net mounted on top of a slow-moving vehicle). The trapping method employed depends upon the purpose of the research as each method enjoys some advantages not shared by the others. Their uses, and the merits and disadvantages of each, are briefly reviewed below.

Blacklight traps

These traps are preferred over those with whitelight as they apparently attract larger numbers and varieties of insects. This, and their flexibility in application, make the blacklight trap the tool of choice when biting midge abundance levels are to be determined during outbreaks of disease, and when large-scale faunistic surveys are being conducted. The *Culicoides* are captured in a bowl of liquid (suspended below a suction fan, which in turn is below the light source) in which they die. In a matter of hours these 'wet' captures make available large numbers of midges suitable for a number of purposes that include virus isolation, blood meal identification, age-grading, and taxonomic studies. When the light trap is set near livestock (usually at a height varying between 1 and 2 m) a few small drops of detergent are added to the bowl of water to break the surface tension. This is necessary because midges are too small and light to drown and sink to the bottom, with the result that in the morning, when the trap is switched off, a number of them may recover and fly off. With minor modification the same trap can be used to catch large numbers of live midges for vector competence studies in the laboratory. In this instance a gauzed cage replaces the bowl of liquid. Alternatively, the bowl may be retained but is partially filled with loosely crumpled tissue paper so that the live, captured midges can creep into recesses away from the incessant wind created by the suction fan. Another advantage of the blacklight trap is that it is easy to use as it can be rigged in a short space of time and, furthermore, does not have to be monitored until morning, when it can be dismantled equally quickly. What is time-consuming, however, is the journey to disease outbreak points and choices to be made as to which sites will produce optimal catches. In addition, the journey has to be made twice as the traps must be retrieved the following morning. Light traps cannot be left in position for more than one night, and should not be allowed to hang in sunlight as the captured midges, especially those in a weak soapy detergent solution, start to decompose very quickly in tropical or subtropical daytime temperatures. Another disadvantage is that most light traps require electricity, which greatly restricts their use in rural situations. Whilst it is possible to run these traps using a generator, the transport and

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movement of such heavy equipment means that very few geographic points can be sampled per night. Light traps can be adapted to run on 12-volt batteries but here, too, the transport and maintenance of heavy batteries can be burdensome and impractical. In this context it must also be borne in mind that the various modifications made to traps to capture *Culicoides* in out of the way places must not result in their lowered performance as this will provide data of limited comparative value. A further disadvantage is that the traps are equipped with a strong suction fan, which can damage *Culicoides*. This can be particularly vexing when rare or new species have been collected which must be in a suitable state for specialized taxonomic studies. Being powerful, these traps also capture large numbers of other insects including moths and, under certain conditions, this can result in collections that are extremely dirty and that take a long time to clean. Larger insects can also damage the smaller midges and is another reason why blacklight traps should be covered with a fine gauze mesh to exclude the capture of all insects larger than 2 mm. As mentioned, exceptionally large numbers of *Culicoides* can be captured in blacklight traps. To be able to determine this total, and the exact number of each species collected, it is essential to be able to subsample catches easily and rapidly as hundreds of collections may be made during a large survey. A method of subsampling *Culicoides* has been developed[325] and is widely employed. Because of their strong attractant power, blacklight traps are considered biased samplers and therefore in more specialized studies, such as those concerned with host preference, other more 'objective' collecting techniques must be employed.

Vehicle-mounted traps

For obvious reasons the blacklight trap is useless for the study of *Culicoides* that may be active by day. For this, vehicle-mounted traps (commonly referred to as 'truck traps') can be used to capture flying *Culicoides* throughout the diel, and in this way hourly activity rates may be determined; the results can also be related to prevailing meteorological conditions such as temperature, relative humidity and windiness. Vehicle-mounted traps are particularly successful for the collection of large numbers of biting midges during the hours of dusk and dawn. In addition, male swarms, which may not be attracted to light traps, can be captured in this way, at times in large numbers. If collection periods are limited to 15 minutes, the midges captured will be in pristine condition and therefore suitable for the preparation of material on glass slides for taxonomic study. Because *Culicoides* are host-orientated, truck trapping greatly enhances the possibility of capturing *Culicoides* near large groups of wildlife that are either dangerous and/or scatter when approached on foot. It is, in addition, very difficult to set light traps near wildlife; this being compounded by the fact that herds are constantly on the move. Disadvantages of truck trapping are the cost of fuel for the vehicle and the time required for driving and trapping.

Aspirators and sweep nets

Aspirators (or 'pooters') and hand-operated sweep nets are used in specialized host preference studies when live *Culicoides* must be captured off tethered animals or humans. A hand-held pooter can be used but this requires that each individual midge be located, using a red torchlight, prior to capture, which is time-consuming. More commonly the 'sweeping' of marked areas of the host with a small hand-held domestic vacuum cleaner is the preferred method. In this way more *Culicoides* may be captured more rapidly, which is necessary if it has to be established exactly which areas of the host are being attacked within a specified time slot. In life cycle studies in which the capture of gravid females for oviposition determinations is required, the midges are best obtained by pootering those attracted to a source of light.

Traps baited with carbon dioxide have also been used to collect *Culicoides*. The particular advantage of these traps is that they enable the collection of diurnal species. Limitations of carbon dioxide traps are that they require dry ice and that the unregulated release of carbon dioxide gas may provide concentrations that are attractive to some species and repellent to others.

Emergence traps

These are made of fine netting, are conical in shape, and have a collection bottle at the apex that is lined with a sticky substance or contains a liquid.[276] An emergence trap (built to cover a specific unit area) is placed over a suspected *Culicoides* larval habitat and remains *in situ* where it can be monitored hourly, daily, or weekly. In this way emergence rates and species-association profiles can be obtained. The chemical composition and structure of the soil, and the amount of water it holds can, in addition, also be determined for a particular emergence site. Furthermore, during emergence, if larvae or pupae must be retrieved, samples of the substrate can be extracted and taken to the laboratory where a saturated sugar solution is added to them to alter the specific gravity in order to induce the larvae and pupae to float to the surface. The pupae are retrieved with a spatula and placed in individual vials for eclosion. The resultant adult and its associated pupal pelt can then be mounted on a single glass slide for identification. The rearing of individual larvae so obtained is more complicated but can be achieved on agarose media to which cultured micro-organisms are added. This, or a similar, rearing strategy is essential towards understanding more precisely the ecology of individual *Culicoides* spp. as it is the availability of the larval habitat that determines primarily the prevalence of a species in any given area.

It should be noted that much more research is still required towards more clearly defining the larval habitats and food requirements of the immature stages of the vector species of *Culicoides*, including *C. imicola* and *C. bolitinos*.

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Table 5.1 Micro-organisms associated with the genus *Culicoides* worldwide

CULICOIDES SP.	MICRO-ORGANISM	REFERENCE
C. amamiensis	*Macacanema formosana*	27, 28
C. arubae	*Onchocerca* sp.	31, 347
C. arakawae	*Leucocytozoon (Akiba) caulleryi*	1, 2, 123, 170, 199, 245–250, 284, 317
	Lemdaninae	318
	Onchocerca gutturosa	98, 113, 201, 318
C. arboricola	*Haemoproteus meleagridis*	7
C. adersi	*Hepatocystis (Plasmodium) kochi*	137–139, 146, 147
C. austeni	*Dipetalonema perstans*	161, 162, 270
C. circumscriptus	*Leucocytozoon (Akiba) caulleryi*	145
C. crepuscularis	*Haemoproteus danilewskyi*	146
	H. fringillae	146
	Chandlerella quiscali	166
C. downesi	*Haemoproteus nettionis*	130, 146, 295
C. edeni	*Haemoproteus meleagridis*	7, 8, 10
	Vavraia sp.	9
C. fulvithorax	*Hepatocystis (Plasmodium) kochi*	137–139, 146, 147
	Onchocerca gutturosa	98, 113, 201, 318
C. furens	*Dipetalonema marmosetae*	204
	D. ozzardi	58, 59, 132, 206
C. grahamii	*Dipetalonema perstans*	114, 156, 270, 271, 292, 293
	D. streptocercum	68, 69, 156
C. haematopotus	*Haemoproteus meleagridis*	7
C. hinmani	*Haemoproteus meleagridis*	7
C. hollensis	*Dipetalonema gracile*	119
	D. caudispina	119
	D. llewellyni	350
	D. marmosetae	204
C. inornatipennis	*Dipetalonema perstans*	114, 156, 270, 271, 292, 293
C. insinuatus	*Dipetalonema ozzardi*	205, 232, 257, 312, 322
C. kingi	*Onchocerca gutturosa*	98, 113, 201, 318
C. knowltoni	*Haemoproteus meleagridis*	7
C. krameri	*Onchocerca gutturosa*	98, 113, 201, 318
C. marksi	*Onchocerca* sp.	31, 348
	O. gibsoni	31, 60, 124, 201
C. multidenatus	*Splendidofilaria californiensis*	341
C. nubeculosus	*Trypanosoma bakeri*	238
	T. chabaudi	67
	T. davidmolyneuxi	67
	T. everetti	67
	T. gentilinii	67
	Hepatocystis brayi	239
	H. levinei	40, 195
	Haemoproteus handai	240
	Eufilariella bartlettae	11
	E. delicata	11
	E. kalifai	237
	Onchocerca cervicalis	133, 134, 157, 229, 230
	O. gibsoni	31, 60, 124, 201
	O. gutturosa	99, 113, 201, 318
	O. reticulata	244, 245
	Dipetalonema ozzardi	205, 232, 257, 312, 322
C. obsoletus	*Onchocerca cervicalis*	76, 151, 157, 180, 201, 231, 301
C. odibilis	*Leucocytozoon (Akiba) caulleryi*	146
C. oxystoma	*Onchocerca gibsoni*	31, 60, 124, 201
C. orientalis	*Onchocerca gibsoni*	31, 60, 124, 201
C. parroti	*Onchocerca cervicalis*	76, 151, 157, 180, 201, 231, 301
C. phlebotomus	*Dipetalonema ozzardi*	205, 232, 257, 312, 322
C. pulicaris	*Francisella tularensis*	176, 273
C. pungens	*Onchocerca gibsoni*	31, 60, 124, 201
C. riethi	*Dipetalonema ozzardi*	205, 232, 257, 312, 322
C. schultzei	*Leucocytozoon (Akiba) caulleryi*	146
C. shortti	*Onchocerca gibsoni*	31, 60, 124, 201
C. sphagnumensis	*Trypanosoma avium*	25
	Haemoproteus canachites	146
	H. danilewskyi	146
	H. mansoni	7
	H. (Parahaemoproteus) velans	146
C. stilobezzioides	*Trypanosoma avium*	25
	Haemoproteus fringillae	146
	H. (Parahaemoproteus) velans	146
	Chandlerella chitwoodae	17
C. travisi	*Chandlerella chitwoodae*	17
C. trifasciellus	*Onchocerca gutturosa*	98, 113, 201, 318
C. variipennis	*Trypanosoma* sp.	161
	Hepatocystis brayi	239
	Onchocerca cervicalis	133, 134, 157, 229, 230
	Dipetalonema ozzardi	205, 232, 257, 312, 322
Culicoides spp.	*Leishmania* sp.	70
	Plasmodium malariae	91
	Leucocytozoon neavei	129
	L. schoutedeni	129
	L. (Akiba) sp.	12, 126, 127, 187, 294
	Haemoproteus canachites	147
	H. sp.	26, 79, 125–128, 130, 144, 147
	Ornithofilaria fallisensis	5
	Onchocerca gibsoni	31, 60, 124, 201
	O. volvulus	141, 326

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Livestock and wildlife diseases/infections associated with *Culicoides*

Summer seasonal recurrent dermatitis

Summer seasonal recurrent dermatitis is most commonly referred to as sweet itch, but is also known as Queensland itch, 'dhobie itch', kasen disease, allergic dermatitis and sommerekzem. It is a chronic, seasonally superficial allergic dermatitis of horses that usually affects the skin of the mane, tail and withers resulting from the bites of *Culicoides* midges. Various species are involved. This condition was first recognized in France in 1840, but has since been investigated in many countries.[55, 56, 78, 148, 188, 256, 323, 349] In Israel *C. imicola* was implicated as the principal species involved as 70 per cent of 195 specimens caught on a horse showed a clear preference for feeding on the dorsal ridge, which overlaps the itch zone.[42] In the UK a significant and rapid reduction in the prevalence of sweet itch was demonstrated once affected horses were more regularly stabled.[234] Virtually no studies have been conducted on the condition in Africa but it is suspected to occur in South Africa.[324]

Diseases associated with Nematoda: Filarioidea

Some 20 species of filarial nematodes are transmitted by *Culicoides* midges and include five species of *Onchocerca* in cattle, horses and water buffalo (*Bubalis bubalus*), and two species of *Dipetalonema* in monkeys (**Table 5.1**).[201]

Diseases associated with Protozoa

Worldwide, at least 33 species of protozoa in the orders Eucoccida and Kinetoplastida are known to be transmitted only by *Culicoides* (**Table 5.1**).[201] Except for a species of *Hepatocystis* found in several species of monkeys in Africa and another in the Malaysian sciurid squirrels of the genera *Callosciurus* and *Sundasciurus*, the other 10 protozoa occur in birds. One of these, *Leucocytozoon caulleryi*, is an economically important disease of poultry in south-east Asia.

Diseases associated with viruses

Of all the pathogens transmitted by *Culicoides*, viruses are of the greatest veterinary importance, and, more especially, those that cause BT, AHS, EE and Akabane disease. Other viruses closely related to Akabane virus have also been isolated from *Culicoides* (**Table 5.2**). Although many of the viruses that are *Culicoides*-associated have been isolated from, but occur more commonly in, other arthropods such as mosquitoes, argasid and ixodid ticks, and phlebotomines, there remain 45 per cent which have been isolated only from *Culicoides* midges.

Worldwide, some 64 named and 11 unnamed arboviruses are *Culicoides*-associated. Of these, 25 are from Australia and 23 from Africa. Of the 75 viruses listed in **Table 5.2**, 46 have been isolated from pools of identified *Culicoides* spp. rather than from pools containing mixed species. Twenty-three of these viruses are from the *Culicoides* subgenus *Avaritia* alone, 15 from *C. brevitarsis* in Australia, and eight from *C. imicola* in Africa, with BTV being common to both continents. Although these figures may be biased in that they reflect both the specific directions taken in *Culicoides* research and the particular drive of the research team involved, they do show that the tropics and subtropics are particularly rich in both bunya- and orbiviruses.

Bunyaviridae The Simbu group viruses (**see Chapter 94: Diseases caused by Akabane and related Simbu-group viruses**) that are found in southern Africa[90] include Akabane virus, the cause of abortion and congenital deformities in domestic ruminants. Akabane virus is widespread in southern Africa occurring in at least 25 species of wildlife,[3] and the prevalence of antibodies to it in African buffalo (*Syncerus caffer*), blue wildebeest (*Connochaetes taurinus*) (as high as 69 per cent in Namibia) and African elephant (*Loxodonta africana*) strongly implicates them in its maintenance in nature. Sabo virus,[90, 321] closely related to Akabane virus, also occurs in cattle and *Culicoides* midges in South Africa, whilst Shamonda virus has been isolated from *C. imicola* collected near cattle at the Onderstepoort Veterinary Institute in South Africa.[211] In addition, in South Africa a strain of Shuni virus has been recovered from the brain of an adult mare that died after showing nervous signs. Subsequent serological surveys revealed positive antibody titres in horses to Akabane, Shuni, Shamonda and Sabo viruses, with the latter two predominant. Their presence may explain some of the febrile reactions and other vague clinical signs observed in horses seasonally.[165] Although they have not yet been tested for pathogenicity in ruminants, South African strains of Sabo, Shamonda and Shuni viruses induce marked teratogenic effects in chicken embryos.[90] Aino virus, which is closely related antigenically to Shuni virus, has, in Japan and Australia, been implicated as the cause of congenital anomalies in cattle and sheep.[110, 316]

Reoviridae Within this family, the orbiviruses of BT, AHS, and EE are commonly associated with causes of livestock diseases in southern Africa. Other members of this family include EHD, Nyabira, Gweru and Kasba (= Chuzan) viruses. Both Nyabira and Gweru are little understood Palyam-serogroup viruses (**see Chapter 107: Palyam serogroup orbivirus infections**) that have been found only in southern Africa but, like Akabane virus, are linked to abortions and teratology in cattle, goats and sheep.[314, 344] Viruses of the Palyam serogroup have a particular association with cattle, and the many isolations made from *Culicoides* suggest that they are competent vectors for Palyam viruses in southern Africa.[345] Nyabira virus, for instance, replicates well in *C. imicola* and *C. zuluensis*, but transmission trials have proved inconclusive.[54] There is serological evidence for the widespread distribution of Palyam-serogroup viruses throughout southern Africa except for the cooler winter rainfall region of the Western Cape Province of South Africa. Since they may be

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Table 5.2 Viruses isolated from the genus *Culicoides* worldwide

CULICOIDES SP.	SUBGENUS	VIRUS	ABBREVIATION	REFERENCE
C. actoni	*Avaritia*	Warrego	WAR	149
C. agathensis	Unassigned	Alphavirus sp.		53
C. algecirensis	*Monoculicoides*	Bovine ephemeral fever	BEF	296
C. austropalpalis	Similis grp.	Kununurra	KNA	149
		Wongabel		225
C. bedfordi		Unidentified		266
C. bolitinos	*Avaritia*	African horse sickness	AHS	226
		Bluetongue		16
		*Letsitele		266
C. brevitarsis	*Avaritia*	Aino	AINO	111
		Akabane	AKA	87, 99, 108, 111, 308
		Bluetongue	BT	302, 307
		Bovine ephemeral fever	BEF	87
		Bunyip Creek	BC	87, 88
		CSIRO Village	CVG	298
		D'Aguilar	DAG	110, 111
		Douglas	DOU	304
		Epizootic haemorrhagic disease of deer	EHD	277, 306
		Kimberley	KIM	351
		Ngaingan	NGA	110
		Peaton	PEA	310
		Tibrogargan	TIB	89
		Tinaroo	TIN	86, 304
		Wallal	WAL	175
		Unidentified		111, 305
*C. brevitarsis + C. schultzei***	Mixed	Unidentified		305
C. bundyensis	Unassigned	Belmont	BEL	149
C. coarctatus	Unassigned	Bovine ephemeral fever	BEF	34
C. cockerellii	*Sylvicola*	Bluetongue	BT	190
C. carnutus	*Monoculicoides*	Epizootic haemorrhagic disease of deer	EHD	16
C. dycei	Unassigned	Wallal	WAL	110, 111
		Warrego	WAR	110
C. exspectator	Similis grp.	Bluetongue	BT	266
C. filarifer	Unassigned	Bluetongue	BT	242
C. filarifer + C. pusillus	Mixed	Bluetongue	BT	149
C. fulvus	*Avaritia*	Bluetongue	BT	298
C. fulvus + C. orientalis	Mixed	Bluetongue	BT	291
C. gulbenkiani	*Avaritia*	Letsitele		266
C. histrio	*Meijerehelea*	Thimiri	TIM	298
C. imicola	*Avaritia*	Akabane	AKA	4, 34
		African horse sickness	AHS	34, 266
		Bluetongue	BT	16, 34, 53, 143, 235, 266, 339
		Bovine ephemeral fever	BEF	34
		Equine encephalosis	EE	266
		*Letsitele		266
		*Nyabira		34
		Sabo	SABO	66, 175, 197
		Shamonda	SHA	175, 197
		Simbu		266
		Unidentified		97
*C. imicola + C. schultzei***	Mixed	Unidentified		97
C. insignis	*Hoffmania*	*Bivens Arm		140
		Bluetongue	BT	77, 145, 242
		Sweetwater Branch		225
C. kingi	*Remmia*	Epizootic haemorrhagic disease of deer	EHD	235
		Unidentified		235
C. magnus	*Culicoides*	*Letsitele		266
C. marksi	Unassigned	Belmont	BEL	298

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Table 5.2 *(continued)*

CULICOIDES SP.	SUBGENUS	VIRUS	ABBREVIATION	REFERENCE
		Barmah	BF	298
		Eubenangee	EUB	109, 298
		*Humpty Doo		298
		*Leanyer		298
		*Mudjinbarry		112
		*Parker's Farm		298
		Wallal	WAL	110, 298
		Warrego	WAR	110, 175
		Unidentified		300
C. milnei	*Hoffmania*	Akabane	AKA	34
		Bluetongue	BT	339
C. nevilli	*Remmia*	Epizootic haemorrhagic disease of deer	EHD	16
C. newsteadi	*Culicoides*	Alphavirus sp.		53
C. nivosus	*Meijerehelea*	Thimiri	TIM	225
C. obsoletus	*Avaritia*	Bluetongue	BT	236
C. oxystoma	*Remmia*	Akabane	AKA	192, 193
		Bunyip Creek	BC	225
		Kasba	KAS	194
C. pallidothorax	Unassigned	Wongorr	WGR	298
C. paraensis	Unassigned	Ananindeua	ANU	175
		Oropouche	ORO	196
C. peregrinus	*Hoffmania*	*Beatrice Hill		298
C. peregrinus + *C. schultzei***	Mixed	Unidentified		88, 305
C. punctatus	*Culicoides*	Barur	BAR	174
C. pusillus	*Avaritia*	Bluetongue	BT	242
C. pycnostictus	*Meijerehelea*	Bluetongue	BT	266
*C. schultzei***	*Remmia*	Bovine ephemeral fever	BEF	97
		Bunyip Creek	BC	298
		D'Aguilar	DAG	111
		Epizootic haemorrhagic disease of deer	EHD	196, 198
		Kasba	KAS	194, 197, 198, 241
		Keterah	KTR	259
		Marrakai	MAR	298
*C. schultzei*** + *C. imicola*	Mixed	Kasba	KAS	97
*C. schultzei*** + *C. peregrinus*	Mixed	Marrakai	MAR	88
C. schultzei grp	*Remmia*	Unidentified		266
C. stellifer	Unassigned	Vesicular stomatitis New Jersey	VSNJ	190, 340
C. tororoensis	*Avaritia*	Nairobi sheep disease	NSD	97, 339
		Bluetongue	BT	340
C. variipennis	*Monoculicoides*	Bluetongue	BT	135, 136, 190, 203, 285, 346
		Bunyavirus sp.		154
		Buttonwillow	BUT	153, 154, 175, 190, 258, 287
		Epizootic haemorrhagic disease of deer	EHD	145, 172, 354
		Lokern	LOK	85, 190, 258
		Main Drain	MD	85, 121, 258
		Vesicular stomatitis New Jersey	VSNJ	175, 190, 340
C. wadai	*Avaritia*	Akabane	AKA	311
C. zuluensis	*Hoffmania*	Letsitele		266
		Unidentified		97
C. zuluensis + *C. imicola*	Mixed	Unidentified		97
Culicoides spp.	*Selfia*	Lokern	LOK	190
		Main Drain	MD	190
		Vesicular stomatitis New Jersey	VSNJ	190, 340
Culicoides spp.	Mixed	African horse sickness	AHS	97, 175, 233, 266
		Aino	AINO	175
		Akabane	AKA	175, 321

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Table 5.2 *(continued)*

CULICOIDES SP.	SUBGENUS	VIRUS	ABBREVIATION	REFERENCE
		Bluetongue	BT	32, 44, 197, 198, 243, 266, 283, 309
		Bovine ephemeral fever	BEF	95, 97
		*Buritirana		150
		Buttonwillow	BUT	175, 190
		Congo-Crimean haemorrhagic fever	CCHF	197
		CSIRO Village	CVG	88
		D'Aguilar	DAG	300
		Douglas	DOU	298
		Dugbe	DUG	197
		Eastern equine encephalomyelitis	EEE	177
		Epizootic haemorrhagic disease of deer	EHD	197, 198, 266
		Equine encephalosis	EE	266
		*Gweru		345
		Israel turkey meningoencephalitis	IT	53, 175
		*Itacaiunas		149
		Kasba	KAS	197, 198
		Kotonkan	KOT	179, 197
		*Letsitele		266
		Marrakai	MAR	298
		Mitchell River	MR	110
		Nairobi sheep disease	NSD	197
		Ngaingan	NGA	110, 178
		*Nyabira		345
		Palyam	PAL	266
		Rift Valley fever	RVF	92, 94, 96
		Sabo	SABO	66, 175, 197
		Sango	SAN	66, 197
		Sathuperi	SAT	66, 175, 197
		Shamonda	SHA	175, 197
		Shuni	SHU	66, 197
		Simbu		266
		Tahyna	TAH	150
		Utinga	UTI	186
		Wallal	WAL	110
		Warrego	WAR	111
		*Weldona		63
		Unidentified		197, 266, 300, 321

* Arboviruses not registered in the International Catalogue[176]

** Most, if not all, of these virus isolations should be attributed to the Schultzei group (= subgenus *Remmia*) as the species *C. schultzei sensu stricto* does not occur outside Africa

abortigenic in cattle, there is clearly a need for pathogenicity trials in livestock.[345] A third Palyam-serogroup virus (Kasba virus) has been associated with congenital abnormalities in calves in Japan and has been isolated from *C. oxystoma*.[241] This *Culicoides* is a member of the subgenus *Remmia* and is widely and abundantly found in south-east Asia and the eastern Palaearctic. Similarly, in Africa, the Mediterranean region and the Near and Middle East seven or eight species of *Remmia* can also be found in some abundance, particularly in hotter, drier regions; these therefore deserve consideration as potential vectors of various reoviruses. It was from a mixed pool of two *Remmia* spp. (including *C. kingi*) that an EHD virus was isolated in the Sudan.[235]

The first isolation of equine encephalosis virus (EEV) was made from a mixed pool of *Culicoides* consisting mainly of *C. imicola* collected at the Onderstepoort Veterinary Institute.[321]

Rhabdoviridae In this family is included bovine ephemeral fever virus (BEFV). The seasonal occurrence of BEF (**see Chapter 100: Bovine ephemeral fever**) suggested insect transmission to early investigators in Africa[29, 30] although

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firm evidence of insect involvement was not provided until the causative virus was isolated from a pool of several species of *Culicoides* in Kenya in 1971.[95, 97] It has been recovered from *Culicoides* only twice in southern Africa, namely from *C. imicola* and *C. coarctatus* in Zimbabwe.[34] Its low isolation rate is possibly due to it being particularly difficult to isolate using standard cell culture techniques, and is further complicated by the fact that there are many other rhabdoviruses closely related antigenically to BEFV.[303] In Australia BEFV has been isolated from *C. brevitarsis*,[253] the sister species to the African *C. bolitinos*. Both species breed in cattle dung, with *C. bolitinos* also being found in the dung of the African buffalo and blue wildebeest.[213]

Two other rhabdoviruses, Bivens Arm and Sweetwater Branch, have been isolated from *C. (Hoffmania) insignis* collected near a water buffalo imported into North America from Trinidad[140] but have not been linked to naturally occurring or experimentally induced disease. These viruses are related to Tibrogargan virus, which is associated with water buffalo, cattle and horses in Australia and has also not, as yet, been shown to be pathogenic.[90] The *Culicoides milnei* complex, which, in Africa, is represented by at least 16 species, is very closely related to species of the subgenus *Hoffmania*. They can occur in large numbers and must therefore be considered as potential vectors of rhabdoviruses.

Viral maintenance and transmission

Vector capacity

Vector capacity is defined as the average number of infective bites that will be delivered by a *Culicoides* midge feeding on a single host animal in one day, and is a combination of all of the following:

- midge density in relation to the host animal,
- host preference,
- midge biting frequency,
- vector competence (reviewed below), and
- life span of the infected midge and duration of infectivity.

The successful transmission of an insect-borne virus from an infected to a susceptible host is dependent upon the complex relationship that exists between the virus, its insect vector and the vertebrate host, with each being influenced by particular environmental conditions.[152] Four criteria determine the competence of a particular vector species:[6]

- the virus must be recoverable from field-collected arthropods whose abdomens are free of fresh blood,
- the ability of the arthropod to become systemically infected by feeding on a viraemic host or an artificial substitute must be demonstrated,
- similarly, its ability to transmit the infection biologically by bite must be demonstrated, and
- there should be field evidence confirming the association of infected arthropods with diseased vertebrates.

Vector competence

Vector competence is a measure of the number of midges that actually become infective after feeding on a viraemic host. This competence is dependent upon the genetic makeup of the vector midge and external environmental influences.[315, 342, 348]

The virus vector competency of a *Culicoides* sp. can be experimentally assessed by first allowing a number of midges to feed on a viraemic animal or, in the laboratory, on a blood-virus suspension through a membrane. The engorged midges are then kept alive for the extrinsic incubation period, i.e. the period between feeding on infected blood and the appearance of virus in the saliva of the midge and is in the order of one to two weeks. Following ingestion by a susceptible arthropod, most arboviruses infect and replicate in cells of the mesenteron before penetrating the basal lamina to be released into the haemolymph to set up more cycles of infection and replication. Further barriers to infection appear to exist at the levels of organs such as the ovaries and salivary glands. A virus must infect and replicate in the salivary glands before the extrinsic incubation period of the virus is successfully concluded and the arthropod is able to transmit the virus by bite. It is possible for arthropods to obtain an infection with great efficiency by ingestion of a viraemic blood meal, yet they may still fail to transmit virus because the infection has not spread to the salivary glands. Hence it is important in vector studies in the laboratory to distinguish between arthropod infection rates and transmission rates.[313] The ability of these midges to transmit virus is then assessed by allowing them to feed on susceptible animal hosts or on suitable substitutes.

After a series of failed experiments involving mosquitoes,[272] Du Toit in 1943 conducted the first successful *Culicoides* vector competence studies at the Onderstepoort Veterinary Institute.[115] He fed field-collected *Culicoides* on BTV-infected sheep, and after an extrinsic incubation period of 10 days, was able to transmit the disease to susceptible sheep, but he did not specifically identify the species of *Culicoides* used in his studies. It is most likely to have been *C. imicola* as it is by far the most abundant species collected around Onderstepoort. He is reported to have similarly infected a horse with African horse sickness virus (AHSV) by *Culicoides* bite.[334]

These seminal findings by Du Toit that *Culicoides* are involved in the transmission of orbiviruses were later confirmed elsewhere in the world, i.e. in North America, Australia, and the UK, and then again in South Africa.[329] The latter study involved *C. imicola*. In the laboratory,[329] *C. imicola* was fed, through latex and chicken skin membranes, on blood containing BTV serotypes 3 and 6 and AHSV serotype 1. After an incubation period of 10 days at 25 to 27 °C, the infection prevalences for *C. imicola* for BTV serotypes 3 and 6 were established at 31 and 24 per cent respectively, but no AHSV could be recovered.

In 1998, in the laboratory, a second species of the *Imicola*

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complex, C. bolitinos, was incriminated as a vector of BTV.[335] Significantly, for the three serotypes of BTV tested, higher infection prevalences, and higher virus titres per midge, were found in *C. bolitinos* than in *C. imicola*. In addition, it was shown that these higher infection prevalences and mean virus concentrations per midge in *C. bolitinos* were not influenced by the incubation period (2 to 20 days), nor by incubation temperature (10 to 30 °C) at which the midges were held.[281] These findings suggest that *C. bolitinos* may be a more competent vector of BTV than *C. imicola*. In a subsequent study[282, 334] three more species of *Culicoides* were shown to replicate BTV, and so broadens our understanding of the epidemiology of BT. In the same trial the workers were unable to demonstrate virus infection, after incubation, in 14 of the 22 *Culicoides* spp. examined.

Artificial feeding of 17 species of field-collected *Culicoides* on blood containing three serotypes of AHSV succeeded in infecting only *C. imicola* and *C. bolitinos*.[327] The findings suggest species-specific serotype dependence. It appears cogent that no AHSV replication could be demonstrated in any of the remaining 15 species assayed, but for most of them only low numbers of midges were available. It was shown also that of seven species (but not including *C. bolitinos*) fed artificially on blood infected with the Bryanston serotype of EEV, only *C. imicola* (22,3 per cent) became infected.[328]

Available data clearly indicate that considerable variation, in terms of susceptibility and prevalence of infection with different orbivirus species and serotypes, appears to exist amongst the various *Culicoides* spp. Species of the Imicola complex appear to be the *Culicoides* most susceptible to infection in the laboratory, and are therefore the likely vectors of these orbiviruses in the field. Other data, such as high levels of abundance, seasonal and geographic prevalence, and field isolations of virus, support this contention (see below).

Biosystematics of *Culicoides*

Most *Culicoides* have a wing pattern that is composed of grey and white spots that is unique to each species. Although this pattern may vary only subtly between species, and so complicates the delineation of intraspecific variation, it remains an extremely useful aid to the identification of the midge. The patterns can be fairly easily observed under a dissecting microscope; those of the 14 species of *Culicoides* most commonly found associated with livestock in Africa appear in **Figure 5.3**. As is evident in the figure, the species are quite easily separable on wing pattern but this is because they are representative of six subgenera. It is within subgenera that the patterns become more broadly similar, but what hampers rapid, easy identification is that subgenera can often comprise five or more species complexes. It is within complexes that identifications based on wing patterns alone are unreliable, even in the hands of the most experienced person. Furthermore, 10 per cent of African *Culicoides* spp., e.g. *C. ravus*, lack a wing pattern, and for reliable identification such species (indeed for all species in all complexes) must be dissected and mounted on glass slides and examined by light microscopy at 100 to 400 x magnification. In slide-mounted specimens identification of the female *Culicoides* to species level is based on the precise shape and number of the spermathecae (**Figure 5.4**), and the shape of the third palpal segment and the distribution of the sensillae on it (**Figure 5.5**), the conformation of the space between the eyes, and whether the chitinous areas between the ocelli are, or are not, adorned with hairs (**Figure 5.6**). Perhaps the most useful taxonomic aid for the identification of females to species level is the precise number and arrangement of each of the seven types of sensillae on the antenna (**Figure 5.7**), but unfortunately, in the majority of taxonomic studies published on *Culicoides* worldwide, these arrangements are not completely enumerated or described. This arrangement and number of sensillae on the antenna are also very useful for identification of the males to a species level but they are also a neglected source of taxonomic information (**Figure 5.8**). The shapes of the various parts of the male genitalia are, in addition, highly species-specific and are always used in identification (**Figures 5.9 and 5.10**). Unfortunately the preparation of such material on glass slides for taxonomic study requires extensive dissection, which is both laborious and time-consuming but is essential if the small differences that exist between species are to be discovered and used in descriptions. Whilst the more modern approaches, such as mtDNA extraction and RAPD-PCR, have recently been employed to separate species of the Imicola complex,[290] it is not foreseen that they will ultimately replace the cheaper and more rapid morphological method of identification. Rather, they will be harnessed to solve the more elusive issues surrounding the identification of particular species within specific vector complexes.

Taxonomy

Historical perspective and problems Nearly 100 years have passed since the first *Culicoides* was described from Africa and still no regional monograph has appeared that treats the Afrotropical fauna as a whole. There are, however, a number of taxonomic publications from South Africa,[74, 131] Angola,[62] Kenya[142, 181] and Nigeria.[37] There are also landmark studies that deal with significant parts of the fauna such as those by Carter, Ingram and Macfie,[65] Clastrier,[71, 73] Clastrier and Wirth,[74] Cornet,[80] Cornet and Chateau,[82] Cornet and Brunhes,[81] Cornet and Nevill,[83] Cornet, Nevill and Walker,[84] de Meillon,[100, 107] Itoua and Cornet,[171] Meiswinkel and Dyce,[224] Meiswinkel,[213–215, 217] and Nevill and Dyce.[269]

The taxonomy of South African *Culicoides* for the larval, pupal, and adult male and female stages is shown in the diagram in **Figure 5.11**. Only where the relevant areas are coloured in the diagram has the particular life stage been

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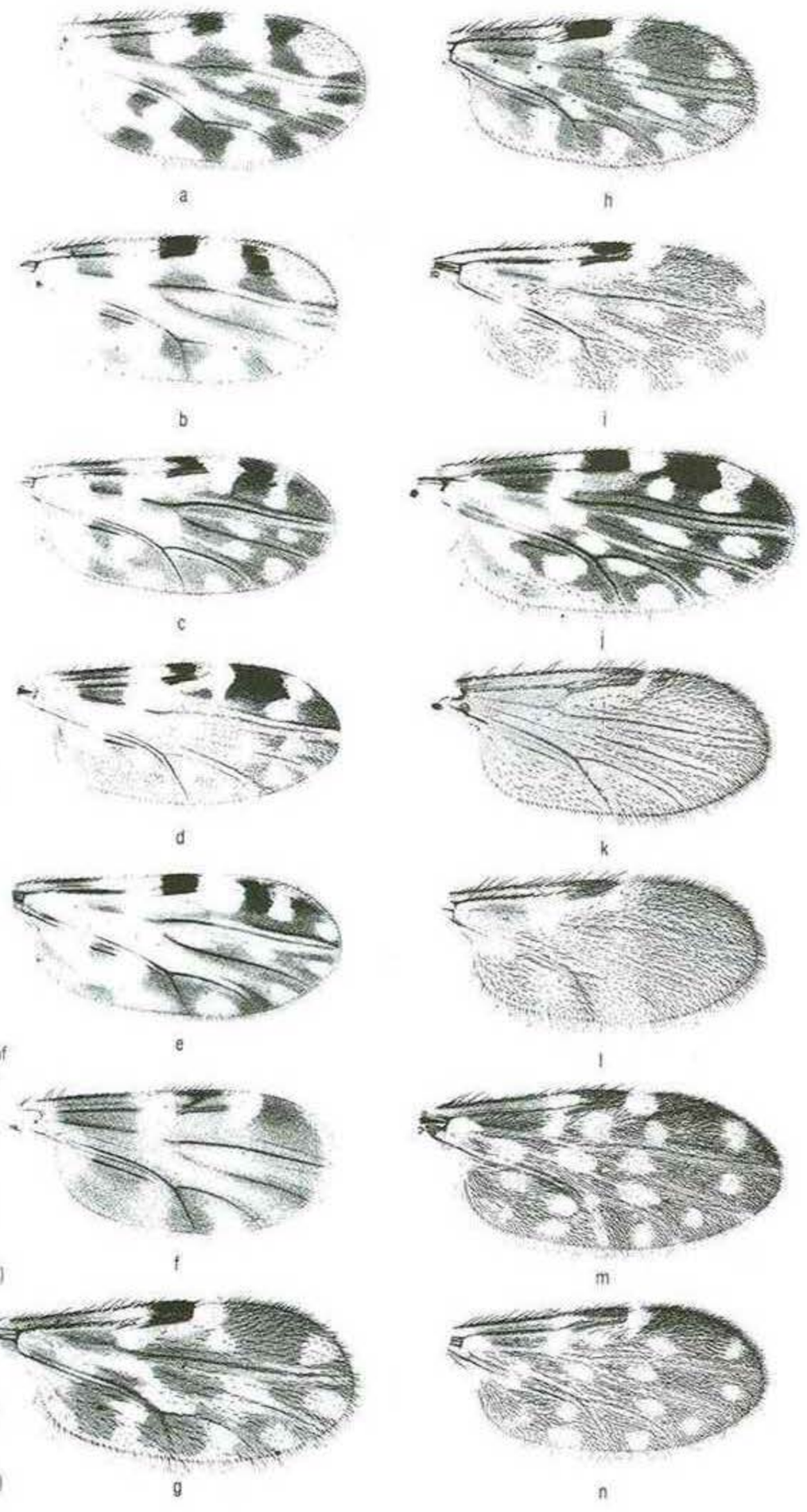


Figure 5.3 A wing of each of the 14 species of *Culicoides* most commonly found associated with livestock in southern Africa

a = *C. imicola* (Zimb. 5)
b = *C. bolitinos* (Afr. OP32)
c = *C. zuluensis* (OP69)
d = *C. magnus* (Zimb. 11)
e = *C. gulbenkiani* (Afr. OP37)
f = *C. fulvithorax* (Zimb. 8)
g = *C. similis* (Afr. OP34)
h = *C. tropicalis* (S.Afr. 6)
i = *C. neavei* (OP17)
j = *C. schultzei* (Afr. OP27)
k = *C. engubandei* (Zimb. 14)
l = *C. bedfordi* (S.Afr. 21)
m = *C. pycnostictus* (Afr. 9)
n = *C. leucostictus* (S.Afr. 10)

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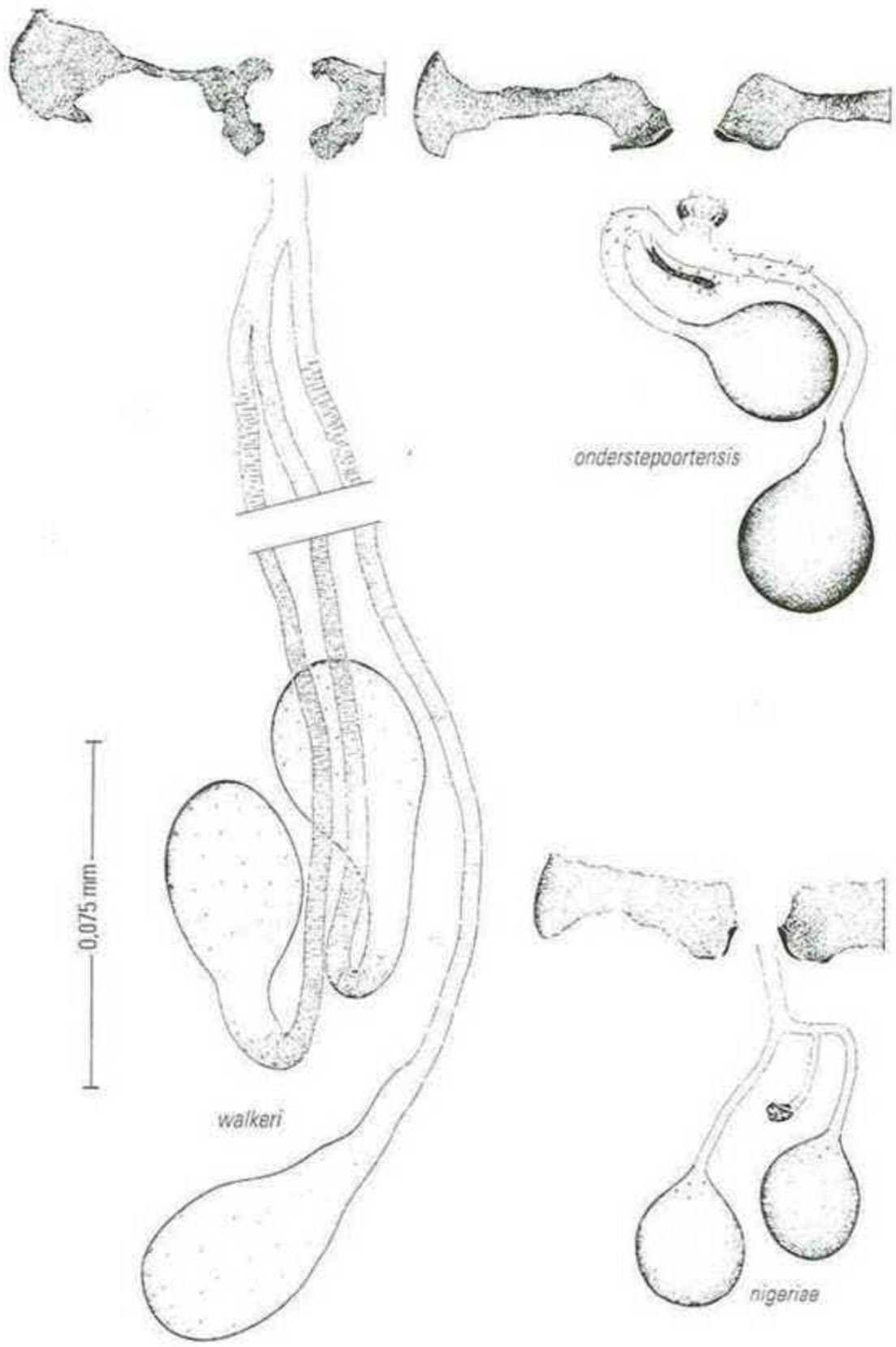


Figure 5.4 The spermathecae of the females of three South African species of *Culicoides*.

described and published. Yet undescribed species are numbered in the diagram; they comprise a third of the 112 species collected over the last 20 years in South Africa. The diagram shows too that the immature stages of at least 70 per cent of the species still await discovery and description. Annually, two or three species are added to this list,[216] and if this is representative of the situation in the rest of Africa, it illustrates the amount of taxonomic research still to be done on Afrotropical *Culicoides*.

The first Afrotropical species described were *C. schultzei* (**Figure 5.3j**) and *C. herero* from Namibia in 1908;[122] by 1925 nearly a third of the 156 Afrotropical species recognized today had been described. An unavoidable result of the disparate collecting by early French, German and English workers is that the holotypes of many of their species are today either lost or are scattered amongst a number of European museums or research institutions; of the holotypes that are extant most are in a poor state of preservation. Exacerbating matters for practising systematists today is that most early descriptions were too brief, were based on a single specimen (usually female), and were often not accompanied by illustrations. This means that many character traits, crucial to identification and assignation of a species to the correct subgenus or species complex, are mostly lacking from these early descriptions.

One of the most prolific students of African *Culicoides* was

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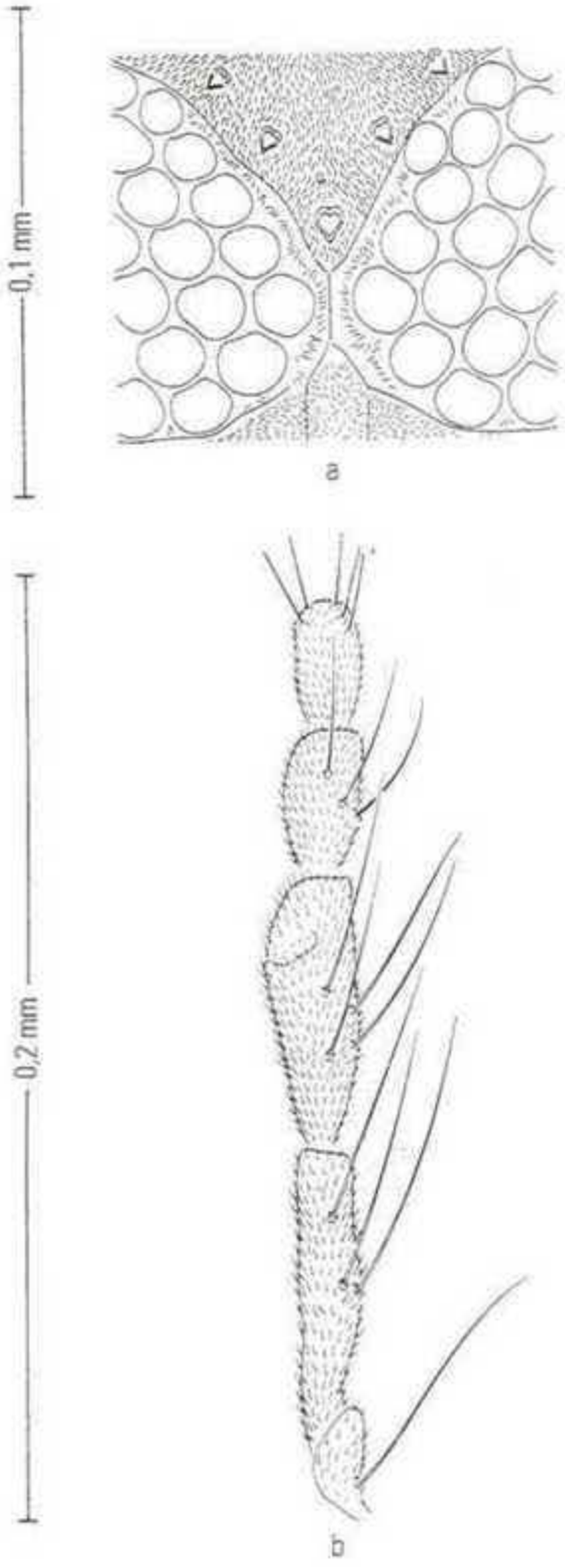


Figure 5.5 The eye-space (a) and five-segmented palp (b) of a female *Culicoides imicola*. The third segment bears a sensory pit

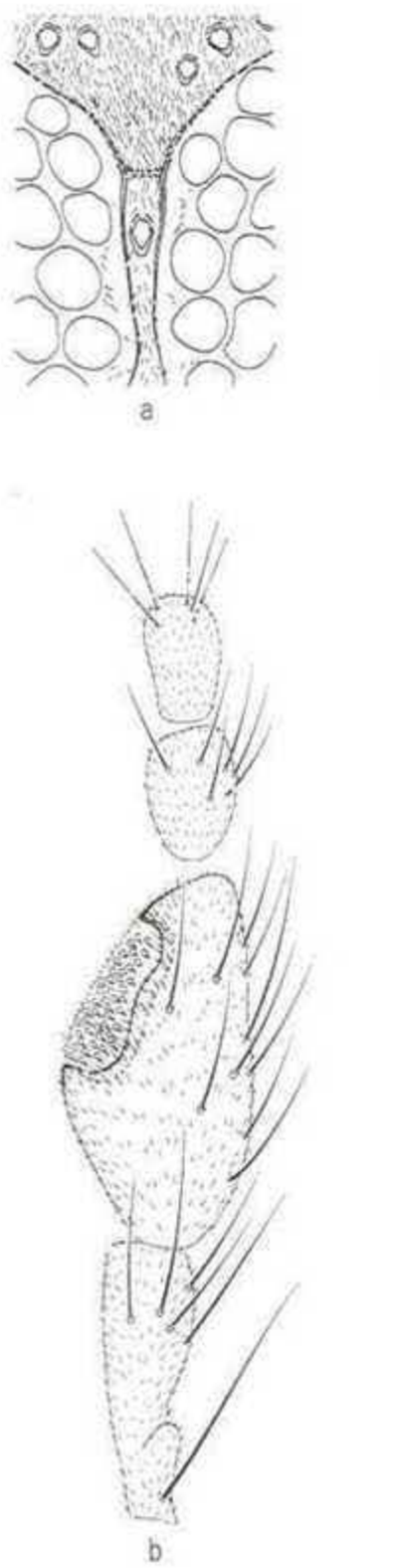


Figure 5.6 The eye-space (a) and five-segmented palp (b) of a female *Culicoides ravus*. The third segment bears a sensory pit

the French abbé, J.J. Kieffer, who described 20 species between 1911 and 1921 from diverse localities that included the Seychelles, Kenya, the Cameroon (most species), Sudan and South Africa. To a great extent the research of Kieffer[182–185] has remained esoteric. For example, 14 of the 20 *Culicoides* names made available by him are not in use today even though the species he described are probably common. His 'floating' species are *albosparsus, bisignatus, dentatus, guineensis, kribiensis, nilogenus, nilophilus, octosignatus, quadrisignatus, remotus, signatus, silvestrii, trisignatus* and *xanthogaster*. Indeed, the names of a further three species, *tropicalis, imicola* and *alticola*, had suffered a similar fate, but were resurrected when their respective holotypes were discovered in the Paris Museum 60 years later.[191]

Contemporaneously (1919 to 1925, 1947), 21 species of *Culicoides* were described by Carter,[64] Carter, Ingram and Macfie,[65] Ingram and Macfie,[167–169] and Macfie.[207, 208] Most of their material emanated from Ghana but also included new species from the Sudan and Malawi. The taxonomic studies of Carter, Ingram and Macfie were of a high quality and, importantly, included the first descriptions of *Culicoides* larval and pupal stages, and their habitats. Influenced by Ingram, De Meillon (1936 and 1937)[100, 101] commenced the first biological studies on South African *Culicoides* that culminated in the description of new species and their pupae, and the

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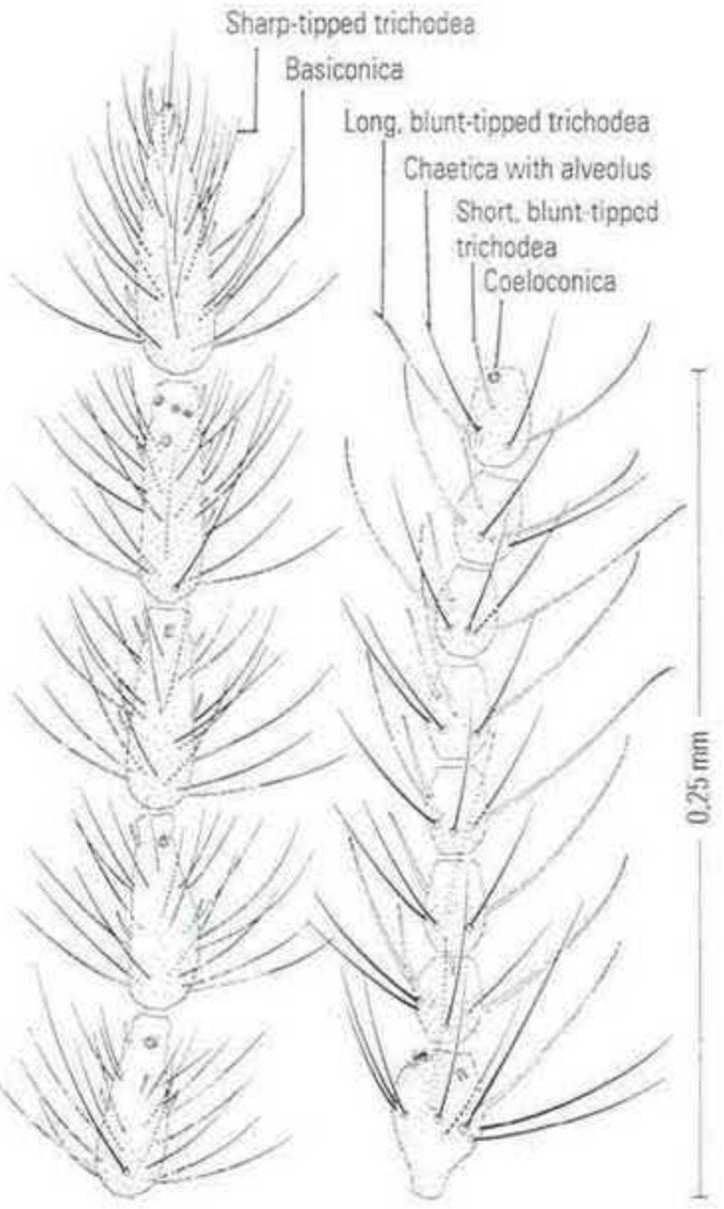


Figure 5.7 The various sensillae that occur on the distal 13 flagellar segments ('antennomeres') of a female *Culicoides*

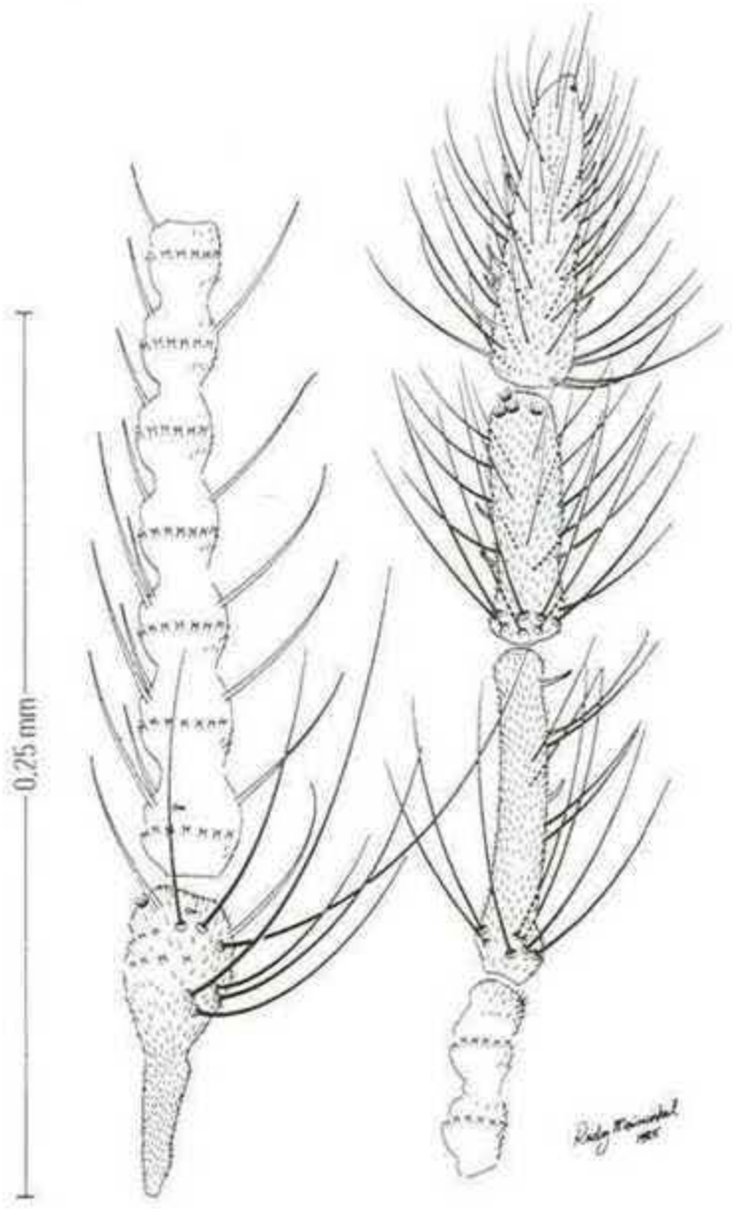
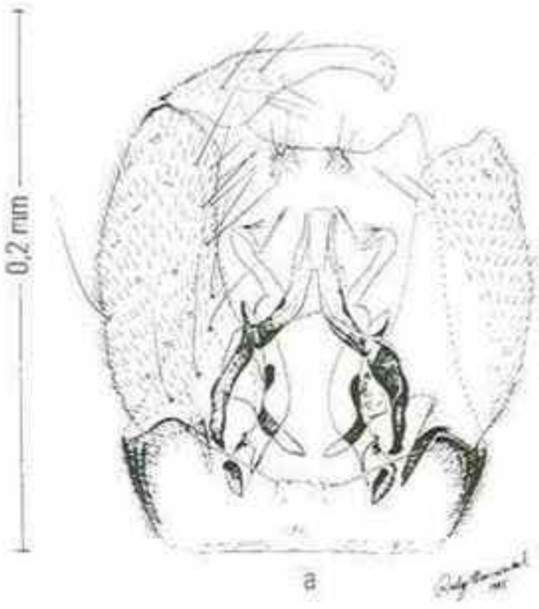


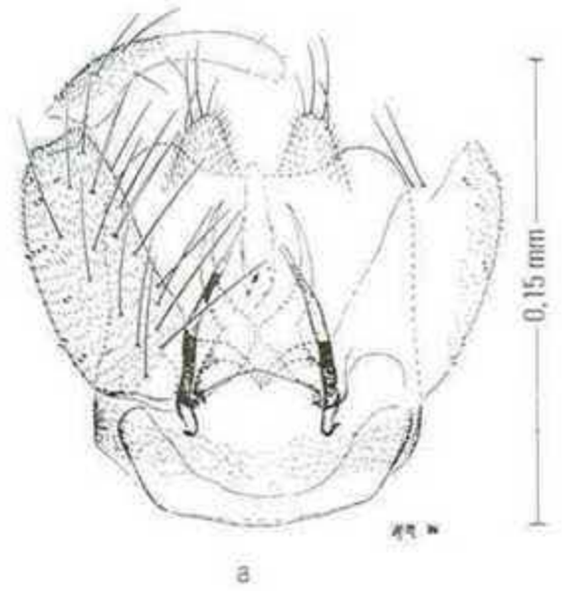
Figure 5.8 The various sensillae that occur on the distal 13 flagellar segments of a male *Culicoides*

first observations on breeding sites. Unlike the situation with Kieffer, many of the Carter, Ingram and Macfie species names, such as *similis, accraensis, brucei, pycnostictus, punctithorax, nigeriae* and *bedfordi*, are in use today. However, it must be noted that, like some of those of Kieffer, a number of their species are also both rarely recorded and poorly understood. These include *adersi, austeni, ochrothorax, eriodendroni, inornatipennis, nigripennis, citroneus, clarkei, confusus, rutilus, arenarius, corsoni* and *lamborni*. One explanation is that the countries from which most species were originally described have since only been rarely sampled for *Culicoides*. More cogent is that these authors collected mostly in the high-forested tropics, an area harbouring only a particular subset of the Afrotropical *Culicoides* fauna, and one that by all accounts appears to be relatively depauperate. It is pertinent that Kieffer and Carter, and Ingram and Macfie described most of their species from the equatorial coastal towns of Kribi (Cameroons) and Accra (Ghana) respectively. It is thus likely that these authors, who seldom referred to each other's work, have to some extent described the same species. An obvious and significant example is *C. pallidipennis*, which was subsequently shown to be a synonym of *C. imicola* Kieffer, 1913.

Besides the possibility of further synonyms the most vexing taxonomic problem to remain is that most of the species mentioned above represent species complexes, the most notable being the Accraensis, Grahamii and Nigripennis complexes. Each complex is represented by 10 or more species that are rarely collected and are very difficult to identify. One consequence is that precise delineation of the nominate species of each complex eludes us to this day, which means that the description of new members of these complexes are either held in abeyance or are being published as *C.* sp. # A, B, etc.[37] or are given numbers.[142, 223] Ultimately these systematic problems require resolution as each species within a complex fills a niche that should be defined as a first step towards establishing whether it includes the transmission of pathogens harmful to livestock. The range of niches is almost infinite and includes differences in geographic distribution, seasonal prevalence and abundance, host preferences and vector capacity. Evolutionary radiation into a particular subset of ecological conditions is

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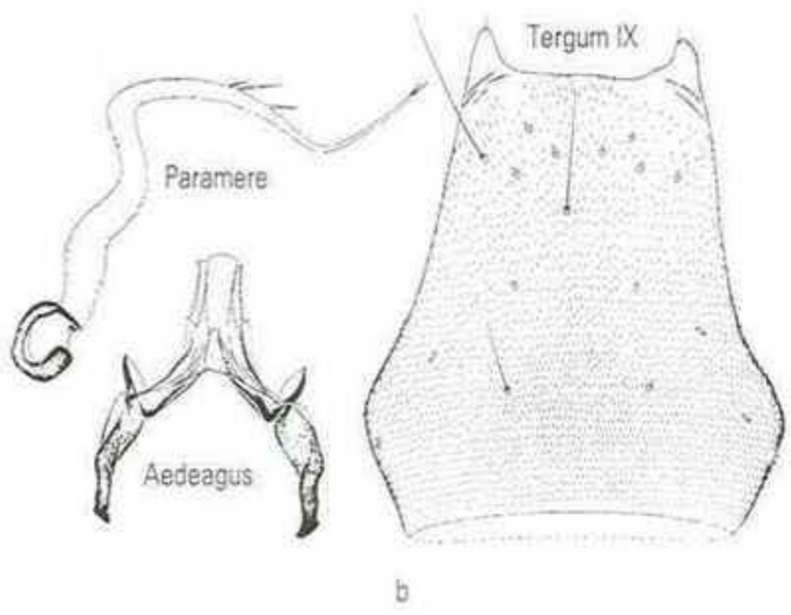


Figure 5.9 The entire male genitalia (a) and tergum IX, aedeagus and paramere (b) of *Culicoides onderstepoortensis*

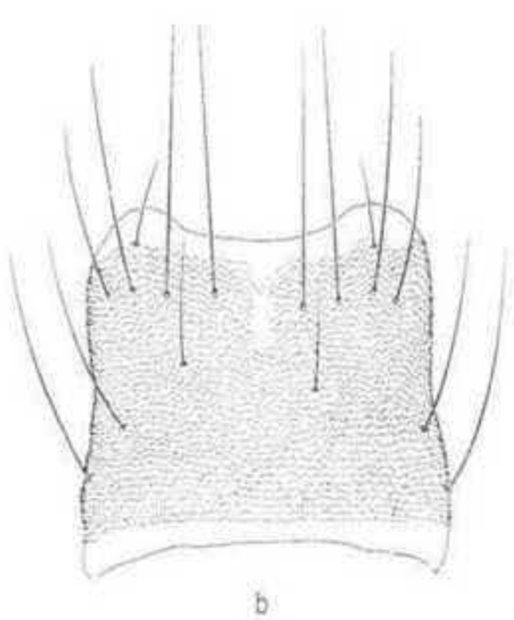


Figure 5.10 The entire male genitalia (a) and tergum IX (b) of *Culicoides imicola*

a hallmark of species comprising the recently discovered Imicola complex, and because this complex contains the most important transmitters of orbiviruses known today in Africa and beyond, it is discussed below in greater detail with special emphasis on the two vector species *C. imicola* and *C. bolitinos*.

Vector species

Of the more than 1 210 species of *Culicoides* that have been described, about 70 belong in the subgenus *Avaritia*. At least 30 species of this subgenus occur in Africa; of these nearly half remain undescribed and so would bring the world total to 85. Worldwide a mere 10 species of *Culicoides* are involved in the transmission of orbiviral diseases that affect livestock; fully half of these belong in *Avaritia*. The most important vectors in Africa are *Avaritia* spp., namely *C. imicola* and *C. bolitinos*. Both transmit BT and AHS (**Table 5.2**),[115] and are the suspected vectors of EE, but less is known about their possible involvement in the transmission of Akabane and BEF. Bluetongue virus has also been isolated from *C. gulbenkiani*, another species of *Avaritia*, which, like *C. bolitinos*, breeds in the dung of cattle and is therefore closely associated with livestock. However, it probably plays only a minor role as it seldom becomes abundant and is restricted in its geographic distribution.

In addition to the three livestock-associated species mentioned above, there are seven species of *Avaritia* particularly closely associated with larger African herbivores such as the elephant, both rhinoceros species (*Ceratotherium simum* and *Diceros bicornis*), buffalo, blue wildebeest and the plains zebra (*Equus burchellii*). Not only do these *Avaritia* spp. feed on these hosts but also use their dung as a larval habitat.[41, 118, 212, 213, 223, 261, 267] In the case of *C. bolitinos*, it has broadened its resource range to now use not only buffalo but also cattle dung as a breeding medium. This has enabled it to maintain a widespread prevalence in Africa.

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which is in contrast to the elephant-associated *C. loxodontis*, which remains restricted in its distribution presumably due to its inability to invade other dung types. With a burgeoning game farming industry in southern Africa, the translocation of wild herbivores can be accompanied by the establishment of pathogens to livestock in hitherto 'clean' farming areas. For example, zebra exported from Namibia were indubitably linked to the outbreaks of AHS that occurred in Spain between 1987 and 1991, in which several hundred horses died or were destroyed as a result.[288] In this instance *C. imicola* played the principal vectorial role.[233] Subsequently, it was confirmed in South Africa that cycles of AHSV occur in zebra and that this can proceed in the driest of environments when *Culicoides* populations are depressed.[13, 217] There is also evidence that where new populations of wildlife are established on game farms, the associated *Culicoides* spp. will also become established in the long term, and in this way new vector foci are created.[216]

It may not be circumspect to single out species of the subgenus *Avaritia* as the sole vectors of orbiviruses in Africa as potential candidates from other subgenera have been discussed and listed in the literature.[330] As noted above there have been sporadic isolations of viruses from a number of other species (**Table 5.2**). In addition, in laboratory infection studies,[335] evidence has emerged that more than two species of *Culicoides* may be capable of replicating BTV; this is the first indication that there may be other vectors of greater or lesser importance in the field. One of the strongest candidates is *C. magnus*, which is a member of the subgenus *Culicoides* and which can at times become quite abundant in the cooler central and southern regions of South Africa. It has been recorded widely from the continent, and is closely related to the European Pulicaris complex, which is also suspected to play a role in the transmission of orbiviruses.[233]

Below is summarized what is known about *C. imicola* and *C. bolitinos* as regards their taxonomy, geographic distribution and seasonality, vectorial competence, host preferences, gonotrophic cycle, dispersal, and association with outbreaks of disease. In **Table 5.3** the broadscale larval habitat differences between these two vector species are compared, and the resultant impact on adult distribution, prevalence and abundance indicated.

Culicoides imicola Kieffer, 1913 (Figure 5.3a)

Taxonomy As a taxon, *C. imicola* is most closely related to *C. nudipalpis* Delfinado, 1961, the latter known only from south-east Asian islands east of the Wallace line. *Culicoides imicola* is a member of the Imicola complex, which comprises 10 species, all restricted to the Old World; eight of these species occur in Africa. Detailed studies have done much towards clarifying their taxonomy, biology and distribution.[213–215, 220] The complex was reviewed *in toto* in 1995.[217] The taxonomy of *C. imicola* in the Mediterranean region was comprehensively reviewed in 1991 by Boorman,[36] who noted only one difference between Mediterranean and South African populations. This difference was due to an error that appeared in a publication of Meiswinkel,[213] and so confirms that *C. imicola sensu stricto* is morphologically homogeneous across its large geographic range; this homogenicity being supported subsequently.[220] The independent species status of the various Imicola complex taxa *sensu* Meiswinkel[217] was recently validated by RAPD–PCR analyses;[289] DNA (RAPD) patterns for seven species of the complex are shown in **Figure 5.12**. In **Figure 5.13** an unrooted neighbour-joining consensus tree shows the relationship and discrete clustering of 19 populations of these seven species collected in South Africa, Botswana, Madagascar and the Ivory Coast. Until 1972 studies on the taxonomy and biology of *C. imicola sensu stricto* appeared under a variety of names that include *C. pallidipennis*, *C. iraqensis*, *C. minutus* and *C. pseudoturgidus*.

Vector competence Incriminated in 1944[115] as a vector for BT and AHS, *C. imicola* is still regarded as the most important Old World vector of these livestock diseases. It is also considered a potential vector for EE.[328] Depending on the environmental temperature, the extrinsic incubation period of BTV in *C. imicola* is 7 to 12 days.[50, 115] More detailed information on serotype-specific infection rates and virogenesis is given above in the section on 'Vector competence'.

Host preferences *Culicoides imicola* has been found to feed on cattle, sheep, horses, pigs, goats and poultry.[42, 45, 52, 57, 163, 265, 267, 338, 339] In Israel it is inclined to bite cattle and horses along the backline; in cattle it prefers feeding in the darker coloured areas of the animal.[43] There are indications that *C. imicola* is somewhat exophilic in its feeding habits showing some reluctance to enter buildings[221] but these require validation in situations where very large populations abound. As regards wildlife, eight females have been collected off a darted African buffalo.[213]

Gonotrophic cycle It has been estimated[338] that, with a gonotrophic cycle of four days, *C. imicola* females might take five blood meals during their lifetimes; it was similarly calculated subsequently[50] that the mean period between blood meals was 3.3 to 4.6 days. In Israel[49] *C. imicola* was found to complete 11 generations per annum with each generation being approximately 23 days in duration. It is generally accepted that under warmer environmental conditions the life cycle is shortened, and thus there is a greater number of generations and adults produced per annum.

Larval habitat Using emergence traps, hundreds of *C. imicola* were first reared at the Onderstepoort Veterinary Institute in 1950 by du Toit and Fiedler[260] in a swampy kikuyu grass (*Pennisetum clandistenum*)-covered area next to a leaking cement dam. Nevill[260] was the first to show that the pupae are susceptible to drowning as they are unable to

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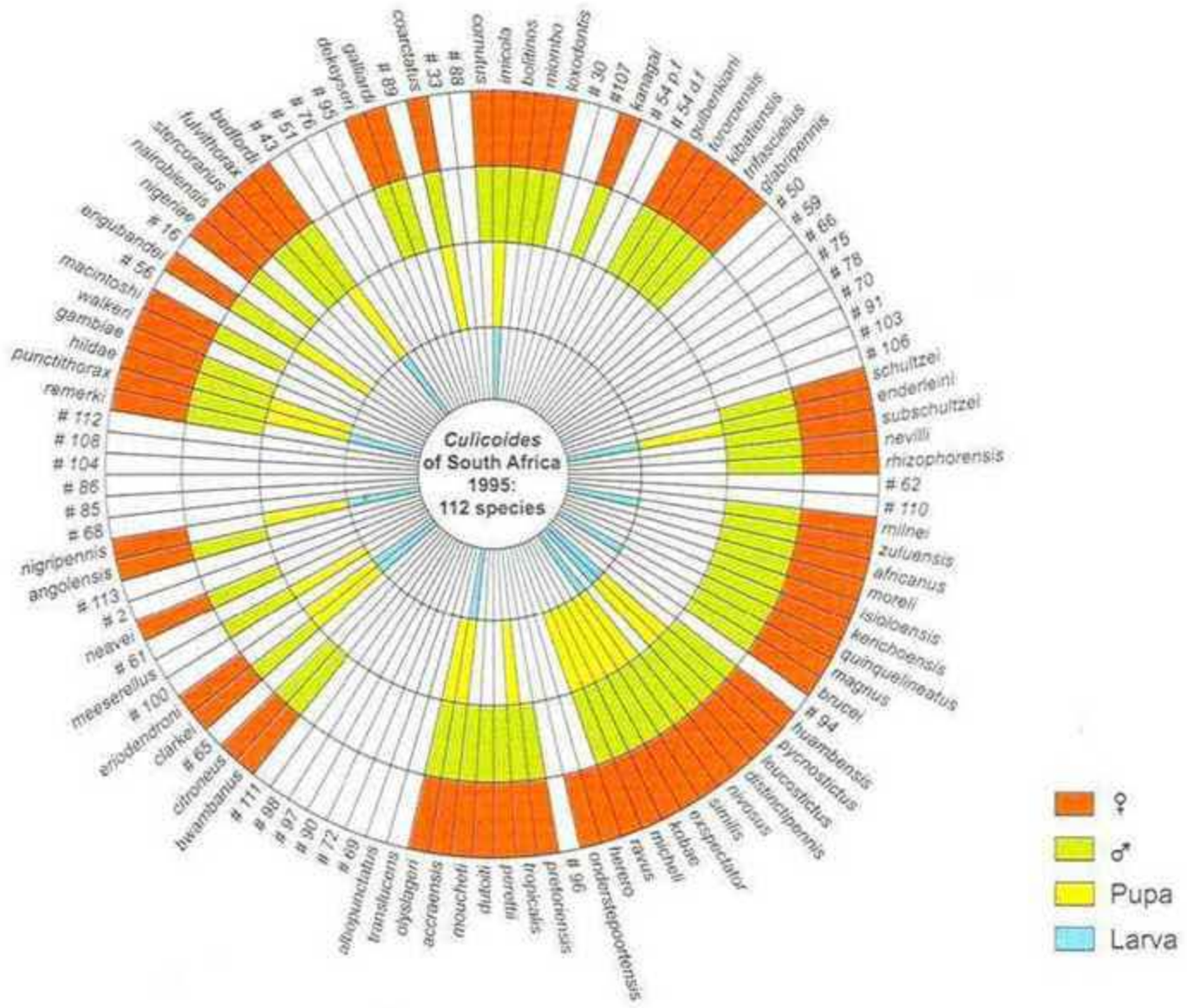


Figure 5.11 A diagram of the taxonomy of South African *Culicoides* for the larval, pupal, and adult male and female stages

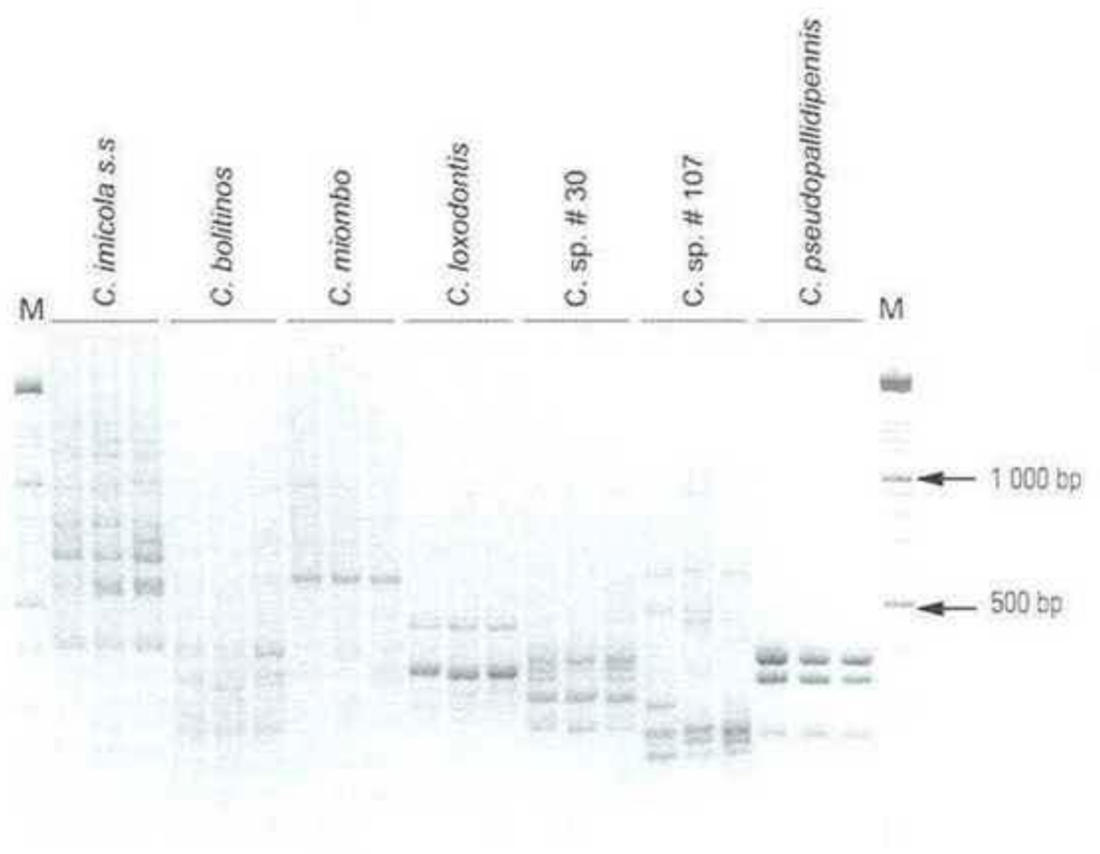


Figure 5.12 Representative individual random amplified polymorphic DNA (RAPD) patterns for the seven morphological species of the *Culicoides imicola* complex, using the primer D17: *C. imicola* ss from Onderstepoort, *C. bolitinos* from Rhinocerosfontein, *C. miombo* from Maun, *C. loxodontis* from Phalaborwa, *C.* sp. #30 from Port Edward, *C.* sp. #107 from Mabula Lodge, and *C. pseudopallidipennis* from Abokouamekro, Ivory Coast

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Table 5.3 Broad-scale larval habitat differences between *Culicoides imicola* and *C. bolitinos*, and resultant impact on adult distribution, prevalence and abundance[216]

CULICOIDES IMICOLA	*CULICOIDES BOLITINOS*
IMMATURE STAGES – Inhabit moist soil that are organically enriched but not waterlogged; predominate in moisture-retentive clayey soils which may be unvegetated or covered in short grass as in pastures – Absent from quick-draining, micronutrient-poor, sandy coastal soils – Upper layer of soils must remain moist for a minimum of seven days to sustain larvae through four moults; this restricts the species, at any altitude, to flat, low-lying, slow-draining areas – Numbers drop dramatically in sloping terrain likely due to water run-off and leaching of soils – During episodic periods of above-average precipitation *C. imicola* will disperse and increase in numbers as moist soils become more widely available – Restricted to 300 to 700 mm rainfall isohyets as soils become leached of nutrients at higher precipitation levels	IMMATURE STAGES – Inhabit exclusively the dung of herbivores including cattle, African buffalo and the blue wildebeest – Following deposition of eggs by adults in fresh dung the larvae require 7 to 10 days to reach maturity – Will be found in any terrain in which the above herbivores may occur, irrespective of degrees of landscape slope – Achieve great abundance in areas where dung is deposited on sandy soils – The larvae, being coprophilic, are independent of soil moisture and quality; can thus occur in both flat and sloping terrain – Average annual precipitation does not appear to affect its geographic distribution
ADULTS – Most widespread and abundant *Culicoides* species in South Africa; feeds on a wide variety of domesticated livestock – Show large variation in abundance; 200-fold increase in years of above-average rainfall – Largest single light trap collection recorded: > 1 000 000 – Widespread in sub-Saharan Africa, and on offshore islands; also occurs in North Africa, and thence up to the 43rd parallel in European countries bordering the Mediterranean Sea; occurs also in the Near, Middle and Far East up to southern China and Vietnam	ADULTS – Almost as widespread; on average one order of magnitude less abundant than *C. imicola* and thus very rarely found in co-dominance – Like *C. imicola* are catholic in their choice of hosts – Adult abundance variable but proportional to the amount of dung available, in turn dictated by animal biomass/unit area – Largest single light trap collection recorded: > 3 000 – Currently understood to be restricted to sub-Saharan Africa; recorded from only eight continental countries (South Africa, Lesotho, Zimbabwe, Malawi, Kenya, Nigeria, Ivory Coast and Gambia), and from the islands of Mauritius and Madagascar

float on the surface of water as do many *Culicoides* spp. pupae. The requirement for a semi-moist larval habitat by *C. imicola* appears to be a characteristic shared by all species of the subgenus *Avaritia* and, for *C. imicola*, was partly confirmed in Israel[47] where it was reared in rich mixtures of organic matter and water-saturated soils, but despite this it was also the species adapted to the driest breeding conditions. The breeding habitat of *C. imicola* in Israel has been summarized as being 'in and around animal pens in water trough overflow, at the margins of animal sewage and drainage canals and puddles created by leakage from water pipes.'[33] In Kenya[340] high concentrations of larvae were found in mud and dung mixtures surrounding cattle pens and in associated effluent ditches. In Cyprus[236] *C. imicola* was found breeding where there were leaks from irrigation pipes. These were small seepages with little free surface water. They were generally drier than the type of habitat preferred by other species of *Culicoides* and were usually covered by a growth of fresh grass. There was almost no contamination by animal droppings, but organic matter was present in the soil in the form of decaying vegetation. In Laos[163] it was reared in mud mixed with dung. In an unpublished study conducted at the Onderstepoort Veterinary Institute up to 382 *C. imicola*/m^2 were reared in islands of short kikuyu grass in the middle of effluent-enriched drainage canals.[275] They were, in addition, reared throughout the year with their numbers peaking in late summer and autumn precisely when the numbers of adult *C. imicola* are at their highest, and when diseases such as BT and AHS are most prevalent. In another study[267] low numbers of *C. imicola* were reared in dry as well as in wet kikuyu paddocks especially where the soil was clay-like and medium moist. Midges were also reared in continuously moist areas where the substrate consisted of sandy soil covered by vegetation and a layer of organic matter, and where the moist but silty soil of irrigated pastures had very little surface organic matter except for some rotting plant particles and/or diluted manure. It is now fairly well established that *C. imicola* is most abundantly encountered where the soils are clayey, such as the black cotton soils at the Onderstepoort Veterinary Institute and those of the Laikipia plateau and the Masai Mara in Kenya, but it is virtually absent where the soils are overly sandy.[222] It is postulated that sandy soils, in draining too quickly of moisture and being nutrient-poor (especially those found along the coast), are simply unable to sustain the larval stages of *C. imicola* for the required 7- to 10-day developmental period.[218, 219] The proposed relationships between soil type, prevalence and abundance of *C. imicola*, and the prevalence of AHS during the 1996 South African outbreak of the disease is illustrated graphically in **Figure 5.14**. It is probable that the rearing records[74, 161] of *C. imicola*, documented under the name *C. pallidipennis*, from rotting banana and plantain stems do not refer to *C. imicola*.

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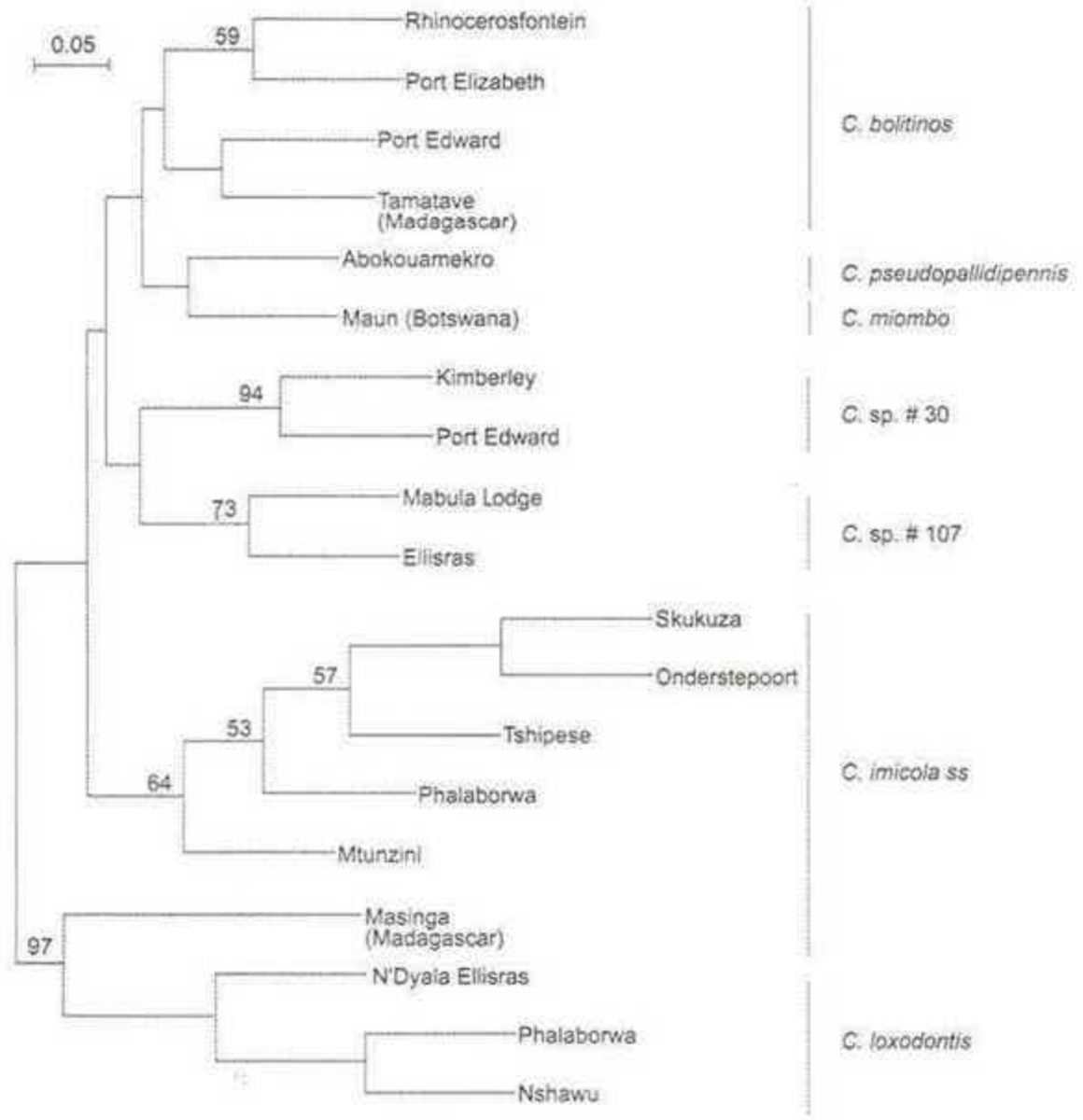


Figure 5.13 Unrooted neighbour-joining (NJ) consensus tree of 19 populations of the seven morphological species of the *Culicoides imicola* complex, based on the dissimilarity values inferred from 196 RAPD fragments, amplified using four arbitrary primers. Only bootstrap values greater than 50 per cent obtained from 100 bootstrap resamplings of the original dataset are shown

Immature stages Contrary to a published statement[142] and the questioning of their possible identity,[36] the descriptions[260, 262] of the larva and pupa of *C. pallidipennis* are indeed those of *C. imicola* and not of *C. bolitinos*. A study of the pupae of five taxa of the Imicola complex has revealed that they differ markedly in morphology from one another.[268]

Geographic distribution *Culicoides imicola* was originally described from a single female specimen collected at Tiwi on the southern coast of Kenya. Today, *C. imicola* is known to be one of the most widely distributed *Culicoides* in the world and is found in at least 35 countries throughout Africa, in most countries surrounding the Mediterranean Sea, and in the Near, Middle and Far East as far as southern China, Laos and Vietnam. Only in South Africa, Morocco and the Iberian Peninsula has its distribution been mapped in some detail.[274, 286, 332]

Seasonal abundance and prevalence Only in South Africa, Morocco and Spain has the abundance and prevalence of *C. imicola* been studied and mapped on a national scale.[20, 23, 332, 333] On a smaller, but more intensive scale are the area-based studies in South Africa, of the Western Cape Province,[267] eastern Free State Province,[330] the Kruger National Park and adjoining areas,[217] the greater Onderstepoort area in Gauteng Province[331] and the Port Elizabeth area in the Eastern Cape Province,[218] and in Lesotho.[336] In addition, its abundance and prevalence were detailed during an outbreak of AHS in the central and eastern regions of South Africa.[219] These studies all took into account the existence of sibling species and in this way do not conflate data from various Imicola complex taxa. They also demonstrate the extreme variability in the prevalence and abundance of *C. imicola* in the vicinity of livestock ranging from it being totally absent[218] to widespread and superabundant.[219] In each study an attempt was made to explain this variation in terms of climatic or edaphic factors. It is emerging from these studies that other than extreme cold and aridity, the degree of topographic slope (inducing water run-off), soil type (whether drainage is slow or rapid) and soil fertility

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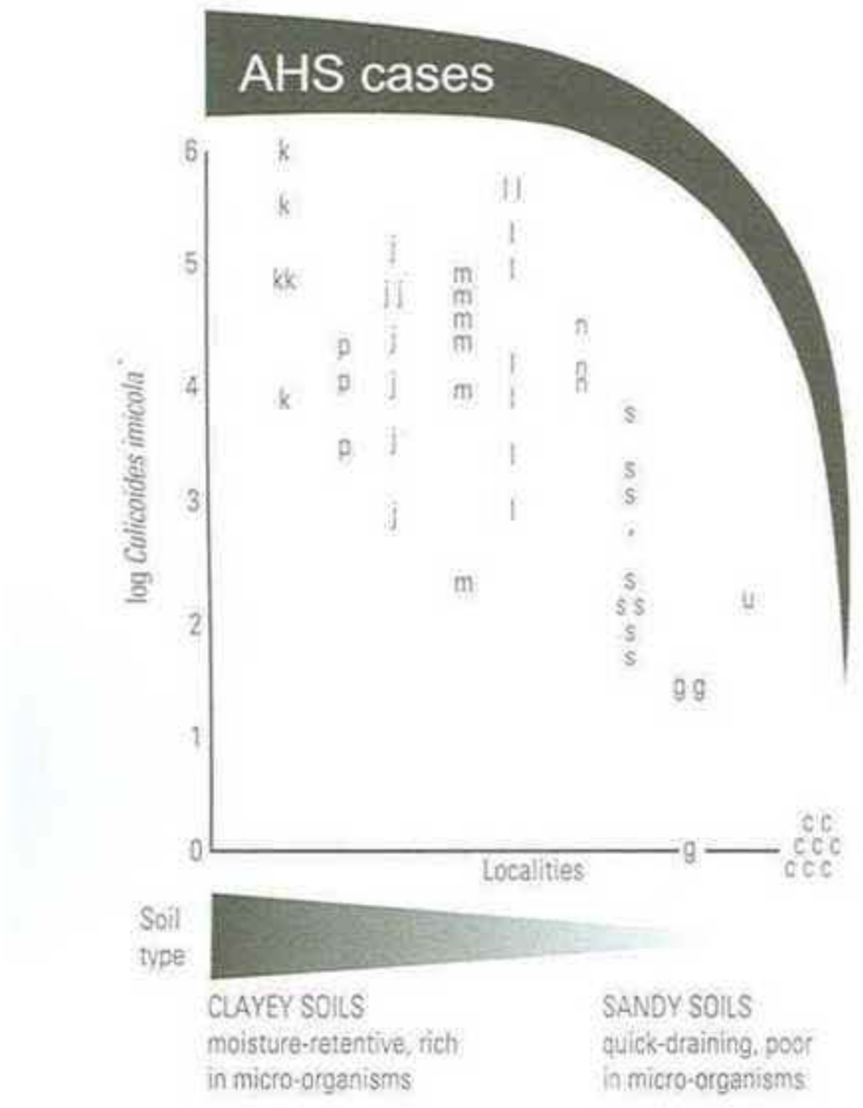


Figure 5.14 A schematic representation of the proposed relationships between soil type, numbers of *Culicoides imicola* and the number of cases of AHS during the 1996 outbreak in South Africa based on 52 insect collections at 47 sites. Sites were allocated to ten region-groups which, moving from left to right, show a decrease in AHS cases and an increase in soil sand content. Region-groups are: Kaalplaas farm, Onderstepoort Veterinary Institute (k); Pretoria (p); Johannesburg (j); south-central Mpumalanga (m); eastern Mpumalanga lowveld (l); KwaZulu-Natal (n); Free State and northern Eastern Cape (s); Graaff-Reinet area of Eastern Cape (g); Uitenhage, 30 km inland from the southern coast (u); and south-eastern Cape coast (c)

(availability of micro-organisms) are additional important factors affecting the distribution and abundance of this species. As to its detailed seasonal prevalence at various sites throughout South Africa reference should be made to two studies[332, 333] whose data were used in conjunction with various climate variables and satellite imagery, to model the prevalence and abundance of *C. imicola*.[21] Whilst the model explained 67 per cent of the observed variances it also shows some anomalies which are probably the result of too heavy reliance on the effects of rain and temperature. This was further demonstrated in an unpublished preliminary African model in which *C. imicola* was predicted to be most abundantly encountered in equatorial Africa, i.e. precisely where it is known that *C. imicola* is virtually absent.[171, 214] In a separate section (see below) the more precise seasonal and geographic distribution of *C. imicola* and of *C. bolitinos*, as revealed by weekly collections made over a full year at 40 sites spread throughout South Africa, are discussed in greater detail, and the various implications of the findings assessed. At the periphery of its range in the Middle and the Far East the distribution and abundance of *C. imicola* are mostly unknown.

Dispersal Under normal conditions the flight range of *C. imicola* probably varies in the range of a few hundred metres to a few kilometres. Clearly this is dependent upon the local availability of hosts for feeding and larval substrates for breeding. It is generally accepted that *Culicoides* are reluctant to fly if the wind is above a certain speed, which is when numbers caught in light traps diminish, but it is apparent too that light traps become increasingly inefficient as wind-speeds increase and do not then accurately reflect biting midge activity rates.[221, 319] There is compelling evidence that *C. imicola* can be transported upon prevailing winds for hundreds of kilometres. This is considered the most likely way in which outbreaks of BT are being precipitated in countries around the Mediterranean Sea,[290] but it is difficult to prove as the clandestine movement of infected animals also merits consideration. Similarly, can the occurrence of *C. imicola* on islands off the African east and west coasts (Madagascar, Reunion, Mauritius and the Cape Verde) be explained by wind transport or were they moved together with livestock? Nevertheless, the evidence to unequivocally support wind dispersal remains largely unsubstantiated as the capture of large numbers of *Culicoides* at various altitudes over sea or land has yet to be made. Another, as yet unsubstantiated, mode of dispersal for *C. imicola*, is the postulation that *Culicoides* may be moved over long distances in enclosed horse transport floats.[219]

Association with outbreaks of disease Since Du Toit's incrimination of *C. imicola* as a vector of both BT and AHS in 1944, it is remarkable how few attempts have been made to isolate one or the other virus from this species during field outbreaks of these diseases. The various isolations made from *C. imicola* (**Table 5.2**) were invariably the result of random surveys for *Culicoides*-associated orbiviruses and were often not linked to an outbreak of a disease. A number of these isolations cannot be linked to a specific species but emanated rather from mixed *Culicoides* spp. pools. As far as can be established, the first isolations of AHSV from identified pools of *C. imicola* not containing freshly blood-engorged females were made during the 1987 to 1991 outbreak of the disease in Spain.[233] Four serotypes of BTV were isolated from *C. imicola* in Israel[44] before these dates. In Africa it is equally remarkable to note that only in 1996 was the first investigation made to establish which species of *Culicoides* (**Table 5.4**) were most abundant at stables in which losses during an extensive outbreak of AHS were occurring.[219] It was clear that *C. imicola* was the most abundant and prevalent species and thus, for the first time in Africa, it

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Table 5.4 Prevalence and ranked abundance of the 13 *Culicoides* species most commonly encountered in 66 light trap collections made at 47 sites during the 1996 outbreak of African horse sickness in South Africa.[219] The last column is the abundance ranking of these species according to an earlier study[332]

ABUNDANCE RANK[219]	*CULICOIDES* SP.	% OF ALL *CULICOIDES*	PREVALENCE IN 66 LIGHT TRAPS	ABUNDANCE RANK[332]
1	*C. imicola*	94,2	60	1
2	*C. zuluensis*	3,0	44	5
3	*C. magnus*	0,7	25	10
4	*C. bolitinos*	0,6	34	15
5	*C. leucostictus*	0,4	33	7
6	*C. pycnostictus*	0,1	28	6
7	*C. nivosus*	< 0,1	15	8
8	*C. milnei*	< 0,1	13	17
9	*C. neavei*	< 0,1	11	16
10	*C. enderleini*	< 0,1	10	2
11	*C. subschultzei*	< 0,1	9	2
12	*C. onderstepoortensis*	< 0,1	9	20
13	*C. gulbenkiani*	< 0,1	9	11

Table 5.5 Summary of blood meal identifications for *Culicoides* species in South Africa and Zimbabwe

CULICOIDES SP.	CATTLE Ref. [57, 225, 265, 267]	SHEEP Ref. [57, 225, 265, 267]	PIGS Ref. [51, 57, 265, 267]	HORSES Ref. [51, 57, 265, 267]	CATTLE/ SHEEP Ref. [57, 225, 265, 267]	BOVIDS Ref. [51]	HORSES/ CATTLE Ref. [57]	HORSES/ SHEEP Ref. [57]	HORSES/ SHEEP/ CATTLE Ref. [57]	MAMMALS Ref. [51]	AVIANS/ CHICKENS Ref. [51, 57, 225, 265, 267]	TOTAL
C. imicola	247	459	270	371	111	11	1	16		126	9	1 621
C. milnei		4	7	120	2	16			1	18	273	441
C. sp. near *glabripennis*	16	139	58	77		8				38		336
C. fulvithorax			28	167		5				5		205
C. zuluensis	11	15	34	74						7	40	181
C. leucostictus											61	61
C. bedfordi		40										40
C. magnus	17	14	6	1	1							39
C. brucei	1	2	20	4						3	1	31
C. pycnostictus	1		1	2		2					16	22
C. gulbenkiani	10	5	2	2						2		21
C. bolitinos	10	5		5			1					21
Schultzei group	16			2	1							19
C. coarctatus	1	1	2									4
C. ravus				2								2
C. nivosus	1				1						1	3
C. engubandei	1			1								2
C. neavei		1									1	2
C. similis		1										1
C. enderleini		1										1
Total	332	687	428	828	116	42	2	16	1	199	402	3 053

was positively linked geographically and temporally to an outbreak of AHS. Even in this investigation no attempt was made to isolate virus from identified pools as the age-grading and selection of non-engorged females from the millions that were captured were just too overwhelming. More recently, a single isolate of AHSV serotype 7 was made from pools of *C. imicola* collected during the 1999 outbreak of the disease in the Western Cape Province.[189] A recent analysis of the 14 largest epidemics of AHS to affect southern Africa since 1719 were shown in 13 instances to be linked to warm-phase El Niño weather events.[22] These usually bring above-average precipitation and it is this factor that strongly points to *C. imicola* having been principally involved in past epidemics of the disease. As shown in **Table 5.4** *C. imicola* was the only species of *Culicoides* found to increase explosively in numbers during, and following, the heavy rains of 1996. This pattern of outbreaks and the occurrence of AHS as a plague following excessive rains was noted as long ago as 1863[278] and was subsequently corroborated in 1921.[320]

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Culicoides bolitinos Meiswinkel, 1989 (Figure 5.3b)

Taxonomy *Culicoides bolitinos* was only recognized as a species separate from *C. imicola* in 1989 when the type series was reared from African buffalo dung in the Kruger National Park, South Africa. It is most closely related taxonomically to the Oriental/Australasian *C. brevitarsis* Kieffer, 1917, which is regarded as the most important vector of BTV in Australia. In the past *C. bolitinos* has been confused with both *C. imicola* and *C. brevitarsis*; these misidentifications have been corrected.[213] Before 1989 *C. bolitinos* was recorded in the South African literature as *C.* sp. # 49. *Culicoides bolitinos* is a member of the Imicola complex but, although it is morphologically very similar to *C. imicola*, the two species differ markedly in various aspects of their respective ecologies. These differences are listed in **Table 5.3**.

Vector competence *Culicoides bolitinos* was first incriminated as a vector of AHS in an outbreak of the disease in South Africa in 1998.[226] Almost simultaneously it was shown in the laboratory to be a competent replicator of BTV and is therefore also a likely vector of the disease in the field.[335] In addition, it is considered to be the likely principal vector of EEV in parts of the Eastern Cape Province, South Africa, where *C. imicola* is absent.[280]

Host preferences Blood meal analyses show that *C. bolitinos* feeds on cattle, horses and sheep (**Table 5.5**). It appears to be more endophilic than *C. imicola* as it can be captured in large numbers in horse stables,[221] thus disputing the long-held belief that *Culicoides* do not enter buildings.

Gonotrophic cycle This is unknown but is probably very similar to that of *C. imicola*.

Larval habitat *Culicoides bolitinos* was first reared from cattle dung in 1968 but at the time was mistakenly identified as *C. pallidipennis* (= *C. imicola*).[261] The larval habitat of *C. bolitinos* was described as dry cattle dung pats with a very hard crust, a dry sponge-like centre, and a moist lower layer about 25 mm thick in direct contact with moist soil underneath. This describes a pat that has aged to some extent. It is probable that *C. bolitinos* oviposits only in very fresh dung still emitting the necessary volatile attractants, but this supposition remains to be substantiated. It has been speculated that in Australia the closely related *C. brevitarsis* oviposits in ageing cattle dung over a number of days. *Culicoides bolitinos* has also been reared from the dung of African buffalo and blue wildebeest.[118, 213] On occasion more than 1 000 of these midges can be reared from a single buffalo dung pat.[216] The duration of the larval and pupal stages in dung has been estimated to vary between 8 and 30 days.[213] In South Africa, buffalo dung can produce *C. bolitinos* for up to 90 days especially during the cooler months when it does not desiccate as rapidly as during the hot summer months.[216]

Immature stages The pupa has been retrieved from cattle dung, and morphological studies have confirmed that it differs from that of *C. imicola*.[268]

Geographic distribution *Culicoides bolitinos* occurs in African tropical and subtropical regions south of the Sahara Desert. It has been recorded from 11 countries, namely South Africa, Botswana, Zimbabwe, Lesotho, Malawi, Kenya, Nigeria, the Ivory Coast, Gambia, Madagascar, and Mauritius (misidentified as *C. brevitarsis*). It is likely that it will be found to occur throughout Africa where cattle are kept. In South Africa it is almost as widespread as *C. imicola*, and has been found in all of the area-intensive *Culicoides* surveys referred to above under *C. imicola*. Further details of its distribution, as determined between 1996 and 1997, are given below (**see Detailed distribution and seasonality in South Africa**). Its occurrence on offshore African islands begs the question as to why *C. bolitinos* has not also penetrated into the Mediterranean region where cattle are found in abundance, or has it been overlooked being taxonomically very closely related to *C. imicola*?

Seasonal abundance and prevalence Only in South Africa has the seasonal abundance and prevalence of *C. bolitinos* been studied.[222] Before 1989 most studies on *Culicoides* in South Africa failed to recognize the existence of *C. bolitinos* with the result that the data on these two species are conflated. This is also partially true for another study conducted through much of South Africa in 1992.[332] However, the area-intensive studies mentioned above under *C. imicola*, and published between 1988 and 1998, are reliable as regards the information on *C. bolitinos*.

Dispersal It is presumed that the dispersal of *C. bolitinos* is similar to that of *C. imicola*. Of interest is the record of thousands of females of *C. bolitinos* captured at dusk in the presence of a slow-moving herd of 60 grazing African buffalo.[217] The specimens consisted of nulliparous, parous and gravid females, which implies that the first two categories were accompanying the buffalo in search of a blood meal for the development of their eggs and that the gravid females were seeking fresh dung for oviposition. In this way *C. bolitinos* may disperse a number of kilometres in a short space of time. The results of an investigation concerning the protective stabling of horses against the attacks of *C. bolitinos* implied that, in the absence of cattle dung in a 500-m radius of the stable, *C. bolitinos* dispersed nightly from neighbouring farms over distances greater than 500 m to attack the stabled horses.[221] The question remains as to how *C. bolitinos* arrived on the islands of Madagascar and Mauritius. A molecular study comparing a Madagascan population of *C. bolitinos* with three populations from mainland Africa failed to reveal any degree of genetic differentiation,[289] and therefore suggests the invasion of Madagascar by *C. bolitinos* to be relatively recent. Whether the introduction of the midge

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occurred at the time cattle were introduced to the island or whether it predated this, cannot be answered.

Association with outbreaks of disease The recent outbreak of AHS in the eastern Free State Province of South Africa involved *C. bolitinos*, and is the first substantive proof of its involvement in field outbreaks of disease.[226, 227] In this outbreak five isolations of AHSV serotype 6 were made from identified pools of *C. bolitinos* as were four isolations of an unidentified serotype of BTV. Furthermore, *C. bolitinos* was the dominant *Culicoides* captured and was the only species to be found at all affected stables. In the same outbreak *C. imicola* was rare in numbers, and had a lowered prevalence rate of 71 per cent. Large outbreaks of AHS have in the past been reported from Lesotho, which immediately adjoins the eastern Free State, such as the one in 1953 when an estimated 5 000 horses died. It has been shown that *C. imicola* is rare in Lesotho, and it can only be assumed that *C. bolitinos* could have been the species involved. *Culicoides bolitinos* is also to be the likely vector of EEV in the Eastern Cape Province,[280] specifically in the Port Elizabeth area, where it comprises up to 90 per cent of all *Culicoides* captured.[218]

Detailed distribution and seasonality of *Culicoides imicola* and *C. bolitinos* in South Africa[222]

From September 1996 to June 1999 the distribution and seasonality of these two vectors was determined by weekly collections made at 40 livestock holdings spread almost equidistantly across South Africa and the results are listed vertically in **Figures 5.15 and 5.16**. The data show that:

- *Culicoides imicola* is most abundant in the warmer northern and eastern parts of South Africa where it can be captured throughout the year, including winter. Sites include Tshipese, Polokwane (formerly Pietersburg), Phalaborwa, Komatipoort, Lydenburg, Middelburg and Onderstepoort Veterinary Institute. These areas have long been considered endemic and so may act as source areas for the southward spread of *Culicoides*-borne orbiviral diseases. However, the data also show clearly that *C. imicola* can be found in some abundance, and also for prolonged periods, in the southern half of the country (e.g. Kimberley, Joubertina, Laingsburg and Porterville); in the past these areas have also experienced devastating outbreaks of disease, those of AHS being especially well documented.[19, 120] The figures also reveal that *C. imicola* can be found in most, but not all, parts of South Africa, which means that there is probably constant cycling of BTV, AHSV and EEV in many areas, and that when circumstances become particularly favourable for *C. imicola*, outbreaks will occur.
- As regards *C. bolitinos* the data in **Figure 5.16** reveal it to be an order of magnitude less abundant than *C. imicola*, and with the most numbers captured in cooler, higher rainfall areas (e.g. Middelburg, Ficksburg, Port Elizabeth and George). This indication that *C. bolitinos* is a wet- and cool-adapted species must be treated with caution as cattle, not present at all sites sampled, determine primarily the prevalence of *C. bolitinos*. Nevertheless, it is clear that significant populations of *C. bolitinos* are encountered in areas somewhat depauperate in *C. imicola*, a fact that increases the total area at risk to orbivirus transmission in South Africa. As revealed in **Figure 5.16** (Thabazimbi) it is clear that *C. bolitinos* can also occur abundantly in warmer, drier areas.
- The lowest annual abundances of both *C. imicola* and *C. bolitinos* occur in winter and spring (or weeks 22 to 48 of the year) (**Figures 5.15 and 5.16**). Their abundances increase gradually in the early summer to peak eventually during late summer and autumn (weeks 10 to 20). During this period the number of older, parous midges is also at its greatest, and so increases further the risk of virus transmission. This perfectly mirrors the late seasonal appearance of BT and AHS, which corroborates the maxim that the geographical and seasonal occurrence of an insect vector must correlate with that of the disease.
- It is generally believed that areas experiencing the coldest winters, including snow, are also those with the smallest populations of *C. imicola*. This holds true for the sites of Giant's Castle in the Drakensberg, and Elliot but, even here, *C. imicola* will appear briefly in late summer (weeks 5 to 10). Unexpectedly, it was revealed (**Figure 5.15**) that *C. imicola* is rarer at, or absent from, three sites which have a far milder, frost-free climate (Port Elizabeth, Struisbaai and Alexander Bay). These sites, situated on the sandy southern and western coastline, demonstrate that within certain limits, climate is secondary in determining the abundance and distribution of *C. imicola*, and that the availability of a suitable breeding habitat is therefore paramount. Where known, it has been found that *Culicoides* larvae inhabit the upper layers immediately below the surface of the soil,[35] where they require adequate levels of moisture and nutrients to complete their 7- to 14-day developmental period. However, if these upper layers of the soil drain too rapidly of moisture, as in sandy coastal soils, or in sloping topography, the larvae desiccate and die. This means that overly dry soils most effectively control the numbers of *C. imicola*. Similarly, it is assumed that soils too poor in microorganisms will also impede larval development. Such poor soils are commonly found in coastal regions as well as in areas where nutrients are leached by higher rainfall. Such barriers do not apply to the replication of *C. bolitinos*. The fact that it breeds in cattle dung ensures adequate levels of moisture and an abundance of microorganisms. Thus soil type and quality play no role in determining its prevalence, which, in turn, means that *C. bolitinos* can invade areas free of *C. imicola*, but only if cattle are present.

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- It has been argued that winds (such as prevail along the coastline) induce high mortality rates in *C. imicola*;[20] this may be another reason to explain its absence along parts of the South African coastline. However, the persistent prevalence of *C. bolitinos* in an area such as Port Elizabeth, which is known to be the second windiest point in South Africa and where some of the largest captures of *C. bolitinos* have been made,[228] reveals that windiness may only affect midge activity on particular nights, and does not impact upon overall feeding and breeding success. Presumably the same could be said for its sibling species, *C. imicola*; this therefore points to factors other than windiness inhibiting its establishment in the Port Elizabeth area.
- Of significance is that these data clearly show that both vectors of AHSV occur in some abundance in the AHS control zone of the Western Cape Province of South Africa. This means that the zone is always at risk to incursions by the virus, as was amply demonstrated by the death of some 30 horses from the disease in the Stellenbosch area in 1999.[24] The prospect that this can recur at irregular and unpredictable intervals is real. Vector-free points (**Figures 5.15 and 5.16**) occur along the southern and western coastline of South Africa.

As noted, an earlier data set[332] similar to the one above was used to model the distribution and abundance of *C. imicola* in South Africa.[21] Whilst the model satisfactorily explained 67 per cent of the observed variance, significant anomalies appeared as regards the geographic distribution of *C. imicola* and its predicted abundances. This may be due to the data being representative of climatically 'average' years, but which fail to model for the superabundance of *C. imicola* following excessive rains.[219] This would seem essential when one considers that its superabundance at such times correlates perfectly with the statement by Theiler[320] 'that horse sickness appears as a plague following heavy rains'. Interestingly this observation appears to be contradicted by the central clumping of abundance data in **Figure 5.15** where it is clear that the largest numbers of *C. imicola* appear in those areas experiencing a more moderate annual average rainfall varying between 300 and 700 mm per annum. It remains to be demonstrated whether this apparent contradiction is explicable in terms of soil fertility, i.e. in areas experiencing a persistent annual average rainfall of above 800 mm, do the soils become too leached of nutrients to sustain the larvae of *C. imicola*? If so this may explain the virtual absence of *C. imicola* in the equatorial regions of Africa where suitably high environmental temperatures prevail, but where rainfall is excessive and soil fertility is notoriously poor. Another factor difficult to incorporate into any model is the extent to which humans aid the establishment of large populations of *C. imicola* by maintaining livestock in confined spaces and by irrigating pastures. Whilst a similar model has yet to be developed for *C. bolitinos* it would require the inclusion of other parameters such as the presence or absence of cattle.

Host preferences

Understandably, nearly all studies on African *Culicoides* are strongly biased towards the welfare of exotic breeds of horses, cattle and sheep. As several of the orbiviral diseases affecting domesticated livestock have their origin in African wildlife, it would seem appropriate to study the relationship between African *Culicoides* and game. As has been reviewed,[223] nine studies in Africa have purported to deal with this relationship but nearly all were conducted in unnatural systems in which wildlife had been removed from their habitats and maintained in subverted conditions. The relationship between some species of *Culicoides* and the African elephant, zebra and buffalo are briefly reviewed below. These herbivores serve as hosts for three species of the Imicola complex, and for other species of *Avaritia* whose role as vectors remains unknown.

***Culicoides* and the African elephant** There is only one study in which an attempt to clarify the life cycle of *Culicoides* found associated with African wildlife was made.[223] This dealt with five species of the subgenus *Avaritia*, which were discovered feeding in considerable numbers behind the ears of the African elephant (**Figure 5.18**) and to breed in its dung. The midges were collected off culled elephants in broad daylight and included specimens that were fully and freshly engorged; this clearly shows that certain species of *Culicoides* feed throughout the diel, and not only at night as is commonly believed. It was learned too that all the *Culicoides* spp. collected on the ears of elephant were also those that breed in elephant dung, indicating their co-evolvement, a fact that is further substantiated by the observation that these mostly new species are exceptionally rarely collected outside the range of elephants. It was observed that gravid females of these species oviposit in fresh dung in broad daylight, and that *Culicoides* are among the first insects to arrive at freshly evacuated dung. Gravid *Culicoides* females must therefore track elephants in small swarms, and when dung is evacuated they immediately descend upon it to lay their eggs. Oviposition is rapid and is a likely part of an evolutionary adaptation enabling the *Culicoides* to relocate the elephant, which will have proceeded little more than 50 m from the original point of dung deposition. By once again attaching themselves behind the ear to obtain a fresh blood meal these elephant-associated *Culicoides* repeat the feeding cycle needed for the development of their next batch of eggs.

It appears that, after blood-engorgement, the *Culicoides* do not stay on the elephant, but rather abandon the host to reside on vegetation during the two or three days required for egg development. Here they will await the return of elephant to once again track them but only if they are fully gravid. It is well known that elephant herds, led by

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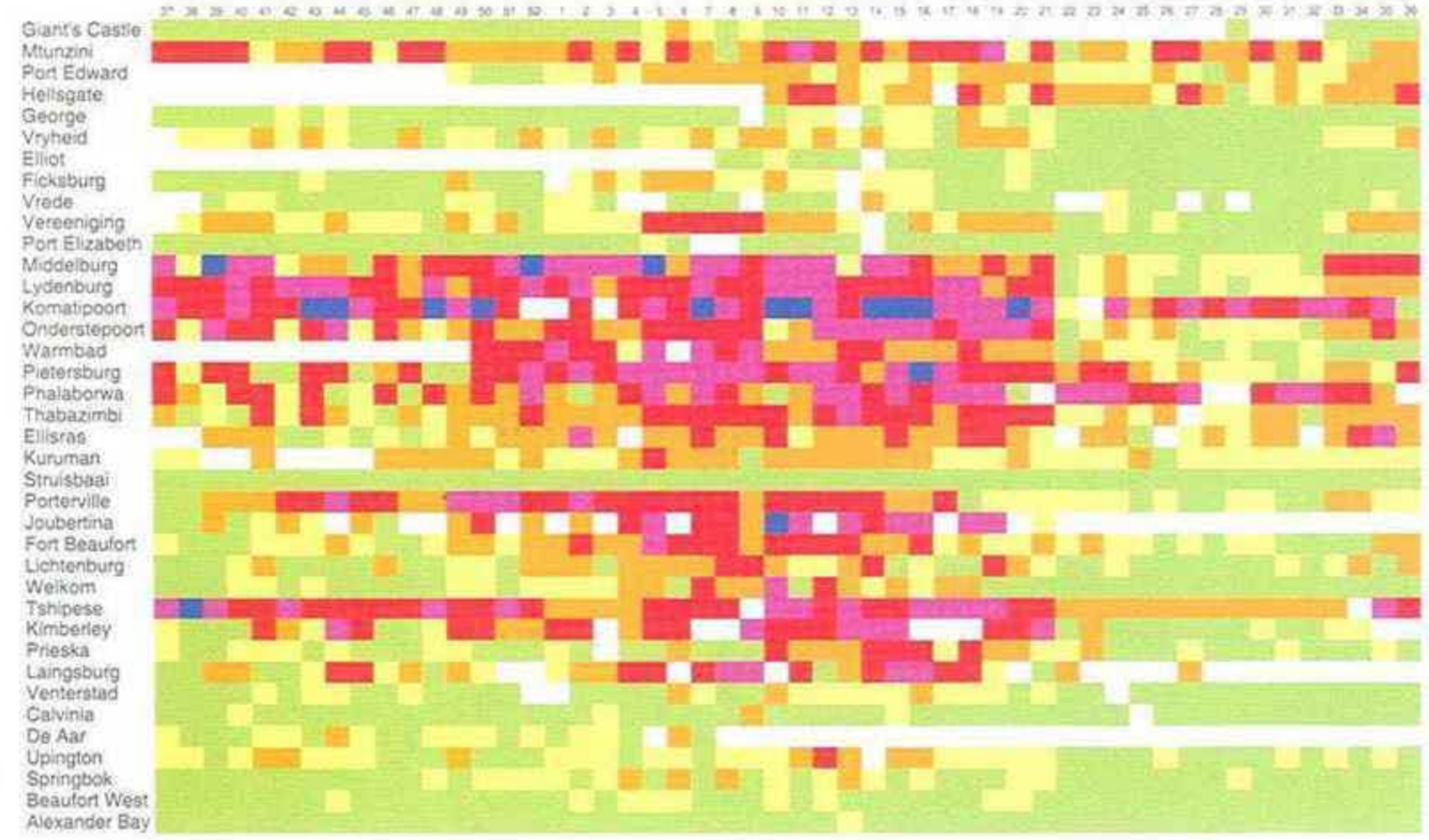


Figure 5.15 *Culicoides imicola*: log (n+1) numbers captured weekly at 39 livestock holdings spread across South Africa between September 1996 (week 37) and September 1997 (week 36); sites are ranked from highest to lowest rainfall (1 200 mm to 250 mm/annum). The weekly *Culicoides imicola* totals are colour-coded: white (no collection made), green (0 *C. imicola*), yellow (1 to 9), orange (10 to 99), red (100 to 999), pink (1 000 to 9 999) and dark blue (10 000 to 99 999)

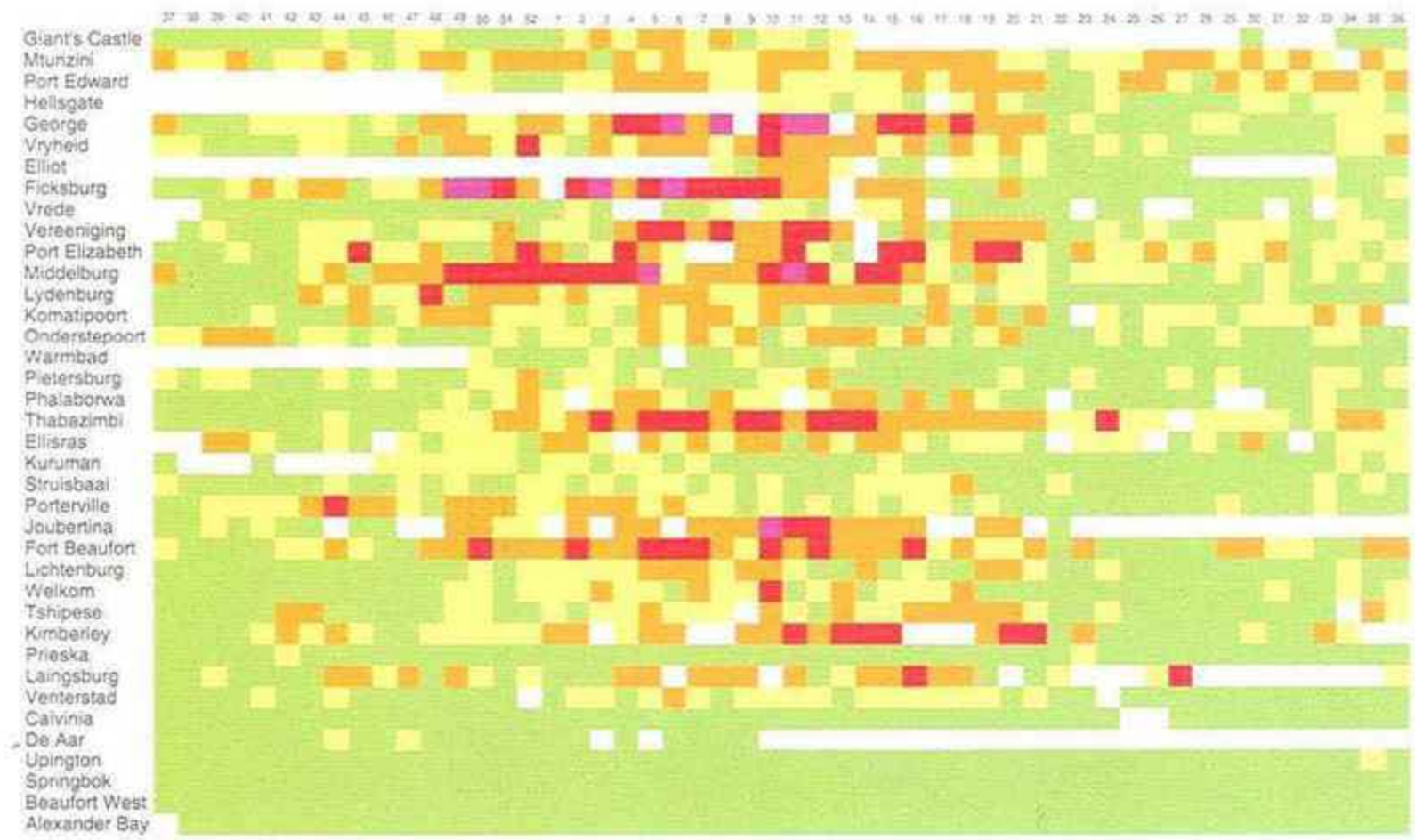


Figure 5.16 *Culicoides bolitinos*: log (n+1) numbers captured weekly at 39 livestock holdings spread across South Africa between September 1996 (week 37) and September 1997 (week 36); sites are ranked from highest to lowest rainfall (1 200 mm to 250 mm/annum). The weekly *Culicoides bolitinos* totals are colour-coded: white (no collection made), green (0 *C. imicola*), yellow (1 to 9), orange (10 to 99), red (100 to 999), pink (1 000 to 9 999) and dark blue (10 000 to 99 999)

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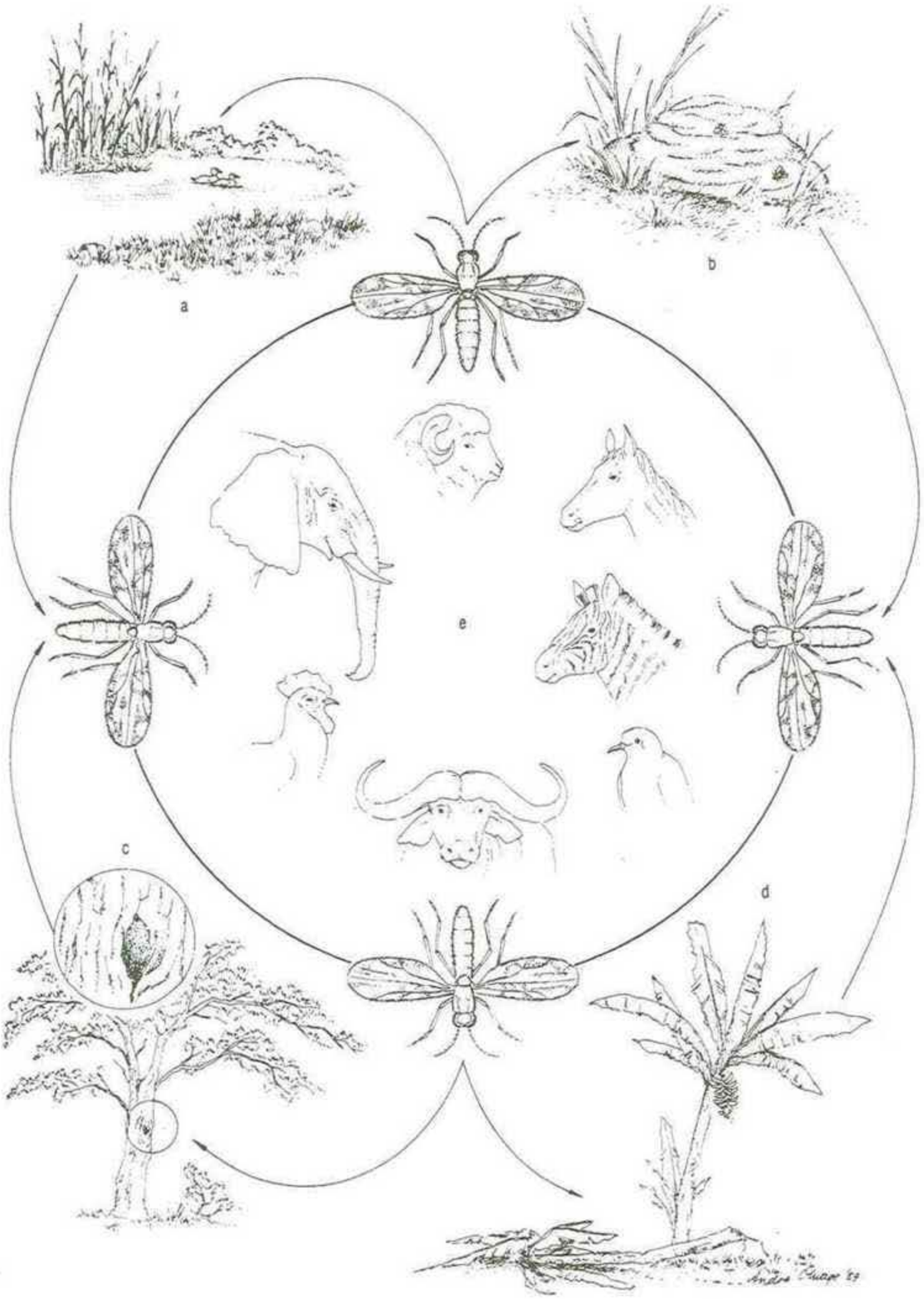


Figure 5.17 Diagrammatic representation of the interaction between *Culicoides* spp., their hosts and preferred habitat types. Each *Culicoides* sp. requires a specific substrate for the development of its larvae; these larval habitats may be divided into four main types:
a = surface water and soil interface (organically enriched or not)
b = dung pats from large game or livestock
c = tree-holes; plant and rock cavities
d = rotting plant stems, fruit and fungi
Culicoides spp. require the blood of specific host animals (e) in order to develop their eggs

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Figure 5.18 The elephant possesses no sweat glands and so cools its blood by pumping large amounts (5 to 12 ℓ/min.) through the ears. The skin at the back of these heavily veined 'radiators' is smooth, and is where hundreds of *Culicoides* feed. This photograph of eight *Culicoides* on the ear of an elephant was taken at 15:00 and includes a freshly blood-fed female

matriarchs, move in well-defined home ranges, and will daily traverse the same well-worn paths for feeding and watering. This would facilitate the development of sizeable populations of elephant-associated *Culicoides* in the immediate vicinity of these routes. An elephant bull daily consumes 300 kg of vegetation and evacuates 100 to 150 kg of dung.[223] More than 3 500 *Culicoides* have been found to hatch from 5,75 kg of elephant dung.[216] Thus the daily accrual in numbers of elephant-associated *Culcoides* amongst the 7 500 elephants of the Kruger National Park, in South Africa, could total roughly 500 million individuals.

One of the species associated with elephant is *C. loxodontis*, a member of the Imicola complex.[214] Because it is closely allied to both *C. imicola* and *C. bolitinos* the question arises as to whether *C. loxodontis* is not also involved in the transmission of orbiviruses. It was suggested that the elephant may be a natural host for AHSV[93] but subsequent studies have failed to bear this out.[15] It would seem that in South Africa at least the elephant plays no part in the epidemiology of AHSV as the disease appears in large areas from which elephants are absent. Elephant-associated *Culicoides* are easily identifiable and they occur exceptionally rarely elsewhere in South Africa. This indicates that their mass movements, if any, over large distances, either do not occur or have yet to be observed.[217, 222] Furthermore, an intensive two-year survey of the *Culicoides* that occur in and around the Kruger National Park which yielded over five million midges, failed to reveal the presence of significant numbers of elephant-associated *Culicoides* on livestock farms immediately adjoining the Park.[217]

***Culicoides* and the plains zebra** Recently, another new species of the Imicola complex, *C.* sp. # 107, was discovered,[217] almost solely restricted to the warmer and drier central-northern, northern and eastern parts of South Africa. In the Kruger National Park it enjoys a close association with the plains zebra (**Figure 5.19**) which is a host of AHSV.[13] Significantly *C.* sp. # 107 breeds in the dung of zebra and, unusually, is most prevalent during the cooler seasons but only in frost-free areas. Whilst *C.* sp. # 107 never becomes abundant, no doubt due to the limited availability of its breeding medium, its occurrence during winter raises the

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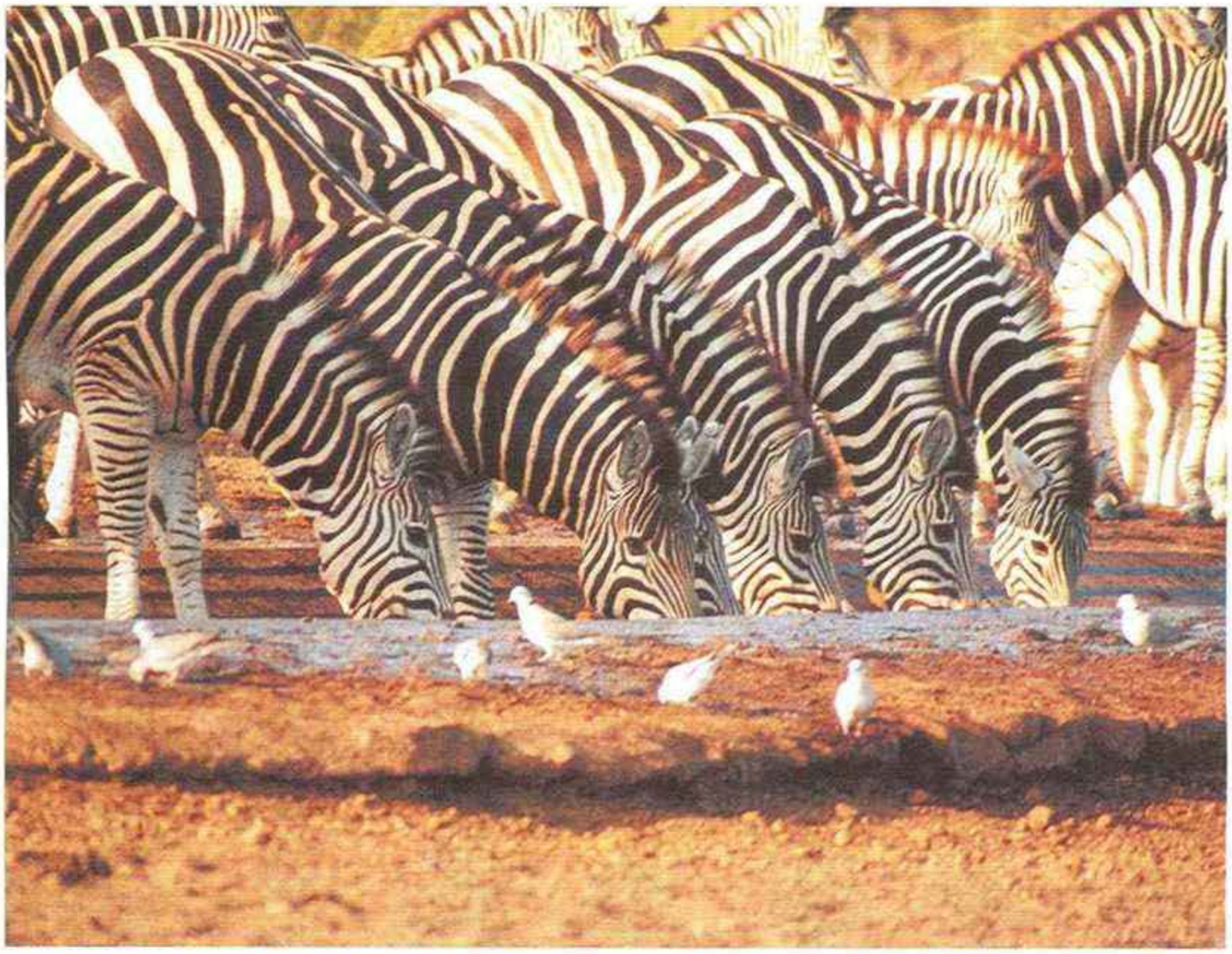


Figure 5.19 The plains zebra (*Equus burchelli*); it has been shown that all nine known serotypes of AHSV circulate in zebra. Even under very dry conditions in the Kruger National Park considerable numbers of *Culicoides* (*C. imicola*) can still be captured

question as to whether or not *C.* sp. # 107 is capable of transmitting AHS at a time of the year when the disease is not considered a problem but has been shown still to be circulating in zebra.[13]

The question is potentially significant as *C.* sp. # 107 can be found in some abundance around horse stables where it in all probability breeds in the dung of horses.[217] It also breeds abundantly in the dung of the white (square-lipped) rhinoceros (*C. simum*).[217]

***Culicoides* and the African buffalo** Both *C. imicola* and *C. bolitinos* have been collected live off a darted African buffalo (**Figure 5.20**).[213] There is little doubt that *C. bolitinos* especially is closely associated with this host; in the Kruger National Park more than 1 000 specimens of *C. bolitinos* have been reared from a single buffalo dung pat.[216] Of veterinary importance is that *C. bolitinos* also uses the dung of domestic cattle for breeding. This ensures that this seemingly efficient vector of BTV occurs almost throughout South Africa, and probably plays a major role in the infection of cattle with the virus. In support of this is the report that a 90 per cent BTV-infection rate has been found in some buffalo herds in the Kruger National Park,[279] but this does not exclude the involvement of other *Culicoides* spp. in their infection. Of the hundreds of light trap collections made in the Kruger National Park the largest numbers of *C. bolitinos*, numbering in their thousands, were captured in the immediate vicinity of grazing buffalo herds. In these collections *C. bolitinos* was the dominant species, comprising more than 80 per cent of the captures: *C. imicola* comprised less than 3 per cent.[217] The only other species of *Avaritia* that is likely to be associated with the buffalo is *C. gulbenkiani* but this has yet to be demonstrated.

***Culicoides* and domesticated livestock** Because of their minute size and generally nocturnal habits it is difficult to collect or identify *Culicoides* on any particular host. Virtually no bait-trapping has been done in Africa, and therefore the presence of large numbers of a particular species near specific livestock species is generally assumed to indicate that the *Culicoides* feed on these hosts.[265] The wing patterns of the 14 species of *Culicoides* most commonly encountered

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Figure 5.20 The African buffalo (*Syncerus caffer*): in the Kruger National Park thousands of *Culicoides* (>80 per cent *C. bolitinos*) have been captured around such herds at dusk. More than 1 000 adults of *C. bolitinos* can be reared from a single buffalo dung pat

near livestock in South Africa are shown in **Figure 5.3**. These, ranked in order of abundance, are *C. imicola, C. subschultzei, C. magnus, C. zuluensis, C. bolitinos, C. pycnostictus, C. leucostictus* (= *C. distinctipennis* in earlier South African studies), *C. nivosus, C. schultzei,* and *C. enderleini*. These species made up 90 per cent of the 86 *Culicoides* spp. collected countrywide.[222] Although close on 4 000 collections were made in the particular survey only 75 per cent of the *Culicoides* known to occur in South Africa were captured, indicating that many species either have highly specialized feeding preferences or larval habitats. A similar pattern of species prevalence and abundance was recently reported from Zimbabwe and Botswana.[254, 255]

Another approach used to determine the host preferences of *Culicoides* spp. is to count the number of sensilla coeloconica ('pits') found on 13 of the 15 antennal flagellar segments ('antennomeres') of the female.[48] It is considered that a preference for mammals is indicated by six or fewer pit-bearing 'antennomeres', while those with 12 or 13 show a preference for birds. Although this approach may have much merit, reliance for its verification is still placed on blood meal identification.

In southern Africa *Culicoides* blood meal identification using various types of precipitin tests and an enzyme-linked immunosorbent assay (ELISA) has been limited to studies conducted in Zimbabwe and South Africa (**Table 5.5**).[57, 265, 267] Blood meal identification can sometimes give a biased impression of the potential vector importance of certain *Culicoides* spp., especially if these species are collected in large numbers at isolated sites. For example, 215 *Culicoides* spp. near *glabripennis* tested positive for the blood of four host animals at one site, yet this species was rare in catches made at the other 23 sites in the Onderstepoort Veterinary Institute area (**Table 5.5**).

Culicoides spp. which usually feed on mammals will occasionally feed on birds if large numbers of them are present in an area and their primary source of blood is scarce. For example, in Zimbabwe, a small proportion of the large numbers of *C. imicola, C. zuluensis* and *C. milnei* collected on a poultry farm of 75 000 birds had fed on the poultry (**Table 5.5**), which indicates that under specific circumstances they

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may become opportunistic feeders.[50] Conversely, known bird-feeders, such as *C. leucostictus* (= *C. distinctipennis* in early studies on South African *Culicoides*) and *C. pycnostictus*, must occasionally feed on mammals (**Table 5.5**), as suggested by the fact that in South Africa two BTV serotypes have been isolated from *C. pycnostictus* (**Table 5.2**). Epidemiologically this could be significant in the transmission of virus from birds to mammals and *vice versa*. *Culicoides pycnostictus* was recently also infected in the laboratory with BTV.[282] However, the data remain confusing as in a more recent study[221] it was found that at least five bird-associated species (*C. leucostictus, C. pycnostictus, C. neavei, C. bedfordi* and the Onderstepoortensis complex) would not enter horse stables whereas seven mammalophilic species did so readily. Whether this reflects a strong exophilic tendency, or whether mammals are truly unattractive to *C. pycnostictus* and thus very seldom attacked, remains to be clarified.

Life cycle

The life cycle of *Culicoides* spp. worldwide was reviewed in 1984 by Kettle.[180] In South Africa an early attempt to clarify the life cycle of some species was made when *C. imicola, C. pycnostictus, C. nivosus, C. leucostictus* (as *C. distinctipennis*), *C. enderleini* (as *C. schultzei*), *C. zuluensis, C. bedfordi* and *C. magnus* were maintained in the laboratory under controlled conditions.[260] After a blood meal had been taken, it was found that the maturation of eggs in *C. imicola* females took two days at 27 °C and three to four days at 22 °C. The 'sausage-shaped' brown eggs were 400 µm long by 50 µm wide and were laid in a staggered double-rowed pattern somewhat resembling footprints. At 21 to 24 °C the eggs of five South African *Culicoides* spp. took three days to hatch. The number of eggs per batch varied from a mean of 69 for *C. imicola* to 162 for *C. nivosus*, but the number of batches was not determined. In *C. variipennis*, a North American species, up to four batches may be produced in a life-time.[251] A blood meal must be taken by the female in between each batch of eggs.

Larvae of *Culicoides* go through four stages, are eel-like in their movements and burrow in and out of their breeding medium. As is shown by the variations in the structure of the epipharynges of different species,[252] the food preferences of larvae vary; many are carnivorous and feed on protozoa, rotifers and nematodes.[200] The fourth stage larvae of *C. zuluensis* and *C. nivosus* have been reported to be cannibalistic on second stage larvae.[260] In the laboratory at 21 to 24 °C, larvae of *C. nivosus, C. leucostictus* (= *C. distinctipennis* in earlier South African studies), *C. pycnostictus* and *C. bedfordi* developed readily in a mixture of moist soil and cow dung and reached the pupal stage in 10 to 20 days. The following developmental times for six South African *Culicoides* spp. were: larval period 7 to 25 days, pupal period four days, oviposition to first adult 20 days. A generation (egg to egg) requires a minimum of 25 days. Adult *Culicoides* survive for between 14 and 21 days if fed on water containing 10 per cent honey.[260] Further details on the life cycle of five species of *Avaritia* found in association with the African elephant have been reported.[223]

In attempts to explain the possible effect of various climatic factors on the survival and seasonal prevalence of *Culicoides* spp., especially *C. imicola*, the effect of desiccation, submersion in water and exposure to low temperatures on different life stages have been investigated.[260] Eggs showed some resistance to desiccation, for example, 1 per cent of eggs allowed to dry at 85 per cent relative humidity did not collapse and later hatched on immersion in water. Low temperatures adversely affected egg viability. If the eggs were kept at 6.5 °C, viability started to decrease after seven days while none hatched after 37 days. The immersion in water of thousands of eggs, mostly of *C. imicola*, had no adverse effect on hatching — the larvae of seven species, including *C. imicola*, survived immersion in water at 22 °C for 6 to 13 days. Fourth instar larvae of *C. nivosus, C. pycnostictus* and *C. zuluensis* present in breeding medium survived refrigeration at 6.5 °C for 10 to 14 days, and completed their development normally on return to room temperature. On immersion, the pupae of all species, except *C. imicola*, wriggled free of the breeding medium and floated to the surface. *Culicoides imicola* pupae, however, lay on the substrate below the water surface and drowned within two days at room temperature. At 6.5 °C they survived for at least six days under water. The effect of low temperatures on adult survival and longevity was also tested.[263] This showed that at temperatures as low as −1.5 °C, 15 per cent of *C. imicola* adults lived beyond 15 days, whereas the last *C. pycnostictus* died on the 54th day.

The results of these laboratory studies indicate that low winter temperatures do not kill any stage of *Culicoides* but merely slow down development or induce diapause. It can also be deduced that soaking rains have no adverse effect on the eggs, larvae and pupae of most species, but that they do drown *C. imicola* pupae; the larvae of *C. imicola* will not pupate until conditions are sufficiently dry. A seven-year study of the seasonal abundance of *C. imicola* at the Onderstepoort Veterinary Institute showed a drop in adult numbers during sustained rainy periods followed by a sharp increase in populations during the drier periods that followed.[263] The greater tolerance of *C. pycnostictus* adults to low temperatures may partially explain why it was the most common species found in two studies in the Free State Province of South Africa, and why *C. imicola* comprised less than 1 per cent of the total *Culicoides* collected.[173]

Larval habitats Knowledge about the habitats of *Culicoides* biting midges has increased greatly since the early 1960s as a result of studies involving the collection of live pupae from moist situations in the field and rearing them through to the adult stage.[262, 268] Breeding material suspected to contain immature stages has also been collected and placed in special emergence boxes or cages,[118, 212, 213, 261] or allowed to emerge *in situ* in specially constructed tent-type emergence traps

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placed over breeding grounds or materials.[276] There is a common tendency to believe that *Culicoides* breed only in moist low-lying areas but, although this is true for some species, others have more specialized larval habitats. The basic requirements for *Culicoides* larvae are moisture and a medium containing organic matter. Thus, *Culicoides* may breed in situations varying from those which are almost aquatic in nature (such as pond margins) to those where no free water is present but the humidity is close to 100 per cent (such as the interior of dung pats). The various larval habitats can be grouped into four main types (**Figure 5.17**).

- Surface water and soil interface situations (**Figure 5.17a**). About half the known *Culicoides* spp. in southern Africa make use of various combinations of soil and water as a medium in which to lay their eggs. Soil may vary from coarse sand to the finest clay, and the basic medium may be enriched to a greater or lesser extent with decomposing plant matter, varying from intact material to humus, or with fresh to well-decomposed dung, such as is often found on irrigated pastures. The water may range from fresh flowing streams to polluted stagnant pools with varying degrees of acidity, alkalinity or salinity. The degree of light and the presence or absence of plant cover, which may be either tall or kept short by grazing animals, are additional important factors that govern the larval habitat of certain *Culicoides* spp. In southern Africa most of the major stock-associated species (*C. imicola, C. zuluensis, C. magnus, C. schultzei* group, *C. pycnostictus, C. leucostictus*, and *C. nivosus*) use one or another of the above combinations as their larval habitat.[275] Similarly, in Zimbabwe,[41] thousands of specimens of 16 species of *Culicoides* were reared from mud samples taken at the edge of water bodies. Short notes on the larval habitats of six South African 'groundwater' species have been published.[269]
- Dung pats of large animals (**Figure 5.17b**). In Africa these larval habitats were only recognized in 1968, and their importance appreciated since 1985.[118, 212, 213, 261] At least 10 *Culicoides* spp., all in the subgenus *Avaritia*, require the fresh dung of certain animals to complete their life cycles. For example, the dung of the African buffalo, cattle and the blue wildebeest is used by *C. bolitinos*, which apparently also feeds on these hosts.[213] Other species, most of them still to be described, breed in the dung of the elephant, the black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*), and the plains zebra.[212]
- Tree-holes, plant and rock cavities (**Figure 5.17c**). These larval habitats vary from deep, dark, water-filled holes to shallow, exposed but moist hollows which may contain various amounts of water, decomposing leaf litter and sediment. Tree-holes are not restricted to dense forests, as even the more sparsely treed savannahs are rich in tree-hole-associated *Culicoides*. About 15 per cent of African *Culicoides* spp. (including *C. accraensis, C. clarkei, C. olysterni, C. eriodendroni, C. punctithorax* and *C. nigripennis*) are known or suspected to breed in these habitats,[269] but owing to the restricted size and availability of such rain-dependent habitats these species never become abundant and are rarely collected. Birds are thought to be their primary source of blood. From the wet boles or crotches of 11 genera of trees in Zimbabwe more than 500 specimens of *Culicoides* representing the Nigripennis, Accraensis and Eriodendroni species groups were reared.[41] As noted above species from these complexes are very difficult to identify.
- Rotting fruits and plants (**Figure 5.17d**). These larval habitats have still to be investigated thoroughly. In South Africa a new *Avaritia* sp., closely related to *C. pseudopallidipennis* from West Africa, has been reared from the rotting fallen fruits of the sausage tree (*Kigelia africana*) and the marula tree (*Sclerocarya birrea*).[117] In West Africa the larvae of *C. grahamii* (also of the subgenus *Avaritia*) have been found in the rotting stems of the banana plant.[209] The cultivation of bananas by humans may thus lead to the creation of larval habitats for this vector of the human filarial worm, *Mansonella perstans*. A concerted effort to rear *Culicoides* from rotting fruits, flower bracts, plant stems, and large fungal fruiting bodies should lead to the discovery of the breeding habits of several known and, perhaps, even new species. Some of these habitats, and many others, were sampled in Zimbabwe but without success.[41]

Control of *Culicoides*

Around livestock, *Culicoides* can occur in stupendous numbers especially on warmer nights and during periods of above average rainfall. At such times more than a million *C. imicola* can be captured in a single light trap in one night (**Figure 5.21**),[219] and, if the estimate that this may represent a mere 1 per cent of the number of midges active on a particular night in the area concerned is correct, it graphically illustrates the intensity of attacks that must on occasion be endured nightly by exposed animals. It is impossible to eradicate such numbers of *Culicoides* and in Africa specifically, the first line of defence against *Culicoides*-borne orbiviruses must remain vaccination. However, even in the case of a notifiable disease such as the highly fatal AHS, and despite the social and commercial value of horses, it appears that less than half the horse population in southern Africa is being vaccinated annually. Recourse to additional methods of disease control, such as the elimination of the larval habitat of *C. imicola*, has received no attention in Africa perhaps because little success has been achieved with other species of *Culicoides* elsewhere in the world. Under restricted situations it may be feasible to reduce *Culicoides* adult numbers by treatment of their breeding sites with compounds such as Temephos.[158] However, *C. imicola*'s potential for an explosive increase in numbers and its rapid radiation over large

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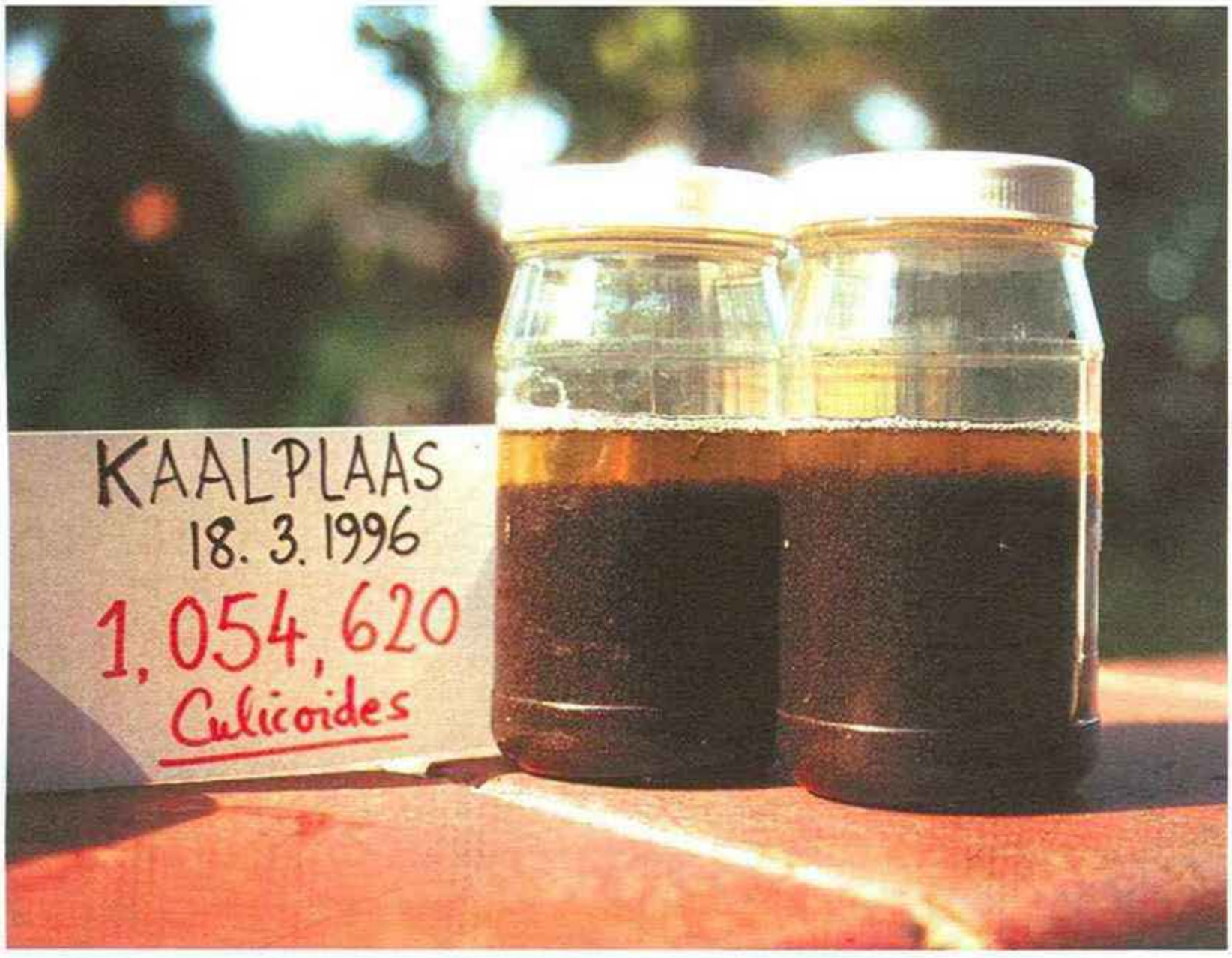


Figure 5.21 The cumulative total in these two bottles amounts to >1 000 000 *Culicoides* (99.4 per cent *C. imicola*) captured in one night in a single blacklight trap set near horses on the Kaalplaas farm of the Onderstepoort Veterinary Institute (March 1996)

areas as soils become suitably moist under continuous rains, would make the widespread application of Temephos not only expensive but impracticable. For the same reasons, the biological control of *C. imicola* larvae would also not be feasible even if such a measure were available. Similarly, the daily treatment of animals with pyrethroids to reduce adult biting rates is labour intensive and expensive. Despite limited successes reported worldwide,[252] and reviewed in some detail below, they merit further study, particularly in Africa. In Israel certain extracts of plants from the families Meliaceae and the Labiatae (including oils derived from sage, rosemary and oregano) were found to have a weak to negligible repellent effect against *C. imicola*.[46] Recourse to more traditional or esoteric remedies such as the smoking of stables and the feeding of garlic would appear equally ineffective, but such control strategies have received little serious attention from researchers.

In the early years of animal husbandry, farmers soon noted that distancing their animals from low-lying moist areas reduced the prevalence of disease, and it became customary to move stock to higher-lying terrain at night or even for entire seasons.[61] However, the modern era has seen a reduction in farm size and a concomitant increase in stocking rates. Sustained irrigation further increases the numbers of *Culicoides* breeding sites and, in the case of the moist soil-loving *C. imicola* and the cattle-dung breeding *C. bolitinos*, it is obvious that few areas will escape being colonized by one, or both, of these vector species.

Besides vaccination only stabling appears to have the potential of more reliably and permanently protecting animals from the attacks of *Culicoides*. More than 100 years ago horse owners in South Africa noted that stabled horses rarely succumbed to AHS, but despite these early and persistent observations only in two studies in Africa,[14, 221] and not many more elsewhere in the world, have the merits of protective housing been explored. The results of the African studies revealed that both *C. imicola* and *C. bolitinos* readily enter stables, and indicated that stables were only of value if well sealed. The extent of sealing required was assessed:[221] an 80 per cent shadecloth induced a 14-fold reduction in the numbers of midges entering stables baited with horses. Since *Culicoides* midges of most species are active mainly

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during twilight hours and at night, valuable or young animals, whose immunity is not established, should be stabled at night. Because it is now evident that *Culicoides* will enter stables, it is essential that stables be well built and as many of the small openings as possible closed. Thousands of *C. bolitinos* (and *C. imicola* but in lesser numbers) have been captured at night in an open stable in the Clarens district of the eastern Free State Province of South Africa. The exclusion of *Culicoides* can be more successfully achieved by screening stables with insect gauze or shadecloth,[221] and will be made more effective if their surfaces are treated with a pyrethroid insecticide. For exceptionally valuable animals a stable can be made virtually insect-free by the installation of extractor fans which maintain a negative pressure so that odours from the animals that remain inside go largely undetected by the *Culicoides* outside. It does not seem that light traps operated outside a stable at night will collect *Culicoides* in large enough numbers to significantly reduce the attack rate on animals inside.

Another strategy for disease control mooted 40 years ago is to maintain decoy hosts, after it was suggested that the presence of cattle protected nearby sheep from contracting BT.[116, 264] It must be pointed out, however, that because *C. bolitinos* is dependent upon cattle dung its numbers are likely to increase wherever cattle occur, and therefore the presence of cattle may actually increase risk. Indeed the implication of cattle in the epidemiology of AHS only recently became obvious after an outbreak of the disease in the Clarens area of the eastern Free State Province in 1998, when it was revealed that the maintenance of unvaccinated horses in the presence of cattle is risky. Decoy animals are also an added expense and consideration must be given to the fact that any increase in animal biomass on a farm will result in a concomitant increase in local *Culicoides* numbers and in viral transmission.

Since many *Culicoides* spp. breed in moist, organically enriched soil, large concentrations of them can be expected in wet low-lying areas. Such areas should, if possible, be avoided by animals between dusk and dawn, i.e. during the hours of midge activity, and especially in mid- to late summer. However, it must be borne in mind that *Culicoides* in search of a blood meal are quite capable of dispersing over considerable distances. In addition, for the tracking of hosts they are equipped with acute sensory apparatuses (on the antennae) and therefore will locate animals wherever they may be kept on a farm. In the case of *C. bolitinos* the presence and amount of cattle dung will determine its presence and relative abundance. Therefore, the removal of dung should lower the numbers of *C. bolitinos* but this is only practically feasible where cattle do not range extensively. Furthermore, such hygienic practices would have to be implemented over an entire farming community as *C. bolitinos* would be able to disperse from neighbouring farms that might harbour *C. bolitinos* if some owners are less careful about the removal of dung. In the case of *C. imicola*, its capacity to breed explosively under rainy conditions makes it an even more formidable insect to control.

No publications exist concerning the efficacy of repellents against *Culicoides* in the African situation. The chemical control of adult biting midges by direct treatment of livestock with pesticides is not usually practical under extensive farming conditions, but it may be justifiable for valuable animals such as racehorses. Many pyrethroid insecticides are effective against the Diptera, and, depending on the formulation used, have a reasonably long residual effect. Sprays can be used weekly and, in cattle, insecticide-impregnated eartags may be effective for four to six weeks,[18] or even as long as 10 weeks against *Culicoides*.[159] In Australia it has been found that, after Hereford yearling cattle had been given a single subcutaneous injection of ivermectin at a dose of 200 mcg/kg, the mean mortality of engorged *Culicoides* females 48 hours after feeding was 99 per cent for 10 days after treatment.[300] An unfortunate adverse effect is the lethal effect of ivermectin on the dung beetle fauna which are so important for sanitation.

In a recent and thorough study[252] very limited success was reported against *C. sonorensis* in North America with formulations of 5 per cent permethrin or 27 per cent pirimiphos-methyl applied to the dorsum of calves. It was found that engorged *Culicoides* exhibiting sublethal intoxication recovered and subsequently matured average-sized batches of eggs. However, a belly spray of 0,2 per cent permethrin substantially reduced numbers of engorged females, and lowered engorgement levels on the third and seventh day post-treatment, but by the tenth day little effect was noted. In the case of *C. sonorensis* it was shown that most feeding was done on the belly of the calf, but in Israel[41] *C. imicola* was found to feed preferentially on the dorsum (70 per cent) of a horse, with less (28 per cent) feeding on the belly. These studies on the chemical control of *Culicoides* show mixed promise and so merit additional research, and particularly in Africa where attack rates appear to be high.

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6

Vectors: Mosquitoes

P G JUPP

Introduction

Among the pathogens that cause disease in livestock in southern Africa, only a few viruses are transmitted by mosquitoes (**Table 6.1**). These and other arthropod-borne viruses (arboviruses) characteristically multiply in both the vertebrate host and the mosquito vector. Successful transmission is dependent upon the virus producing a viraemia in the vertebrate, while the mosquito which feeds on the vertebrate must develop a salivary gland infection followed by excretion of virus in the saliva. The arboviruses belong to several families and genera and their taxonomic categories and host relations are discussed elsewhere (**see Chapter 7: Classification, epidemiology and control of arthropod-borne viruses**).

Of the mosquito-borne viruses that occur in southern Africa, Rift Valley fever virus (RVFV) is periodically responsible for serious disease in sheep and cattle in the subcontinent, while Wesselsbron virus (WSLV) causes a milder disease, affecting sheep in particular. West Nile virus (WNV) and Banzi virus (BANV), however, have only been experimentally incriminated as causes of congenital abnormalities in lambs in South Africa.[12] In France, WNV has been documented as the cause of encephalomyelitis in horses.[32, 33]

Other mosquito-borne viruses of the families *Togaviridae* and *Flaviviridae* that do not occur in Africa, but which cause important diseases mainly in horses and humans outside Africa include the alphaviruses of Eastern, Western and Venezuelan encephalomyelitides and the flaviviruses of Murray Valley, St. Louis and Japanese B encephalitides (see the chapters on the specific diseases). It is possible that Akabane virus, which is a bunyavirus of the family *Bunyaviridae*, is also present in southern Africa, although this still remains to be demonstrated. This virus has been isolated from *Culicoides* spp. and from mosquitoes in Japan, suggesting it is also mosquito-borne.[98] Akabane virus has been incriminated as the cause of abortion and congenital abnormalities in livestock[64] (**see Chapter 94: Diseases caused by Akabane and related Simbu-group viruses**).

Rift Valley fever, WSL and WN viruses are also important in causing human disease, while two other mosquito-borne viruses, namely chikungunya (CHIK) and Sindbis (SIN) viruses, only cause disease in humans. Their epidemiology and association with human disease have been summarized recently by McIntosh.[78]

Apart from virus isolation from wild-caught mosquitoes, the lines of evidence incriminating a mosquito species as a vector are its susceptibility to infection with, and its ability to transmit, the virus concerned (vector competence) plus its relative density and ecological characteristics. Vector competence is assessed quantitatively in laboratory tests while data on density and ecology are obtained by field observations. Relevant aspects of ecology are feeding behaviour, including host preferences, larval habitat associations and biology of the egg stage.

Climatic factors influence arboviruses both directly and indirectly. Temperature conditions directly affect the multiplication of the virus in the mosquito vector, low temperatures slowing down viral replication and lowering vector competence.[38, 101] However, more importantly, climatic factors operate through their influence on the geographical distribution and prevalence of the viral hosts, both mosquito and vertebrate. In the case of mosquitoes, high rainfall and temperatures can lead to higher population densities of species belonging to all genera, particularly *Aedes*. Climate therefore has a considerable effect on the epidemiology of arbovirus infections.

Apart from some work in the temperate, moist highlands and subtropical, moist lowlands of Zimbabwe, the areas of southern Africa lying outside South Africa have largely only been studied for the presence of mosquito-borne viruses by means of serology, without virus isolation from wild-caught mosquitoes being attempted. There is considerable arbovirus activity in the tropical region of Mozambique and the Zambezi valley, and in the subtropical, moist northern belt that extends westwards from Zimbabwe and embraces the

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Table 6.1 Mosquito-borne viruses causing disease in livestock and/or humans in southern Africa

	VIRUS	MOSQUITO VECTOR	WILD VERTEBRATE HOST	HOSTS SHOWING DISEASE	
				HUMANS	LIVESTOCK
Togaviridae					
Alphavirus	Chikungunya	*Ae. (Dic.) furcifer*	Primates	+	−
	Sindbis	*Cx. (Cux.) univittatus*	Birds	+	−
Flaviviridae					
Flavivirus	Banzi	*Cx. (Eum.) rubinotus*	Rodents	+	+[a]
	Wesselsbron	*Ae. (Neo.) circumluteolus* *Ae. (Neo.) mcintoshi* *Ae. (Och.) caballus/juppi*	Rodents?	+	+
	West Nile	*Cx. (Cux.) univittatus*	Birds	+	+[a]
Bunyaviridae					
Phlebovirus	Rift Valley fever	*Cx. (Cux.) theileri* *Cx. (Cux.) zombaensis* *Ae. (Neo.) circumluteolus* *Ae. (Neo.) mcintoshi* *Ae. (Och.) caballus/juppi*	Antelope?	+	+

a Disease produced after inoculation with virus in laboratory

Okavango Swamps in northern Botswana, and the Caprivi Strip and Etosha Pan in north-eastern Namibia.[13, 58, 62]

Arbovirus activity in South Africa is more or less confined to the subtropical and tropical Lowveld of the Limpopo and Mpumalanga provinces, to the KwaZulu-Natal coastal lowlands, and to the temperate, high inland plateau of the Karoo and Highveld. These regions all fall in the summer rainfall area. The inland plateau has virtually no rain from May to November, while in the winter, from June to August, night temperatures are often near freezing and periodically fall below this. In the Limpopo Province–Mpumalanga Lowveld and KwaZulu-Natal lowlands, winters are dry but mild.

Records of rainfall in the summer rainfall region of South Africa since 1910 reveal a 20-year oscillation between decades of above and below average rainfall.[111] The 1950s was a decade of high average rainfall, the 1960s below average and the 1970s again above average. During the period 1957 to 1979, the major outbreaks of WN, CHIK and RVF virus infections in humans and domestic animals have occurred mainly during periods of above average rainfall.

During times of heavy rain certain parts of the inland plateau, notably the panveld area of the western Free State and northern Eastern Cape provinces, may become so saturated that large pans and areas of pasture are flooded. Such accumulations of water may remain standing on farms for several months, particularly if there is a granite underlay as in the case of the Kaap Plateau. Under these conditions large populations of floodwater *Aedes* mosquitoes are produced initially, which are subsequently replaced by equally large populations of *Culex* mosquitoes. At such times epidemics of RVF may occur, but the extremely high densities of mosquitoes and other biting flies are also detrimental to livestock in other ways. These include reduced milk production by dairy cows and the development of a poor general state of health in sheep and cattle. Sheep may even die from loss of blood as a result of the continual biting by vast numbers of insects. This is probably the main reason why mosquito control in South Africa is necessary. However, effective large-scale mosquito control on farms would be difficult and very expensive to carry out.

Virus isolations from mosquitoes

Table 6.2 lists the six mosquito species which yielded multiple isolations of RVFV when collected on farms during RVF epidemics among sheep and cattle. The largest number of isolations on the inland plateau came from *Culex theileri* (**Figure 6.1**) and in the KwaZulu-Natal lowlands from *Cx. zombaensis*. Fewer isolations were made from aedine mosquitoes because collecting only began once an epidemic was already well under way, by which time the aedine populations were waning. All the *Aedes* spp. given in **Table 6.2** are floodwater mosquitoes belonging to the subgenera *Neomelaniconion* (*Neo.*) and *Ochlerotatus* (*Och*). *Aedes* (*Neo.*) *mcintoshi* and *Ae.* (*Och.*) *juppi* are illustrated in **Figures 6.2 and 6.3** respectively.

As shown in **Table 6.3**, 43 isolations of WSLV have been made from mosquitoes collected in South Africa and 15 from mosquitoes collected in Zimbabwe. Apart from two, all these isolations were made from floodwater aedine mosquitoes. However, RVFV isolations were obtained from numerous *Culex* as well as *Aedes* spp. Like RVFV (**Table 6.2**), the most important *Aedes* spp. involved with WSLV were *Ae.* (*Neo.*) *circumluteolus* (KwaZulu-Natal lowlands), *Ae.* (*Och.*) *caballus/juppi* (inland plateau), *Ae.* (*Neo.*) *mcintoshi/luridus* (inland plateau) and *Ae.* (*Neo.*) *mcintoshi* (Zimbabwe highlands).

From 1956 to 1980, 139 isolations of WNV were made from mosquitoes in South Africa. Of these, 136 came from mosquitoes collected on the inland plateau and three from

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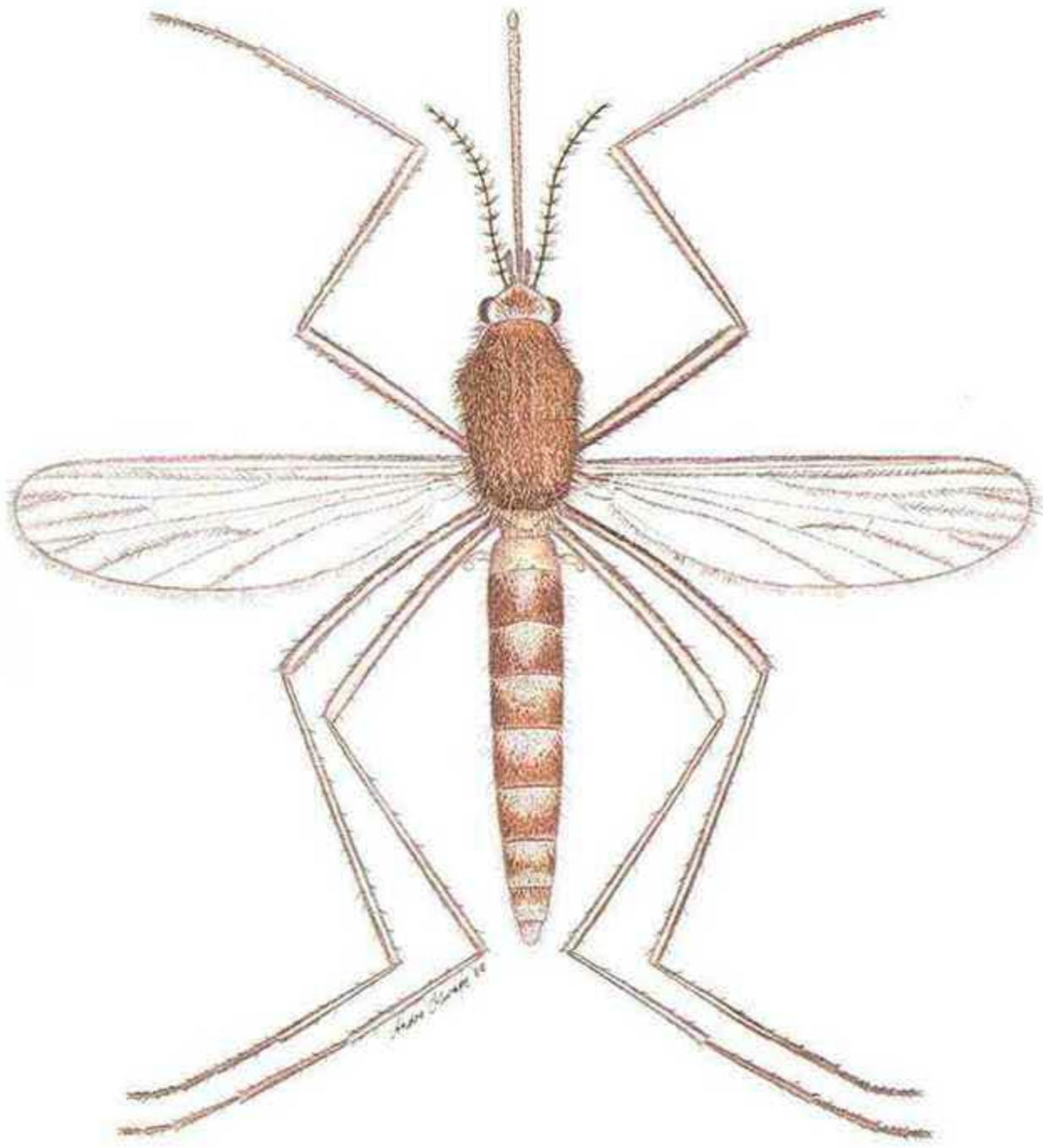


Figure 6.1 *Culex theileri*

the KwaZulu-Natal coastal lowlands. On the plateau, 128 of the isolations were from *Culex univittatus* which occurs throughout this region.[83] There was a large epidemic of WN fever in the Karoo and Northern Cape Province in 1974, when the infection rate of the *Cx. univittatus* population reached 39 infected mosquitoes per 1 000 at Upington.[90] The low incidence of WNV in the KwaZulu-Natal coastal lowlands is apparently due to the poor susceptibility of *Culex neavei* to infection.[52] *Culex neavei* replaces *Cx. univittatus* allopatrically in the KwaZulu-Natal lowlands, where it is apparently the main vector.[36, 83, 85, 113]

Banzi virus has been isolated almost exclusively from *Culex* (*Eumelanomyia*) *rubinotus* at Harare in Zimbabwe and at Port Shepstone, Empangeni and Mtubatuba on the KwaZulu-Natal coast. Infection rates in this species have often been high.[91]

Vector competence

Values for the 50 per cent infection threshold and the transmission rate determined in horizontal biological transmission tests for six species of mosquito with RVFV are given in **Table 6.2**. Data indicating the approximate length of the extrinsic incubation period for each species are also shown. On the basis of these results the order of vectorial importance is *Cx. theileri*, *Ae. circumluteolus*, *Cx. zombaensis*, *Ae. mcintoshi* and *Ae. juppi*.

Table 6.4 gives the results for preliminary tests on three more mosquito species, all of which had high infection rates after feeds of high titre of virus, and which subsequently transmitted the virus. Comparison of the results for *Aedes unidentatus* (**Table 6.4**) with those for *Ae. mcintoshi* (summarized in **Table 6.2**) shows that *Ae. unidentatus* is a more efficient vector.

Field studies during RVF epidemics have shown only low infection rates in the mosquito vectors, often no more than one infected mosquito per 1 000.[75, 89] In addition, high vector competence evidently requires high levels of viraemia in the vertebrate host.[45, 84, 89] Despite this, a striking feature of RVF epidemics among sheep on the inland plateau of South Africa is the rapidity with which infection spreads through flocks, causing heavy losses. To account

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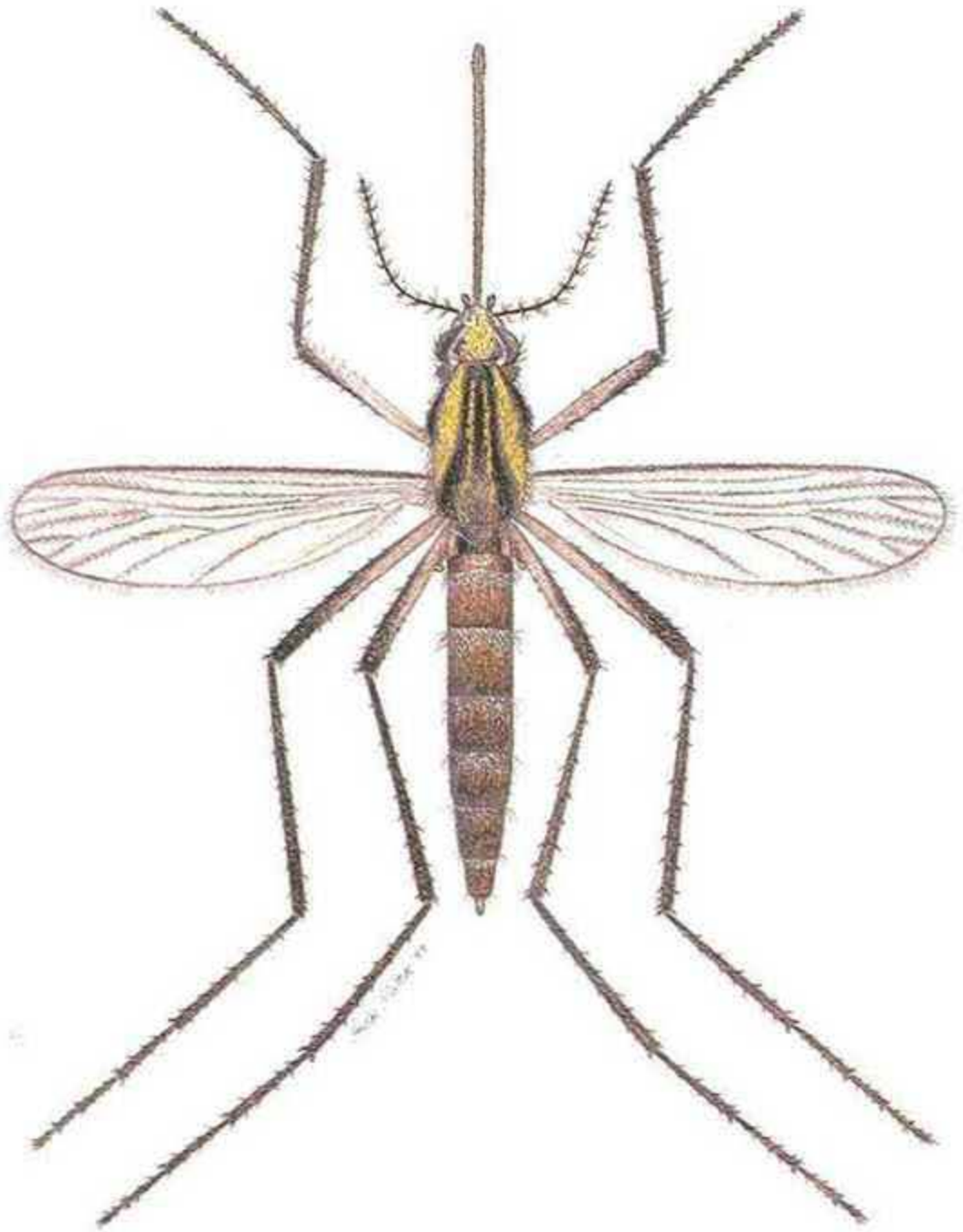


Figure 6.2 *Aedes (Neo.) mcintoshi*

Table 6.2 Southern African mosquito species yielding multiple isolations of Rift Valley fever virus from 1953 to 1981: number of isolations and results of transmission experiments

REGION	SPECIES	NO. ISOLATIONS	50% INFECTION THRESHOLD[a] $LOG_{10}LD_{50}$/ml	TRANSMISSION RATE[b] (DAYS POST-INFECTION)	REFERENCES
SOUTH AFRICA:					
KwaZulu-Natal	*Cx. (Cux.) zombaensis*	7	*c.* 7,5	3%, 41%, 42%, (14, 22, 29)	56, 92
coastal lowlands	*Ae. (Neo.) circumluteolus*	3	*c.* 7,5	32–38% (16–31)	54, 56, 92
SOUTH AFRICA:					
Inland plateau	*Cx. (Cux.) theileri*	22	*c.* 5,7	13–35% (15–22)	22, 41, 75, 84, 89
	Ae. (Och.) juppi	3	8,7	6% (17)	89
	Ae. (Och.) caballus s.l.	6	8,0[c]	0%[c] (12, 13)	22, 89
	Ae. (Neo.) mcintoshi[d]	2	8,1	17% (10,17)	89
ZIMBABWE HIGHLANDS	*Ae. (Neo.) mcintoshi*[d]	2			75

a The titre of virus needed to infect 50 per cent of the mosquitoes
b The proportion of infected mosquitoes transmitting virus to hamsters
c Experiments done with *Ae. caballus* s.s.
d Reported as *Ae. lineatopennis*

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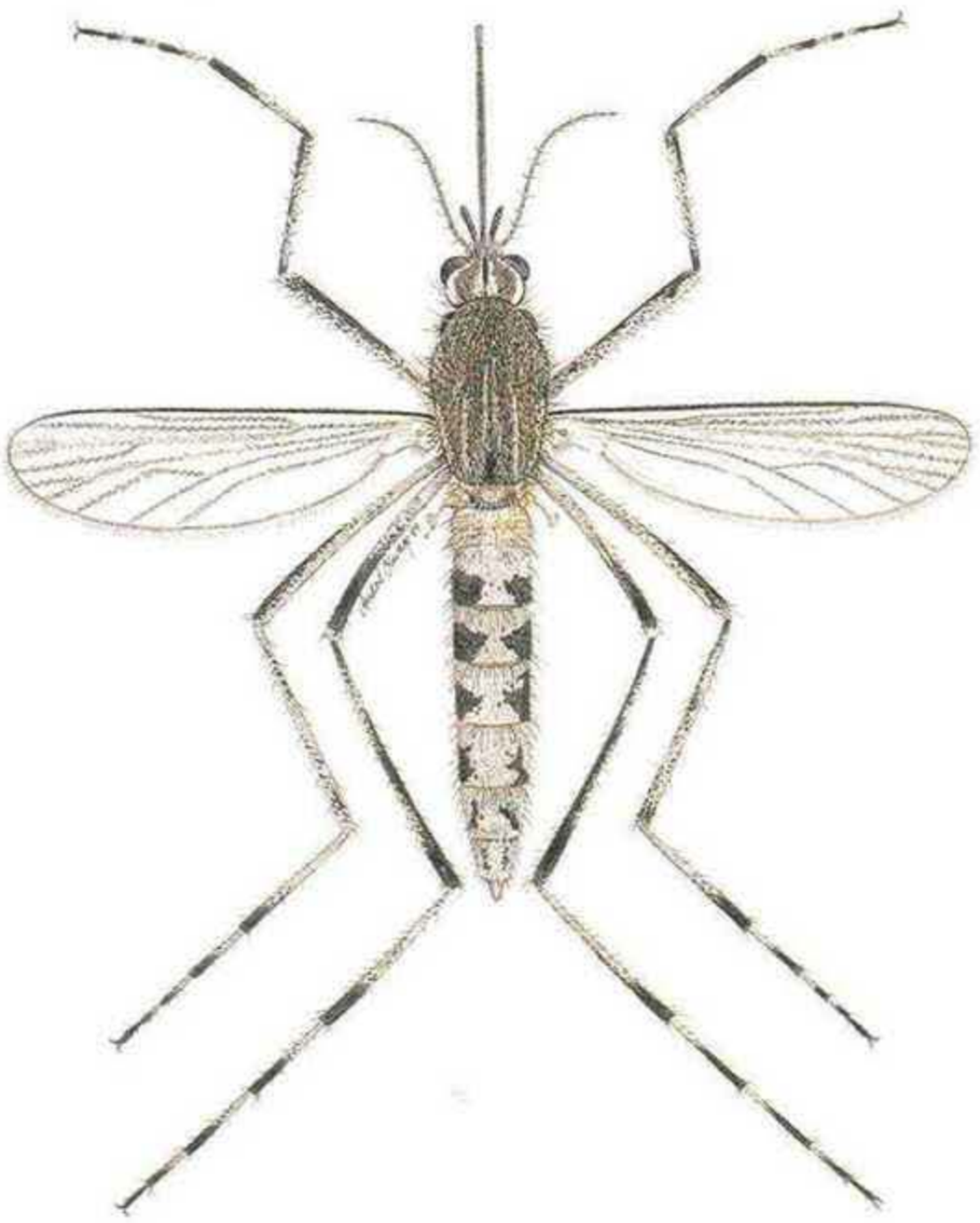


Figure 6.3 *Aedes (Cch.) juppi*

for the high level of infection, biological infection by mosquitoes is almost certainly augmented by mechanical transmission by various biting flies. In mechanical transmission there is no viral replication in the vector, only a passive transfer of virus. Experimental mechanical transmission of RVFV has been accomplished by various haematophagous Diptera: *Glossina morsitans, Stomoxys calcitrans, Lutzomyia longipalpis, Culicoides variipennis,* and several species of *Aedes* and *Culex*.[26, 55] In interrupted transmission tests from viraemic to susceptible hamsters by the bite of *Aedes aegypti formosus*,[55] transmission was achieved by groups of mosquitoes for up to 25 minutes after the infective feed but not after 5,5 hours. After shorter intervals of 3 to 5 minutes, single
mosquitoes were also shown to transmit the virus on three occasions.

The results of earlier vector competence tests with WSLV are shown in **Table 6.5**. They indicate that *Cx. theileri* can be excluded as a vector, while *Ae. circumluteolus, Ae. caballus* s.l. and *Cx. zombaensis* are potential vectors.

Laboratory evaluation of all the common mosquito species occurring in the Highveld as vectors of WNV showed that *Cx. univittatus* is by far the most efficient vector, with a low 50 per cent infection threshold of about 2,3 log LD_{50}/ml and a high transmission rate of 97 per cent.[38, 40]

Owing to the high infection rates of BANV in natural populations of *Cx. rubinotus* at Port Shepstone, it was possible to conduct a transmission experiment in the field in which individual hamsters were exposed to 16 groups of between 4 and 19 wild-caught mosquitoes. Banzi virus isolates were made from the mosquito groups that had fed on 11 out of 16 hamsters, which suggested a high degree of vector efficiency.[81]

Ecology of mosquito vectors

Culex (Cux.) theileri

Culex spp. deposit their egg rafts on the water surface, where, depending on the temperature, they hatch within two or three days. These eggs cannot resist drying, hence the preferred types of larval habitat are permanent or semi-permanent ground pools, although temporary pools are sometimes used. *Culex theileri* is the most abundant and consistently prevalent mosquito throughout the inland

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plateau region of South Africa, but it is usually rare on the KwaZulu-Natal coast and in the Limpopo Province–Mpumalanga Lowveld.[48, 53, 89, 112] This species feeds on goats, cattle, sheep and humans, mainly at ground level during the first three hours after sunset. It also feeds to a limited extent in the tree canopy as it is moderately ornithophilic.[1, 37, 48, 53] The larvae are found in a wide range of ground pools varying from clear vleis with emergent vegetation to pools highly polluted with organic waste from cattle.[28, 34]

Culex (*Cux.*) *zombaensis*

This species is common in the northern KwaZulu-Natal coastal plain,[18, 85, 86, 92] but rarer in the southern part.[49] It is mainly a ground frequenting species which feeds nocturnally on cattle, sheep, goats, antelope and humans.[86, 92, 99] *Culex zombaensis* seems more selective in its choice of larval habitat than *Cx. theileri* and is probably more confined to cleaner water with emergent vegetation, such as river margins and permanent pans.

Culex (*Cux.*) *univittatus*

This mosquito occurs throughout the Highveld and Karoo regions and is usually one of the most abundant *Culex* spp. taken in catches.[48, 53, 112] It is predominantly ornithophilic but sometimes feeds on humans and other mammals.[1, 37, 48, 53] It feeds nocturnally, particularly in the tree canopy but also at ground level. While this species is only slightly anthropophilic, it has been shown to feed on humans indoors to some extent (endophagy).[37] Epidemics of WN fever are probably partly a result of increased feeding on humans when particularly high densities of the vector occur, as they did at Upington in 1974.[90] Precipitin tests on blood meals of *Cx. univittatus* collected on a cattle farm near Johannesburg showed that 37 per cent of them were of mammalian origin, and 55 per cent of these mammalian meals were from cattle. *Culex univittatus* will utilize temporary, semi-permanent and permanent ground pools as larval habitats, although it prefers sites with clear water and emergent vegetation.[28, 34] Reed beds at the margins of the Gariep (formerly Orange) River were probably utilized at Upington in 1974 and 1976 when densities of *Cx. univittatus* were high there. It appears to be important for *Cx. univittatus* adults and larvae to enter a state of quiescence to overwinter, although the available evidence indicates that adults pass the winter in a nulliparous rather than a parous condition.[35, 39]

Table 6.3 Number of isolations of Wesselsbron virus from mosquitoes collected in southern Africa

REGION	SPECIES	NO. ISOLATIONS	REFERENCES
South Africa: KwaZulu-Natal coastal lowlands	*Ae. (Neo.) circumluteolus*	13	5, 77, 106, 113
	Ae. (Adm.) minutus	1	113
	Ae. (Adm.) veeniae	1	9
	Mansonia (Mnd.) uniformis	1	60, 113
	Cx. (Cux.) neavei[a]	1	113
South Africa: Inland plateau	*Ae. (Neo.) mcintoshi*[b]/*albothorax*[c]	3	60
	Ae. (Neo) mcintoshi/luridus	5	46
	Ae. (Och.) caballus s.l.[d]	13	60
	Ae. (Och.) juppi/caballus	5	46
Zimbabwe highlands	*Ae. (Neo.) mcintoshi*[b]	15	7, 75, 77

a Reported as *Cx. (Cux.) univittatus*
b Reported as *Ae. (Neo.) lineatopennis*
c Probably *Ae. (Neo.) luridus*
d Could be *Ae. caballus* s.s. or *Ae. juppi*

Culex (*Eum.*) *rubinotus*

Culex rubinotus can be collected in reasonable numbers only within or near its preferred habitat. This is dense marshland vegetation, always associated with permanent water such as lagoons, rivers or streams, and dams or lakes. Rank reeds, papyrus or grass are always present but trees are either few or absent. Although this type of habitat occurs on the inland plateau, it is particularly common on the KwaZulu-Natal coast, and large populations of the mosquito have been sampled at Mtubatuba and Empangeni.[91] The mosquito probably seeks out this dense vegetation because of its sensitivity to wind and low humidity. Large numbers have also been taken in mature sugar cane fields when these adjoined the natural habitat.[51]

Culex rubinotus feeds primarily on rodents, to a moderate degree on domestic ungulates and rarely on humans.[51] The feeding rate is high on hamsters in mosquito traps placed on the ground. Female mosquitoes confine their activity almost entirely to ground level and rarely enter traps in the tree canopy.[51]

Larvae of *Cx. rubinotus* have been collected in reed beds in shallow water.[51] Hopkins[28] recorded the larval habitats as being papyrus swamps and weedy or *Pistia*-covered borrow-pits.

Aedes (*Neo.*) *circumluteolus*

This is the predominant member of the subgenus *Neomelaniconion* to be found in the tropical coastal lowlands from northern KwaZulu-Natal in South Africa northwards into Mozambique. In Tongaland in northern KwaZulu-Natal it is often one of the most prevalent of all mosquitoes during the summer.[16, 50, 74, 85, 86, 113] It has been collected in much smaller numbers in other parts of northern KwaZulu-Natal at river margins or in marshes after rain, although it can apparently become quite prevalent at Lake St Lucia.[17] This is a floodwater species which oviposits at the edge of rivers and pans within the flood plain. Aedine eggs are resistant to

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Table 6.4 Results of vector competence tests with Rift Valley fever virus and three mosquito species: infective feeds on viraemic hamsters but transmission by the *in vitro* capillary method[45]

SPECIES	TITRE OF INFECTIVE FEED IN $LOG_{10}CPD_{50}$ PFU/ml[c]	MOSQUITO INFECTION		TRANSMISSION	
		DAYS AFTER INFECTIVE MEAL	RATE[a]	DAYS AFTER INFECTIVE MEAL	RATE[b]
Ae. (Neo.) unidentatus	6.8–7.8	11	12/14 (86%)	11	7/12 (58%)
Ae. (Adm.) dentatus	6.8–7.8	11	28/31 (90%)	11	9/28 (32%)
		17	6/8 (75%)	18	3/6 (50%)
Cx. (Cux.) poicilipes	7.8	15	26/29 (90%)	15	4/26 (15%)
				30	24/30 (80%)

a Numerator = no. mosquitoes infected; denominator = no. mosquitoes tested
b Numerator = no. mosquitoes transmitting; denominator = no. infected mosquitoes feeding
c Cytopathic dose (CPD); Plaque forming units (PFU)

Table 6.5 Results of vector competence tests with various mosquito species and Wesselsbron virus

SPECIES	VIRAEMIC INFECTING HOST	BLOODMEAL TITRE $LOG_{10}LD_{50}$/ml	INFECTION RATE	SUSCEPTIBLE HOST/S	TRANSMISSION ATTEMPTS	REFERENCE
Ae. (Neo.) circumluteolus	Artificial	7.2	7/9	Mice	16/18[a]	97
Ae. (Och.) caballus s.l.	Mosquitoes collected in epidemic		NT	Lambs	1/1[a]	57
Ae. (Och.) caballus s.l.	Mosquitoes collected in epidemic	NT	Mice	1/31[a]	57	
Ae. (Och.) caballus s.l.	Lambs	NT	NT	Mice	2/2[a]	57
Cx. (Cux.) zombaensis	Hamsters	8.4	13/26	Hamsters	1/2[b]	8
Cx. (Cux.) theileri	Hamsters	6.7–7.2	21/52	Mice	0/15	8

a Numerator = no. successful transmissions; denominator = no. attempts with groups of mosquitoes
b Transmission rates
NT Not tested

desiccation as long as a high level of humidity is maintained within their microhabitat under the soil where, like *Ae. juppi*, they are probably deposited within the top 50 mm.[21] They do not hatch until they are inundated at the next river flooding. Local rainfall apparently has to be heavy and concentrated to cause surface flooding similar to that associated with river flooding.[50]

A study at Ndumu in KwaZulu-Natal showed that during the dry winter and spring months (June to October) *Ae. circumluteolus* declined to very low numbers, but it never completely disappeared. Blood-feeding and ovarian development occurred throughout the year.[50] *Aedes circumluteolus* is a ground feeder feeding during the day and also at night from about half an hour after sunset. It feeds avidly on cattle, sheep, antelopes and humans.[50, 86, 99]

Aedes (*Neo.*) *unidentatus* and *Aedes* (*Neo.*) *mcintoshi*

In 1971 McIntosh revised the subgenus *Neomelaniconion* in southern Africa.[74] He named two new species, *Ae. unidentatus* and *Ae. luridus*, making a total of eight species including *Ae. circumluteolus* and *Ae. lineatopennis*. Prior to this revision *Ae. unidentatus* may have been called *Ae. lineatopennis* in Johannesburg and at Lake Chrissie for example,[48] while *Ae. luridus* may have been confused with *Ae. albothorax*. In 1985 Huang described a new species, *Ae.* (*Neo.*) *mcintoshi*, and showed that African mosquitoes previously reported as *Ae. lineatopennis* really belonged to this species.[31] *Aedes lineatopennis* itself was shown to be confined to the Philippines. Thus, reports of *Ae. lineatopennis* in the literature after 1971 refer to *Ae. mcintoshi* but before 1971 could have been either *Ae. unidentatus* or *Ae. mcintoshi*.

Aedes unidentatus is probably more common in the moister eastern part of the Highveld and the middle veld of the Free State Province, where it is probably the numerically dominant species of the subgenus.[74] *Aedes mcintoshi* is a more widely distributed species adapted to regions with

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markedly different climates: it occurs in the Karoo, Highveld, Limpopo–Mpumalanga Lowveld, and the KwaZulu-Natal coastal lowlands of South Africa, and in the Zimbabwe highlands. In the northern part of the KwaZulu-Natal lowlands this species appears to be dominated by *Ae. circumluteolus*. Its prevalence relative to *Ae. unidentatus* and *Ae. luridus* in the Highveld and Karoo respectively is not yet properly known because of insufficient sampling. Large numbers of *Ae. mcintoshi* have recently been collected at Villiers in the north-eastern Highveld, where it was about as common as *Ae. unidentatus*. In southern Africa, the distribution of *Ae. mcintoshi* indicates that it is probably best adapted to regions of intermediate temperature.

Aedes unidentatus feeds both by day and night, with peak biting commencing about half an hour after sunset in Johannesburg. It feeds voraciously on goats, cattle, sheep and humans.[1, 48, 74] *Aedes mcintoshi* has the same host preferences but its peak biting in the eastern Highveld at Bultfontein in the Free State Province was from late afternoon until sunset, with a lesser peak at dawn.[46]

Larvae of *Ae. unidentatus* have been collected near Johannesburg in temporary rainwater pools in flooded pasture.[34] Both *Ae. unidentatus* and *Ae. mcintoshi* larvae occur in large numbers in flooded pans and, to a lesser extent, in vleis in South Africa.[4]

Aedes (*Och.*) *juppi* and *Aedes* (*Och.*) *caballus*

McIntosh made a taxonomic reassessment of *Aedes* (*Ochlerotatus*) *caballus* and found that mosquitoes that had been identified as this species before 1973 could have been either *Ae. caballus* s.s. or a new species, *Ae. juppi*, or a mixture of both these species.[76] From the limited number of locality records available for these two species[76] it appears that *Ae. juppi* is adapted to temperate conditions and is endemic to the temperate regions of southern Africa. It probably outnumbers *Ae. caballus* in the Highveld, and seems to be the only species in the western and coastal regions of the Western Cape Province. It is sympatric with *Ae. caballus* over a large area, including the Karoo and southern Free State, but the relative proportions of the two species require further elucidation. *Aedes caballus* is apparently adapted to higher temperatures and a lower rainfall than *Ae. juppi* and is absent from the coastal regions of KwaZulu-Natal and Mozambique.

Aedes juppi and *Ae. caballus* have very similar ecologies. Both feed avidly in the light and just before and after sunrise on goats, sheep, cattle and humans.[43, 53, 89] In the eastern Highveld *Ae. juppi* has its biting peak from late afternoon until sunset with a lesser peak at dawn.[46] Larvae of *Ae. caballus* s.l. have been recorded from small or large temporary pools or pans.[96] More recent studies have shown that the eggs of both species are laid in sedge or grassland within low-lying depressions (pans) and hatch to produce enormous numbers of larvae when these pans fill after heavy rainfall.[21, 42] Hence, larvae of the subgenus *Ochlerotatus* are found in similar habitats to those of the subgenus *Neomelaniconion*, and often species belonging to both subgenera are collected together at the same aquatic site on the inland plateau. Recent work at Bultfontein in the eastern Highveld has shown that in the case of eggs of *Ae. juppi* and *Ae. mcintoshi* in pans, the development cycle from egg to adult can be as short as five days after inundation.[46, 47]

Viral maintenance and occurrence of epidemics

Many years of work in southern Africa and in other African countries have failed to provide evidence supporting a continuous cryptic vertebrate–mosquito virus transmission cycle that serves to maintain RVFV during the inter-epidemic period. Evidence has, however, been obtained in southern Africa and Egypt that transmission by various mosquito species, notably *Culex* spp., occurs during epidemics in sheep and cattle.[22, 27, 89, 92] Virus isolation attempts over 20 years from some 40 000 mosquitoes trapped in RVF areas in KwaZulu-Natal during the inter-epidemic period failed to incriminate mosquitoes.[2]

Culex theileri and *Cx. zombaensis* are the important species on the inland plateau and KwaZulu-Natal coastal lowlands respectively. There is strong evidence that these species transmit RVFV biologically between sheep and cattle, and that mechanical transmission by several mosquito species and other biting flies is responsible for the maintenance of the epidemic. Humans are also thought to become infected on occasion through transmission by *Cx. theileri* and *Cx. zombaensis*, although some human infections during epidemics are undoubtedly caused by contagion during the slaughtering of infected animals.

In South Africa, transovarial transmission tests and testing of mosquitoes reared from field-collected eggs have been undertaken to try to incriminate various floodwater *Aedes* spp. as reservoir vectors of RVFV.[21, 45, 89] Such studies have so far been directed mainly at *A. juppi* and have indicated that this species has a low vector potential, that it is only a subsidiary epidemic vector, and that it is not involved in vertical transmission of the virus.

The paucity of isolations from floodwater *Aedes* during epidemics is partly because sampling of the mosquito populations for virus assay invariably began late in the course of an epidemic. Despite this, isolations have been made from *Ae. mcintoshi* and *Ae. juppi* on the inland plateau and from *Ae. circumluteolus* on the KwaZulu-Natal coastal lowlands. If one or more of these species should be responsible for transovarial transmission in the way that *Ae. mcintoshi* apparently is in Kenya (see further on), such infected mosquitoes probably occur only in a few endemic foci where RVFV erupts to trigger a new epidemic in sheep and cattle when climatic conditions are favourable. **Figure 6.4** summarizes the known epidemic cycle and the hypothetical maintenance cycle for RVFV in South Africa. Theoretically it might also be possible for some infected *Culex* spp. to over-

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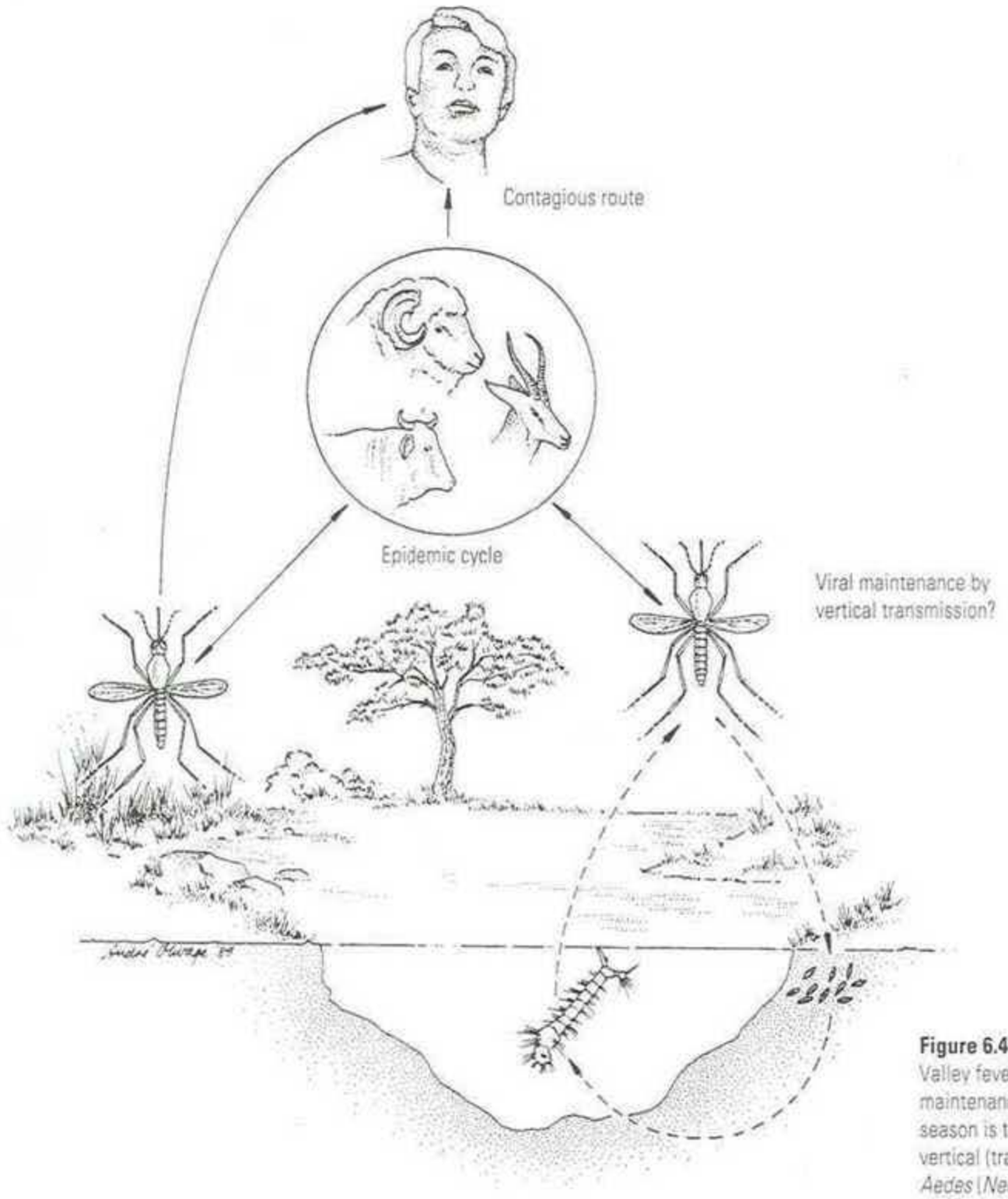


Figure 6.4 Transmission cycles of Rift Valley fever in southern Africa and Kenya; maintenance of virus through the dry season is thought to be by means of vertical (transovarial) transmission by *Aedes* (*Neomelaniconion*) species

winter and initiate transmission the next spring, especially as small numbers of *Cx. theileri* adults have been collected in the winter in Johannesburg.[35, 39]

Recently RVFV has emerged as a small outbreak in African buffalo (*Syncerus caffer*) at Skukuza in the Kruger National Park, Mpumalanga,[23] and the retrospective testing of buffalo sera from this game reserve has indicated the presence of the virus in this area at times when no epidemics were occurring in livestock in South Africa.[30] This suggests that RVF remains endemic in the reserve over extended periods.

Antibody surveys in humans and livestock have shown that WSLV is more common in the moist tropical part of southern Africa — northern coastal KwaZulu-Natal, Mozambique and Namibia — than on the inland plateau, the southern KwaZulu-Natal lowlands and the Cape provinces of South Africa.[58, 59, 80, 93, 105]

On the temperate South African plateau the *Ae. caballus-juppi* and *Ae. mcintoshi-luridus-unidentatus* group of mosquitoes are vectors. Along the tropical, eastern coastal plain of northern KwaZulu-Natal and Mozambique, *Ae. circumluteolus* is the most likely vector. Because the tropical areas of northern KwaZulu-Natal and Mozambique receive a higher rainfall, and are therefore moister than the drier temperate areas of South Africa, *Aedes* spp. are probably active there over longer periods. This would account for the higher level of WSLV activity in the tropical areas.[77]

The only evidence for the possible existence of a wild vertebrate host for WSLV is the single isolation of virus from the gerbil (*Desmodillus auriculatus*).[6] However, the absence of *Culex* vectors suggests that viral maintenance would be through transovarial transmission from one generation of floodwater *Aedes* to the next by vertical transmission, the

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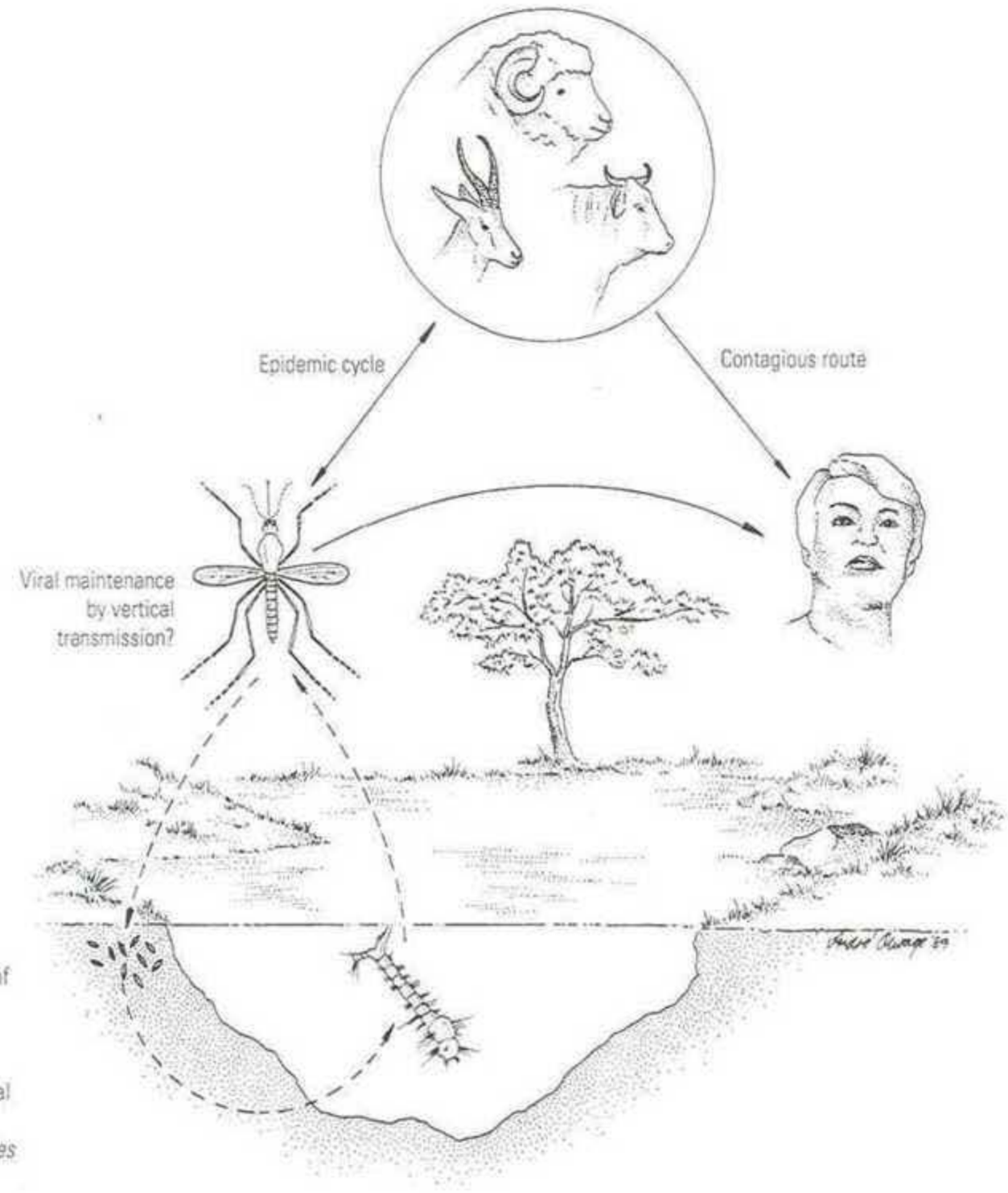


Figure 6.5 Transmission cycles of Wesselsbron virus in southern Africa: maintenance of virus through the dry winter is hypothetically by means of vertical (transovarial) transmission by *Aedes* (*Neomelaniconion*) or *Aedes* (*Ochlerotatus*) mosquito species

virus surviving dry periods in the eggs of these mosquitoes. Only one attempt has so far been made to show the presence of infected floodwater *Aedes* mosquitoes among those emerging from an inundated pan.[47] This was unsuccessful, probably because insufficient mosquitoes were tested. Domestic ungulates are important incidental hosts with which the vector could probably maintain the virus for quite long periods in the absence of a primary vertebrate maintenance host. Livestock, particularly adult sheep and cattle, are often inapparently infected. Humans can be infected either by contagion through handling infected carcasses or by mosquito bite (**Figure 6.5**).

During each summer on the inland plateau of South Africa, WN and SIN viruses are maintained in a feral cycle between *Cx. univittatus* and various avian species. The same mosquito, *Cx. univittatus*, also transfers infection to humans.[38, 44, 46, 87, 88, 90] An outbreak of WN fever in the human population will depend upon an epidemic situation first developing in the avian population. The exposure of sentinel pigeons at Bethulie in the Free State Province indicated the occurrence of such epidemics in April 1970 and April 1971.[82]

Serological surveys for neutralizing antibodies in livestock have shown a prevalence of 16 per cent for WNV in sheep and cattle on the Highveld generally, and rates of 21 to 47 per cent in older cattle, oxen and horses in Johannesburg.[61, 80] It appears, therefore, that livestock also become infected with WNV in the field, although calves show only a trace of viraemia after inoculation with virus.[3] Such infections might be expected sometimes to lead to the development of abnormalities in the foetuses of pregnant animals.[12]

Culex rubinotus maintains BANV among wild rodents. That rodents are indeed the feral vertebrate maintenance hosts is clear from aspects of the studies referred to above. Additionally, there is the isolation of virus from, and the presence of antibodies in, *Mastomys natalensis*,[79] plus the

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viraemic response of wild rodents to inoculation with the H336 strain of the virus.[73] The occurrence of *Cx. rubinotus* in other parts of Africa, with associated viral isolations, suggests that it is probably the vector of BANV over a large part of the continent.[24, 95]

Serological surveys in the KwaZulu-Natal coastal lowlands showed that neutralizing antibodies were present in cattle at rates of 2 to 16 per cent and in sheep at rates of 0 to 7 per cent.[59] This evidence, together with the moderate feeding of *Cx. rubinotus* on domestic ungulates, suggests that pregnant cattle, for example, could become infected, particularly if they were to browse near the marshy reed beds where *Cx. rubinotus* is prevalent. It would seem worthwhile to examine foetal material arising from natural abortions on the KwaZulu-Natal coast for the presence of the virus and developmental abnormalities. Whether bovine infections ever attain the level of an epidemic is not known. Banzi virus has been isolated from humans and is known to cause at least mild illness,[107] but the extent to which it causes morbidity in humans is uncertain.

Mosquito control

From the point of view of controlling RVF, prior immunization of livestock would be more cost-effective than vector control. However, for other reasons mentioned above, it may on occasion be advisable to institute some mosquito control measures to reduce the degree of exposure of stock to mosquito bites. Since the peak period for biting is from just before sunset until sunrise, and because mosquitoes are always active at or close to their breeding sites, stock should, if possible, be moved away from pans and vleis to higher ground before nightfall. In the case of dairy farms, the milking sheds should be made mosquito proof, and perhaps *Culicoides*-proof, and monthly sprays of their interior walls should be carried out with a residual insecticide other than an organochlorine. Organophosphate insecticides, such as malathion, fenthion (Baytex), fenitrothion (Sumithion), or carbamates such as carbaryl (Sevin) and propoxur (Arprocarb), can be used at rates of 1,5 to 2,0 g/m^2.[104]

Control of adult mosquitoes outdoors would probably be neither effective nor economical. Outside control could, however, be directed at larvae in the main breeding pans, vleis and dams. Larvicides such as Temephos (Abate) can be incorporated into sand granules or pellets and sprinkled into the breeding place. In the case of pans, a dry pan could be seeded with such pellets just before a flood is expected so that the insecticide is released into the water as the aedine eggs hatch. An alternative larvicide is the bacterium, *Bacillus thuringiensis israelensis*, whose spores can be incorporated into pellets. This may become practicable if its present high cost is reduced. The emphasis should be on a control programme with measures that are selective and integrated.

Recently a technology was developed using polar-orbiting meteorological satellites whereby data on green-leaf vegetation dynamics, ground moisture and rainfall patterns can be collected over wide areas of Kenya.[66, 67] It is hoped such data will enable predictions to be made as to when and where dambos (pans) would be flooded. This would allow veterinary authorities time to vaccinate livestock before an outbreak of RVF occurred.

Sub-Saharan Africa, excluding southern Africa

Introduction

In African countries outside southern Africa and south of the Sahara, RVF has periodically caused epidemics in livestock in Kenya and in the Senegal River Basin of northern Senegal and southern Mauritania. The most recent outbreak in Kenya was a very large epidemic which occurred both in the Garissa region of north-east Kenya and in Somalia in 1998, which subsequently spread to the plains of the Great Rift Valley and northern Tanzania.[11] These outbreaks have followed unusually heavy rainfalls except in the Senegal River Basin, where damming the river caused widespread flooding. During active surveillance for arboviruses in mosquitoes in West Africa and the Central African Republic, multiple isolations of WSLV have been made (**Table 6.7**) although the mosquito species concerned have yet to be incriminated in the transmission of WSLV to cause disease in sheep or goats. Only occasional isolation of WSLV have been from mosquitoes in Uganda,[25] Kenya[95] and the Cameroon[102] that have not been associated with any livestock disease.

The only other mosquito-borne virus that appears to have veterinary importance is the Semliki Forest virus, which is an *Alphavirus* (Togaviridae) that has been the cause of encephalitis in horses in Senegal.[14]

Virus isolations from mosquitoes and their vector competence

Table 6.6 lists the eight mosquito species which yielded multiple isolations of RVFV together with the results of vector competence experiments where these have been undertaken. *Culex pipiens* from Egypt is included as its level of vector importance appears to be similar to *Cx. theileri* in South Africa. It is likely that the epidemic vector involved in the 1978 Egyptian epidemic was *Cx. pipiens molestus* — this subspecies not only feeds on livestock but is also highly anthropophilic, which could account for some of the human cases that occurred. The other species listed were either collected during epidemics of RVF or during interepidemic periods in Kenya and Senegal. Two of the three species implicated as vectors in Kenya, *Ae. mcintoshi* and *Cx. zombaensis*, were the same as those important in southern Africa

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Table 6.6 Mosquito species from African countries outside southern Africa yielding multiple isolations of Rift Valley fever virus; number of isolations and results of transmission experiments

COUNTRY	SPECIES	NO. ISOLATIONS	50% INFECTION THRESHOLD $LOG_{10}LD_{50}$/ml	TRANSMISSION (DAYS POST-INFECTION)	REFERENCE
Egypt	*Cx. (Cux.) pipiens*	3	<6.5	+ (7–12)	27, 94
Kenya	*Ae. (Neo.) mcintoshi*	10^{a}	<5.7	+ (7)	15, 70, 100
	Cx. (Cux.) antennatus	3	<5.3	+ (7–21)	70, 110
	Cx. (Cux.) zombaensis	6	ND	ND	70, 72
Senegal	*Ae. (Adm.) dalzieli*	4	ND	ND	20
	Ae. (Adm.) fowleri	–	c. 6.3	+ (7)	70, 109
	Ae. (Adm.) ochraceus	3	ND	ND	19
	Ae. (Adm.) vexans	10	ND	ND	115

a pools of female mosquitoes collected directly in the field;
6 pools of reared female mosquitoes
2 pools of reared male mosquitoes
All isolations from other species were from female mosquitoes
ND Not done

(**Table 6.2**). Of the 10 isolations made from *Ae. mcintoshi*, one was made during an epidemic[15] while the remainder were made during an interepidemic period.[70] In the latter study, eight of the nine isolations came from mosquitoes reared from field-collected larvae (**see Table 6.6, footnote**). *Culex antennatus*, the remaining species, has never been prevalent during outbreaks of RVF in southern Africa. The vector competence tests done on Kenyan *Ae. mcintoshi* and *Cx. antennatus* showed they were both highly susceptible to RVFV. The higher susceptibility of Kenyan over South African *Ae. mcintoshi* may indicate that the mosquitoes tested from the two different countries represent different species. This would seem likely as members of the *Neomelaniconion* subgenus are morphologically very similar. All the isolations of RVFV from Senegal were obtained from floodwater *Aedes* mosquitoes belonging to the subgenus *Aedimorphus* (*Adm.*). *Aedes vexans* is apparently the most important of these vectors, although none of the three species concerned has been evaluated for vector competence. However, another *Aedimorphus* species, *Ae. fowleri*, has been shown to be susceptible and able to transmit RVFV, which suggests that other members of the subgenus could be shown to be laboratory vectors.

Although WSLV occurs in a number of sub-Saharan African countries outside southern Africa, only single isolations have been made in Kenya from a pool of *Aedes* (*Adm.*) *dentatus* mosquitoes[95] and in Cameroon and Guinea from various *Aedes* spp.[108] Multiple isolations of the virus have, however, been obtained from nine different mosquito species collected in the Ivory Coast, Senegal and the Central African Republic[10] (**Table 6.7**). Out of these 59 isolations, most (45 isolates) came from *Aedimorphus* species and it is thought that these floodwater mosquitoes parallel the *Neomelaniconion* and *Ochlerotatus* floodwater *Aedes* spp., which are the vectors in South Africa.

Table 6.7 Multiple isolations of Wesselsbron virus from mosquitoes collected in West Africa and the Central African Republic (CAR)[10]

COUNTRY	SPECIES	NO. ISOLATIONS
Ivory Coast, CAR	*Ae. (Adm.) abnormalis* grp	11
Ivory Coast, Senegal	*Ae. (Adm.) dalzieli*	13
Senegal	*Ae. (Adm.) minutus*	7
Ivory Coast, CAR	*Ae. (Adm.) tarsalis* grp	11
Senegal, CAR	*Ae. (Adm.) vittatus*	3
Ivory Coast, CAR	*Ae. (Stg.) africanus*	5
Ivory Coast, Senegal	*Ae. (Stg.) luteocephalus*	3
CAR	*Ae. (Stg.) opok*	3
Ivory Coast, CAR	*Cx. (Cux.) perfuscus*	3

Ecology of mosquito vectors

Aedes (*Neo.*) *mcintoshi*

Recent studies on the ecology of *Ae. mcintoshi* near Nairobi in the Kenya highlands have indicated a behaviour pattern similar to that of South African populations. Larvae have been collected in very large numbers in dambos (pans) on bushed grassland.[68] Adult females feed avidly on cattle and humans, with peaks just before sunset and just after sunrise.[69, 71] The dispersal distance from a dambo was found to be 0.07 and 0.15 km for males and females respectively, and the daily survival rate 8.82 and 0.85.[65]

Aedes (*Aedimorphus*) spp.

Little information has been published on the breeding places of the Senegalese *Aedimorphus* floodwater *Aedes* mosquitoes, although Zeller and co-workers state that *Ae. dalzieli* occurred in small ground pools located in the flood plain of temporary rivers in the gallery forests.[115] In the USA, *Ae. vexans* larvae also occur in the small ground pools as well as

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in the larger bodies of water formed in the flood plain of rivers.[29] Apparently all three species of *Aedimorphus* that have been incriminated as vectors are mosquitoes that breed in temporary ground water, although whether they utilize the dambo, or are confined to small pools, is not known. They feed on cattle and sheep[19] and their behaviour is generally similar to that of the *Neomelaniconion* and *Ochlerotatus* subgenera including a very short development period from egg to adult. Fontenille and co-workers observed that *Ae. vexans* deposited eggs on the soil of temporary ground pools in the dry Sahelian regions of Senegal and that adult mosquitoes appeared only four days after the rain.[19]

Viral maintenance and occurrence of epidemics

As mentioned above, RVF virus has been isolated from mosquitoes collected in Kenya during an interepidemic period.[70] These included eight isolations from adult *Ae. mcintoshi* (reported as *Ae. lineatopennis*) that had been collected as larvae or pupae reared to adulthood in the laboratory. These immatures came from newly flooded dambos in bushed grassland near Nairobi. The only other isolations from aedine mosquitoes were from wild-caught *Ae. cumminsi* adults and another from wild-caught *Ae. mcintoshi* adults. This important finding provides evidence that RVFV can be maintained throughout interepidemic periods in a state of dormancy in the eggs of *Ae. mcintoshi* and can then be transmitted transovarially to the next generation of mosquitoes. *Aedes mcintoshi* is therefore considered to be important in the generation of epidemics by seeding primary virus in cattle and sheep and susceptible wildlife species. Subsequently, other mosquitoes, *Culex* and *Anopheles* spp., colonizing temporary and permanent water in the epidemic areas, are able to propagate the virus in a mosquito-to-sheep/cattle transmission cycle to maintain an epidemic.

In Senegal, West Africa, some of the virus isolations included in **Table 6.6** — those from *Ae. ochraceus* and *Ae. Vexans*, both *Aedimorphus* species — were made when no epidemic of RVF was in progress. This evidence, together with other evidence from serological studies in livestock led Zeller and his colleagues[115] to conclude that there was some endemic maintenance of this virus in the Sahelian region and that these two *Aedimorphus* species should be regarded as interepidemic vectors. They considered that these species fulfil this role in West Africa, which the *Neomelaniconion, Ae. Mcintoshi*, fulfils in Kenya. They pointed out, however, that while epidemics in East Africa are associated with increased rainfall, this same relationship has not been established in the Sahelian areas.[115] For example, the RVF activity described in southern Mauritania in 1982 to 1985 occurred during a period of drought, and the 1993 epidemic was not associated with extensive rainfall.[103, 114] It is noteworthy that Zeller *et al.*[115] made no isolations from *Culex* spp. during the interepidemic period as it strengthens the conclusion that *Cx. theileri* in South Africa and *Cx. pipiens* in Egypt are only epidemic vectors and probably do not have a viral maintenance function.

There have been no field studies on WSLV outside southern Africa that implicate particularly mosquito vectors in the maintenance of the virus or in the transmission of virus during epidemics. However, it is likely that the *Aedimorphus* floodwater *Aedes* contribute towards maintenance of WSLV by transovarial transmission. Experiments need to be carried out with *Aedimorphus* species, such as *Ae. dalzieli*, to see whether such transmission can be demonstrated in the laboratory.

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7

Classification, epidemiology and control of arthropod-borne viruses

R SWANEPOEL

Introduction

As recently as the late 1960s the term arboviruses, a partial acronym derived from arthropod-borne viruses, was used in a taxonomic sense to denote the existence of an inferred underlying relationship between viruses with similar modes of transmission. After morphological and physicochemical characterization, it became evident that the viruses actually belonged to a range of widely disparate families, and since then there has been a tendency to stress the diversity of the viruses, at the cost of overlooking shared epidemiological features which dictate a common approach to the investigation of arbovirus diseases. Indeed, the study of the arthropod-borne viruses has always been a specialized branch of virology in which there is, nevertheless, a need for co-operation between such varied investigators as virologists, medical and veterinary clinicians, entomologists, zoologists, ecologists and climatologists, among others. Except where otherwise indicated, the following outline of the subject is drawn from a number of general accounts of arboviruses.[22, 109, 228, 240]

For centuries it was suspected that certain diseases were transmitted by arthropods, but it was not until 1878 that Patrick Manson demonstrated in China that mosquitoes serve as vectors for the filarial worm *Wuchereria bancrofti*. In 1893 Smith and Kilborne established that ticks transmit the babesia, which causes Texas redwater fever of cattle, and between 1895 and 1898 Ronald Ross performed investigations in India that proved that mosquitoes transmit the parasite of malaria. Shortly thereafter, in 1900, Walter Reed and his associates in Cuba demonstrated human to human transmission of the virus of yellow fever by mosquitoes, as had been postulated earlier by Carlos Finlay. Not only did this constitute the first proof of transmission of a virus by arthropods, but it was also the first occasion on which evidence was produced to indicate that an agent causing disease in humans, as opposed to lower animals, was caused by a virus. However, it was not until 1927 that Stokes and his co-workers in Nigeria succeeded in maintaining yellow fever virus by serial passage in monkeys. By then, several arboviruses of veterinary significance had been isolated, the first two being African horse sickness and bluetongue viruses in South Africa. Although the exact dates and authorship of isolation of these two viruses are debatable, they had been maintained by subinoculation in horses and sheep respectively, long before they were shown to be filterable agents or were adapted to laboratory hosts.[4, 65, 164, 176, 216, 217, 239]

By 1939, 16 arboviruses had been isolated (**Table 7.1**), although this figure is somewhat misleading as it does not take into account that African horse sickness and bluetongue viruses are each made up of multiple serotypes. Of the 19 arboviruses isolated between 1940 to 1949 (**Table 7.2**) eight were human pathogens from various parts of the world, but 11 were viruses of unknown significance that had been isolated from mosquitoes and a sentinel monkey in the course of yellow fever investigations in which the Rockefeller Foundation Virus Laboratories (RFVL) of New York played a leading role. During the Second World War, RFVL concentrated on the investigation of yellow fever, but during the 1950s it seconded staff to laboratories in many parts of the world, including what is now known as the National Institute for Communicable Diseases in Johannesburg, to establish and foster arbovirus units which were intended to continue operating as regional centres with locally trained staff.

The newly established arbovirus unit in South Africa, initially under the leadership of Smithburn of RFVL, conducted surveys for antibodies to a range of arboviruses on sera collected from humans and domestic animals in South Africa,[62, 124, 127, 148, 155, 213] Mozambique,[123] Angola,[116] Namibia and Botswana.[129] The highest prevalence of antibodies was found in sera from Tongaland, a subtropical area occupying the coastal plain of the northern part of KwaZulu-Natal Province in South Africa, and consequent efforts to isolate new viruses were concentrated mainly on this area. In the decade from 1950 to 1959, members of the Onderstepoort Veterinary Institute isolated Wesselsbron virus from a lamb in the Wesselsbron district of the Free State Province, while

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Table 7.1 Arthropod-borne viruses isolated throughout the world prior to 1940. Information adapted from Karabatsos (1985)[109]

VIRUS	ORIGINAL SOURCE	YEAR
Bluetongue	Sheep blood	1904*
African horse sickness	Horse blood	1905*
African swine fever	Pig blood	1910
Nairobi sheep disease	Sheep blood	1910
Vesicular stomatitis – Indiana	Cow tongue epithelium	1925
Yellow fever	Human blood	1927
Louping ill	Sheep brain	1929
Rift Valley fever	Sheep blood	1930
Western encephalitis	Horse brain	1930
Eastern encephalitis	Horse brain	1933
St Louis encephalitis	Human brain	1933
Japanese encephalitis	Human brain	1935
Sofjin **	Human brain	1937
Bwamba	Human blood	1937
West Nile	Human blood	1937
Venezuelan encephalitis	Horse brain	1938

* See text for derivation of the dates given here for isolation of bluetongue and African horse sickness viruses

** Sofjin = prototype strain of Russian spring-summer encephalitis virus

Table 7.2 New arthropod-borne viruses isolated throughout the world from 1940 to 1949. Information adapted from Karabatsos (1985)[109]

VIRUS	ORIGINAL SOURCE	YEAR	COUNTRY
Wyeomyia	Mosquitoes	1940	Colombia
Anopheles A	Mosquitoes	1940	Colombia
Anopheles B	Mosquitoes	1940	Colombia
Semliki Forest	Mosquitoes	1942	Uganda
Ntaya	Mosquitoes	1943	Uganda
California encephalitis	Mosquitoes	1943	USA
Bunyamwere	Mosquitoes	1943	Uganda
Sandfly fever – Sicily	Human blood	1943	Italy
Colorado tick fever	Human blood	1943	USA
Dengue 1	Human blood	1944	Hawaii
Ilheus	Mosquitoes	1944	Brazil
Dengue 2	Human blood	1944	New Guinea
Sandfly fever – Naples	Human blood	1944	Italy
Zika	Sentinel monkey blood	1947	Uganda
Uganda S	Mosquitoes	1947	Uganda
Omsk haemorrhagic fever	Human blood	1947	Soviet Union
Hanzalova*	Human brain	1948	Czechoslovakia
Trivittatus	Mosquitoes	1948	USA
Negishi	Human cerebrospinal fluid	1948	Japan

* Hanzalova = first strain of the virus of Central European encephalitis to have been isolated; indistinguishable by cross-neutralization from the virus of Russian spring-summer encephalitis

personnel of the arbovirus unit in Johannesburg isolated 10 new viruses from Tongaland and four from elsewhere in southern African (**Table 7.3**). Most of these viruses were originally isolated from mosquitoes or birds collected in surveys and have not been found to be of great medical or veterinary significance, although a few of the same viruses were later isolated from the blood of persons with febrile disease. Since 1959, there has been less emphasis on surveys and only three new viruses have been isolated from arthropods, although several potential veterinary pathogens have been isolated in southern Africa (**Table 7.4**). A number of arboviruses that had originally been isolated elsewhere, were also encountered in southern Africa. These included Rift Valley fever and Akabane viruses and human pathogens such as chikungunya, West Nile and Sindbis viruses (**Table 7.5**).

There was a need to collate information on the wealth of new arboviruses isolated worldwide from 1950 onwards and in 1959 the RFVL organized meetings that led to the formation of the American Committee on Arthropod-borne Viruses (ACAV). In 1960 a Subcommittee on Information Exchange (SIE) produced the 'Catalogue of Arthropod-borne Viruses of the World', which is continually updated by the mailing of registration cards for new viruses. However, many of the more than 500 registered viruses have been proven not to be arthropod-borne but are nevertheless retained in the catalogue as they are likely to be encountered by arbovirologists in the course of testing materials such as organs and sera from small mammals and birds. The title of the catalogue has been altered to 'International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates'.

Classification

The system of classifying arboviruses on the basis of immunological relationships, which was largely developed by Jordi Casals of RFVL and its successor, the Yale Arbovirus Research Unit (YARU), has been borne out by modern morphological and physicochemical characterization of the viruses. Serologically related viruses were first placed into groups A and B,[35] and then later a group C and a Bunyamwera group were added.[33, 34, 36, 37] Weak serological cross-reactions which were observed to exist between group C, the Bunyamwera group and certain smaller groups led to the inclusion of these groups in a Bunyamwera supergroup.[7] Many other unclassified arboviruses and rabies virus were found to be rod- or bullet-shaped and to share certain physicochemical properties. These were then placed together in a rhabdovirus group (*rhabdos* Gr. = rod).[42, 100, 103, 115, 145, 163] As African horse sickness and bluetongue viruses were found to be resistant to lipid solvents and had a similar morphology to reoviruses,[28, 173] it was suggested that they should be placed in an orbivirus group,[28] named for the ring-shaped capsomeres seen on the surface of virus particles (*orbis* L. = ring).

The overwhelming majority of arthropod-borne viruses were thus placed into A, B, rhabdovirus and orbivirus groups plus the Bunyamwera supergroup. The 'groups' were initially

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Table 7.3 New arthropod-borne viruses isolated in southern Africa from 1950 to 1959

VIRUS	ORIGINAL SOURCE	LOCATION	YEAR	REFERENCE
Wesselsbron	Lamb liver	Wesselsbron, RSA*	1955	264
Pongola	Mosquitoes	Lake Simbu, RSA	1955	128
Simbu	Mosquitoes	Lake Simbu, RSA	1955	262
Spordweni	Mosquitoes	Lake Simbu, RSA	1955	125
Lebombo	Mosquitoes	Ndumu, RSA	1956	22
Banzi	Human blood	Ndumu, RSA	1956	214
Middelburg	Mosquitoes	Middelburg, Cape, RSA	1957	117
Nyamanini	Bird blood	Nyamanini Pan, RSA	1957	228
Germiston	Mosquitoes	Germiston, RSA	1958	126
Witwatersrand	Mosquitoes	Germiston, RSA	1958	152
Usutu	Mosquitoes	Ndumu, RSA	1959	228
Tete	Bird viscera	Ndumu, RSA	1959	SIE, 1970**
Mossuril	Mosquitoes	Lumbo, MOZ*	1959	123
Ndumu	Mosquitoes	Ndumu, RSA	1959	120
Ingwavuma	Bird viscera	Ndumu, RSA	1959	153

* RSA = Republic of South Africa; MOZ = Mozambique
** SIE, 1970 = Subcommittee on Information Exchange of the American Committee on Arthropod-borne Viruses, 1970

Table 7.4 New arthropod-borne viruses isolated in southern Africa subsequent to 1959

VIRUS	ORIGINAL SOURCE	LOCATION	YEAR	REFERENCE
Shokwe	Mosquitoes	Ndumu, RSA*	1962	147
Olifantsvlei	Mosquitoes	Johannesburg, RSA	1963	109
Cascara	Horse brain, viscera	Kimberley, RSA	1967	70
Apies River	Cattle blood	Onderstepoort, RSA	1967	266
Bovine ephemeral fever**	Cattle blood	Onderstepoort, RSA	1967	253
Gamil	Horse blood	Naboomspruit, RSA	1971	71
Pretoria	Argasid ticks	Pretoria, RSA	1973	50
Nyabira	Cattle foetus	Nyabira, ZIM*	1973	223
Kaalplaas	Horse blood	Onderstepoort, RSA	1974	71
Kyalami	Horse viscera	Onderstepoort, RSA	1974	69
Gweru***	Cattle foetus	Gweru, ZIM	1976	265
Bryanston	Horse viscera	Johannesburg, RSA	1976	71
Langeberg	Horse foetus	Parys, RSA	1977	69
Marondera	Cow viscera	Marondera, ZIM	1978	265

* RSA = Republic of South Africa; ZIM = Zimbabwe
** Virus isolated in 1967 from cattle blood stored in 1958
*** Results of recent tests on unidentified viruses in storage at the National Institute for Communicable Diseases, indicate that Gweru virus was isolated from *Culicoides* midges in South Africa in 1969 and 1970, prior to its recognition as a new virus in Zimbabwe in 1976[265]

raised to the status of genera,[5, 269] and some were later designated as families as the general classification system of viruses evolved. On the grounds that they possessed similar morphology with lipoprotein envelopes, groups A and B were initially included in the new *Togavirus* genus (*toga* L. = cloak),[5] but were later designated as the *Alphavirus* and *Flavivirus* genera of a new family *Togaviridae* ('flavi' referring to the yellow of yellow fever virus, the type species for the genus *Flavivirus*).[74] Subsequently, the elucidation of fundamental differences, including differences in replication strategy, led to the genus *Flavivirus* being placed in a new family *Flaviviridae*.[30] However, the genus *Alphavirus* remained in the *Togaviridae* along with a few other genera containing non-arthropod-borne viruses such as rubella and those of hog cholera, bovine virus diarrhoea and equine viral arteritis.

Bunyamwera supergroup viruses, and serologically ungrouped viruses with similar morphology, were placed in the new family *Bunyaviridae*,[73, 174, 181] which initially contained the single genus *Bunyavirus*, but to which was later added *Nairovirus*, *Phlebovirus* and *Uukuvirus* genera (named for Nairobi sheep disease, phlebotomus fever and Uukuniemi viruses respectively).[24, 146] More recently, the genus *Uukuvirus* has been dropped and its members incorporated in the genus *Phlebovirus* on the basis of biochemical similarities, while a new genus *Hantavirus* (named for Hantaan virus), which includes a group of non-arthropod-borne, rodent-associated viruses producing haemorrhagic

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Table 7.5 Arthropod-borne viruses originally isolated elsewhere and subsequently isolated in southern Africa

VIRUS	SOURCE	LOCATION	YEAR	REFERENCE
Rift Valley fever	Cattle liver	Johannesburg, RSA*	1951	3
Sindbis	Mosquitoes	Springs, RSA	1954	252
Bunyamwera	Mosquitoes	Ndumu, RSA	1955	118
Chikungunya	Human blood	Letaba, RSA	1956	84
West Nile	Human blood	Ndumu, RSA	1958	119
Tahyna	Mosquitoes	Lumbo, MOZ*	1959	122
Semliki Forest	Mosquitoes	Namacurra, MOZ	1959	156
Quaranfil	Bird viscera	Naboomspruit, RSA	1960	22
Chenuda	Argasid ticks	Hartebeespoort, RSA	1960	147**
Shaamonda	Midges	Onderstepoort, RSA	1967	147**
Sabo	Cattle blood	Onderstepoort, RSA	1967	56
Shuni	Cattle blood	Onderstepoort, RSA	1967	56
Gomoka***	Mosquitoes	Harare, ZIM*	1969	25
Thimiri	Midges	Lake Chrissie, RSA	1971	25
Arumowot	Mosquitoes	Port Shepstone, RSA	1972	150
Soldado	Argasid ticks	Lambert's Bay, RSA	1973	147**
Abadina	Cattle foetus	Nyabira, ZIM	1976	265
Israel turkey meningoencephalitis	Turkey ovaries	Krugersdorp, RSA	1978	15
Bagaza	Cattle foetus	Ofavi, NAM*	1978	16
Uganda S	Mosquitoes	Port Shepstone, RSA	1979	147
Crimean-Congo haemorrhagic fever	Human blood	Bloemhof, RSA	1981	224
Akabane	Midges	Mazowe, ZIM	1982	26

* RSA = Republic of South Africa; MOZ = Mozambique; ZIM = Zimbabwe; NAM = Namibia
** Additional information from unpublished laboratory records, National Institute for Communicable Diseases
*** Gomoka and Thimiri viruses were recently identified in tests on unidentified isolates in storage at the National Institute for Communicable Diseases

fever with an associated renal syndrome in humans has been added to the *Bunyaviridae*.[96]

The family *Reoviridae*, as originally established, included arthropod-borne members in a single genus *Orbivirus*, but later it was found that Colorado tick fever and related viruses differed from orbiviruses in the number of RNA segments in the genome, and they were moved to a new genus *Coltivirus*. Arthropod-borne members of the family *Rhabdoviridae* were placed in a genus *Vesiculovirus* (named for vesicular stomatitis virus).[74]

Altogether 497 of the 534 viruses (93 per cent) registered in the working catalogue at the end of December 2000 belonged to five families (*Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae* and *Rhabdoviridae*) (**Table 7.6**) and, although not all of these viruses are known for certain to be arthropod-borne, very few of the remaining 37 viruses in the catalogue are considered to be definitely or probably arthropod-borne. New viruses are continually being registered in the catalogue and it is believed that only a small proportion of the arboviruses existing in nature have yet been discovered. Although it can be assumed that virtually all viruses that cause major epidemic diseases in livestock and humans are known, it is possible that many arboviruses that cause less obvious 'erosive' diseases such as sporadic abortion and teratology, remain to be discovered. Furthermore, it occasionally happens that pathogenic roles are discovered for viruses registered in the catalogue and which had no previously known medical or veterinary significance.

Names of arboviruses with a long history of general usage were accepted unaltered for registration in the catalogue. New viruses are named by the authors reporting the isolation, it being common usage to name arboviruses after the geographic location in which they are first discovered. Geographic names are spelt with capital initial letters, but arboviruses named for diseases are not capitalized — for example: Rift Valley fever, African horse sickness, and bluetongue.

Viruses registered in the catalogue are accorded abbreviations derived in a specified manner from their names.[8]

Epidemiology

Arboviruses are defined as viruses that are maintained in nature principally, or to a significant extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods. They replicate and produce viraemia in at least one species of vertebrate in order to facilitate infection of haematophagous vectors. Following an extrinsic incubation period lasting several days and involving replication of the virus in the tissues of the arthropod, arboviruses are transmitted to new vertebrates by the bites of the arthropod vector. By contrast, mechanical transmission of arboviruses involves the direct subinoculation into a second vertebrate host of infected blood, contaminating the mouth parts of a biting arthropod during the feeding process. Robust tabanid flies, which are painful biters and are consequently

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Table 7.6 Taxonomic status of 534 viruses registered in the 'International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates', at the end of December 2000

FAMILY	GENUS	NUMBER OF SEROGROUPS	NUMBER OF VIRUSES
Togaviridae	*Alphavirus*	1 (formerly=group A arboviruses)	27
	Unassigned	Ungrouped	1
Flaviviridae	*Flavivirus*	1 (formerly=group A arboviruses)	68
Bunyaviridae	*Bunyavirus*	18	138
	Nairovirus	6	24
	Phlebovirus	2	43
	Hantavirus	1	7
	Unassigned	8	41
Reoviridae	*Orbivirus*	15	69
	Coltivirus	1	2
	Orthoreovirus	Ungrouped	1
	Unassigned	Ungrouped	5
Rhabdoviridae	*Lyssavirus*	3	16
	Vesiculovirus	1	18
	Unassigned	6	37
Arenaviridae	*Arenavirus*	1	13
Arteriviridae	*Arterivirus*	1	1
Asfarviridae	*Asfivirus*	1	1
Filoviridae	*Filovirus*	2	2
Nodaviridae	*Alphanodavirus*	Ungrouped	1
Orthomyxoviridae	*Thogotovirus*	1	1
	Ungrouped	Ungrouped	2
Paramyxoviridae	*Paramyxovirus*	Ungrouped	1
Poxviridae	Unassigned	1	1
	Unassigned	Ungrouped	2
Unclassified		3	12
			534

frequently disturbed during feeding, constitute good mechanical vectors (**see Chapter 4: Vectors: Tabanidae**).

Arthropods that have been incriminated as biological vectors of viruses in animals and humans include dipterous insects of three families: mosquitoes, ceratopogonid midges (*Culicoides*) and phlebotomine flies (sandflies), as well as ticks (**see Chapter 1: Vectors: Ticks, and Chapter 5: Vectors: *Culicoides* spp.**). Of the 534 viruses registered in the arbovirus catalogue at the end of December 2000, 263 have been isolated from wild-caught mosquitoes (predominantly culicine rather than anopheline mosquitoes), 117 have been isolated from argasid and ixodid ticks, 45 from sandflies, 36 from midges and 17 from miscellaneous other arthropods. The figures provide an indication as to the relative importance of the various groups of arthropods as vectors, but definite proof of biological transmission in nature is lacking in most instances. However, much can be gleaned from analysis of supplementary evidence on virus transmission, and for this purpose ACAV established a Subcommittee on the Evaluation of Arthropod-borne Status (SEAS) in 1970.

Criteria used by SEAS when determining whether or not a virus should be regarded as arthropod-borne include:

- the frequency of isolation of the virus from naturally infected arthropods;
- demonstration of natural transmission of infection to suitable sentinel animals exposed under controlled conditions;
- demonstration of transmission by naturally infected, wild-caught arthropods after an appropriate interval in captivity to exclude mechanical transmission;
- demonstration of biological transmission following infection of putative arthropod vectors, either by feeding on an experimentally produced viraemic blood meal, or by direct inoculation of virus into the arthropods;
- demonstration of virus replication in experimentally infected arthropods, including transstadial persistence of infection during the life cycle of hemimetabolous arthropods such as ticks; and
- demonstration of the ability of the virus to produce viraemia in laboratory animals, suitably exposed sentinel animals or in naturally infected, free-ranging animals.

Supporting evidence includes taxonomic relationship to known arboviruses and strong epidemiological associations with arbovirus diseases, such as seasonal occurrence of infection that coincides with the biting activities of potential vectors.

Negative evidence which renders a virus unlikely to be arthropod-borne, includes:

- possession of physicochemical characters that are not typical of known arboviruses;
- failure to replicate in a battery of suspected arthropod vector species, including failure of infection to persist transstadially in hemimetabolous vectors; and
- isolation of the virus from salivary gland, urine or faeces of a vertebrate, since this implies the existence of an alternative mode of transmission.

On the basis of available evidence, SEAS classifies each registered virus as either arbovirus, probable arbovirus, possible arbovirus, probably not arbovirus, or not arbovirus. Of the 534 viruses registered in the catalogue at the end of December 2000, 214 (40 per cent) were rated as definitely or probably arboviruses and 33 (6 per cent) were rated as definitely or probably not arboviruses. In essence, this means that the information available on the remaining 287 viruses (54 per cent) rated as possible arboviruses is too meagre to allow meaningful conclusions to be drawn.

The concept of threshold intensity of viraemia for the infection of haematophagous arthropods was established with instantaneous feeders, namely mosquitoes. Threshold has been arbitrarily defined as the level of viraemia at which 1 to 5 per cent of arthropods taking a blood meal become infected with the virus concerned,[39] and the concept has important epidemiological implications. For instance, the detection of antibodies in a survey would indicate which

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vertebrate species become infected with a virus in nature, but the performance of experimental infections would allow distinctions to be made between vertebrate species in which the intensity of viraemia exceeds the threshold for infection of arthropod vectors and vertebrates which may become infected yet fail to develop sufficient intensity of viraemia to infect arthropods.[113, 114, 178, 193]

It is a general property of arboviruses that they can replicate in a wide range of arthropods after being directly inoculated into the haemocoele, but that they tend to produce natural infection in only a limited range of specific vectors. The proportion of arthropods that becomes infected with a virus generally increases as the intensity of a viraemia rises above threshold value, but for each virus–vector relationship there is a maximum proportion of arthropods that become infected. Intense viraemia with a particular virus may produce infection in a maximum of 30 per cent of one species of mosquito, 80 per cent in another, and yet fail to produce infection in a third species of mosquito, however intense the viraemia. The exact determinants of specificity in vector–virus relationships are imperfectly understood, but intrinsic factors in the arthropod are genetically controlled,[91, 187, 206, 226, 254] and appear to operate as barriers to infection at the level of enzymes, membranes and cell surfaces.[87, 99, 199, 200, 248, 259, 260] Following ingestion by an arthropod, most arboviruses infect and replicate in cells lining the mesenteron, before penetrating the basal lamina to be released into the haemolymph to set up further cycles of infection and replication. In some instances, arboviruses may penetrate the mesenteron without replication,[239] but the concepts of a mesenteronal- or gut-barrier to infection and a threshold level of virus in the blood meal to overcome the barrier, remain valid. Further barriers to infection appear to exist at the levels of organs such as the ovaries and salivary glands. By definition, a virus must infect and replicate in the salivary glands before the extrinsic incubation period is successfully concluded and the arthropod is able to transmit the infection by bites.[38] Arthropods may gain infection with great efficiency from the ingestion of a viraemic blood meal, yet fail to transmit virus because the infection has failed to spread to the salivary glands. Hence, it is important in vector studies in the laboratory to distinguish between arthropod infection rates and transmission rates.

Replication of virus in arthropods is influenced by ambient temperature, and, within optimal limits, increasing temperatures have the effect of shortening the extrinsic incubation period and increasing vector efficiency.[17, 40, 60, 88, 89, 104, 131, 175, 252, 255] It was formerly believed that infection with an arbovirus was harmless for the arthropod vector, but it has been observed that infection with certain arboviruses may produce morphological changes in the salivary gland cells of mosquitoes, reduce ability to refeed, and reduce the longevity of mosquitoes, as well as prolong larval development time after transovarial infection.[18, 72, 86, 92, 169, 218, 227, 230, 250, 252, 267] Viral genetics can also influence transmission by arthropods[251] but differences in geographical origin or limited passaging of virus strains in the laboratory do not necessarily affect vector efficiency.[168, 195, 261]

Holometabolous vectors, such as mosquitoes, midges and phlebotomine flies, undergo marked metamorphosis during their life cycles and the immature instars exist and feed in environments, often aquatic, that are very different from those in which the adults exist. Mosquito larvae can be infected with arboviruses by ingestion[47–49] but the epidemiological significance of this phenomenon is uncertain, and in general, holometabolous vectors must gain and transmit infection during the adult stage. Female mosquitoes may take a blood meal every two to four days and live for several weeks, so that after an extrinsic incubation period of one to two weeks, an infected mosquito is able to transmit infection to several vertebrates. In hemimetabolous vectors, such as ticks, the immature instars resemble and feed as adults, so that infection can be gained as larvae or nymphs, and then transmitted in the succeeding instars as nymphs or adults. Ixodid ticks attach and feed over a period of days so that determining threshold levels of viraemia for infection of these ticks is more complicated than for instantaneous feeders such as mosquitoes. Furthermore, ixodid ticks feed only once in each stage of the life cycle and a period of weeks or months may elapse before the tick has moulted to the succeeding instar and is ready to seek a further blood meal, so that transmission to a second vertebrate may occur long after initial infection of the vector. Argasid ticks tend to have multiple nymphal instars and to feed rapidly and at frequent intervals, so theoretically an argasid tick could transmit infection to numerous vertebrates. They are also able to withstand starvation for months or even years, and can consequently harbour infection for protracted periods.

The phenomenon of transovarial transmission of viruses in arthropods has important implications for the perpetuation of the viruses in nature. Early in the known history of arboviruses, evidence was produced to show that viruses may pass from the ovaries of infected arthropods, through the eggs, to the succeeding generation of mosquitoes,[142, 143, 170] ticks,[58, 172] or sandflies,[171, 179, 268] but investigators were unable to confirm the occurrence of transovarial transmission of virus in mosquitoes.[61, 82, 85, 219] Although some workers failed to demonstrate the phenomenon in ticks, others obtained convincing evidence that it occurred on a significant scale with tick-borne viruses.[94, 95, 101, 105, 130, 135, 140, 180, 184, 192, 215]

The belief that transovarial transmission either did not occur with mosquito-borne viruses, or else that it occurred on an epidemiologically insignificant scale, prevailed for many years.[31, 41, 66, 182, 232, 256] However, the bunyaviruses, and particularly the members of the California encephalitis complex, were investigated most intensively and in recent years many investigators have succeeded in demonstrating transovarial transmission of these viruses in mosquitoes, with filial infection rates generally below 50 per cent.[6, 14, 19–21, 23, 29, 43, 45, 51, 55, 57, 77, 79, 133, 134, 138, 158, 159, 165–167, 206, 230, 231, 244–246, 251, 257, 258]

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In more limited observations, clear evidence has been obtained that transovarial transmission with low filial infection rates, generally below 1 per cent, occurs among flaviviruses in mosquitoes.[1, 18, 52, 54, 63, 78, 80, 88, 89, 102, 111, 112, 175, 185, 188–190] Filial infection rates as high as about 20 per cent were obtained with one flavivirus.[81] There is some evidence that vertical transmission of flaviviruses in mosquitoes may involve the infection of fully formed eggs rather than the passage of virus through the ovaries[186] and it is not known whether this phenomenon occurs with other families of viruses.

Little evidence has yet been produced to show conclusively that transovarial transmission occurs with mosquito-borne alphaviruses and phleboviruses.[41, 110, 137] Intriguing evidence has emerged to suggest that certain mosquito-borne viruses may be maintained by transmission in ticks, including transovarial transmission, but the full significance of this phenomenon has not been determined.[27, 44, 191, 225] Transovarial transmission of viruses by sandflies has been well documented.[2, 67, 197, 233, 234–237]

Evidence of transovarial transmission is obtained either by testing the progeny of arthropods infected in the laboratory, or by isolating virus from eggs or immature arthropods collected in the wild prior to the taking of a blood meal. It has been observed that a period of days may elapse after the infection of female mosquitoes before infection of the ovaries occurs, analagous to the extrinsic incubation period for infection of salivary glands, so that virus may be absent from the progeny of the first ovarian cycle following infection, yet appear in later progeny.[20, 167, 230] Lack of awareness of this phenomenon may account for the failure of many earlier workers to demonstrate transovarial transmission of arboviruses.[232] Since male mosquitoes and sandflies do not take blood meals, isolation of virus from wild-caught males has also been interpreted as evidence of transovarial transmission. As an extension of this observation, it has been shown that infected male mosquitoes can transmit infection venereally to females, and that the females can subsequently transmit virus transovarially to their progeny.[175, 177, 185, 244, 245]

Transovarial transmission would seem to represent an ideal mechanism for the perpetuation of arboviruses in nature. However, mathematical models based on filial infection rates observed in laboratory studies indicate that the viruses concerned would not persist in more than a few generations of arthropods if transovarial transmission were the sole mode of infection.[75, 77]

In an incisive review of transovarial transmission, Tesh[232] points out that the above finding suggests two possibilities:

- as postulated for some viruses, transovarial transmission permits the viruses to survive periods of vector inactivity, such as winters or dry seasons, but amplification of virus is required through the infection of vertebrates during periods of vector activity; or
- laboratory studies have not given an accurate indication of what occurs in nature.

Tesh[232] examines the latter possibility and draws an analogy with inherent viruses of arthropods, in particular the sigma virus of the fruitfly *Drosophila melanogaster*. Inoculation of fruitflies with sigma virus generally results in 'non-stabilized' infections in which there may be transovarial transmission of virus to a limited proportion of progeny for a few generations. Occasionally, so-called stabilized infection arises in which there is infection of germinal cells (oogonia) with sustained transovarial transmission of virus by cytoplasmic inheritance to virtually all progeny of succeeding generations. Most infections produced experimentally in laboratory studies of vector–virus relationships have probably been of the non-stabilized type, but it appears that stabilized infections may have been experimentally produced in mosquitoes with a few members of the California encephalitis complex, with sustained filial infection rates ranging from 71 to 90 per cent.[166, 238, 249] The existence of such subpopulations of arthropods with stabilized infections in nature would serve to perpetuate arboviruses, even though the infection rates in the total vector populations remain low. Arbovirus diseases are characterized by:[66, 157, 182, 207–211, 232]

- a tendency to occur within specific geographic limits, although spread to new areas sometimes occurs with dramatic results;
- seasonal occurrence in periods of vector activity; and
- a propensity to produce major epidemics periodically.

Primary factors affecting the distribution of arboviruses include latitude, altitude, topography, geological features and climate. These determine the nature of the vegetation cover and the availability of surface water, which provide habitats with suitable micro-climates for the breeding and survival of some arthropod vectors (**see Chapter 6: Vectors: Mosquitoes, and Chapter 5: Vectors: *Culicoides* spp.**)

Within areas or foci, arboviruses are maintained by circulation between vertebrate hosts and arthropod vectors. The vertebrates involved in maintenance transmission cycles are often birds, small mammals or, less commonly, large mammals. Possibly as a result of long association with the viruses concerned and the consequent subjection to selection pressure, the vertebrate species involved in maintenance transmission cycles frequently appear to lack susceptibility to the pathogenic effects of the viruses, yet they develop sufficiently intense viraemia to serve as a source of infection for arthropod vectors. In the vast majority of instances, the vertebrates are not true maintenance or reservoir hosts of virus in the sense that they develop chronic infection with persistent viraemia. Instead, the vertebrate and arthropod vector populations collectively maintain virus in transmission cycles in which the vertebrates serve as amplifier and disseminator hosts of virus during the seasons of vector activity. Such basic transmission cycles usually remain cryptic unless susceptible vertebrate species that exhibit disease, including humans and domestic animals, impinge upon them. An example is the circulation of

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viruses of the tick-borne encephalitis complex of the northern hemisphere in forest ticks and rodents. It is generally inferred that vertebrate species that are prone to developing disease have, in evolutionary terms, comparatively recent experience with the virus concerned, and that they are dead-end hosts that play no part in further dissemination of the virus – in other words, the occurrence of infection in them is incidental or tangential to the basic transmission cycles that serve to perpetuate the viruses. This is particularly true of the sporadic diseases most commonly produced in humans by arboviruses, but livestock more often contribute significantly to the perpetuation of pathogenic viruses or to the generation of epidemics. For example, sheep and cattle are thought to play a key role in the dissemination of virus to mosquitoes and mechanical vectors during Rift Valley fever epidemics.[93, 149, 247]

The roles which vertebrates and arthropods play in the epidemiology of an arbovirus disease are compounded by:

- the nature of their infection:
- their behavioural patterns; and
- population factors.

Thus, the duration of viraemia in a vertebrate above threshold intensity for the infection of arthropod vectors affects the number of arthropods which are exposed to infection, while the duration of the incubation period in the vertebrate and extrinsic incubation period in the arthropod contribute to the length of the intervals between successful transmissions. Once a vertebrate has undergone infection, it is usually immunologically 'dead' to further infection with the same virus and, for persistence of infection to occur in the area, it is required that the rate of replenishment of the vertebrate population with susceptible individuals should be commensurate with the rate of transmission of virus. Birds and small mammals such as rodents offer greater opportunities for the circulation of virus as they may occur in large numbers and are liable to population explosions under favourable circumstances. Small mammals often have restricted home ranges with considerable overlap between individuals, and this leads to high population densities,[90] which favour transmission of virus. Furthermore, they attain sexual maturity rapidly, give birth to several young at a time, and may produce several litters per annum, so that there is rapid replenishment of the population with susceptible individuals. Migration of birds and mammals can also serve to replenish the susceptible host population of an area, or to introduce (or seasonally reintroduce) viruses and ectoparasitic vectors such as ticks.[97, 98] Habitat preferences and manifestation of nocturnal or diurnal activity are vertebrate behavioural characteristics which influence their exposure to arthropod vectors.

Haematophagous arthropods manifest host preferences and other behavioural characteristics that serve as mechanisms that isolate them from particular vertebrates while bringing them into contact with others. For instance, there is vertical stratification of habitats in forests as well as horizontal distribution, and it has been shown in Uganda that *Aedes africanus* mosquitoes, which feed in the canopy layer, rarely or never bite at ground level.[212] These mosquitoes, and monkeys in the canopy, can maintain sylvan yellow fever which may be communicated to humans by the arthropodan link host (*Aedes simpsoni*), which bites at intermediate levels. People who enter forests to hunt or fell timber acquire infection from *Ae. simpsoni* and themselves serve as vertebrate link hosts by conveying the infection to centres of human habitation where transmission cycles of urban yellow fever may be initiated between humans and the peridomestic mosquito (*Aedes aegypti*) which enters dwellings. In southern Africa, Rift Valley fever is transmitted by zoophilic mosquitoes, and humans acquire the infection principally from contact with the infected tissues of livestock.[154, 221] Consequently, human infection in the subcontinent is encountered almost exclusively as an occupational hazard of veterinarians, farm workers, slaughtermen and others engaged in the livestock industry. On the other hand, in the first outbreak of Rift Valley fever to be recognized in Egypt in 1977, anthropophilic mosquito vectors were involved in the spread of the disease to humans of diverse occupations.[161, 162] Many haematophagous arthropods, particularly dipterous insects, tend to bite at set times of the day. Some are diurnal feeders, others are nocturnal, while crepuscular insects feed in the twilight hours of dusk and dawn. It is interesting to note that long before it was known that crepuscular *Culicoides* midges were involved as vectors, it had been observed by farmers in southern Africa that African horse sickness and bluetongue could be controlled by stabling animals at night and releasing them after the dew had evaporated in the morning.

The transmission of arboviruses is seasonally interrupted by the suppression of vector activity by the cold winters of temperate zones, or by the dry seasons of the tropics and subtropics. Protracted dry seasons occur in much of Africa and both cold and dry conditions prevail during winters on the inland plateau of southern Africa. Viruses transmitted by arthropods may survive dry seasons through the transmission being sustained by limited numbers of the vectors which continue to breed in restricted sites, such as in the tropics where mosquitoes breed in water conserved in tree-holes.[83] Alternatively, viruses may disappear entirely from an area during winters and dry seasons, to be re-introduced later by migrating or wandering infected vertebrates or arthropods. There is evidence, for instance, that under certain weather conditions involving favourable temperatures, humidity and sustained winds, infected mosquitoes or midges may be carried vast distances at high altitude to be deposited in a viable state at a remote site where they initiate outbreaks of infection and disease in vertebrates.[201] It has been suggested that vectors of African horse sickness, bluetongue and Rift Valley fever could be transported in this manner, and that movement to-and-fro on a north–south

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axis in Africa occurs in association with seasonal movement of the inter-tropical convergence zone.[201-205] A variation of this mechanism, involving movement of virus only, is postulated for the perpetuation of yellow fever in the vast tropical forests of the Amazon River basin of South America, where large populations of mosquitoes and primates constitute a continuous substrate through which the virus can migrate.[208] Within a given area, epidemic infection soon leaves most primates either dead or immune, and yellow fever virus is perpetuated by the extension of infection from the periphery of the focus, to circle back at an interval of years when the slow-breeding primate population is replenished with susceptible animals.

Between the extremes of continuous transmission and complete disappearance of virus from an area, several mechanisms are postulated for the overwintering or hibernation of arboviruses through the occurrence of persistent infection in either arthropods or vertebrates. Infected arthropods may enter a state of diapause as adults, and hibernate in protected niches or resting sites with favourable micro-climates. Alternatively, they may diapause and hibernate as eggs or other immature instars. Overwintering of virus is readily explained in ticks, which even without diapause may spend weeks or months away from hosts between the feeding of successive instars. For female mosquitoes to survive hibernation, they must cease ovarian activity in autumn and deposit reserves in the fat body instead — a physiological state which is triggered in a proportion of the population by being subjected to shortened day lengths and cooler temperatures from the larval stages of development onwards.[66] Failure to understand the physiology of hibernation may have contributed to past failures to demonstrate the phenomenon in the laboratory.

It is a prerequisite that transovarial transmission should have taken place if virus is to hibernate in eggs or immature arthropods. Floodwater-breeding aedine mosquitoes, which hibernate as eggs in dried mud, may be involved in the overwintering of Rift Valley fever virus.[4, 137] Failure to demonstrate virus in hibernating adult or immature arthropods in the past could have been because virus was only present at low concentrations or in forms such as temperature sensitive mutants, which are not readily detectable by conventional methods.[66] It may be necessary to stimulate the preliminary replication of virus by warming hibernating mosquitoes and allowing them to take and digest a non-infected blood meal before attempting to isolate virus.[13] It has even been suggested that, through the mediation of reverse transcriptase, the genomes of RNA arboviruses could become incorporated in arthropod host cell DNA,[270] and, although there is little evidence to support this hypothesis,[32] it fuels speculation that some arboviruses had their origins as components of arthropod cells.

There are few examples of chronically infected vertebrates constituting true maintenance or reservoir hosts of arboviruses with persistent or intermittent viraemia, such as the harbouring of western encephalitis virus by wild birds in North America.[183] However, cattle may harbour bluetongue virus in this manner.[139] A variation of chronic infection is the suspended or latent infection, which appears to result from the lowered body temperature and metabolic processes and delayed immune response, which occur in such animals as bats, hedgehogs and reptiles that hibernate.[53, 132, 220, 241-243] The infections are activated and run their normal course when the animals return to the active state.

Finally, allowance must be made for the possibility that viruses may persist in hitherto unsuspected arthropod and vertebrate hosts, aquatic organisms, protozoan and metazoan endoparasites or even plants, some of which may occur in the food chains of currently accepted primary vectors and vertebrate hosts.[182] There are examples to indicate that viruses may use more than one mechanism to ensure survival during periods of inactivity of primary vectors. As an example, mention has already been made of the possible transmission of mosquito-associated viruses by ticks.

In many instances, arbovirus activity does not merely abate for the duration of a winter or a dry season, but the disease seems to disappear from an area for an interval of many years, only to reappear suddenly in epidemic form. Rift Valley fever is a classic example in which there is debate as to whether the virus persists at an unrecognized, low level of activity in those areas where epidemics occur, or whether the virus disappears to be reintroduced to the areas during epidemics.[137, 149, 205, 221]

Factors which precipitate epidemics include climatic events such as the occurrence of particularly heavy rains which favour the breeding of arthropod vectors,[59, 149, 221] or unduly warm weather which shortens the extrinsic incubation period of viruses in arthropods and increases vector efficiency.[106, 107, 151] Furthermore, good rains may be followed after an appropriate interval by population explosions of rodents or other vertebrate hosts of ectoparasites and viruses, as a result of the availability of increased food supplies. The effects of favourable seasons on virus transmission may be enhanced if interspersed with a succession of unfavourable or drought seasons, creating an unstable situation through the lowering of 'herd immunity'. Although tick-borne virus diseases sometimes occur as epidemics, the long intervals between tick blood meals and the utilization of small mammal hosts with rapid reproduction rates often lead to the situation where tick-borne diseases occur sporadically in fixed foci which remain infected indefinitely.[208] By contrast, the high biting frequency which occurs in outbreaks of mosquito-borne diseases usually leads to explosive epidemics which subside rapidly once most of the susceptible vertebrate population has undergone infection.

Human activities frequently have an over-riding influence on the occurrence of arbovirus diseases, and, of these, land utilization probably has the most profound effect. Primary or indigenous forests tend to have a rich and varied flora and fauna, which become progressively simplified with

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deforestation and diversion of land usage to secondary forests, pastures or monoculture of cereal crops, until a single species of rodent may be the dominant vertebrate.[12] In the process, haematophagous arthropods and arboviruses may transfer to new hosts with potentially serious implications. Edge habitats, such as junctions of forests and fields, have a particularly high potential for the interchange of vectors and viruses.[11]

The final ingredient needed for the occurrence of outbreaks of disease is the introduction of susceptible populations of humans and livestock into the ecosystem. In the past, major socio-economic upheavals, such as wars and land resettlement or reclamation schemes, have sometimes provided the influx of humans and livestock which triggered epidemics or led to the emergence of previously unknown pathogenic viruses.[96, 207, 210, 221] Large civil, industrial and agricultural engineering projects, such as the building of dams and irrigation schemes, sewage plants, power stations and factories with effluent waste water have also brought about changes in the juxtaposition of human, livestock and wild vertebrate populations, and provided breeding sites for arthropod vectors to trigger outbreaks of disease. Air, marine, and even land transport provide the means for the worldwide dissemination of arbovirus-infected vectors and vertebrates, including humans.

At the levels of individual humans and households, occupational or recreational exposure to arthropods, livestock or wild vertebrates will increase the risk of acquiring zoonotic arbovirus diseases, as does living in a rural environment, particularly in housing with poor insect-proofing. Humans gain infection either from the bites of arthropods or from contact with infected blood and other tissues of domestic and wild vertebrates, but viruses which are pathogenic for humans do not necessarily produce disease in other animals. For example, Rift Valley fever virus causes disease in sheep and cattle, while Crimean-Congo haemorrhagic fever virus produces mild or inapparent infection in these animals. Both are important human pathogens. Livestock undergo inapparent infection with tick-borne encephalitis of Europe, but serve as link hosts which transfer virus from cryptic circulation in ticks and rodents to the environment of humans. They also serve to amplify and disseminate infection through human consumption of infected milk.[208] Pigs are link hosts for Japanese encephalitis virus. They infect peridomestic mosquitoes, which in turn infect humans.[194] Arboviruses such as dengue and yellow fevers, which can be propagated by circulation between humans and mosquitoes, can pose a threat even in urban situations, particularly where mosquito breeding sites are afforded by poor drainage or water held in discarded containers.

The population explosion and drift towards urbanization experienced in the developing countries of Central and South America, Africa and Asia over the past few decades, has proved fertile ground for arboviruses and the literature abounds with descriptions of massive epidemics.[10, 207, 210, 211]

Two examples involving Rift Valley fever suffice to indicate the impact that arboviruses may have:

- according to one estimate 500 000 ewes may have aborted in the 1950 to 1951 epidemic in South Africa and 50 000 to 100 000 adult sheep may have died;[198] and
- the official estimate was that there were 18 000 human cases of Rift Valley fever in the 1977 to 1978 epidemic in Egypt, with 598 deaths, but unofficial estimates were much higher.

Outbreaks of arbovirus disease are terminated when:

- most of the susceptible vertebrate population is either dead or immune (that is, when there is so-called immunological exhaustion of the population);
- factors which precipitate or facilitate the outbreak are reversed naturally, such as when the onset of winter frosts in southern Africa terminates outbreaks of Rift Valley fever, bluetongue and African horse sickness through the suppression of vector activity; or
- there is effective human intervention, such as the administration of vaccine.

Control

The planning and institution of effective control measures require a proper understanding of the factors involved in the epidemiology of an arbovirus disease. The complex interrelationships between viruses, vertebrate hosts, arthropod vectors, the environment, climatic factors and acts of human intervention are amenable to logical analysis[141] and mathematical modelling.[76, 208] Frequently, disease represents the end product of a minor and aberrant pathway for the virus concerned, created or facilitated by human actions. It has been stated that for the epidemiologist, in contrast to the clinician, the occurrence of disease has no significance other than the implication of its recurrence.[141] Measures for the control of arthropod-borne virus diseases may be applied to the susceptible vertebrate species, vertebrates which serve as maintenance or link hosts of the virus, and the arthropod vectors.[208]

In many respects, protection of the susceptible vertebrate species through vaccination appears to represent the most practical methods for controlling arbovirus diseases. However, despite the availability of vaccines, arboviruses such as Rift Valley fever continue to produce massive outbreaks of disease. A major part of the problem is that it is difficult to motivate and sustain regular use of vaccine during inter-epidemic periods which may last many years,[221, 222] and it is equally as difficult to predict the occurrence of outbreaks of disease sufficiently early and accurately for immunization to be applied effectively in instances where it has been neglected.[136, 221, 222] Moreover, arbovirus diseases sometimes appear unheralded in areas where they have not previously been known to occur, as when an explosive outbreak of Rift Valley fever occurred in Egypt in 1977.[161] Some

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vaccines have inherent problems of efficacy, such as the difficulties encountered in obtaining immune response to all of the serotypes of virus included in multivalent vaccines like those used for bluetongue and African horse sickness.[68] Some vaccines are only partially attenuated and can cause problems when used incorrectly, such as live Rift Valley fever and Wesselsbron vaccines, which can be teratogenic and abortigenic when used in pregnant sheep.[46] A further problem is that vaccines are not universally available for all arboviruses of medical or veterinary importance. In some instances, such as Crimean-Congo haemorrhagic fever of humans, lack of potential demand inhibits the production of vaccines, particularly in view of the expenses incurred in meeting stringent safety and purity requirements.

Palliative measures for the protection of humans from arthropod-borne viruses include insect-proofing of the home and the use of mosquito nets and insect repellents. The same principles can be applied to livestock, and possible measures include the housing of animals at night and the use of highly effective insecticides. During epidemics which are caused by viruses transmitted by mosquitoes or midges, it may be beneficial to move livestock from low-lying marshy areas to high, windswept ground.

Vertebrates which are classed as vermin or pests, and which serve as maintenance or link hosts of arboviruses, can be subjected to specific population control measures. These include the proper handling and storage of crops and feeds in the vicinity of homesteads and stables. Livestock which serve as link hosts can be immunized to protect humans against zoonotic diseases, an example being the immunization of milk cows and goats against tick-borne encephalitis in eastern Europe.

Control of vectors includes selective clearing and drainage of land to alter habitats that favour the development and survival of haematophagous arthropods. Larvicidal treatment may be applied to water where vectors breed and insecticides may be applied to resting sites of adult vectors in buildings. Acaricides may be used to treat livestock for ticks. However, care should be taken to minimize pollution of the environment with toxic chemicals and their use should be combined with management systems designed to limit vector populations. For example, intensive grazing of pastures over short periods, combined with effective acaricide treatment of livestock, can be used to reduce or eradicate tick populations. However, efficient vector control may lead to a potentially unstable situation through loss of herd immunity. Biological methods for the control of vectors should be considered, such as the use of fish or bacterial agents to control mosquito larvae in water.

Finally, measures to control arbovirus diseases include the promulgation and application of regulations that require the production of health and vaccination certificates, ectoparasite treatment, quarantine and inspection of imported vertebrates. This includes not only susceptible livestock species, but also animals such as cage birds which may serve as reservoir hosts of viruses affecting humans and farm animals. Movement permits, vaccination certificates and ectoparasite control are also common requirements for the translocation of pets and livestock within countries. Inspection for, and control of, arthropod vectors and vermin, may also be applied to aircraft, ships and surface transport vehicles on international routes.

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8

Special factors affecting the control of livestock diseases in sub-Saharan Africa

M-L PENRITH AND G R THOMSON

Introduction

Although livestock production, particularly in the arid and semi-arid regions of sub-Saharan Africa, is of vital importance as a food source and for trade, it is also an integral part of social custom, tradition and wealth, and therefore of greater value than can be reckoned in mere financial terms. Furthermore, because epidemic diseases are more diverse and prevalent in these regions, animal production and household food security are more constrained by diseases than elsewhere,[4] as many of the chapters in this book attest. The fact that many of the Office International des Epizooties (OIE) List A diseases (i.e. those considered most dangerous in terms of international trade) are indigenous to Africa, often makes it difficult for products that originate in Africa to gain acceptance to international markets where the best prices prevail.[2] A result is the perception — one hopes erroneous — that disease control is generally doomed to failure in most parts of the continent.

Successful control or eradication of livestock diseases requires a holistic approach that involves appropriate animal husbandry practices, careful surveillance and control measures such as zoosanitary provisions that are enforced, vaccination, chemoprophylaxis and chemotherapy. To be sustainable these actions must be cost-effective and practical, and be recognized by livestock owners as necessary and appropriate. Ideally, livestock owners and animal health authorities need to co-operate in endeavours aimed at more effective disease control. Unfortunately, in sub-Saharan Africa, this ideal is rarely achieved, which is arguably the major reason why animal disease control is generally less effective in this region than in most other parts of the world.

Another well-recognized constraint is finance, to which there are two aspects. In the first place, there is the question of the return on the investment in controlling animal health. Studies in this area have generally shown that returns on such investment are highly positive (**see Chapter 9: The control of infectious diseases of livestock: Making appropriate decisions in different epidemiological and socio-economic conditions**),[14] although there is a view that the cost of eradicating the 2001 foot-and-mouth disease (FMD) outbreak in the UK was greater than the benefit it achieved. The argument is that a cheaper, albeit more time-consuming, approach would have been more appropriate.[15] The second aspect relates to who pays for control or eradication. In the modern world it is generally accepted that the 'user' — more accurately the 'beneficiary' — pays. This presents two problems because (1) in sub-Saharan Africa most communities dependent upon livestock are impoverished, and (2) when it comes to effective control or eradication of epidemic diseases, the beneficiary is not confined to livestock owners. It is generally accepted therefore that the State has this responsibility but, once again, many states in sub-Saharan Africa have rarely been able to afford large-scale control/eradication programmes. The eradication of contagious bovine pleuropneumonia (CBPP) from Ngamiland (north-west Botswana) in 1996/97 is a remarkable exception. When the disease appeared after an absence of more than 50 years,[1] the government of Botswana devoted more than US $ 350 million to that cause. At present, international donors make very significant contributions to the control/eradication of epidemic animal diseases in sub-Saharan Africa but these are not sustainable in the long term. Nevertheless, donor-assisted programmes such as PARC (Pan-African Rinderpest Campaign) and its successor, PACE (Pan-African Programme for the Control of Epizootics), both of which were funded by the Food and Agriculture Organization, have made vital contributions to the eradication of rinderpest from large parts of Africa as well as to improved surveillance programmes.

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In the event of epidemics, the measures and approaches necessary to control/eradicate them and prevent trans-boundary spread are generally prescribed by international convention based on experience and approaches accepted in the developed world, particularly western Europe and North America. In practice, it is often not possible to apply these measures effectively in sub-Saharan countries because of financial and/or logistical reasons. This sometimes leads to the accusation that the measures applied to control an outbreak were not properly implemented and hence the lack of success. However, it is often not recognized by donors and international agencies that the methods prescribed have no prospect of being successful in the environment in which they are applied.[17] There is therefore a pressing need for control measures of epidemic diseases in sub-Saharan Africa to be more effectively researched in order to develop more practical strategies to overcome the special conditions that prevail.

Even in highly developed countries, the possibility that conventional control measures may not achieve the desired result without excessive financial losses has been demonstrated during the outbreaks of classical swine fever (hog cholera) in the Netherlands in 1996 and FMD in the UK in 2001. Failure to recognize disease early enough to prevent spread usually lies at the root of the problem. After the disease has been diagnosed, logistics, socio-economic factors and politics can all contribute to the failure of control/eradication measures wherever they are applied. However, the nature of the logistical, political and socio-economic problems differs between developed and developing countries.

Apart from the financial constraints discussed above, other factors contributing to the failure of control/eradication programmes include the lack of adequate or adequately trained and motivated human resources, the lack of infrastructure, discipline, political will, and, often, the lack of information. In addition to this, sub-Saharan Africa continues to be afflicted by civil strife, rendering effective animal disease control impossible in regions where insecurity exists. Even when peace prevails and the resources necessary for control can be supplied either by the government or by donor organizations, there are often cogent reasons why conventional control measures cannot work.

Control measures

Quarantine and movement control

In the management of contagious diseases, strict movement control must be enforceable for quarantine and other zoosanitary measures to be effective. In this context it is important to remember that movement control of livestock in most parts of sub-Saharan Africa – some countries in southern Africa being an exception – is difficult because the borders between most countries are 'open', i.e. there is usually no indication on the ground as to where the border is, and they are often not controlled. Members of the same ethnic group frequently live on either side of a border and people and livestock move freely from one side to the other. Most important, however, is the requirement for pastoralists, who live in a wide belt across the continent, to move long distances, irrespective of country boundaries, in search of water and better grazing. Any attempt to stop historic trans-human practices is usually interpreted by pastoralists as an attempt to ensure their demise. In addition, even within countries there is often little differentiation between different land-use areas (e.g. wildlife and farming areas), and frequently livestock are managed on a communal basis rather than by individual farmers.

Where outbreaks of highly contagious diseases are diagnosed, their subsequent control/eradication ideally requires immediate deployment of people and resources to effect movement control so that the affected population is isolated from adjacent susceptible populations and transmission interrupted. Roadblocks are the most obvious means to this end. However, in most sub-Saharan countries our experience has been that roadblocks have been established very late in the course of the outbreak, if at all, and have not been effective in preventing movement of animals and livestock products. Bureaucratic delays and slow mobilization of the necessary funds are at the root of the problem. Although most countries have permanent police and/or army checkpoints along major roads, these fall under ministries unrelated to agriculture. The mechanism to allow them to act as veterinary checkpoints often does not exist, and complicated negotiations at ministerial level are necessary either to allow a veterinary presence at the points or to authorize the regular personnel to search for livestock and/or products. The establishment of a permanent inter-ministerial committee to respond to emergencies offers a possible solution. However, even if it is possible to establish veterinary checkpoints, they are seldom successful in preventing movement out of the infected area. There are several reasons for this. In Africa, movement of livestock is not restricted to roads, even if the movement is across international borders. Many people on foot and on bicycles will bypass any checkpoints placed on roads, particularly when moving illegal material. Much trans-boundary movement takes place in the same way, particularly in areas where the border, which may be a road or a river, cuts through settlements in which people are related to one another. Thorough searches of traffic passing through roadblocks are also impractical. Many roads carry heavy traffic, much of which consists of heavily laden public and long-distance cargo transport. The time required for adequate searches would bring traffic to a standstill for unacceptably long periods and would in fact encourage the use of minor routes. Furthermore, officials manning roadblocks are generally underpaid and poorly motivated, and may not confiscate animals or their products if offered inducements to ignore them.

The pressure to indulge in illegal movements can be very strong among poor livestock owners. Livestock often constitutes their only resource, and they have no reserves on

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which to fall back when the marketing of their animals is prevented. Entire communities are often affected, so that no money other than what can be obtained by selling animals or their products is available. Depending on the extent of the outbreak, this situation may be protracted. Furthermore, it is frequently beyond the power or volition of governments in sub-Saharan Africa to insist on a complete cessation of trade for the duration of the outbreak, particularly if no compensation is possible. For example, when markets are closed by official decree, they are often replaced by unofficial markets that spring up in the vicinity.

It is arguable that the inability of most African countries to effectively control and trace animal movement when it becomes necessary to control outbreaks of contagious diseases such as CBPP, African swine fever (ASF) and FMD, is the major reason for their widespread occurrence in Africa. On the other hand, methods that have been applied in the developed world or even southern Africa are not practical in most regions of Africa. This is particularly so in the vast pastoralist areas of sub-Saharan Africa. Unless a solution to this conundrum can be found, effective control, much less eradication, of major trans-boundary diseases will remain a problem. Perhaps any real progress towards addressing the problem will necessitate frank acceptance of its nature as a first step.

Stamping out

In poor countries, and increasingly in developed ones too, the concept of 'stamping out' (i.e. compulsory slaughter and destruction of animals in the infected area) is accepted only with difficulty by livestock owners and the public at large. It is therefore doubtful whether this is a viable alternative in most situations. It has been applied successfully in Botswana to eradicate CBPP and also in Côte d'Ivoire and Ghana to eradicate ASF. In the latter two countries it is highly likely that the ASF problem will recur because both have largely uncontrolled borders and many countries in the region are infected.

The only way to ensure co-operation by owners, particularly if movement control is inadequate, is to pay market-related compensation for animals slaughtered. When state budgets do not permit such payment, or where payment is retarded, the entire operation is doomed to failure. Concerted efforts to avoid the forces of government are likely to succeed, and may result in even more rapid spread of disease than would otherwise be the case. When compulsory slaughter does take place, strict monitoring of the disposal of carcasses is necessary, and the carcasses should be rendered unusable.[5] In poor countries, destruction of large amounts of edible meat is a very difficult concept, and exhumation of buried carcasses is common.

Vaccination and chemoprophylaxis

Prevention of both serious epidemic diseases and erosive diseases of livestock often depends on either vaccination or chemoprophylaxis, or both. This presupposes access to the necessary products and sufficient financial means, either on the part of government or the owners, to purchase them. In areas far from cities and towns, or, as is the case in many countries, in all regions other than the capital city, products are often simply not available. This is further complicated when products, usually vaccines, require an unbroken cold chain in order to retain viability. Inability to read the instructions supplied in order to use the products correctly is also a problem in many areas in the region. Even when outlets for storage and sale of products are provided, either by the state or by the private sector, storage facilities and back-up advice and service may be less than optimal.[7] In this respect, drugs — both conventional commercial products and traditional remedies — are sometimes more easily obtainable from unofficial traders than through veterinary or pharmaceutical agencies. This means that the authenticity and/or storage of the products supplied may be dubious, which can result in animal owners wasting their hard-earned money on inappropriate or ineffective drugs. To some extent this problem is being countered by community-based animal health workers (see below) who have received some basic training in animal husbandry and health and who are employed by the animal owners in the communities in which they operate.[3, 8] In many countries this approach is largely being advocated and financed by aid agencies, and it remains to be seen how viable the initiative will be in the long run.

In the event of an outbreak of disease, ring vaccination may be indicated to prevent spread. The expense of vaccination, like that of compensation, may prove too great for the government to bear. Even when donor funding and vaccine are available, lack of infrastructure, such as passable roads into the area, serviceable vehicles and the wherewithal to maintain teams in the field, may delay delivery of vaccine and result in the cold chain being broken. Maintaining sufficient trained personnel in the area to administer the vaccine also has cost implications that may not be feasible for the state to cover.

The complexities and interaction of some of the factors outlined above are illustrated by the problem that has arisen with respect to rinderpest eradication in eastern Africa. Since the early 1990s, 'mild rinderpest' has become prevalent in some parts of that region, particularly in the Somali eco-region that encompasses parts of Somalia, Kenya and Ethiopia. The viruses concerned have so far all been members of 'lineage 2'.[16] There is usually little if any evidence — other than residual antibody — for the presence of the infection in cattle while wildlife, chiefly African buffalo (*Syncerus caffer*), may have more obvious disease.[11] The result is uncertainty as to how to go about controlling the problem. Limited ring vaccination is not advocated because it is difficult to be sure of the exact limits of spread of inapparent disease. Mass vaccination over a wider area is also problematic because it becomes expensive and difficult to persuade livestock owners that such resources be devoted to a disease

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they do not recognize as serious. At present, there is no scientific evidence that mild strains of rinderpest virus are able to revert to the virulent form, although it is an obvious danger.

Surveillance and monitoring of diseases

Maintenance of adequate surveillance in order to detect occurrence or an increase in the prevalence of economically important animal diseases at an early stage is vital for effective disease control in any circumstances. However, to retain an effective country-wide surveillance system is expensive and the benefits are usually long-term and not always appreciated by those who control the treasury. Approaches to, and minimum standards for, surveillance are currently under consideration in many African countries and constitute a specialized field in its own right. There are a number of texts that deal with this subject that should be consulted for detailed information (**see Chapter 9: The control of infectious diseases of livestock: Making appropriate decisions in different epidemiological and socio-economic conditions**).[18, 19]

When an outbreak has occurred, surveillance and monitoring both during and after the outbreak are essential to establish the extent of the outbreak, determine how effective control has been, and ascertain its origin. It is frequently necessary that resources in addition to those of the veterinary service of the country concerned are made available during and immediately after a serious outbreak of an epidemic disease. The major additional requirements are usually trained personnel, transport and equipment to mount an effective campaign. However, maintenance of surveillance activities beyond the outbreak to assist in preventing recurrence and to enable early detection is often extremely difficult. The fact that donor resources are usually available during outbreaks has engendered a perception that transboundary disease control is an international rather than a national responsibility. This has serious implications for sustainability of control programmes.

Alternative approaches to disease control/eradication

When considering approaches to disease control, it is important not to lose sight of the reasons for control, facile as this may sound. These may vary, but with the exception of a few countries in the region, the most important reason for control is to enable production to continue at a local level rather than to protect international trade. If livestock diseases can be adequately controlled at local level, commercial production has a chance to reach a level at which formal export becomes a reality. Control measures should therefore be designed primarily to protect the interests of the livestock owners and not to destroy their enterprises to the extent that they will be unable to resume them for a long time by, for example, wholesale stamping out. The most successful control measures will be those that are understood and applied by the livestock owners themselves, since they have the most to gain from successful control. For livestock owners to play either a leading or supporting role in disease control, they need to be informed about the diseases and trained in methods of preventing their occurrence. These methods may demand modification or even complete change of their husbandry system.

In sub-Saharan Africa livestock disease control measures are required for two completely different levels of production. Commercial livestock farming, which is undertaken entirely to generate profit, ranges from small- to large-scale, and includes dairy farming, feedlots, and intensive production of pigs and poultry. These enterprises have increased in number during the last decade. Explosive urban growth has necessitated higher levels of food production on less land, which favours commercial production.

By far the greater numbers of livestock are still produced in traditional systems, where animals range extensively and are given little or no supplementary feed. In the more arid parts of Africa, livestock keepers are often nomadic.

Disease control in commercial production systems Disease control is more easily applied in commercial, more intensive production systems than in extensive systems. It is also crucial, because concentrations or confinement of animals particularly where large numbers of animals are involved, favour rapid spread of contagious pathogens. Additionally, high morbidity and mortality in commercial systems may prompt panic selling of animals through outlets that reach a wider market, and can contribute materially to the spread of the epidemic.

Commercial enterprises are more amenable to effective disease control/eradication for the following reasons:

- owners are often more educated and informed;
- owners are generally more willing to spend money on higher-producing animals;
- access to veterinary services and other resources is generally better;
- quarantine is much easier when animals are restricted to a defined area; and
- marketing of animals and products often takes place through formal outlets where some form of veterinary inspection is available.

Additional advantages are that the number of animals is usually known, or easy to count, and animals may be individually identified. Records of purchases, sales, births and deaths may also be kept. Owners will often seek veterinary assistance when animals are ill or unusual mortality occurs. This encourages rapid reporting of disease emergencies. Owner compliance with control measures to deal with disease emergencies depends strongly upon convincing them that the advantages of control/eradication of the disease outweigh the short-term advantage of evading the control/eradication measures and selling as many infected animals

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as possible. The key factors in successful disease control/eradication in the commercial sector are therefore:

- organization of the industry;
- awareness, information and training;
- a holistic approach to on-farm biosecurity to prevent outbreaks;
- ensuring that when outbreak control is necessary, damage resulting from control measures is limited by assurance of market-related compensation for compulsory slaughter and/or other measures that encourage owner compliance; and
- participation of owners in disease control surveillance and monitoring.

Disease control in traditional production systems Disease control is challenging in traditional production systems as these enterprises do not have ready access to veterinary services. Although the animals themselves represent wealth, their owners frequently have little or no ready cash to spend on animal health care. Because production in these systems is low, large numbers of animals may be kept to offset higher mortality and lower reproduction rates, and the value of the herd is often measured in the number rather than the quality of the animals. If animals are sold, marketing is usually via informal outlets. While cattle owners, in particular, are often highly knowledgeable and skilled stockmen, their attitude to livestock diseases may be stoical rather than pro-active.

The principles of disease control are identical whatever the production system. The challenge in traditional systems lies in how to practically apply control measures given the lack of resources, information and, sometimes, the very different outlook of subsistence livestock owners. Ultimately, however, the aim would be to promote transformation from traditional to commercial systems of production. Given that livestock owners depend upon their animals to provide them with the necessities of life, few of them would refuse the opportunity to improve the income or life support generated by keeping livestock. Historically, development has depended upon the commercialization of agriculture, and there is no doubt that, given the opportunity, sub-Saharan Africa can achieve the necessary level of disease control for optimal animal production. Being a latecomer to the developed world, it may even be possible for Africa to avoid some of the problems that are inherent in excessive intensification of animal production.

Community-based animal health care, although not a new concept, has been developed to address the need for access to veterinary services in rural, frequently pastoral, communities usually far removed from the official veterinary service of countries.[3] It has been formally applied in several African countries for varying lengths of time and with varying degrees of success.[3, 8, 12] Obviously, owners have practised a degree of primary animal health care ever since humans have domesticated animals, and there is no doubt that considerable knowledge exists among those who possess traditionally kept animals. However, the diagnosis of disease is often a problem, as is the availability of suitable remedies.[7, 13, 20] Community-based animal health care systems are most successful when based upon existing knowledge and when there is an obvious gain for the community.[3, 9] Because community-based animal health care increases awareness of and information about animal diseases, and concentrates upon prophylaxis, including vaccination for serious diseases, it can make a vital contribution to improving disease control, particularly in traditional systems of animal husbandry in remote areas where there are currently no other options. The contribution of community-based animal health care programmes to control livestock diseases depends largely upon the perceived importance to the community of those diseases, as well as the approach adopted. Rinderpest control via community animal health workers achieved considerable success in the Afar region of Ethiopia and in Sudan, because livestock owners had no doubts about the effect of the disease.[3] On the other hand, tsetse fly-control programmes that were theoretically community based enjoyed varying degrees of success, which was at least partly ascribed to the fact that the priorities of the technologists and the communities did not coincide.[3] Only when it was recognized that tsetse fly-control provided tangible benefits were communities prepared to commit time and resources to maintaining and, where necessary, replacing targets.

Information and awareness are essential at all levels of livestock production for disease control. The livestock owner or herder is the first line of defence, as he/she is in daily contact with the animals.[10] Two of the essential elements of disease control, namely early warning and surveillance, depend strongly upon disease recognition and reporting by owners or members of the community. In spite of the knowledge that exists among traditional livestock keepers, most are eager to improve on this knowledge and are highly receptive to information, provided it is delivered in a form that they can easily understand. The incorporation of high-quality photographic material showing signs and lesions familiar to them greatly enhances acceptance and learning. Ideally, the information should be transmitted to owners by the veterinary services of the country concerned, who can at the same time identify lines of communication for reporting diseases that require government intervention. However, where this is not possible, the function may be taken over by private or non-governmental organizations, who may also serve as the first link in the line of communication to the veterinary authorities. In a disease emergency, participation of livestock owners in formulating and implementing control activities is vital for the success of the exercise.[6]

Globalization is increasingly becoming an impetus for change in many spheres and livestock production is unlikely to prove an exception, even in the remotest parts of sub-Saharan Africa. Thus the present preoccupation of the

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developed world with diseases such as FMD, bovine spongiform encephalopathy and food safety generally will force any country wishing to export to developed countries or trade blocks to be able to certify their livestock and livestock products accordingly. An example of the difficulties that result has been provided by the events surrounding the apparent spread of Rift Valley fever (RVF) virus from the Horn of Africa to the Arabian Peninsula where, in 2000/1, several hundred people died of RVF in Yemen and Saudi Arabia. As a consequence, a ban was placed on the traditional annual export of millions of sheep and goats to the Arabian Peninsula — particularly at the time of the *Haj* — causing great hardship to the pastoralists of the Somali ecoregion who rely on this trade as their major source of income. The internationally accepted guidelines on the requirements for ensuring freedom from RVF contained in the OIE's International Animal Health Code[2] require updating, particularly from the African perspective. Additionally, so far African countries have not managed to bring their collective resources to bear in addressing such problems. It is therefore vital that the African perspective is conveyed more successfully into international forums such as the OIE and the World Trade Organization, where trade-related decisions are made, to ensure that developing countries are not 'victimized', albeit unintentionally.

Change from traditional to commercial livestock production systems seems unrealistic, especially when one considers the nomadic livestock systems practised widely in arid parts of Africa. Nevertheless, there is no doubt that in some areas change has taken place, at least with respect to the marketing of cattle. Expanding cities have increased the demand for meat by people who cannot keep livestock themselves. Large numbers of cattle, sheep and goats from the Sahel find their way to coastal markets such as Abidjan, Cotonou and other coastal cities, indicating that their owners are willing to undertake commercial enterprises. Conditions under which livestock are kept and marketed are dictated largely by feasibility and by the advantages perceived by the owners. Given sufficient incentive through increasing market demand and the dwindling land available for migration, there is little reason to suppose that Africa would not respond, as other regions have, by adopting more sophisticated husbandry systems.

Initially, changes in production systems are most easily effected with the short-cycle species, in particular pigs and poultry. Currently, large numbers of scavenging pigs and chickens are an indispensable part of life in villages in many African countries. The survival of these animals is crucial to their owners, to whom they represent household food security and, in the case of pigs, a mobile bank to meet unexpected expenses. Scavenging systems require no input from the owners and serve additional useful purposes such as environmental cleaning, but production is generally very low and mortality unacceptably high from causes unrelated to disease, such as road accidents, predation, malnutrition, and eradication because of damaged crops. The productive potential of pigs is such that relatively small inputs can result in sizeable gains. Convincing owners to change from extensive to more intensive production systems should therefore be relatively easy, particularly if local 'champions' can be identified who, by precept and example, can convince other owners of the profitability of improved enterprises.

The inherent resistance of indigenous breeds of domestic livestock diseases endemic to the region should not be under-estimated as a means for disease control.[12] Much research is still needed in this field. However, upgrading production systems by the wholesale introduction of animals of exotic breeds that are not suited to African production systems and are highly susceptible to the diseases that are endemic in the region, is a recipe for disaster. Such animals can usually only be successful in artificial systems where complete protection from vectors and diseases, and an appropriate level of nutrition and management can be achieved. Retaining and improving the genetic resources that exist in the region is vital for improving production and control of livestock diseases in sub-Saharan Africa.

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9

The control of infectious diseases of livestock: Making appropriate decisions in different epidemiological and socioeconomic conditions

B D PERRY, J J MCDERMOTT AND T F RANDOLPH

Introduction

This book describes the many infectious diseases that affect livestock in sub-Saharan Africa, and indeed in many other regions of the world, and discusses in detail the technical aspects of their diagnosis, treatment and control/eradication. In an environment with unlimited resources, and with the technical capacity to undertake the appropriate measures, many of the diseases described here could be brought under control or even eradicated. Indeed, in the past, a few of them have been eradicated, but usually at enormous cost. Remarkably, in many cases, these costs are not available in the records, indicating that they may not have been of prime importance in the decision to eradicate. Take for example the eradication of East Coast fever (ECF) from southern Africa. In his detailed account of the eradication programme that spanned the years 1901 to 1960, Lawrence[114] estimated, from what he called 'fragmentary data', that a total of 1,4 million cattle died as a result of the disease, and an additional 100 000 were slaughtered. However, he noted that data on the costs of control were 'non-existent'. One cannot help asking the question: would such an intensive control and eradication programme, involving compulsory weekly dipping of all cattle, strict movement controls, quarantine of affected herds, compulsory examination of spleen smears from all animals that were slaughtered or died, and slaughter of infected animals with compensation, all sustained over decades, be carried out if ECF were to break out in certain southern African countries today?

It is in the answering of this question that one confronts the stark interaction between the technical feasibility and the socioeconomic feasibility of infectious disease control/eradication. There are several important differences in circumstances that would affect the nature of a decision made today. These include changed political and socioeconomic circumstances, different disease-control institutions and authorities, and different decision criteria for allocating public and private funds and other resources to infectious disease control, to name but a few.

Why would we today expect the answer to be 'probably not'? Much has changed in the intervening years since the initiation of ECF control in southern Africa in 1904. The magnitude of the threat presented by even the most virulent of infectious diseases is different, and is probably much smaller now due to our increased understanding of the risks and impacts of different infections, and a greater inventory of measures at our disposal to control them. It is doubtful that ECF would threaten the very existence of the entire agricultural sector today as it did at the beginning of the previous century for settler agricultural development. At the time, the region was recovering from the devastation caused by the rinderpest pandemic, and in an era when the world was preoccupied by the perils of infectious diseases, South Africa was also struggling to recover from the ravages of the Anglo-Boer War (1899 to 1902).

Furthermore, it is difficult to imagine the determined application of a technical solution being successfully implemented today given the political and organizational realities of much of the developing world. Part of this doubt is related to the more complicated nature of decisions on resource allocation and how (and by whom) these decisions are made today. It appears that an extraordinary degree of authority was given to the highly successful group of scientists active in investigating the causes and control of ECF, and this outweighed many of the bigger economic questions. There have been substantial changes in the seat of authority for disease

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control in virtually all corners of the world, from a very centralized, powerful and autocratic government involvement to a decentralized and often impoverished public sector, and this change has accelerated in most countries of the African continent in the last few years. The change has been complicated by a deterioration in the veterinary infrastructures of many African countries, and thus the feasibility of delivering even highly technically efficacious control measures is increasingly questionable. In addition, several decision-support tools (such as cost-benefit analysis, for example) to aid in decision-making are now available and widely used, but did not exist in the early part of the twentieth century.

In summary, decisions regarding disease control have changed radically due to a combination of improved knowledge about infectious diseases and of the need to take into account a whole range of socioeconomic considerations that were not previously considered.

This chapter evaluates how decision-making for the control of infectious diseases can be improved at all levels (e.g. farm, community, country, region) by the consideration of both epidemiological and socioeconomic issues. We review what the impacts of diseases and their control are, how these can be quantified, how such information can be used in the choice of disease control interventions, and how such interventions can be best implemented under different sets of circumstances.

Livestock production and use in Africa

An important perspective on the socioeconomic issues affecting infectious disease control can be gained through an understanding of the concept of different production systems in which diseases have different impacts. These impacts vary in terms of the production losses and control costs incurred, and whether the control costs are borne by private or public coffers. During the colonial era, and for a variable period after depending on the country and region, there were possibly two major production systems (although such terminology was not applied to these) in much of Africa. The first of these was the 'commercial' sector, comprising different scales of beef, dairy, sheep and poultry production on medium-sized to large farms with fairly high levels of inputs (in terms of feed, veterinary drugs and vaccines, and genetically improved breeds), in which many of the financial aspects of disease control were the responsibility of the individual farmer, as currently occurs in Europe, for example. There was some financial support provided by the state to farmers, such as loans for the construction of dips, access to vaccines and remedies at government-subsidized prices, and public veterinary services that were generally state-financed and -run, and delivered at no or a highly subsidized cost to farmers. Socioeconomic issues in this sector very much boiled down to the cost efficacy of different approaches to successful infectious disease control.

The second production system was the peasant sector, which was often in geographically distinct areas known by names such as 'tribal trust lands' or 'African reserves', which have evolved into the so-called 'traditional', 'communal' or 'smallholder sectors', depending on the country and the nomenclature used. These low-producing, often subsistence-orientated systems, relied very much on the state for the provision of veterinary services, which in general were provided at no cost to farmers. This was done for many reasons, both political and technical. A principal concern, not always stated publicly, was the belief that infectious diseases could spread from the peasant sector into the more commercially orientated livestock enterprises, but the responsibility of governments for the welfare of the poorer sectors of society was clearly also an important driving force (**see Table 9.1**).

Both of these systems have evolved substantially, forming a much wider set of production systems in which socioeconomics play a critical role in the choice and efficacy of interventions to control infectious diseases, and as to who pays, and who benefits. Overall, the commercial sectors have shrunk in size considerably, although less so in southern Africa than in other parts of the continent. In most cases, state support in the form of loans and subsidized veterinary services has vanished. In many cases, their efficiency of production has improved, despite the economic and political pressures on them.

The changes in the 'peasant' sector have been much more profound, and it is here that socioeconomic issues usually outweigh technical issues in the optimal control of infectious diseases. Firstly, there has been recognition of the unique nature of certain livestock-centred systems in Africa characterized by pastoralism. These include those of the Maasai, Rendile, Samburu, Boran and others in eastern Africa, the Tuareg and Fulani of West Africa, and the Herero of Namibia, among others. These groups are characterized by their extreme scarcity of financial resources, but by a high priority to disease control for their 'walking bank accounts'. They are highly demanding of veterinary interventions, in terms of the 'priority' of the disease it addresses and the appropriateness of the solution to their circumstances, but if these are correct, they are higher adopters of technologies to improve animal health than other smallholder groups in Africa.

Secondly has been the evolution of market-orientated smallholder systems that are commercial, often intensive in nature, but very small-scale. These include the smallholder dairy system of the Kenyan central highlands, the 'emergent' small-scale commercial farmers of South Africa and Zambia, the 'small-scale commercial' sector of Zimbabwe, and the peri-urban dairies of Addis Ababa, Cairo, and Dakar. Some of these have achieved significant success in terms of their productivity. In the Kenyan context, the smallholder dairy sector now supplies somewhere in the region of 90 per cent of the milk consumed in the country,[145] a dramatic change from the domination of large commercial dairies some 30 years ago. In these sectors there has been a changing pattern of disease priorities, and a tendency for risk avoidance management practices to develop. Again using

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the example of the smallholder dairy system of Kenya, the use of stall-feeding (known locally as zero-grazing) has dramatically reduced the risk of tick-borne diseases, with most of the fodder being cut and carried to the confined animals, thus avoiding heavy tick infestation derived from communal grazing. This has brought greater attention in this production system to the so-called infectious diseases of intensification, such as mastitis.

The other major peasant production system, widely prevalent in southern Africa, is not orientated to the livestock product market, but to the crop market. This sector, characteristic of the 'traditional sector' in Zambia and the 'communal lands' of Zimbabwe, is primarily a staple crop (mainly maize) system in which livestock play many roles. The use of oxen for tilling the land is probably one of the most important functions of cattle in this system, followed by the social (or networking) value of animals. The sale of cattle into the beef sector usually comes in third place, and formal off-takes from this sector have always been considered remarkably low (in the region of 3 to 5 per cent; see for example Perry and associates).[184] As a result, this system has suffered considerably from infectious diseases with the dramatic decline in public sector veterinary services, and the fragile socioeconomic role of cattle in the livestock component of the agricultural sector.

These are very broad production system groupings. Attempts have been made to develop more structured classifications of livestock production that further disaggregate and that can be used for analytical purposes and for priority setting. In 1996, Seré and Steinfeld[223] produced the first global livestock production classification system associated with a detailed data set. For this reason, it has been widely used, and served for example as the basis for the International Livestock Research Institute's (ILRI) recent priority-setting exercise.[203, 238] Seré and Steinfeld's classification is based on the main food source for livestock, and on the agro-ecology of the region. They used the Food and Agriculture Organization's (FAO) agro-ecological zoning classification and produced detailed country tables with disaggregated data by area, human population, livestock numbers, and livestock outputs for each livestock production system category. Thornton and co-workers[237] have since refined and updated the Seré and Steinfeld classification system and database, and plotted a global distribution of the different production systems.

Table 9.1 Examples of general livestock production system categories currently in use in southern Africa and their animal population numbers

The general groupings used for reporting of livestock population numbers in many southern African countries still fall in the broad categories of traditional (communal, *sector familiar*, or Swazi National Lands) and commercial. In these, the 'traditional' sectors dominate in terms of livestock numbers, except in South Africa

COUNTRY	CATTLE (GENERAL)		DAIRY			GOATS		SHEEP	
	TRADITIONAL	COMMERCIAL	TRAD.	SMALL-SCALE	COMM.	TRAD.	COMM.	TRAD.	COMM.
Angola	291 213	59 546	0	0	0	1 685 600[1]	34 400[1]	–	–
Botswana	1 562 200	258 500	0	0	0	1 809 400	28 300	233 300	16 600
Malawi	579 700	23 500	11 600	0	4 000	1 583 400	14 100	98 000	4 800
Mozambique	291 213	59 546	0	0	1 400	518 525[1]	19 874[1]	–	–
South Africa	4 090 791	6 153 720	0	0	1 433 465	4 485 953	1 433 547	3 162 068	23 188 489
Swaziland	491 428	142 367	3 997	0	4 187	428 475[1]	30 887[1]	–	–
Tanzania	15 374 119	58 351	0	187 687	24 645	10 682 434	12 343	3 484 957	8 071
Zambia	1 700 000	970 000[2]	0	20 000	50 000[2]	646 257	71 806[2]	27 038	50 212[2]

Note: [1] Sheep and goats reported together
[2] Also includes 'semi-commercial sector'
Sources: References [8–16, 28]

In Zimbabwe, the livestock populations of the smallholder sector are reported in three categories, communal, resettlement and small-scale commercial

SPECIES	LIVESTOCK SECTOR			
	LARGE-SCALE COMMERCIAL	COMMUNAL	RESETTLEMENT	SMALL-SCALE COMMERCIAL
Cattle	1 679 652	3 689 640	468 056	231 416
Goats	77 667	2 582 463	188 263	61 477
Sheep	136 566	455 774	28 247	19 588
Pigs	135 216	123 095	8 826	11 674
Donkeys	14 468	323 550	26 726	8 378
Horses	9 047	67	22	74

Source: Department of Veterinary Services, Zimbabwe, 2001

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In this production system classification, four main production categories are identified: landless systems (typically found in peri-urban settings), livestock/rangeland-based systems (areas with minimal cropping, often corresponding to pastoral systems), mixed rainfed systems (mostly rainfed cropping combined with livestock, i.e. agro-pastoral systems), and mixed irrigated systems (significant proportion of cropping in which irrigation is used and is interspersed with livestock). All but the landless systems were further disaggregated by agro-ecological potential, as defined by the length of growing period. Three different agro-ecological zones are used: highland/temperate, arid/semi-arid and humid/sub-humid. In summary, the following 10 livestock systems have been defined and mapped across the globe:

- LGA – Livestock only, rangeland-based, arid/semi-arid
- LGH – Livestock only, rangeland-based, humid/sub-humid
- LGT – Livestock only, rangeland-based, highland/temperate
- MIA – Mixed, irrigated, arid/semi-arid
- MIH – Mixed, irrigated, humid/sub-humid
- MIT – Mixed, irrigated, highland/temperate
- MRA – Mixed, rainfed, arid/semi-arid
- MRH – Mixed, rainfed, humid/sub-humid
- MRT – Mixed, rainfed, highland/temperate
- LL – Landless (peri-urban)

The geographical coverages for each livestock production system have been mapped[237] and the distribution of systems for Africa are shown in **Figure 9.1**.

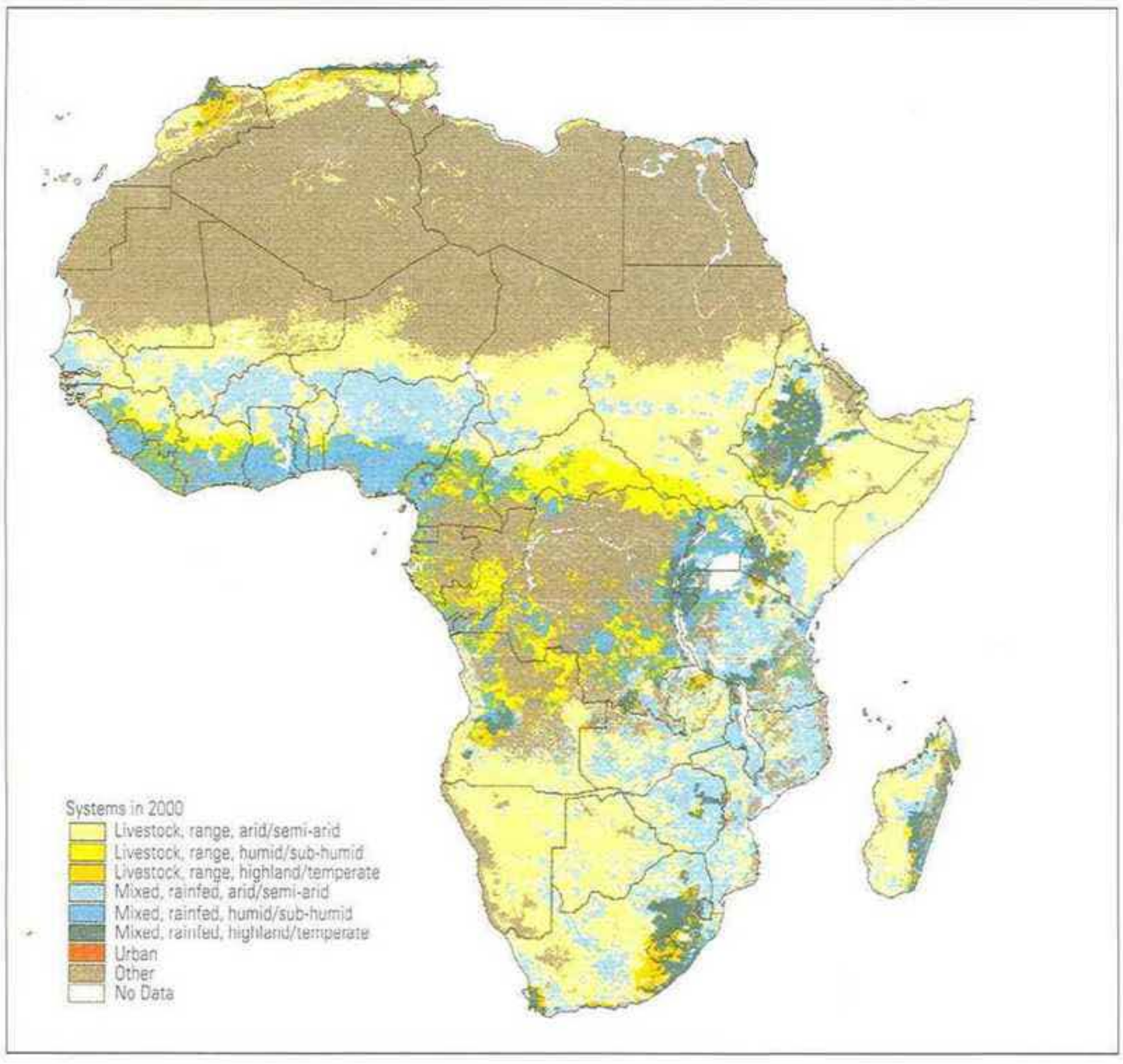


Figure 9.1 The distribution of livestock production systems in Africa (from Thornton *et al.*, 2001[237])

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In each of these systems, disease-control priorities are different, and the efficacy and viability of interventions also differ. Optimal success will be achieved by serious consideration of the socioeconomic issues influencing, indeed favouring, optimal delivery, adoption and impact in the particular target production system.

The role of livestock in African societies

Livestock play many differing roles in agriculture and the livelihoods of virtually all sectors of society in Africa (**see Table 9.2**). They are important sources of animal protein in the form of meat, milk and eggs, their hides and skins contribute to clothing people, the fat from their bones contributes to the manufacture of soap, their work contributes to ploughing maize fields and moving products to and from markets, and their manure fertilizes the soil used for crops. They also play an important role in social networking in many societies, and as a 'walking bank account'. Livestock and their products contribute to the incomes, health and livelihoods of livestock keepers, traders in livestock and their products, labourers on farms and in abattoirs, and of course to the great majority of the human population who consume livestock products in their diet. The export of livestock and their products generates valuable foreign exchange, so contributing to national economies. The health of livestock thus has a crucial influence on the health and well-being of Africa's peoples.

Livestock are particularly important to the poor. It has been estimated that livestock form a component of the livelihoods of 70 per cent of the world's poor.[123] Animal diseases are an every-day occurrence to these people, as animals of the poor are particularly vulnerable to disease (due to many reasons, including lack of knowledge about their management and control, and lack of access to — and resources for — animal health and production inputs and services). Furthermore, poor farmers usually have few animals, so the loss of an individual animal has proportionally greater significance. They also have few reserves on which to survive during lean times, livestock being one of them, which they can use for recovery.

Livestock have not always been the flavour of the moment. Not so many years ago, much publicity was given in the media of the developed world to the woes of consuming meat, and the negative environmental and animal welfare impacts of keeping large numbers of pigs and poultry under intensive management. While these views might have been relevant to the conditions in many developed nations, they totally misrepresented the conditions and demands of the poor in the developing world. In the consumer societies of the developed world, *per capita* consumption of meat and many other livestock products is predicted to decline.[49] In these high-income societies, a wide variety of readily available food products, low levels of malnutrition, and increasing levels of cardiovascular disease, diabetes and other complications of overindulgence have led to a negative view of animal pro-

Table 9.2 Domestic livestock species kept in Africa, and their contribution to national economics and household assets (from Perry *et al.* 2002[190])

SPECIES	CONTRIBUTION TO HOUSEHOLD ASSETS				
	FINANCIAL	SOCIAL	PHYSICAL	NATURAL	HUMAN
Cattle	Sales of milk, meat, hides, animals, draught power services, transport Savings instrument	Networking mechanism Social status indicator	Draught power for crop cultivation Draught power for transport	Manure for maintaining soil fertility	Household consumption of milk, meat
Camels	Sales of milk, meat, hides, animals, transport services Savings instrument	Networking mechanism Social status indicator	Draught power for transport		Household consumption of milk, meat
Donkeys and horses	Sales of animals, draught services, transport (esp. water)		Draught power for crop cultivation Draught power for transport (esp. water)	Manure for maintaining soil fertility	Provision of household water supplies
Goats and sheep	Sales of milk, meat, hides, animals Savings instrument	Networking mechanism Social status indicator		Manure for maintaining soil fertility	Household consumption of milk, meat
Pigs	Sales of meat, animals Savings instrument			Manure for maintaining soil fertility	Household consumption of meat
Poultry	Sales of eggs, meat, fowl	Networking mechanism		Manure for maintaining soil fertility	Household consumption of eggs, meat

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tein. These have been coupled with concern over the environmental threats posed by the need to dispose of large amounts of animal effluent from large-scale and intensive animal production units.

Ironically, these two factors are reversed in much of the developing world. Malnutrition is rife in many regions, and livestock products, particularly meat and milk, provide an important means to overcome this by providing protein, micronutrients and vitamins. The demand for meat and milk is predicted to grow by 2,8 and 3,3 per cent, respectively, per year, dwarfing the growth rates of 0,6 and 0,2 per cent predicted in the developed world.[49] Furthermore, livestock are a powerful means of enhancing the purchasing power of the poor through the sale of livestock products, generating income that can be used for the purchase of food, education and health care. When it comes to the environment, livestock are a most valuable asset, providing the essential fertilizer for crop production, often unavailable or unaffordable in any other form.

The realization of the starkly different roles of livestock in the developed and developing worlds, and the predictions as to how these differences are likely to continue and intensify, has reversed the negative attitudes to supporting agricultural development that involve livestock.

On a global basis, precise estimates of the numbers of poor livestock keepers, traders, labourers and consumers by region and system, and the types of livestock that contribute to their livelihoods, have not been made. Thus, it is necessary to rely on crude aggregate estimates from summary reports. These do provide a picture of the relative importance of livestock to poor people. The Livestock in Development (LID)[123] has developed global estimates of numbers of poor livestock keepers; these are presented in **Table 9.3**.

The impact of infectious diseases and of their control

Disease classification based on disease impact, transmission and control

Diseases can be classified in a variety of ways, based on their aetiology, mode of transmission, impacts on animals and humans, and epidemiology, among other criteria. In terms of their impact, a number of factors are important in determining the scale of impact. These are:

- the number of species affected;
- the proportion of animals affected;
- the size of the geographical area affected or under threat;
- the ability of the disease to spread;
- the losses associated with the disease in both animals and humans; and
- the availability, affordability and efficacy of measures to control it.

We propose a classification of infectious diseases based on the main characteristics of their transmission and on their different impacts. This is presented in **Table 9.4**. This classification contains the following categories:

1 Epidemic diseases, mainly corresponding to the list A diseases of the World Organization for Animal Health or Office International des Epizooties (OIE). These diseases are considered to be an international threat and are usually directly transmitted micro-parasites, for which the risks of epidemics accompanied by high production losses are the main impacts.
2 Diseases from the OIE list B that are directly transmitted. In general these occur sporadically or endemically. They have important impacts on livestock production, and on lost production opportunities.
3 Diseases from the OIE list B that are indirectly transmitted (mainly vector-borne). In general these occur sporadically or endemically. They also have important impacts on livestock production and lost production opportunities.
4 Farm-level endemic diseases — termed 'other diseases' in the OIE classification. Their main impacts are production effects on the farm, but these have an aggregate influence on national productivity.
5 Zoonotic diseases or infections in livestock in which a major impact is the risk of human diseases, this element of risk being important in control decisions (e.g. brucellosis, tuberculosis, *Trypanosoma rhodesiense* sleeping sickness).

Table 9.3 Numbers (in millions) of poor livestock keepers by livestock production system (from Livestock in Development, 1999[123])

AGRO-ECOLOGICAL ZONE	CATEGORY OF POOR LIVESTOCK-KEEPERS		
	EXTENSIVE GRAZIERS	POOR RAINFED MIXED FARMERS	LANDLESS LIVESTOCK KEEPERS
Arid or semi-arid	63	213	156*
Temperate (including tropical highlands)	72	85	
Humid, sub-humid and sub-tropical		89	
Total	135	387	156

* Predominately in irrigated agricultural areas, but also in other densely populated regions

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Table 9.4 Classification of infectious diseases based on their transmission, types of impact, and level of impact

CATEGORY OF INFECTIOUS DISEASE (see text)	EXAMPLES OF DISEASES	MAJOR IMPACTS	LEVEL OF IMPACTS	IMPACT INDICATORS
1 Epidemic, directly transmitted, international importance (OIE list A)	Rinderpest, foot-and-mouth disease, African swine fever, classical swine fever	Costs to minimize epidemic risk Production losses in susceptible animals if epidemic Restrictions to markets and international trade	International, regional, national, local area, farmer	Incidence of disease Control costs
2 Endemic/sporadic, directly transmitted, farm and national importance (OIE list B)	Haemorrhagic septicaemia, infectious bovine rhinotracheitis (IBR), dermatophilosis	Production losses Control costs Restrictions to marketing and trade	National, local area, farmer	Disease occurrence Production losses Control costs
3 Endemic/sporadic, indirectly transmitted, farm and national importance (OIE list B)	Babesiosis, cowdriosis, theileriosis, African animal trypanosomosis	Production losses Production opportunities lost Restrictions to marketing and trade Control costs	National, local area, farmer	Disease occurrence Vector abundance Production losses Control costs
4 Endemic/sporadic, directly transmitted, mainly farm importance (OIE other diseases)	Mastitis (*Staphylococcus aureus*), pneumonia (pasteurellosis), foot rot	Production losses Production opportunities lost Control costs	Farmer (national, local area less important)	Disease occurrence Production losses Control costs
5 Zoonotic diseases transmitted directly or indirectly from animals to humans	Brucellosis, tuberculosis, *T. rhodesiense* sleeping sickness	Impacts on human health Production losses Control costs	National, local area, farmer	Disease occurrence — animals and humans Production losses in animals Burden of disease (DALYs)* in humans Control costs
6 Food-borne infections	*E. coli* O157 infections, salmonellosis, campylobacteriosis	Impacts on human health Costs of food quality control systems	Local area, national	Burden of disease (DALYs)* in humans Control costs

* DALY = Disability Adjusted Life Year, a measure of the cost of human disease

6 Food-borne infections and intoxications, such as salmonellosis, and *Escherichia coli* O157 infections. Such infections are increasingly a problem of intensive livestock production and marketing systems in which there are significant opportunities for cross contamination of, for example, carcasses and milk supplies.

Multiple impacts of animal diseases and their control

Livestock play an important role in many agricultural systems and thus animal diseases that impair their function have multiple impacts at animal, farm, ecological zone, national, regional and international levels. Decisions on disease control depend on a good understanding of these multiple impacts. Focusing only on direct losses of overt disease can lead to a poor appreciation of the relative importance of different diseases and of what should be done, or not done, to control them.

The major impacts of different classes of infectious diseases are greatly influenced by the production system, infrastructure and other features of the socioeconomic milieu in different settings. The impacts of endemic diseases are mainly felt at farm level, while broader economic impacts can occur with epidemic diseases that restrict marketing and trade in livestock and livestock products. The occurrence of epidemic diseases affects both poor and richer livestock producers by marginalizing them from higher-price livestock markets and restricting their capacity for value-added trade.[135] The overall benefits of the control of epidemic diseases are great[188] and in rich countries, where resources are available for their eradication, this option is invariably pursued. However, numerous recent examples highlight how the maintenance of disease-free status, when the disease still occurs elsewhere, is risky (e.g. foot-and-mouth disease (FMD) introductions worldwide, and classical swine fever reoccurrence in the Netherlands). The specific impacts of epidemic diseases and their control include both direct disease effects and potential trade benefits. For the latter, there are usually differential

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benefits and costs to different livestock sectors.[135, 188]

In recent years, a wider perspective on disease impacts has been adopted that considers both the direct effects, as well as a number of non-livestock (indirect) effects. An important consideration is to differentiate impacts associated with the occurrence of disease from those associated with the avoidance of disease risk. These are illustrated in **Figure 9.2**.

The framework in the figure is particularly useful for considering the more profound impacts, beyond direct productivity effects, that livestock diseases have on African production systems. These include the impacts of disease on control costs, the constraints on livestock management imposed by disease risk, including limitations on species and breed choices, as well as impacts on the production of agricultural and other products, natural resource use, and human welfare. The following sections briefly describe the major types of impact.

Direct effects on livestock productivity The direct effects of animal diseases on livestock productivity have been comprehensively described by Morris[153] and are illustrated in **Figure 9.3**. These range from reduced feed intake and changes in digestion and metabolism to increased morbidity and mortality, and decreased reproduction, weight gain and milk production. They influence product quality at animal level, and their aggregate effects influence herd productivity. In addition, they limit economically important herd management decisions such as animal selection and optimal longevity. The interactions between disease, nutrition and genetic selection are important and emphasize the necessity to control the effects of key epidemic and endemic diseases before enhanced nutrition and genetic programmes can make an impact. Likewise, substantial productivity and economic gains will not necessarily be achieved by disease control alone.

Risk management — control costs and lost potential Considerable costs are often incurred in controlling animal diseases and investments in control measures paid for may not always be effective. This is particularly true for smallholder farmers, who often lack information and have limited diagnostic support to make appropriate disease control and treatment decisions.[132] The overall impact of control measures may be constrained by non-compliance of a significant proportion of the community, emphasizing the need for regulatory veterinary services supported by legislation and incentives to comply. An important principle, emphasized by several authors[134, 188] is that it is not the total losses associated with diseases that are important, but rather the benefits relative to costs that can be obtained from different control options. An important loss associated with animal diseases is the cost of not investing in production inputs and adopting less profitable livestock management strategies to avoid disease

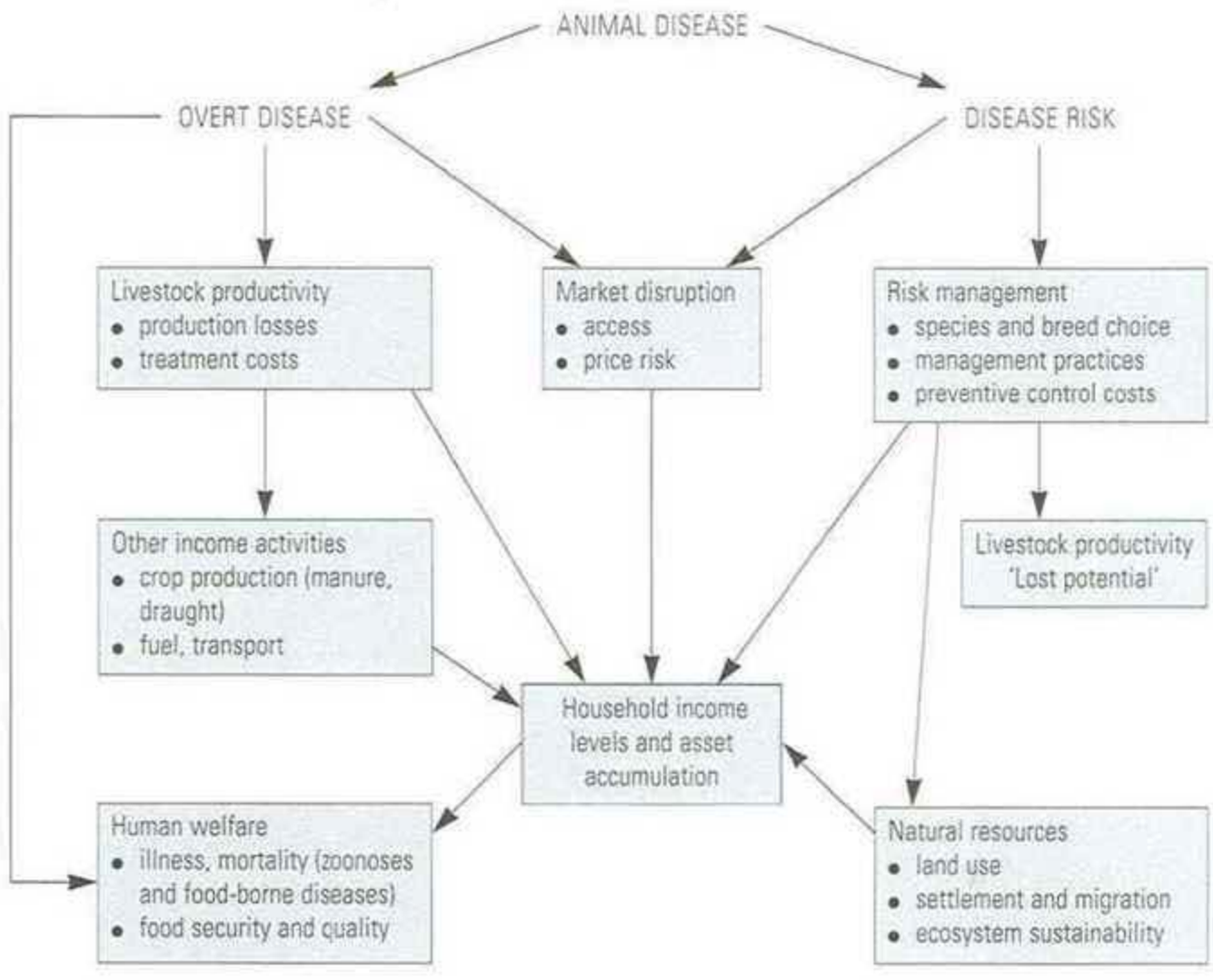


Figure 9.2 Disease impacts (adapted by Perry *et al.*, 2002[190] from Swallow, 2000[232])

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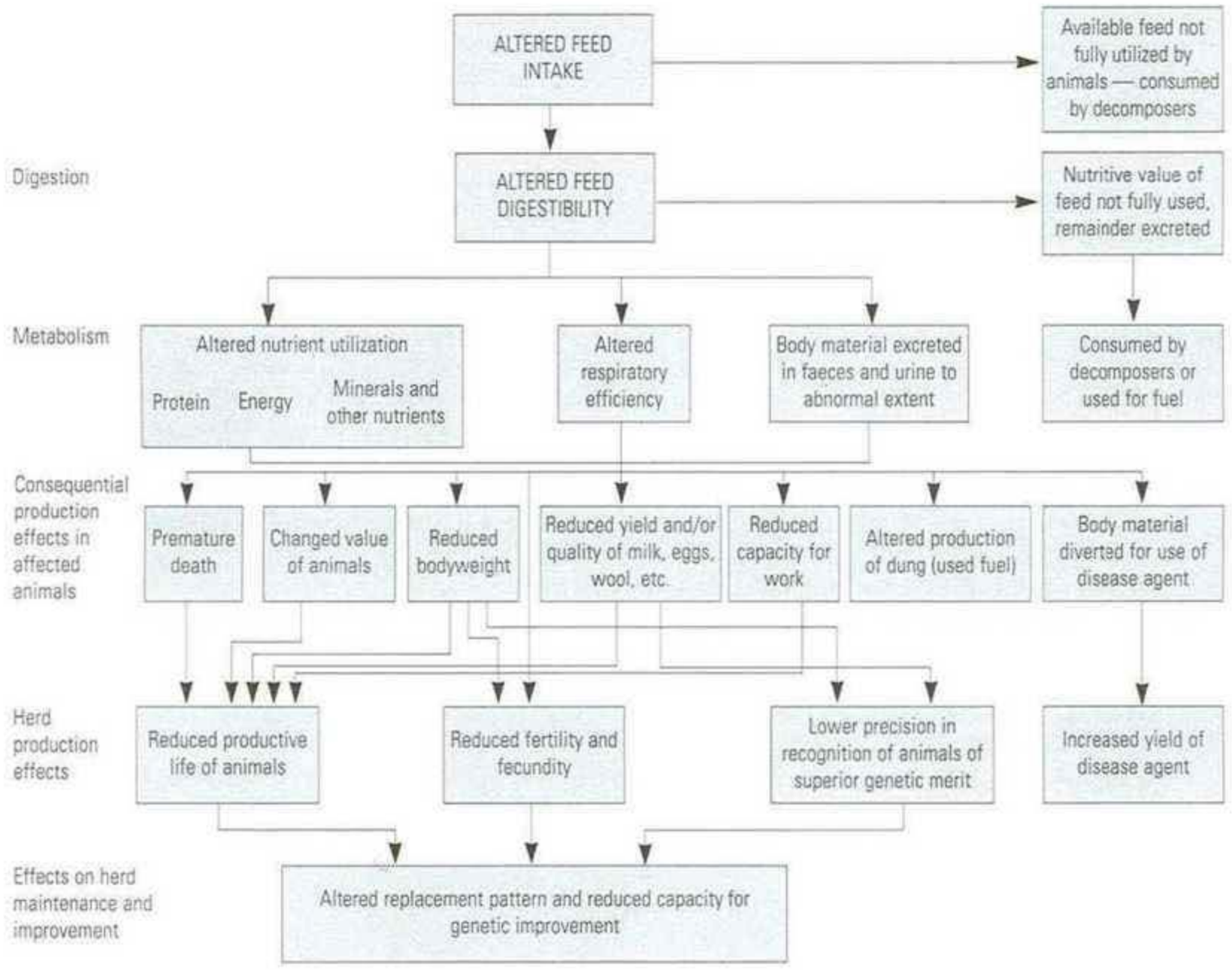


Figure 9.3 The ways in which diseases may affect the productive value of animals (redrawn from Morris & Marsh, 1994[154])

risk.[232] Such strategies may include a reduction in livestock numbers (for example in tsetse-infested versus non-infested areas in sub-Saharan Africa[111]), variations in grazing practices to avoid tsetse challenge,[248] and limitations in breed choices (such as the necessary use of trypanotolerant breeds of cattle and small ruminants in West Africa).[224]

Impacts on crops and other farm enterprises Given the key role of livestock in mixed farming systems, there are numerous effects of animal diseases on other agricultural enterprises beyond livestock themselves. These include impacts on crop production through decreased nutrient cycling and draught power, and loss of other products such as fuel and transport. Intensive smallholder farming systems in many regions of the developing world have integrated livestock into all other agricultural practices to such an extent that livestock diseases, when they occur, will have multiple agricultural production impacts. Good examples of such crop–livestock systems in Africa include the smallholder dairy production system in the East African highlands[130] and the mixed crop–livestock systems in sub-humid West Africa.[130] The multiple impacts of trypanosomosis in this latter system are highlighted in **Table 9.5**. The integration of livestock into mixed farming systems is now widely considered to be an essential component for sustainable agricultural development.[244]

Ecological impacts Another key category of livestock disease impacts is on the utilization of natural resources and ecological impacts. Livestock have been considered harmful to the environment and implicated in a host of environmental sins including desertification, deforestation, global warming, and pollution. Thus, the control of livestock diseases, particularly large-scale disease control efforts, have been the subject of much debate and environmental concerns have had a negative effect on investment in livestock disease control. This has been especially true of tsetse fly and trypanosomosis control in Africa. However, recent evidence suggests that the environmental impacts of disease control efforts are not invariably negative, but can be also positive or neutral, depending largely on how people choose to manage their livestock in response to reduced disease risk (see Reid *et al.*[204] for a fuller discussion related to tsetse fly

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and trypanosomosis control and its effect on land use, settlement and migration, ecological habitat and biodiversity). Considering such inter-linkages is crucial in arid and semi-arid environments, where livestock are usually the key means for utilizing available natural resources and where the risk of animal diseases (among other risks) complicates rationale utilization of available natural resources. This has important implications for poor livestock keepers, who rely almost exclusively on common-property natural resources. These common-property resources are declining in most areas of Africa.

Human welfare and human disease Animal diseases have multiple, important and measurable effects on human welfare. Many of these have been described in considerable detail by Schwabe,[220] particularly the importance of animal products in human nutrition, the social benefits of livestock, and the impact of food-borne infections and zoonotic diseases. Livestock products have been shown to have considerable impacts on improving child nutrition,[113] and animal disease control has also been shown to benefit child nutrition and welfare.[44, 97] This is particularly so in pastoralist communities, in which 75 per cent or more of general and child nutrition is from milk and other livestock products.[37]

Traditional zoonotic diseases, such as brucellosis, bovine tuberculosis, hydatid disease and anthrax, for example, have impacts on both animal and human health. While many of the zoonotic diseases are not among the top impact human diseases globally[159] — where the impact is measured by the Disability Adjusted Life Years (DALYs) lost, they are very important among livestock keepers and workers handling livestock products. A recent study in Mongolia has shown cost:benefit ratios of approximately 10:1 for the control of brucellosis in livestock and 2:1 for the direct health costs such as hospital care and drugs.[212] In addition, it was estimated that one year of human disability (DALY) could be avoided for US$ 34. This study highlights the importance and benefits of policy changes to enhance inter-sectoral assessment and action for controlling zoonotic diseases.

The intensification of livestock systems in many areas of the developing world[49] is expected to increase the transmission and impacts of food-borne infections and intoxications. However, because of poor surveillance and diagnostic facilities, their impacts on consumers, labourers and traders of livestock products in many African and other developing countries are likely to be poorly understood and ineffectively controlled. Currently, low-income consumers face greater risk from food-borne infections such as anthrax and hydatid disease, because they often buy livestock products in parallel markets in which meat inspection is non-existent. In addition to these classic diseases, new zoonotic risks are likely to develop, particularly in the area of food-borne infections in the more industrialized production and processing systems of developing countries. These food-borne infections include *Salmonella* serovars, *Camplyobacter* spp. and enterotoxigenic coliforms. It is also likely that public health will be further compromised by increasing levels of drug residues and antibiotic resistance. For low-income consumers, this will be a serious problem, as they will confront many of the multiple antibiotic-resistant infections occurring in Western countries but without the benefit of newer generation antibiotic treatments.

Impacts of disease on market access Outbreaks of infectious diseases in an area or country may result in local and/or international market disruptions as the movements of animals and livestock products are restricted. Two important examples in Africa in recent years have been the trade restriction on livestock movements from the Horn of Africa to the Arabian Peninsula associated with outbreaks of Rift Valley fever and the restriction of meat exports from southern Africa associated with FMD. The impact of trade restrictions on livestock trade in the Horn of Africa has been particularly devastating and is highlighted below.

In Somaliland alone, the ban associated with Rift Valley fever prevented the export of two million head of sheep and goats, valued at US$ 100 million between February 1998 and May 1999 (**Figure 9.4**). Because of this, local livestock prices decreased by 30 per cent, although these lower prices did

Table 9.5 Multiple impacts of trypanosomosis in crop-livestock systems (mainly from Swallow, 2000[232])

Direct impacts on livestock productivity
– reduced calving rates (1–12% tolerant; 11–20% susceptible)
– increased calf mortality (0–10% tolerant; 10–20% susceptible)
– small ruminants (lambing/kidding rates decreased 4–28% / 37%)
– decreased milk production (cow: 10–26% tolerant; land-area: – 83%)
– decreased animal offtake (herd: 5–31%; land-area: – 97%)
– drug use (SSA – US$ 35 million)
Impacts on livestock risk management
– decreased cattle numbers (arid – 14%; sub-humid – 27%; humid – 77%)
– grazing changes[23, 248]
Impacts on agricultural and other products
– decreased draught efficiency (40%)
– increased crop production (+25–45% per unit land; +140–143% per unit labour)
– decrease in agricultural gross domestic product (GDP) in affected countries (from 5–10%)
Effects on natural resource use
– change in migration/settlement patterns (variable effects)
– limited to moderate changes in biodiversity associated with tsetse fly control
Impacts on human welfare
– loss of income and assets related to impacts above
– livestock reservoir of sleeping sickness, Eastern, Southern and West Africa (limited importance in Central Africa)

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not persist. Unfortunately, resolving the removal of this ban will not be easy.

Sanitary and phyto-sanitary (SPS) requirements often have an even greater effect on market access in local markets. In many African countries, sanitary regulations for livestock products have been adopted from those put in place for industrialized systems in developed countries. These may or may not be reasonable or appropriate, depending on local conditions. In **Table 9.6**, the health risks associated with the marketing of raw and pasteurized milk in Kenya are explored in terms of protection of public health versus market access and income-generating activities.

Impacts on household income and asset accumulation

The benefits of livestock as a regular source of 'income', both for cash and barter, have been detailed in numerous studies.[123] A number of observers (cited in LID, 1999[123]) have shown that poor farmers (both those with small land size or those who are landless) are increasingly relying on livestock as their main income source. In addition to income, livestock are often the main way in which poor farmers can acquire real assets, providing a safety factor when difficulties strike. The asset acquisition pathway usually begins with poultry, followed by small ruminants and/or pigs, with larger stock such as equids, cattle and water buffalo (*Bubalus bubalis*) being acquired at later stages. Animal diseases are a key constraint to both income generation and asset acquisition, especially for the poor, since they usually have limited access to cash to pay for animal health services.[35, 45]

In addition to improving the welfare of livestock keepers, enhancing livestock production through the control of animal diseases can have multiple benefits to consumers, traders and labourers. This is particularly true if disease control and other livestock development benefits are focused on poor livestock keepers such as smallholder mixed farmers and pastoralists. Enhanced livestock production on smallholder farms tends to be labour-intensive, using both surplus family labour and to a lesser extent labour from non-family members.[19] Improved smallholder dairy production has also been shown to increase labour opportunities for small-scale milk traders and to lower transaction costs so that milk prices to poor consumers are considerably lower (**Table 9.6**).[173]

The importance of animal diseases to the poor in sub-Saharan Africa

Because of the impact that animal diseases can have on poor communities, disease control can be an important tool for improving poverty alleviation and enhancing social equity. This topic has been investigated in some detail in a report commissioned by the Inter-Agency Group of Donors Supporting Research on Livestock Production and Health, and sponsored by the Department for International Development (DFID) of the Government of the United Kingdom.[190]

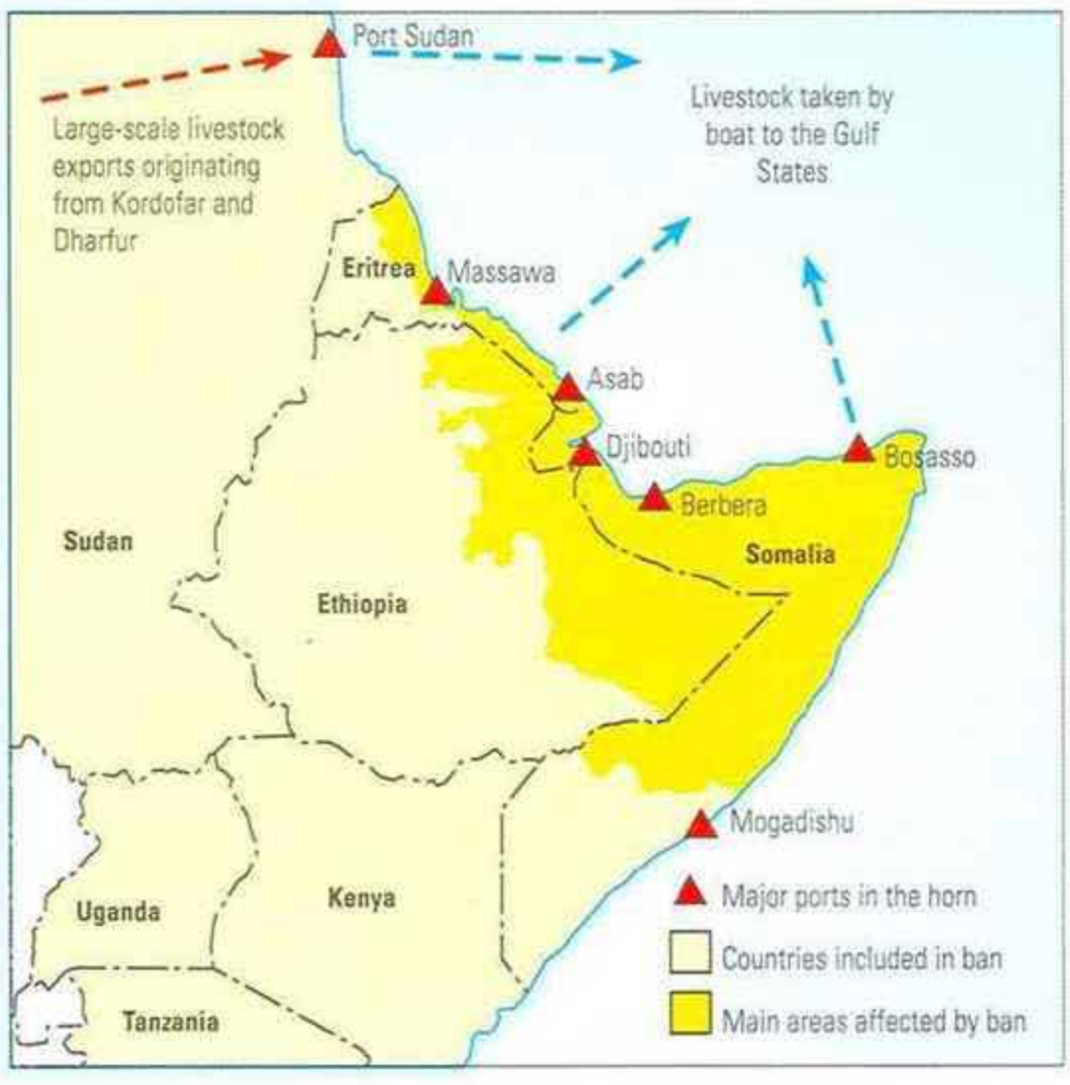


Figure 9.4 Main livestock production areas in the Horn of Africa affected by the Rift Valley fever export ban (source: FEWS NET, 2000[64])

Table 9.6 Assessing and managing milk-borne health risks for the benefit of consumers in Kenya (from Omore *et al.*, 2000[173])

In Kenya, approximately 90 per cent of milk is produced by smallholder farmers. Much of this is consumed locally but increasing amounts are being collected, transported and sold in towns and cities by small- and large-scale traders.
Omore and colleagues[173] assessed a variety of risks associated with different milk marketing systems. Overall, both raw and pasteurized milk rarely met their accepted standards for bacterial counts. Ninety per cent of milk reaching consumers was not pasteurized but 95 per cent of it was boiled before consumption. Worryingly, 5 to 15 per cent of milk sampled had antibacterial residues; however, *Salmonella* and *E. coli* O157 were only rarely detected. Zoonoses (brucellosis and bovine-source tuberculosis) were not detected in milk from smallholder farms but antibodies to brucellosis in milk indicate that there is some risk that increases, as milk is collected and bulked from many sources.
This study highlights the trade-offs that will need to be considered, in weighing both public health and economic benefits to producers, traders and consumers. Two milk marketing systems are emerging — a formal market for high-quality milk and milk products that will require the highest milk quality standards and an informal milk market involving small-scale producers and traders who sell unpasteurized milk at a third less cost to consumers. The informal sector provides employment to thousands of people. The authours recommend measures to improve the quality and safety of milk from the informal sector (advice to consumers on boiling milk, training and improved handling containers for small-scale traders) and maintain economic benefits for poor farmers, traders and consumers.

Many of the points made thus far on the multiple impacts of animal disease are particularly relevant to the poor, who are often at the greatest risk of suffering from the deleterious effects of animal and zoonotic diseases and who have the least capacity to control disease or cope with disease risk. Here, we briefly outline the main pathways in which the control of animal disease can benefit poor people and illustrate this with an example of the control of Newcastle disease. Readers are referred to Perry *et al.*[190] for a fuller account of the impact of animal diseases on the poor.

The control of livestock diseases is likely to improve the livelihoods (see Carney[32] and DFID,[48] for further accounts of livelihoods issues) of the poor in three main ways:

- Secure the current assets (human, financial, social) of poor people who keep livestock, consume livestock products, market livestock and livestock products, and work as wage labourers with livestock, by reducing the risks they experience through animal and zoonotic diseases.
- Enhance the marketing opportunities of the poor by controlling the diseases that affect the movement and marketing of livestock and animal products, primarily locally, but also regionally and internationally.
- Reduce the constraints experienced by the poor to livestock-based pathways out of poverty through intensification (improved productivity and performance efficiency through the use of inputs).

For many diseases, control efforts targeted at poor livestock keepers will simply require the transfer of available knowledge and tools; for other diseases, current tools and delivery systems will need to be adapted and improved so that they are appropriate for local conditions, and in particular for optimal uptake by the poor. In some instances, new technologies, such as vaccines and other disease control tools, will need to be developed based on new scientific methods and approaches.

It is generally acknowledged that poultry are very important to the poorest of the poor. The major disease constraint to poultry keeping throughout the developing world is Newcastle disease.[228] However, greater benefits from Newcastle disease control could be enjoyed if other inputs such as the control of other important diseases (e.g. Gumboro, fowl pox, internal parasites) plus improved housing and feeding were also available as a package. Essentially, some benefits in securing poultry assets will be achieved by providing thermostable Newcastle disease vaccine alone but greater intensification and income generation benefits could be obtained if input packages were delivered. These are expanded upon in **Table 9.7**.

Measuring the impacts of infectious diseases and their control

There is a series of data needs required for the evaluation of disease impacts, and of the impacts of alternative interventions to prevent or control them. These are similar in principle regardless of the scale of assessment (i.e. whether at the level of herd, community, production system, agro-ecological zone, country or region). These fall into the following broad categories:

- Size, structure and composition of animal population under consideration.
- Characteristics of animal population and its environment, particularly in terms of agro-ecology and feeding management.
- Disease occurrence, in terms of prevalence, incidence, distribution and dynamics.

Table 9.7 Assessing options for Newcastle disease (ND) control by vaccination in smallholder subsistence poultry systems of the developing world

Socio-political and environmental context
- Limited input/output systems
- Variety of social networks
- Relatively weak public support institutions for credit, animal health and production inputs
- Markets variable but some local demand

Acquisition of capital
- Village poultry accessible to poor and often provide an effective starting point for acquisition of additional livestock assets (pigs, small ruminants); livestock assets maintain their value in environments with weak banking systems and are crucial in times of crisis (such as health emergencies, etc.)
- Can be instrumental in providing funds for school fees (human capital);[93] enhanced nutrition — particularly important for child development[113]
- Depending on scale, markets for village poultry are relatively robust (physical capital) but will require enhancement for larger-scale production
- Importance of poultry as social capital — ability to fulfil social obligations[228]

Evaluation of opportunities
- 50% mortality rate main reason for low output; 50% of mortalities due to infectious diseases; ND most important and widespread infectious disease of poultry[179] (Annex 12 in Reference[190])

Potential options
A) Distribution of thermostable ND vaccine in feed (S.E. Asia) and by eye drop (Mozambique)[229]
B) Enhanced input-output systems including ND vaccination:[214] vaccination, micro-credit, improved breeds and feeds

Assessment of options

Option A: Appropriate vaccination strategies to secure assets
- success depends on development of appropriate and very inexpensive vaccine production and distribution systems (being developed and assessed by ACIAR and partners)
- limited benefits in terms of aquisition of capital can be captured by poor people in a variety of contexts
- economic benefits (cost:benefit ratio 14:1[104]) important but limited due to constraints from other limiting inputs and market potential depending on market access

Option B: Vaccination and other inputs to improve the intensification of rural poultry keeping
- greater success possible if more integrated support systems developed (housing, feeding, etc) but this is not an easily delivered option everywhere (successfully applied in Bangladesh[214] but needs assessment elsewhere)
- has greatest potential for intensification and helping to reduce poverty but requires enhanced markets for both input delivery and outputs

- Effects of disease (or of interventions) on productivity (or other key indicators).
- Value of productivity losses of disease/incremental gain from intervention.
- Value of control costs of disease/incremental savings from intervention.
- Impacts of zoonotic diseases (or of interventions) on the human population.
- Environmental impacts of disease (or of interventions).

This series combines demographic, epidemiological, productivity and socioeconomic data, illustrating the increasing trend towards a multi-disciplinary approach to disease and disease control impact assessments. Clearly, as a general principle, the broader the range of data included, and the better the quality of the data, the better the resulting decision. However, the collection and processing of data also have a cost in terms of money, time and communication, which must be taken into consideration, and may have a significant effect on the quality and value of any impact assessment study.

For each of these categories of data, there is a set of techniques that can be used for data collection and assembly, and these are listed and discussed in general terms below.

Use of intuitive response

Many impact assessments use intuitive valuations of some or all of the parameters listed. This might sound very non-scientific and obvious, but is the rule rather than the exception at the farm level regardless of the production system. Even at the national level, where resources are inadequate and when impact assessments are demanded within a short time, this technique is common. As a general rule, when made by farmers, this tends to be based on a combination of personal experience and financial commonsense, when made by economists it tends to be on the basis of sound economic criteria regardless of technical considerations, and when made by veterinarians it tends to be on the basis of technical considerations regardless of economics!

Passive surveillance systems

The standard source of quantitative data on livestock populations and infectious disease occurrence in most countries is still from national passive reporting systems. In these, livestock population sizes by administrative boundary (district, province) and cases of a given disease observed by the veterinary department are compiled in periodic reports. The advantage of using such data in an impact assessment is that they are available, and generally at no cost to the user (but of course often at a significant cost to the state in their

compilation and publication). However, there are numerous disadvantages, which usually far outweigh the advantages. The data are often long out of date, with some annual reports of veterinary departments in Africa published 10 years late. Furthermore, there is a tremendous variation in the method of reporting used. As far as livestock population sizes are concerned, there are only a few countries that carry out regular livestock censuses. In many cases, populations seem to mysteriously increase annually by a standard index. There is also a large variation in the categories of livestock quantified, some taking species, some dividing within species (such as beef and dairy), and very few quantifying on the basis of production system.

The continuation by most national veterinary authorities in Africa to report livestock populations on the basis of species rather than production system severely hampers impact assessments. We have, for example, experienced this recently in studies of the impact of FMD and its control in the countries of south-east Asia. The pig populations of many countries in the region are reported as a single entity. Hidden within this are at least two contrasting production systems, in which both the infection dynamics of FMD and the economic impact of its control are entirely different.[188] Medium- and large-scale pig production present in Thailand, the Philippines and to a lesser extent Vietnam is similar to commercial production enterprises in many parts of the world, with intensive hygiene measures, regular preventive FMD vaccination, and little in the way of FMD occurrence. In contrast, the vast village and 'backyard' sectors are highly vulnerable with little vaccination, hygiene and movement control being applied, and as a consequence, frequent outbreaks of the disease occur.

As far as disease reporting in passive surveillance systems is concerned, there is much variation from country to country, and several different indices are used. For example, some use number of cases, while others use number of outbreaks. In both instances, there is often a mixture of clinical and laboratory records contributing to this index, which may go undifferentiated. This is particularly important with infections in which the mere presence of an organism does not necessarily mean it has anything to do with the mortality, disease or production loss under investigation. Mere identification of haemo- or gastrointestinal parasites, for example, is often taken as diagnostic when their presence may have been merely coincidental.

Generally, passively derived systems have been at their best when not applied nationally, but rather strategically and demand-driven for specific diseases. The overall decline of passively derived disease data has probably been less in developed countries and in the more commercial livestock enterprises of southern Africa. In these areas, there has been a significant shift in data collection techniques, through greater focus and more use of active methods. These changes have been supported by improved technologies for animal identification and more effective trace-back procedures, enhanced by the information and communications explosion, and the Internet. There has been a greater focus on priority diseases or disease complexes, on greater privatization of the data collection and analysis, which then become a marketable product, as well on the greater use of active surveillance techniques.

The decision as to whether or not to use official reports of disease occurrence based on a passive reporting system will depend on a deeper knowledge of the quality of the data, and on whether standard reporting procedures are being followed. This can vary dramatically from place to place, and from year to year, depending on the enthusiasm and diligence of individuals, and of course on the resources available for such a service.

In passive surveillance, consideration is rarely given to reporting the productivity effects of a disease (or a control intervention), productivity losses/incremental gains from an intervention, or control costs/incremental savings from an intervention.

Active surveillance systems

As a result of an expert consultation, FAO[60] developed a framework for animal health information systems in developing countries that could serve the varying needs these countries face. A key component of this report was a recognition that the passive disease information systems that have been in place for so many decades are often of little value, frequently being out of touch and highly inaccurate. Strategic active surveillance, and strategic sample surveys and studies with focus, were considered to be much more useful. It has been encouraging to see this approach adopted in some regions; for example, the southern African countries recently held a workshop in Pretoria on demand-driven animal health information systems in collaboration with the OIE, FAO and ILRI.

Rabies surveillance in Kenya provides a good illustrative example of the problems of passive collection of data and the potential opportunities for strategic active data collection. For many years, passive data collection for rabies was considered to provide a reasonable indication of rabies occurrence in Kenya, albeit an underestimated one, particularly in non-endemic rabies areas. Potential rabies exposures were reported to veterinary offices for follow-up and any humans considered exposed were referred to government medical officers for free/subsidized post-exposure treatment. However, over time, the latter form of post-exposure treatment was withdrawn, without alternative suppliers being established, and many people gradually stopped reporting cases as there was no incentive to do so. In order to better assess the rabies situation with a view to improving control, Kitala *et al.*[106] established an active surveillance system using stratified random sampling for human and animal rabies, linked to a network of private-sector vaccine suppliers. Active surveillance through community rabies workers uncovered 40 times more rabies

cases than the passive surveillance system, as well as providing data on dog demographics and transmission parameters for the development of models to assess rabies transmission and the potential impact of control measures.[107] Depending on resource availability and priorities, countries could consider mixes of passive surveillance (if it can be linked to animal or human health service provision) and active surveillance from sites chosen using probability-based sampling methods.

Observational studies

There is a large number of different types of observational surveys and studies that can be used to gain more precise data on livestock populations, their characteristics, infection and disease occurrence, and disease causality. Below is outlined the general characteristics of such studies, but for more detailed discussion of their relative merits, and design and analysis issues, consult the numerous texts on the subject.[143, 180, 201, 221, 227, 239, 240]

Surveys The tool used to gather further information from the field on a particular subject is often termed a 'survey'. Thrusfield,[239] a stickler for detail, differentiated between surveys and studies as follows: He defines surveys as counting the occurrence of something (of a disease, for example), whereas studies involve the comparison of the occurrence of the event of interest in different groups of animals, usually undertaken to elucidate causal associations. The major characteristics and useful features of different survey and study types are outlined in **Table 9.8**.

Observational studies are structured comparisons of the risk or rates (depending on the study) of disease (or of other parameters indicative of disease, such as infection, or decreased productivity) between different subsets of the animal population being studied. Successful studies depend on the accurate identification of 1) the presence of disease (or infection) in the study group, and 2) the presence or absence of attributes that may be determinants of the disease (or infection, or deviation from expected performance). Thus in its simplest form, this results in the generation of a 2 × 2 contingency table, as illustrated in **Table 9.9**.

Cross-sectional studies In the process of better understanding diseases, their causes, their impacts and methods to optimally control them, a sensible starting place is to describe disease prevalence and what factors or attributes may be associated with it. For common diseases, cross-

Table 9.8 A summary of the main features of surveys, observational studies and clinical trials in veterinary epidemiology

	RETROSPECTIVE			PROSPECTIVE		
TYPE	SURVEY	CROSS-SECTIONAL STUDY	CASE-CONTROL STUDY	LONGITUDINAL OBSERVATION STUDY	COHORT STUDY	CLINICAL TRIAL
Disease or infection frequency unit measured	Prevalence (sometimes incidence)	Prevalence	Prevalence	Prevalence and incidence	Incidence	Incidence
Comparison group	None (counting cases)	Non-affected animals in the study	Selected controls, matched with 'cases' for factors (e.g. breed, age)	Usually none	Selected animals not exposed to potential causal determinant	Matched animals exposed to infection or disease, but not to treatment
Primary data analysis procedure	Sum, mean, median	Relative risk, attributable risk	Odds ratio, attributable risk	Depends on parameter(s) being monitored	Relative risk, attributable risk	Relative risk, attributable risk
Ease of conduct	Very easy and cheap	Easy and cheap	Moderately easy and cheap	Hard and expensive	Hard and expensive	Hard and expensive
Level of causal proof	Low	Low	Low to moderate	High	High	High
Degree of investigator control	None	Very low	Low	Moderate	Moderate	High
Relevance to the field situation	Moderate	High but poor for impact measures	Moderate and poor for impact measures	High and good for impact if study population is representative	High and good for impact if study population is representative	High and good for impact if study population is representative

sectional studies are the logical, quickest and easiest first step. For rare diseases, when disease records are available, case-control studies to compare cases with different non-case groups are commonly employed. After preliminary information on a disease has been collected, more powerful but costly study designs, such as cohort studies or clinical trials, need to be employed to assess the impact of risk factors on the incidence of the disease and to assess the impact of control factors on direct and indirect losses associated with the disease.

Thus, the commonest study used in the field in sub-Saharan Africa has been the cross-sectional. This has been the starting point for almost all efforts to study infectious disease occurrence in the field. It can be used to establish the prevalence of a given disease or infection, and the association with different natural (such as agro-ecological) or human-made variables (such as management or feeding). It has been quite extensively used to study the epidemiology of ECF over the last decade or so.[47, 75, 138] At the commencement of a cross-sectional study, the only number that is known is the total animal population under study (or the number of the random or otherwise selected sample of that population). The number with and without the disease, with and without the attributes under investigation that might be associated with the disease, and any quantities to be estimated in the study, are unknown. Importantly, it will usually not be possible to differentiate between old and new cases (of disease or incidents of infection in the case of antibody prevalence being taken as the indicator of disease) in this type of study. The cross-sectional study therefore measures point prevalence. The attributes being studied as influencing disease risk may have been acquired at different times than the disease itself, and thus causal relationships can be difficult to establish. However, causal relationships are somewhat easier to establish for attributes that are relatively unchanging and characteristic of the production system in which the animals are found. On balance, the principal benefits of cross-sectional studies are their ability to estimate prevalence of disease (which may be a rough guide to its impact) and how prevalence cases are distributed by potential risk groups.

Case-control studies are also relatively easy and cheap to perform and have been used as a first step in trying to assess disease impact and factors associated with disease occurrence. The initial step is to identify 'cases' of the disease within a study population. Strict diagnostic guidelines must be established to define the case; the value of this type of study hinges on the quality of the diagnostic criteria, particularly with respect to their specificity. The same guidelines are then used to select non-cases, or controls, from the same population. Depending on the rarity of the disease, multiple controls per case may be selected. Case-control designs were initially responsible for linking smoking to lung cancer in humans,[53] and have been used in the veterinary field to study a variety of conditions including feline urolithiasis,[253] respiratory disease outbreaks in cattle[200] and an acute and highly fatal intestinal syndrome in horses.[185] While often very useful, case-control studies are susceptible to multiple biases, which has led them to be dismissed by many.[61] Some main drawbacks include trying to extrapolate results from a specific control population (based on hospital or slaughterhouse records) and changes in the case population after their detection

Table 9.9 Basic data analysis for assessing the association between a two-category disease risk factor and disease or infection occurrence in different types of observational studies

		DISEASE (OR INFECTION) STATUS		
		PRESENT (D+)	ABSENT (D−)	TOTAL
RISK FACTOR STATUS	PRESENT (F+)	a	b	a + b
	ABSENT (F−)	c	d	c + d
	TOTAL	a + c	b + d	N = a + b + c + d

TYPE OF STUDY	SEQUENCE OF RISK FACTOR AND DISEASE STATUS DEFINITION	CALCULATION OF RISK MEASURE
Cross-sectional	F+ D+ F− D− Information on risk factors and disease status collected at one time	$\frac{P(D+/F+)}{P(D+/F-)}$ (Relative risk from prevalence data)
Case-control	F+/F− ← D+ D− Disease cases and controls chosen and retrospectively checked for risk factor status	$\frac{P(D+/F+) / P(D-/F+)}{P(C+/F-) / P(D-/F-)}$ (Odds ratio)
Cohort	F+ → D+/D− F− Disease-free populations with and without the risk factor are followed to determine disease incidence	$\frac{P(D+/F+)}{P(D+/F-)}$ (Relative risk from incidence data)

(e.g. dairy farmers with antibiotic residue violations who subsequently are better informed of sound antibiotic administration practices). These biases can be reduced to some extent, for example by investigating multiple control groups (often a control group in which the disease has been definitively excluded and a control group from the general population).

Longitudinal studies Both cross-sectional and case-control studies provide a 'snapshot' of disease in a population, providing useful spatial data but lacking in temporal information. Investigation of what causal factors precede infection or disease incidence and the sequence of disease and control impacts are best investigated in longitudinal studies, in which study populations are monitored over time. There are three main variants of longitudinal study design. The first does not (necessarily) involve a comparison group, and is simply the monitoring of particular disease features over time. This may be simply to determine the dynamics of a particular infectious disease under defined field conditions over time, or to determine the relationship between levels of infection challenge and host response (in terms of immune response, morbidity, mortality and other productivity parameters). An important feature is the ability to measure infection and disease incidence, and disease-specific morbidity and mortality, all crucial components of impact assessment studies. However, longitudinal studies are expensive to set up and are logistically demanding, particularly if the study size is large. In addition, because of their expense and complexity, they are usually carried out under very defined local conditions, and it may not be appropriate to extrapolate the results to other circumstances.

Two other study types using a longitudinal structure, beginning with a disease-free population and monitoring the incidence of disease over time, are cohort studies (an observation study type) and clinical trials (an experimental study type). In cohort studies, the starting point is identifying a group of animals exposed to a particular suspect causal determinant, and then the identification of a matched group of animals not exposed. Both groups are monitored for the development of disease. This design of study is commonly used in human medicine with registered members of the population with certain characteristics (e.g. located in a high-risk radiation area, or with diabetes) to monitor their subsequent health compared to groups without those characteristics. In veterinary medicine, this design is primarily used where detailed herd monitoring schemes are in place. One example of a cohort study used to assess the incidence of ECF in different risk cohorts of female calves was conducted by Gitau *et al.*[77]

Clinical and field trials In assessing the impact of diseases and their control, the most important aspect is not what the total losses associated with a disease are but what benefits can be obtained using specific strategies to control the disease.[183] Clinical and field trials provide the optimal method for assessing the impact of control strategies on disease incidence, livestock production, reduction in control costs and other direct and indirect disease impacts (see above). The key advantage of these trials is that they are conducted in real populations of interest, either individual animals, farms or disease control areas. The more the study groups resemble the diseased populations of interest, the greater the ability to extrapolate results widely. Another useful feature is that new control strategies can be compared to the current standard strategy, thus avoiding the difficult situation of trying to force farmers to apply no control in the face of disease risk. Finally, because their design requires the random allocation of animals or farms into treatment groups, clinical or field trials are extremely powerful, providing for statistical comparisons to be made without the worry of confounding, which is a constant problem in observational studies. Essentially, clinical trials have a very basic design. A study population, representing the reference population of interest, is assembled and randomly allocated into different treatment groups, depending on the treatment or control comparisons to be made. Treatments are made according to a standard protocol and, when possible, some degree of blinding, in which those who assess the disease and productivity outcomes are unaware of the treatment status. Following treatment, standard follow-up protocols are implemented to record loss-to-follow-up and clinical and production parameters of interest. Because individuals or herds are randomly assigned to treatment groups, the statistical comparison to assess differences between groups is usually straightforward unless there has been differential loss-to-follow-up. Further details on clinical and field trials can be found in standard veterinary epidemiology texts, as well as in Nordhuizen *et al.*[164] and Meinert.[148]

Important considerations in conducting epidemiological studies in the field Standard survey and study designs invariably need to be adapted to field conditions. The challenge is to maintain the essential features of observational or experimental designs that will lead to valid, meaningful and interpretable results. In **Table 9.10** a checklist of key generic steps that need to be followed when planning a survey or study is provided.

Proper sampling is crucial to designing a study that provides maximum information for minimum cost and to obtaining valid results. Given its importance, there are surprisingly few comprehensive textbooks on sampling that can be read by non-mathematicians. Exceptions include Cochran.[41] Levy and Lemeshow,[121] and Yates.[258] However, there are a number of papers, reports and books that describe useful methods for important sampling issues in veterinary field studies.[29, 30, 31, 117, 143] The important principle in all sampling is that every animal has an equal or measurable probability of selection.

As part of the different field studies discussed above, the following practical questions usually need to be addressed:

- estimation of the prevalence or incidence of infection or disease;
- estimation of the distribution of risk factors, implementation of control methods and other factors associated with disease;
- comparison of disease rates, survival, mortality and productivity effects for different risk groups or under different control strategies; and
- detection of disease presence.

For all of these questions, the sample size will depend on the level of precision desired for the statistical estimate of interest, the level of confidence required that the estimate of interest lies within a given bound, the independence of sampling units from each other, and the performance (sensitivity and specificity) of tests used to measure disease. For small (finite) animal populations, the sample size may be reduced as a function of the total population size.[143] Standard sample size calculations assume sampling from an infinite (10 000) population. Often in complex surveys or studies with multiple risk factors, it is difficult, *a priori*, to estimate the size of important comparison groups. In the end, as with most things in life, practical and logistical questions will play an important role in arriving at a final sample size.

A starting point for estimating sample sizes in cross-sectional studies is to estimate the likely prevalence of the disease or infection in the study population. Well, you might say, if we knew that, we wouldn't be doing the study! The largest sample sizes are required when the estimated prevalence is around 50 per cent, and the smallest when the estimated prevalence is very high or very low. There is a reason for this apparently paradoxical situation. The estimation of prevalence requires an estimation of the proportion of positive to negative animals, rather than the absolute number of positives. Thus a larger number is required to demonstrate a 50:50 relationship than one of 90:10 (or 10:90). Thus, if no estimate of prevalence can be obtained, the sample size needed to determine a prevalence of 50 per cent will define the upper limit.

Depending on the distribution of disease, simple random sampling procedures may not be very efficient and more complex sampling methodologies, such as stratified, cluster and multi-stage sampling, may greatly decrease costs, increase sampling efficiency and provide estimates of disease occurrence at varying levels of interest. For example, a well-designed study to determine the prevalence of an infectious disease in a district may be of little value in determining the impact, or in deciding on an intervention, if there are significant differences within the district. These differences may depend on factors such as agro-ecological zone, management and feeding practices and different cattle types, and it may be more effective to stratify the district on the basis of these variables. Standard statistical texts[41] and epidemiology texts[143] describe the sampling options available under different circumstances.

For infectious diseases, it is usual that the risks of disease for animals in a herd, agro-ecological zone or other grouping are not independent. While non-independence requires a modification of standard sample size formulae (an increase in sample size if the risk in a group is positively correlated), and is thus considered an extreme nuisance by most, it actually provides key information on the variability of disease risk and transmission. Highly contagious diseases, for example, tend to infect almost all animals of the susceptible species on a farm. The clustering patterns of other diseases can provide information on whether within- or between-herd risk factors are more important or whether factors associated with ecological areas are most influential (as happens with many vector-borne diseases.[128] Methods to account for disease clustering, both in time and space, have been an active research area in recent years and there are a variety of techniques that can be used, ranging from simple to complex.

Table 9.10 Steps in planning a field survey or study

1 Clearly state the OBJECTIVES of the study.
   - what parameters are to be estimated?
   - what is the UNIT OF CONCERN (animal, farm, area)?

2 What is the TARGET POPULATION?
   - all animals
   - an age, sex or other subgroup

3 Can a SAMPLING FRAME (LIST) of the TARGET POPULATION be obtained?
   - if YES, collect it.
   - if NO
     - what lists are available?
     - does this list represent the TARGET POPULATION?
   - Is there a listing of the UNITS OF CONCERN or larger UNITS from which lists of primary sample units can be obtained at lower costs?

4 To meet your objectives, what DATA and BIOLOGICAL SAMPLES need to be collected?
   - State the data needs explicitly
   - Consider the logistic difficulties

5 Select a SAMPLE SIZE consistent with your objectives
   - What PRECISION (e.g. within 5%) is required
   - How CONFIDENT (95%, 99%) do you wish to be with your estimate?
   - Can you adjust your sample size because the target population is FINITE?
   - Are responses of individual units in a herd or other cluster independent or correlated with responses of other individuals in that herd or cluster?

6 Write out a detailed WORKPLAN

7 PRETEST your survey or study instruments (questionnaires, diagnostic tools, etc.)

A simple and usually adequate procedure to apply if disease risk varies between herds is to inflate the sample size estimate by a factor of $1 + (m - 1)\rho$, where m is the median herd size and ρ is the intra-herd-correlation coefficient (which measures the non-independence of cluster mates). A practical example of the use of this method in a study of antibody prevalence to tick-borne pathogens in three different agro-ecological zones of coastal Kenya can be found in the publication of Deem *et al.*[47] More complex methods for two (such as animal and herd) and multiple (such as animal, herd, community, ecological area) levels of potential correlation have been described and applied to a variety of veterinary and medical situations.[122, 126, 127, 129]

Another important disease sampling application is to assess the presence of disease (or vectors) in large-scale control and eradication campaigns.[125] Cameron and Baldock[29, 30] have developed very useful methods for assessing the probability of disease presence using tests of varying sensitivity and specificity. These programmes are available and easy to use.[65]

As noted in **Table 9.10**, the most important and first task in developing any plan for a field survey or study is to carefully define the study objectives and from these identify important details such as the parameters to be measured, unit of concern and target population. Invariably, good studies result from appropriate and well-defined questions being asked and poor studies result when the questions to be addressed by the study are not correctly formulated.

Evidence from the published and unpublished literature

In assessing the impact of diseases and their control, much of the impact information required may be available from previously conducted studies. Unfortunately, study validity and quality vary greatly. Some studies can be relied on to provide good quality impact information while others are best left unread. Deciding on study quality is a daunting task for the uninitiated; however, with common sense and some basic guidelines on how to review papers, most readers can become very discriminating as to the value of most published studies. In **Table 9.11** an outline of the criteria that need to be considered in evaluating observational studies, and in **Table 9.12** an outline for the evaluation of clinical or field trials, are given. These checklists have been developed and refined over many years with a number of colleagues at the University of California, Davis, USA, and the University of Guelph, Canada.

Given that most information is imperfect, persons embarking on a large-scale review of evidence on the impacts of a disease and its control will probably wish to consider information from as many sources as possible, but to weight the information sources by the strength of the evidence provided. Meta-analysis of multiple study outcomes has been widely applied in human medicine to synthesize the results of multiple studies.[50, 213] There are only a few examples of formal meta-analyses in veterinary studies.[24] More common are impact and scenario development studies that informally evaluate and combine data from previous studies.[183] In both sets of circumstances, an objective evaluation of the evidence presented by different studies is an important step in impact assessment.

Models

While evidence based on empirical field studies is critical to assessing the impact of a disease and its control, the wide range of field situations, control scenarios and other unique disease circumstances mean that all the empirical impact data required are unlikely to be available. Because of the wide variety of disease scenarios possible, models have been used to build a plausible framework that can be evaluated and validated with empirical field data, and if reasonable, used to make impact predictions under different disease and control scenarios. A wide selection of models has been used for different purposes to assess livestock populations, disease dynamics or combinations thereof. Based on their mathematical formulation, models are either:

- deterministic — in which a system of equations with an equilibrium solution is solved;
- stochastic — in which random probability elements are involved that can lead to extinction or outbreaks at various points; or
- simulation — in which computational algorithms are used to incorporate a wide variety of influences on different outcomes or states.

Livestock population dynamics models have been used to predict herd growth and productivity for different systems in Africa. One of the earliest models was developed by Texas A & M University[215, 216] and subsequently applied to beef cattle production in Botswana.[98] The model accounted for the interactive effects of genotype, breeding system, nutrition, reproduction, growth, milk production and nutrient intake. Animal performance was simulated by various sub-routines, the most important of which were animal growth, cow fertility and mortality. The model was used to predict the productivity of beef production systems in Botswana. Although it permitted a very detailed analysis, it was correspondingly very demanding in its data requirements. The model has not been used to assess the effects of disease on productivity.

A herd simulation model was developed by ILCA to provide the user with an array of potential policy options, such as for weaning, breeding, milking, buying and selling livestock, so that herd performance could be evaluated under different production regimes.[110] A further model at ILCA, originally designed to assess interventions of fodder cultivation and feed supplementation in Mali and Niger, has been developed[246] and subsequently modified to assess animal health interventions, specifically the economic impacts of chemoprophylaxis to control trypanosomosis in the Coast Province of Kenya.[100]

Other approaches to herd dynamics modelling have been taken[20, 144] and models of the herd structure and dynamics of cattle in the traditional sector of Zambia have been developed.[144] These models were used to assess the effects of varying calving rates, and calf and adult mortality on herd structure and dynamics.

For infectious diseases, mathematical models assessing transmission and infection dynamics have proved very useful in assessing and predicting the impact of changes in key risk factors and the impact of vaccination and other control strategies. The main concepts and major applications for infectious disease modelling have been set out by Anderson and May[7] in their authoritative text on the subject. The essential feature of mathematical transmission models is quite simple, particularly for microparasites (e.g. viruses and bacteria). Transmission persists if each case can produce one or more secondary cases. The rate (or ratio) at which primary cases become secondary cases is known as the reproductive rate of the disease, measured as either the effective reproductive rate (R) or the basic reproductive rate (R_0), and transmission persists if this rate is greater than one. The basic reproductive ratio represents the reproductive potential of an infection while the effective reproductive rate, influenced by several factors, is less than or equal to the basic rate. If by circumstance or control effort the reproductive rate falls below one, then transmission ceases. The basic reproductive rate can be estimated in a number of ways, including:

- as a function of the number or density of susceptibles;[5]
- as a function of the birth rate, average age at infection and the duration of maternal immunity;[7] or
- directly from outbreak data in closed populations when secondary cases due to a primary initial case can be determined.[42]

A variety of influences on disease transmission can be estimated via R_0. Of key interest for control programmes is the critical proportion of the population to be immunized ($p_c = 1 - (1/R_0)$).[7] R_0 can also be evaluated for other control options.

Mathematical modelling approaches have been applied to a number of infectious diseases, including brucellosis,[96] tuberculosis,[196, 241] sheep blowfly infestation,[66] FMD,[124, 155, 188, 256, 257] rinderpest,[102, 211] rabies,[6, 18, 42, 199, 245] theileriosis,[70, 146, 147] heartwater,[168, 169] fascioliosis,[71, 73, 172] ostertagiosis,[72, 176] and trypanosomosis.[86, 87, 88, 150, 208]

In general, mathematical models have been very useful tools for assessing many infectious diseases, with most disease transmission and impact of major control strategies reasonably well predicted by relatively simple models.[7]

Key socioeconomic information required

In most cases, socioeconomic factors will be a critical component for understanding the impact of disease and its control. The epidemiological investigations described above generally incorporate some aspect of socioeconomic data collection, and this becomes increasingly important in smallholder production systems where livestock activities are less commercialized. While the emphasis has been on epidemiological methods, there also exist a number of design issues and methodologies specific to socioeconomic data collection and analysis. Refer to Poate and Daplyn[197] for an overview.

Two practical points are worth stressing. First, deciding the sample size for socioeconomic data is more likely to be based on a rule of thumb conditioned by logistical constraints rather than on the standard formula. For epidemiological studies, the focus is often on a key parameter of interest, which drives the sample size. In socioeconomic

Table 9.11 Outline for the evaluation of observational studies

Were the objectives of the study clearly stated?	Y/?/N
Was this a data snooping/preliminary study? (Relates to multiple comparisons and interpretation of results)	Y/?/N
Was the study group likely to be representative of a larger (target) population? (Only concerned with extrapolation)	Y/?/N
Type of study?	Case-control ( ), Cohort ( ), Cross-sectional ( ), Longitudinal ( ), Hybrid ( )
GROUP FORMATION	
Were exclusion/inclusion criteria well specified?	Y/?/N*
Was sample size considered *a priori*?	Y/?/N
Was the F+ (D+) group well defined?	Y/?/N*
Was the F- (D-) group from the same source?	Y/?/N
Was the F- (D-) group well defined?	Y/?/N*
FOLLOW-UP/TRACEBACK	
Was the *follow-up* (traceback) performed	
a) completely?	Y/?/N*
b) blind to F+ (D+) status?	Y/?/N
Was response bias within tolerable limits?	Y/?/N*
OUTCOME and ANALYSES	
Was the outcome D+ (F+) status:	Y/?/N*
well defined?	
defined independently of F+ (D+) status?	Y/?/N*
If testing a specific hypothesis, were confounders defined *a priori*?	Y/?/N
Was confounder control adequate?	Y/?/N*
Were there adequate efforts to control/explain possible biases?	Y/?/N
Were appropriate analyses done?	Y/?/N
Were analyses done (apparently) correctly?	Y/?/N*
Was power likely to be adequate?	Y/?/N
If used, was multiple testing managed/explained well?	Y/?/N
Is there evidence of a dose-response relationship?	Y/?/N
PRESENTATION and CONCLUSIONS	
Were the *Methods* presented clearly?	Y/?/N
Were the *Results* and *Analysis* presented clearly?	Y/?/N
Was the *Discussion* summarized clearly?	Y/?/N
Are the author's conclusions supported by the data?	Y/?/N*
Was the study design appropriate to answer the objectives?	Y/?/N*

The more '?' and 'N's', the less valid the study.
* Indicates a serious problem if 'no'.

Table 9.12 Outline for the evaluation of clinical trials

OBJECTIVES AND EXPERIMENTAL POPULATION	
Were the objectives of the trial clearly stated?	Y/?/N
Was the trial group likely to be representative of a larger (target) population? (Only concerned with extrapolation.)	Y/?/N
GROUP FORMATION	
Was trial size (i.e. 'n') considered *a priori*?	Y/?/N
Were exclusion/inclusion criteria well specified?	Y/?/N*
What was the stated method of allocating treatments: ———	
Is there evidence that formal randomization was actually used?	Y/?/N*
Were the treatment and procedure(s) well defined?	Y/?/N
What was the comparison group(s)? (tick one) other treatments ——— other levels of same treatment ——— no treatment ———	
FOLLOW-UP PERIOD	
Was adequate follow-up performed: a) completely?	Y/?/N*
b) blind to treatment status?	Y/?/N
Were the managers adequately blinded about treatment?	Y/?/N
Was compliance within adequate limits?	Y/?/N*
OUTCOME ANALYSES	
Was the outcome(s) well defined?	Y/?/N*
Assessed independently of treatment status?	Y/?/N*
Was the outcome(s) appropriate for the stated objectives?	Y/?/N
Were there adequate efforts to control/explain possible biases?	Y/?/N
Were appropriate analyses done (ie. consistent within design)?	Y/?/N*
Were analyses done (apparently) correctly?	Y/?/N*
Was power likely to be adequate for main outcome(s)?	Y/?/N
Is there evidence of a dose-response relationship?	Y/?/N
PRESENTATION AND CONCLUSIONS	
Were the *Methods* presented clearly?	Y/?/N
Were the *Results* and *Analysis* presented clearly?	Y/?/N
Was the *Discussion* summarized clearly?	Y/?/N
Are the author's conclusions supported by the data?	Y/?/N*
Was the trial design appropriate to answer the objectives?	Y/?/N*

The more '?' and 'Ns', the less valid the trial.
* Indicates a serious problem if 'no'.

studies, there is usually no single parameter of interest, but rather a number of potentially influential factors. The rule of thumb, therefore, is to try to have sufficient numbers of units of analysis (e.g. farmers, households and villages) for each major subgroup by which the sample has been stratified, to permit statistical comparisons across groups (e.g. t-tests). Social scientists tend to be comfortable, therefore, with a sample size of 25 to 30 units of analysis per subgroup.

Second, researchers often underestimate the care that is needed during the design and implementation of instruments (questionnaires) for socioeconomic surveys. Questions must be appropriately formulated and asked if they are to solicit effectively the information sought by the researcher. The error that can be introduced simply in the translation process has been highlighted by Lee *et al.*[116] Since many of the types of data collected are standard (e.g. farmer characteristics, livestock management practices, and expenditures on health inputs), it is important therefore to review and draw upon existing proven questionnaire strategies from standard socioeconomic surveys and borrow as needed rather than trying to reinvent the wheel and repeat past mistakes. Allowing adequate time for pre-testing questionnaires and thorough training of enumerators are both essential for ensuring quality data.

Measurement of other indirect impacts of disease and their control As noted above, animal disease and its control generate a wide range of other outcomes beyond the obvious direct impacts on livestock and human health. Both productivity gains and expenditures on control have implications for farm and national-level income. Secondary economic outcomes such as these are discussed in the following section on economic approaches since their evaluation often serves as the basis for decision-making about control. Within the framework of such analyses, innovative approaches often must be developed to address specific indirect impacts. When evaluating a potential vaccine against bovine trypanosomosis, for example, Kristjanson *et al.*[111] attempted to value the lost production potential — cattle production activities not undertaken due to the disease — that could be recovered by the introduction of a vaccine. They estimated this value by assuming that cattle densities in tsetse areas would rise to equal those found in non-tsetse areas, and in this manner predicted the added value of production.

Indirect impacts of epidemic diseases, in particular, may be much more severe than the direct impacts. Effective control of diseases that restrict international trade can offer access to new, higher-value export markets for livestock and their products, generating added value and employment not only within the livestock sector, but also through the rest of the national economy due to multiplier effects. The substantial impacts that an outbreak of FMD would create in Australia in terms of loss of exports and secondary effects beyond the livestock sector have been demonstrated.[68] The 2001 outbreak of FMD in the UK highlights the magnitudes of these impacts, including revenues lost to tourism. Mullins *et al.*[158] report a similar, more modest, effect of tsetse control on tourism in Botswana, though no attempt is made to value the impact. In each case, the measurement of indirect impacts will require the development of appropriate economic models adapted to the specific impact, once the impact has been properly identified.

Geographical information systems

The reporting and analysis of disease occurrence in a spatial context can be a useful tool in impact assessment. For years, large maps peppered with coloured pins denoting

the sources of reports of diseases and vectors have adorned the offices of veterinary departments throughout Africa. With the advent of computer power, these have developed rapidly into geographical information systems (GIS). What do they do that the pins did not?

Geographical information systems have computerized procedures to allow input, storage, manipulation, analysis and display of spatial data.[207] They have been applied to the reporting and study of many infectious diseases, particularly the vector-borne infections in Africa.[91] We do not intend to cover this huge area in any detail in this chapter, but will summarize the main features of this useful tool.

Spatial data require that a location be assigned to the recording of a disease event, in the form of latitude/longitude for example. This then allows this piece of data to be aggregated with other data describing the environment in which that event occurred. Commonly, the first step is to express the disease occurrence in relation to administrative boundary data (such as province, district), and this is how diseases are generally reported. However, while this serves to locate where a disease incident has occurred, it does little to evaluate disease risk and impact on productivity and other parameters. Therefore, the next set of data commonly included in any spatial reporting of disease is usually agro-ecological zone, land use category, vegetation and communications infrastructure (such as roads). Remarkably, in most African countries, these types of data are now much easier to acquire, generally at quite high levels of resolution, than data on animal diseases or on the hosts they affect. It is also possible to access, through the Internet and other sources, high-quality satellite-derived data on climate and vegetation cover.

In many African countries, GIS units have been established within the cartography departments of government ministries and planning departments that have assembled much of the natural and infrastructural features of their countries. However, there are relatively few that have established the same degree of capacity in the livestock production or animal disease sectors. On a continental scale, the International Livestock Research Institute (ILRI) developed a map of cattle density for the continent of Africa (**Figure 9.5**). In doing this, ILRI used the most recently available data at the highest resolution from published sources. The result combines some densities that are at secondary administrative boundary level, and possibly 10 years old, with new data at a very high level of resolution (such as Zimbabwe, with cattle data down to the dip tank level, revised and published annually).

Not only are there difficulties with the quality of data on numbers and distribution in space for a given species, but when it comes to impact assessment, these livestock distributions do not differentiate between the different production systems in which any one species is used, and so the different impacts that animal diseases and their control will have. In the case of the cattle example, this will be particularly important in the differentiation of dairy, pastoral, mixed agro-pastoral and ranching systems, for example.

Given the inadequacies of available data on the distributions of host populations, some attempts have been made to model their distributions using GIS, and most notable is the work of Wint *et al.*[255] These authors used a combination of available data, including those assembled by ILRI, and modelling techniques (**Figure 9.6**).

Similar to data on the distribution of host species, available spatial data on animal diseases in Africa are in the categories of recorded observations and model predictions. Given the inadequacies of many disease-reporting systems, there is no continental distribution of the main diseases based on recorded observations. However, these do exist for several countries in the continent. A notable example is Zimbabwe, which developed a GIS capacity in the Department of Veterinary Services in the late 1980s. This unit produces distribution maps of disease outbreaks and of intervention programmes (such as vaccination campaigns against Newcastle disease) at regular intervals, which are used to keep stakeholders informed, but also as feedback to veterinary staff to encourage better disease reporting (**Figure 9.7**).

Considerable progress has been made using GIS for predicting the distributions of disease vectors. Most notable in this field is work on the distributions of ticks and tsetse flies. For the latter, pioneering work was done by Rogers and colleagues at Oxford.[209] This has recently been followed up by McDermott *et al.*,[131] who used models of predicted climate change on the length of the growing period to predict how the distribution of tsetse species might change over the next 50 years (**Figure 9.8**).

The use of GIS has also extended to mapping disease risk. Considerable effort was devoted to predicting the current and potential distribution of ECF and its vector, the brown ear tick (*Rhipicephalus appendiculatus*), in Africa through the use of climate matching models,[120, 186, 187] but more recently the focus has been on predicting risk of the disease through the incorporation of a wider set of variables including the satellite-derived vegetation indices. This has been done both at a country level (in Zimbabwe)[55] and at a continental level[152] (**Figures 9.9 and 9.10**).

Combination of models, observational and experimental studies

In the above sections an array of tools that can be used to determine the impacts of diseases and of methods to control them have been described. More likely than not, no single tool will suffice in providing the answer. As a result, should resources be available, a combination of tools can be used, and the results normally render more than the sum of the component tools included. Epidemiological tools have played a particularly important role in studying the dynamics and impact of tick-borne diseases.[182] A practice we have advocated in several investigations has

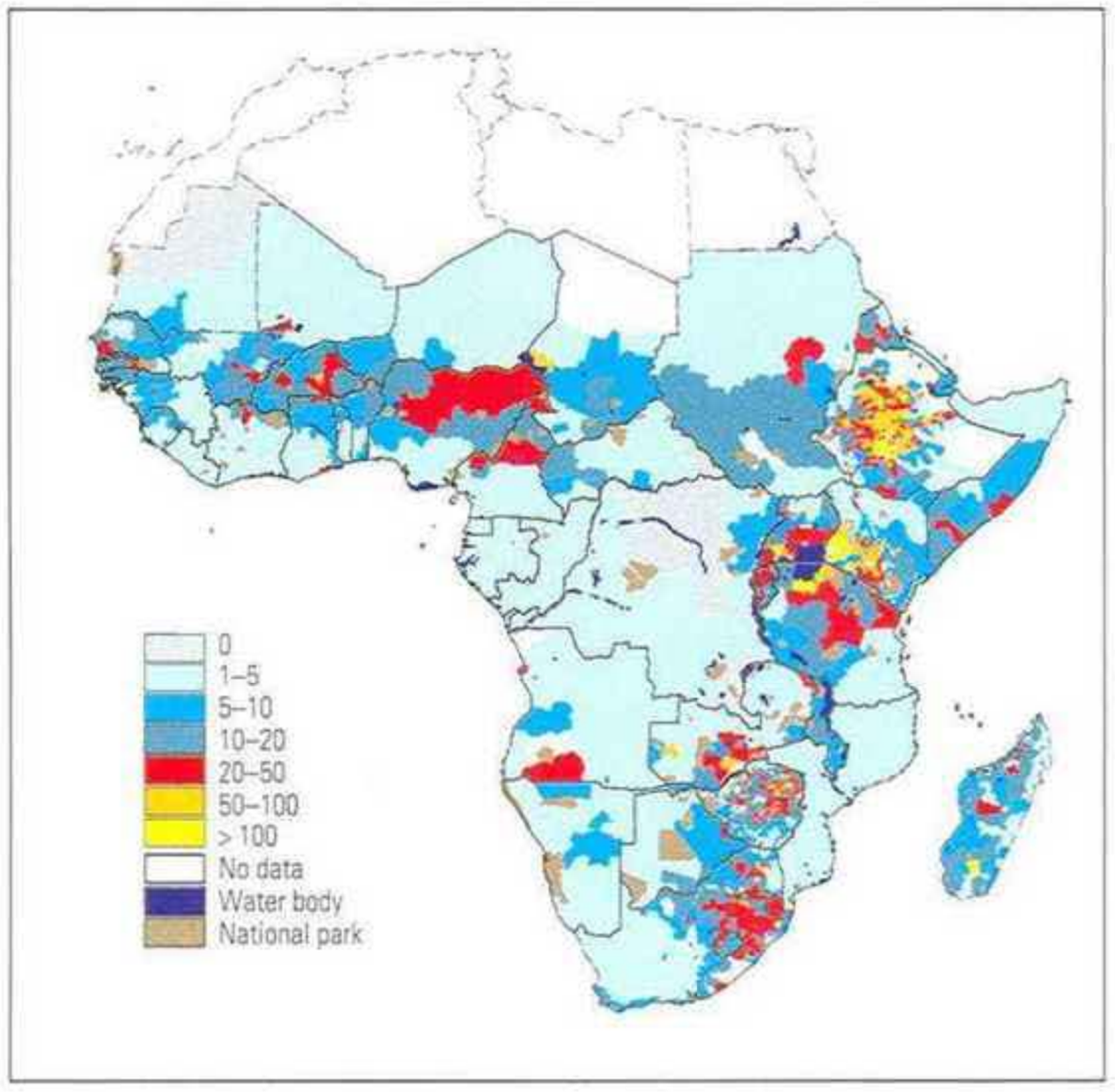


Figure 9.5 The distribution of cattle density in Africa (source: Kruska *et al.*, 1995[112])

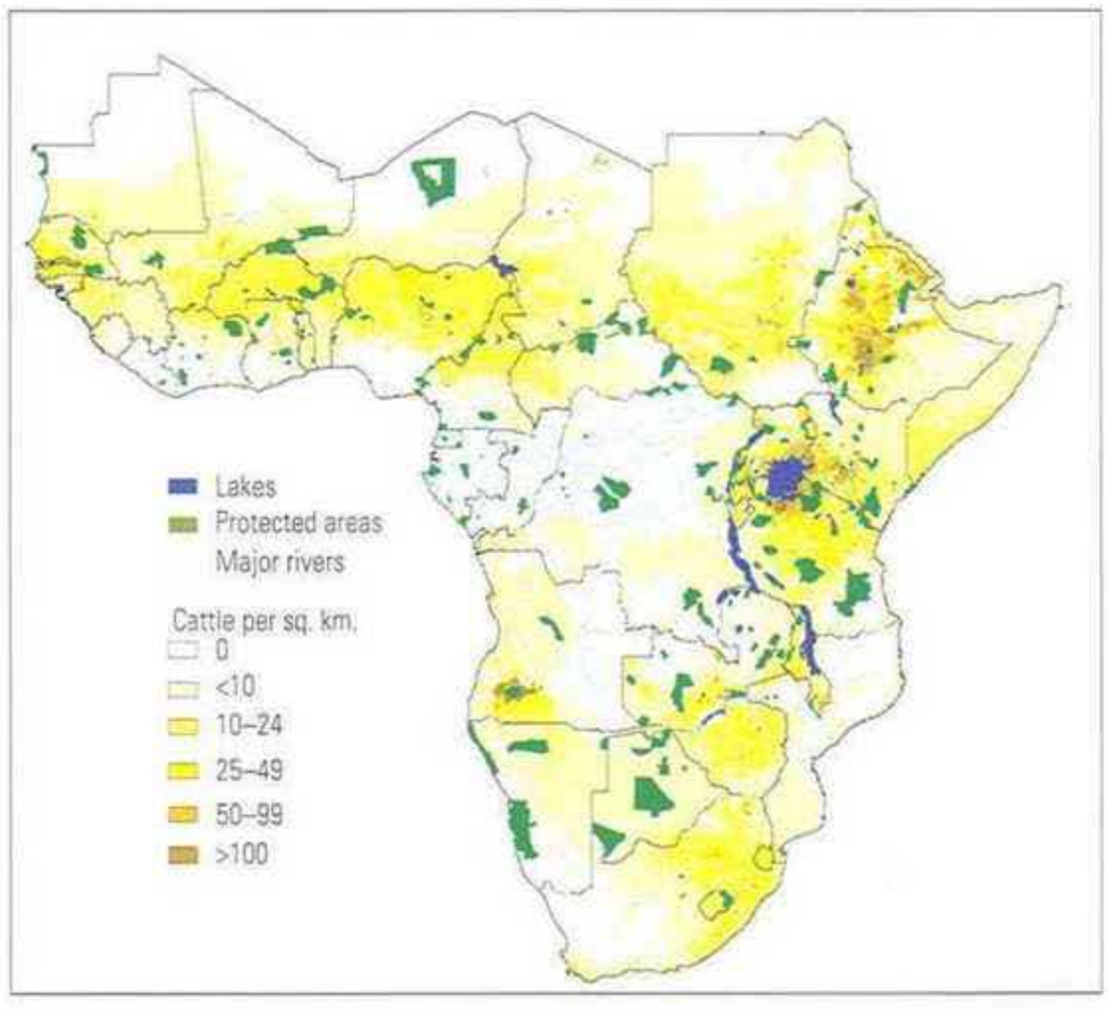


Figure 9.6 The predicted density of cattle (from Wint *et al.*, 2000[255])

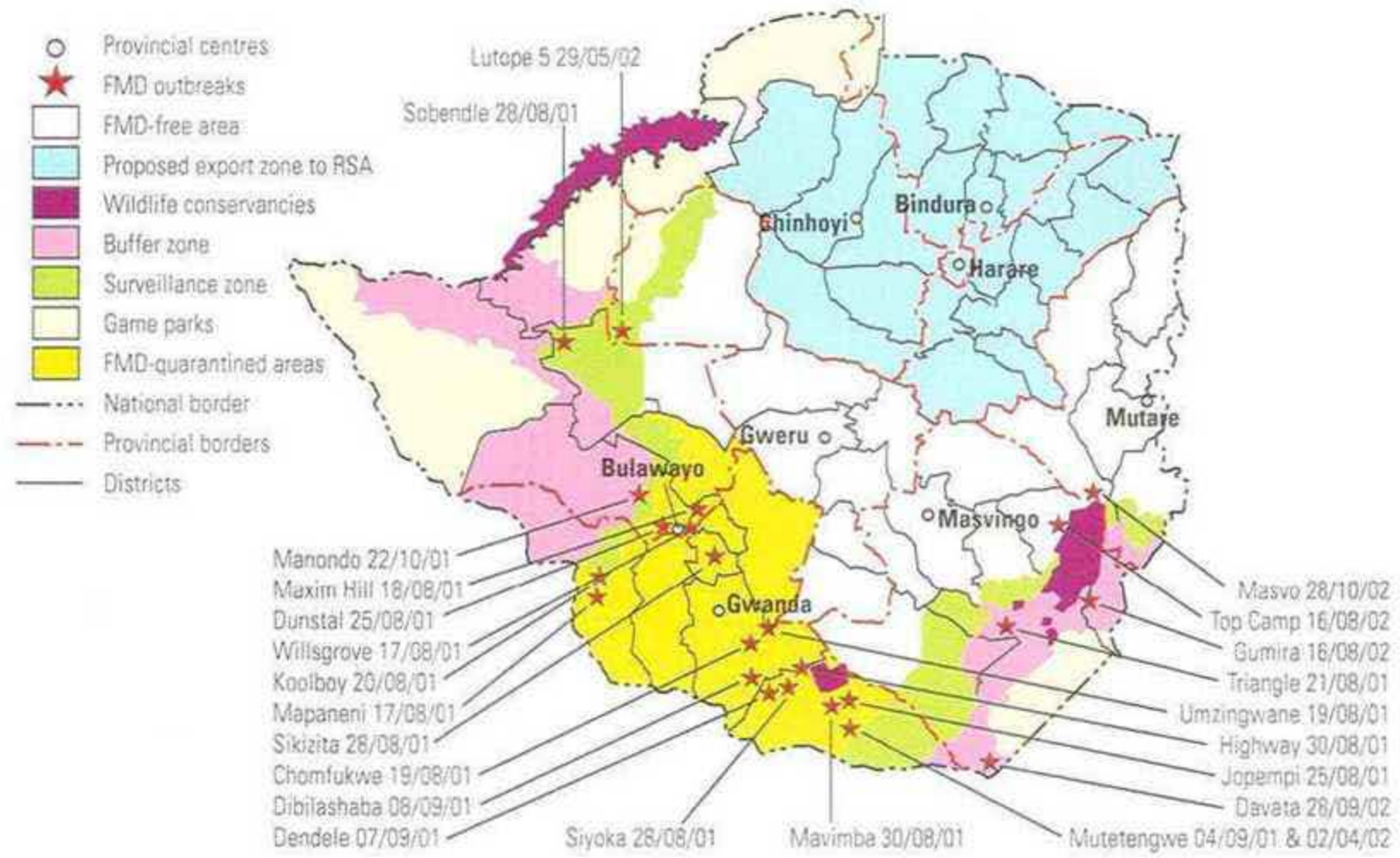


Figure 9.7 An example of output from the GIS unit at the Department of Veterinary Services, Zimbabwe. In this case the location of foot-and-mouth disease outbreaks from August 2001 to October 2002, in the context of the country's disease control zoning, is illustrated[260]

been a combination of cross-sectional and longitudinal (usually cohort) studies. The cross-sectional is carried out initially, stratifying the population by key variables to determine major differences in prevalence within a parent population of animals, farms or households, followed by a longitudinal study to determine incidence and impact in the contrasting prevalence populations. Such was the design of two sets of studies on the impact of ECF in two different regions of Kenya: the central highlands[75, 76, 77, 78] and the coastal region.[138, 139, 140] Both of these series of studies ended with robust estimates of infection prevalence, disease incidence, case-morbidity and case-fatality in the different agro-ecological zones, cattle types and feeding management regimens prevalent in the study areas. The studies in the central highlands also derived impacts of infections on daily weight gains. Neither series progressed into evaluations of economic impacts.

In another series of studies, carried out on the impact of heartwater and its control in Zimbabwe, two additional components were added to this framework for impact assessment. The first was a mathematical model of the infection dynamics of the disease, which served as a starting point for the entire study (**Figure 9.11**).[168] Using initially a conceptual model framework, the major parameters and pathways in the maintenance of the infection were proposed, and supplemented where possible by parameter values in an attempt at understanding the dynamics of infections. Major gaps in knowledge, and in parameter values, were identified, and these formed the basis for the objectives of a series of experimental and observational studies. Experimental studies were used to investigate principles and, in some cases, quantify infection processes under experimental conditions, and observational studies, principally longitudinal studies in clearly defined areas, were used to determine parameter values under different ecological and farming systems conditions in Zimbabwe.[191, 192, 193, 194, 195] Concurrently, surveys were set up to characterize the different livestock production systems in which heartwater occurs, and their economics.[38, 39] Armed with the results from this wide range of studies, it was back to the models. The first task was to use the models to predict heartwater incidence and mortality under the variety of production systems in the country, based on the outputs of the cross-sectional and longitudinal studies. The second task was to use these estimates to calculate the economic impact of the disease, and of different control scenarios contemplated by the Department of Veterinary Services.[157]

These economic impact studies were subsequently expanded to include the other countries of the Southern African Development Community (SADC) region,[151] although with the large region and multitude of production systems involved, considerable reliance was placed in this study on secondary data. Finally, it was back to the models again to

evaluate how new vaccines under development might work under the different control scenarios contemplated.[169]

These examples illustrate the need for a good conceptual framework behind impact assessment studies, the important role of good epidemiological data in economic impact assessments, and the advantages of combining investigative tools for maximum effect.

Risk assessment

The introduction, spread and control of diseases all depend on a sequence of biological events that are uncertain. Rules regulating trade in animals and animal products (e.g. the sanitary and phyto-sanitary (SPS) regulations of the World Trade Organization (WTO)/OIE, and decisions regarding what disease control and food safety strategies should be adopted all depend on assessing and managing disease risks. Hazard Analysis and Critical Control Point (HACCP) methods used in industrial food safety is one example of risk analysis methods that have been widely adopted. Research and development in risk analysis, both technical and policy issues, have greatly expanded in recent years.[90, 137, 165, 247, 249, 259]

A risk-assessment process for potential hazards (such as disease introduction) and methods to control them needs to be considered by various categories of decision-makers in animal health. This should be a formal process but may be either qualitative or quantitative. A key initial step is to identify and characterize the potential hazard both in terms of its causes and consequences. The likelihood of a hazard (or hazards) occurring is then estimated. The impact or consequences of hazards then need to be assessed from a biological, economic and environmental perspective. Then an overall assessment of both risk and the uncertainty of the risk-assessment process is made by combining the likelihood and consequence analyses with judgements on the quality of data available. Once a risk assessment has been conducted, its results need to be communicated to and discussed with all stakeholders so that it can be incorporated into risk-management practices. As disease control and food safety become increasingly more complicated, formal and informal risk assessment and management will be an important feature of decision-making in animal disease control.

Measuring the impact of zoonotic diseases on the human population

Within human health, the disability adjusted life year (DALY) is an instrument recently developed to allow the relative burden imposed on humans to be determined. This was used as a common health measure in the first Global Burden of Disease study commissioned by the World Health Organization (WHO)[159] that documented the human health status, worldwide, for the year 1990. It has subsequently been used in revised global disease burden estimates, most recently in the WHO World Health Report 2000, in which the health status for 1999 is described.[252]

As well as being used as a means of ranking the overall burden of individual disease, the DALY is also particularly useful as an outcome measure in economic evaluations of interventions, including cost-effectiveness analysis.[80, 81] Consensus cost-effectiveness ratios of US $150 per DALY and US $25 per DALY averted are agreed thresholds for disease control interventions to be considered 'attractive' or 'highly attractive',

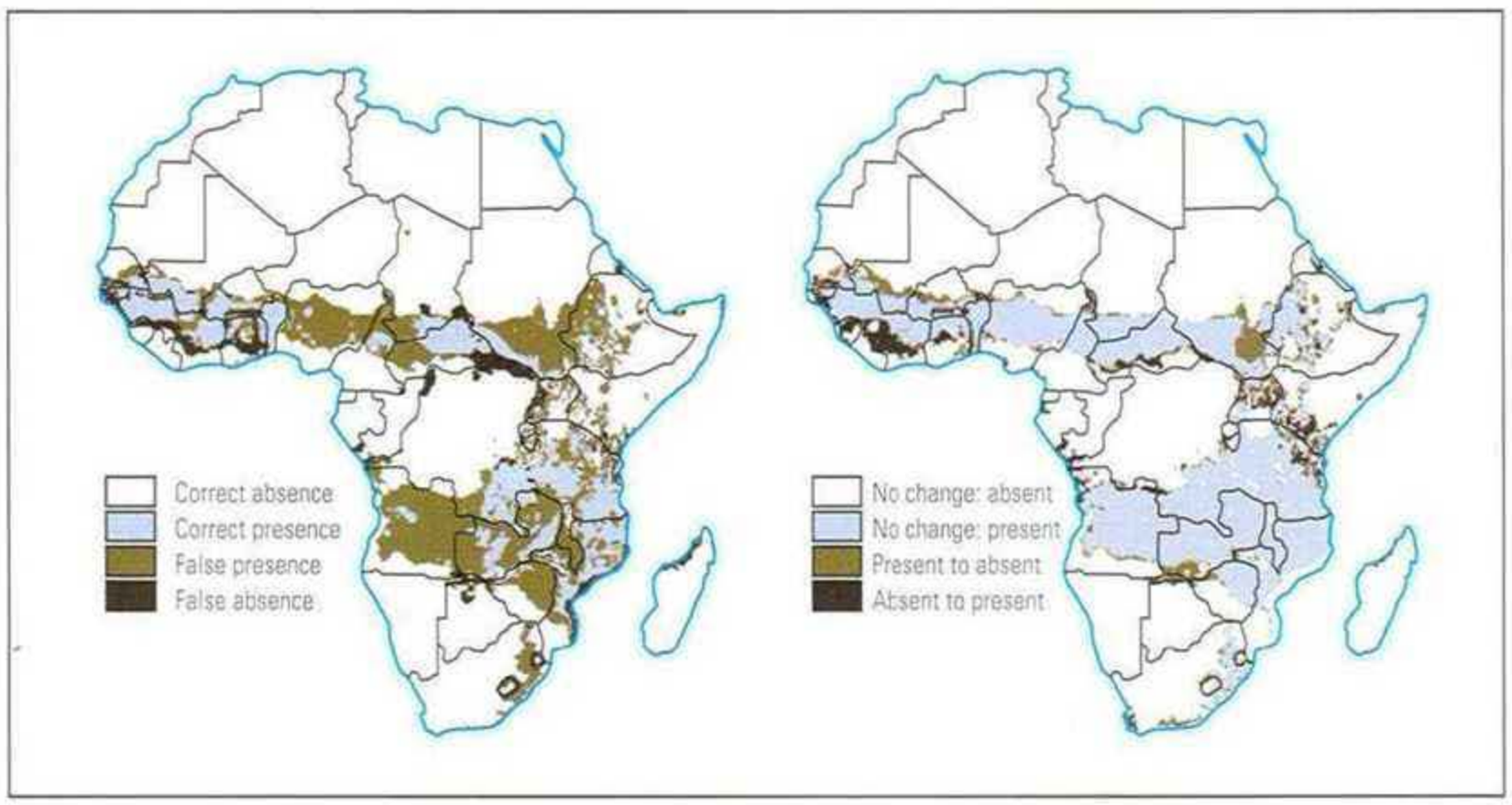


Figure 9.8 Predicted distribution of the *Glossina morsitans* group of tsetse flies compared to known distribution (left), and predictions of how this might be affected by climate change in the year 2050 (from McDermott *et al.*, 2000[131])

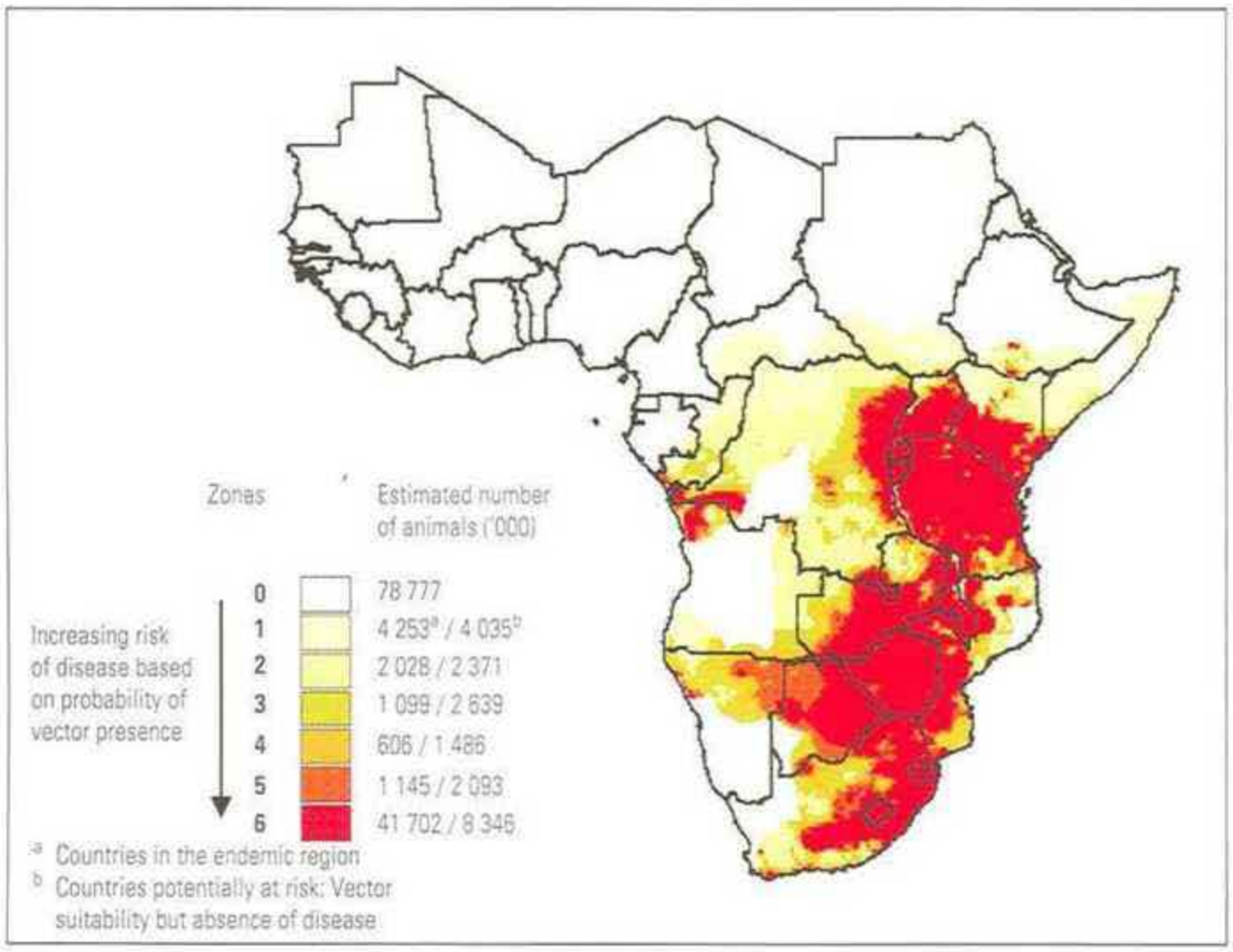


Figure 9.9 The predicted distribution of risk of East Coast fever (from Minjauw *et al.*, 2001,[151] unpublished data,[152] and based on Cumming, 1999[43])

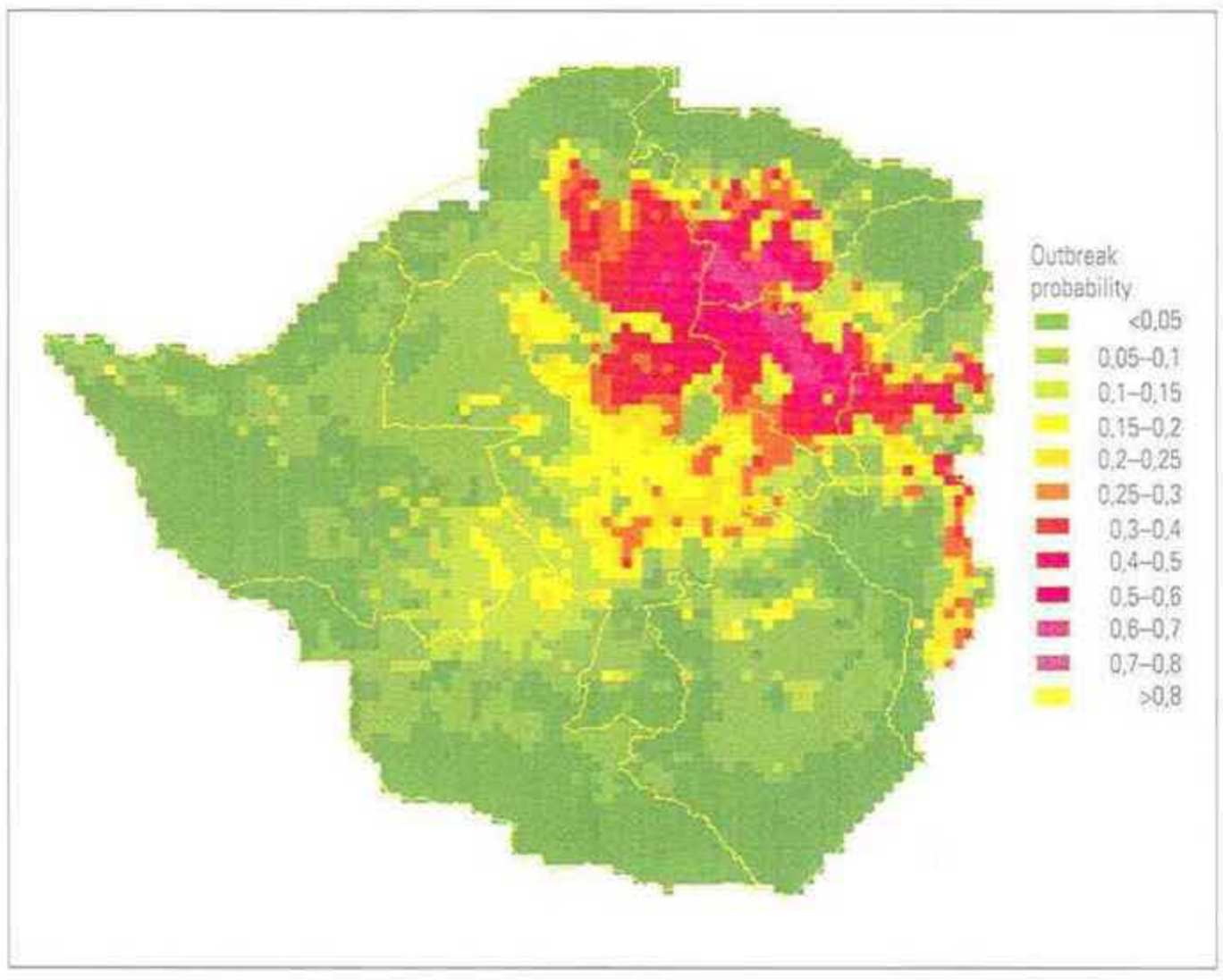


Figure 9.10 The predicted distribution of risk of theileriosis in Zimbabwe (from Duchateau *et al.*, 1997[55])

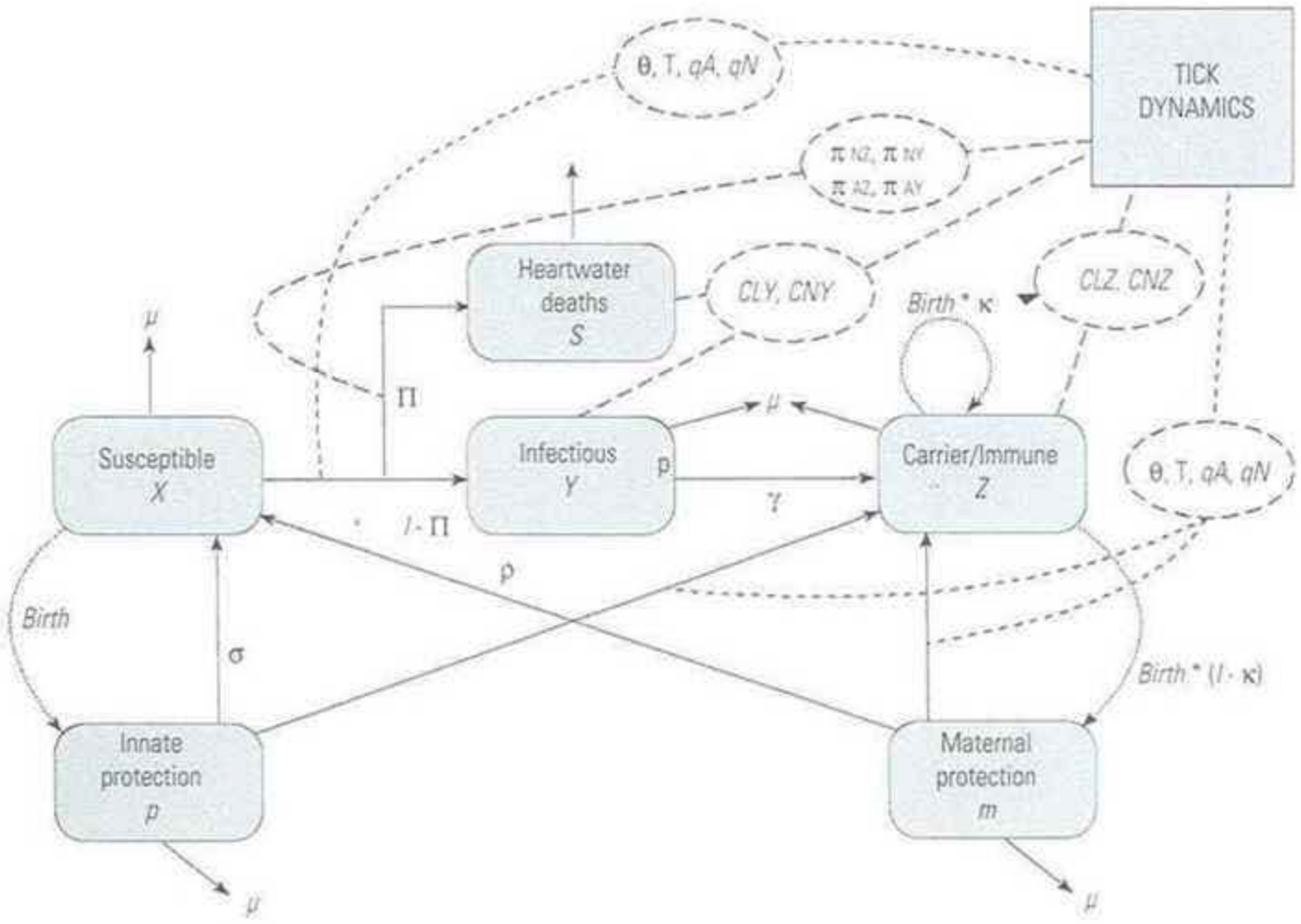


Figure 9.11 A model of the infection dynamics of *Ehrlichia ruminantium* infection (from O'Callaghan *et al.*, 1998[168])

respectively, in a developing country setting, and used to advocate one strategy over another.[251]

The DALY is a measure of health outcome that incorporates both premature death and morbidity/disability. The DALYs caused by a given disease consist of the years of life lost (YLLs) and the years of life lived with disability (YLDs). In quantifying the cost-effectiveness of an intervention, deaths averted due to the implementation of the intervention are converted to YLLs using the age-specific life expectancies found from appropriate life tables. In comparing the DALY burden across different settings a common life table is used (e.g. for the global burden of disease studies a Japanese life table with a life expectancy at birth of 82.5 years for females and 80 years for males). For cost-effectiveness studies, comparing different interventions in one setting, a local life table should be used.[159]

The years of life lived with disability are calculated based on the duration of disability/morbidity, and disability weights given to each condition. The weights ranged between 0 and 1, with 0 representing full health and 1 representing death. The weights for each condition used in the Global Burden of Disease study[159] were derived using the person trade-off method developed at an international meeting held in Geneva in 1995. For the vast majority of zoonotic diseases no such consensus of disability weights exists.

The YLLs and YLDs are summed to give the DALYs score. The DALYs may be discounted to account for time preference and the risk premium. The discounting of health benefits, which gives a lower weight to health benefits that occur further into the future, has been criticized as inequitable[4] and has attracted considerable controversy and debate. Age weighting of DALYs is also highly controversial and should be avoided as no consensus on the use of age weights has yet emerged, and the impact of the age weighting function used in the Global Burden of Disease has been shown to be counter-intuitive.[21, 175]

The use of a generic measure such as the DALY, rather than intermediate measures of benefits such as cases prevented or cases cured, allows for comparison of preventive and treatment interventions. Moreover, as DALYs incorporate both mortality and morbidity/disability, it is possible to make comparisons with other interventions that improve the quality of life, as well as with those that save lives. Whilst several limitations of DALYs have been identified in recent critiques,[4, 22, 58, 175, 242] they do have several important advantages, particularly in cost-effectiveness analysis,[81] and their use facilitates comparison with the results of other recent cost-effectiveness studies in developing countries.[103]

Official global estimates exist for 27 infectious diseases of humans, with the most recent values calculated for 1999[252] as shown in **Table 9.13**. Using a strict definition of documented natural transmission between animals and humans, 20 of the 27 diseases are classed as zoonoses.[236] However, from a control perspective, targeting the animal reservoir is unlikely to have a significant impact on the burden of human disease in

13 of these 20 strictly defined zoonoses (**Table 9.13**), as the anthroponotic transmission cycle (from human to human) is relatively much more important in these than any zoonotic cycle. For the remaining seven zoonotic diseases — trypanosomosis,[74, 208] schistosomosis,[235] leishmaniosis,[17, 206] Chagas disease,[83] Japanese B encephalitis,[115] hookworm[198] and hepatitis (caused by hepatitis E virus)[226] — there is evidence suggesting that the animal transmission cycle is important and that veterinary interventions could have a profound effect on the global DALY burden of the disease. However, it is debatable whether any of these diseases falls in the most important category of zoonoses, in which the animal host is the sole maintenance reservoir of human disease, and where blocking the transmission between animals and humans will eliminate the disease in the human population. The conclusion is that DALYs are yet to be calculated for many of the important zoonotic diseases of impact in Africa.

Measuring environmental impacts of disease and its control

Livestock have been considered harmful to the environment and implicated in a host of environmental sins including desertification, deforestation and global warming. The control of livestock diseases, particularly large-scale disease control efforts, have been the subject of debate from an environmental impact perspective. The greatest environmental concerns have been in the large-scale control of tsetse fly and trypanosomosis in Africa, both the indirect effects on habitat and wildlife[174] and the direct effects of chemicals used for tsetse fly control on non-target organisms (including people), water quality and nutrient cycling.[161]

A number of studies have assessed direct impacts of chemicals.[54, 59, 109] In general, newer insecticides and the methods by which they are applied are considered to be less persistent and have no long-term, irreversible effects on non-target species.[222] However, risks always exist for personnel handling chemicals. Rola and Pingali[210] provide a framework and examples for assessing the economic implications of such risk in a related application for crop pesticides. Another potential impact is the development of resistance to pesticides or drugs, such as in the case of trypanocides in sub-Saharan Africa.[69] Fleischer and Waibel[63] describe an approach based on natural resource economics for assessing the social value of resistance for crop protection in Germany; the approach has yet to be applied to the field of animal

Table 9.13 Burden of infectious diseases in disability-adjusted life years (DALYs) by cause in WHO regions; estimates for 1999. The zoonotic status of each disease is indicated (from WHO 2000[252])

CAUSE	GLOBAL	AFRICA	THE AMERICAs	EASTERN MEDITER-RANEAN	EUROPE	SOUTH-EAST ASIA	WESTERN PACIFIC	ZOONOTIC
HIV/AIDS	89819	74449	2812	2172	725	8866	795	No
Diarrhoeal diseases	72063	24321	2514	10123	1131	30017	3955	Yes
Malaria	44998	36838	76	2774	2	3071	2235	Yes
Tuberculosis	33287	8721	1114	2260	1258	14101	5832	Yes
Measles	29838	17463	31	3293	148	8308	594	Yes
Tetanus	12020	3039	160	1821	131	6302	566	Yes
Pertussis	10905	4937	189	1415	197	3634	533	No
Meningitis	9824	3620	722	1472	438	2567	1005	Yes
Chlamydia	7969	2233	779	250	581	3978	145	Yes
Syphilis	6081	3189	113	648	6	2067	59	No
Gonorrhoea	5686	2425	313	101	126	675	45	No
Lymphatic filariosis	4918	1834	8	11	0	2788	278	Yes
Hepatitis*	2790	1172	212	109	57	706	535	Yes
Trypanosomosis*	2048	1991	1	56	0	0	0	Yes
Leishmaniosis*	1983	256	50	210	0	1467	0	Yes
Schistosomosis*	1932	1637	133	98	15	18	30	Yes
Poliomyelitis	1725	279	152	19	1	1095	178	No
Hookworm*	1699	406	173	93	0	862	166	Yes
Trachoma	1239	434	0	237	0	62	505	No
Onchocercosis	1085	1083	2	0	0	0	0	Yes
Japanese encephalitis*	1046	0	0	1	0	218	828	Yes
Chagas disease*	676	0	677	0	0	0	0	Yes
Ascarosis	505	202	43	43	0	186	31	Yes
Trichurosis	481	198	155	3	0	120	5	Yes
Leprosy	476	79	60	10	0	318	9	Yes
Dengue	465	24	0	1	0	440	0	Yes
Diphtheria	151	24	2	11	1	110	2	No

* Disease in which the zoonotic component is considered significant compared to anthroponotic transmission

health. Various indirect environmental impacts of vector control of tsetse fly on wildlife, biodiversity and land use have been studied and found not to be invariably negative[204, 205, 254] but to depend on ecological circumstances and people's response to reduced disease risk. Approaches have yet to be adapted from natural resource economics for valuing these types of impacts.

Identifying and deciding between intervention options

In this section we focus on tools and methods for deciding between different control strategies for diseases judged to be of high priority. A much more extensive review of the relevant issues and methods, and from which much of the material in this section is drawn, can be found in the August 1999 OIE *Revue Scientifique et Technique*.[181] The textbook on animal health economics by Dijkhuizen and Morris[52] is the most up-to-date source for details on how to apply many of the standard analytical methods.

Steps in evaluating disease control interventions

Four major steps are generally involved in determining the most appropriate, technically feasible and economically sound control or intervention strategy. These are: scenario development; quantification of the intervention options and their effects in epidemiological and economic terms; development of clear decision criteria; and *ex post* evaluation of the results of the decision-making process.

Scenario development In scenario development the framework for decision-making is constructed by listing the intervention options that could be considered and the different consequences of such options are described. The scenarios might be based on different geographical expansions of a disease (such as that described by Mukhebi *et al.*, 1999,[157] when considering the spread of heartwater in Zimbabwe), different technologies used for disease control (such as that of Minjauw *et al.*, 2000[151] when comparing acaricide-based and vaccine-based strategies for heartwater control in southern Africa), a combination of the two (such as in the Mukhebi *et al.*, 1999[157] example cited), or different lengths of time for a disease eradication programme to succeed (such as that of Perry *et al.*, 1999,[188] when considering future FMD eradication in Thailand).

There are many important features of scenario development, but the most important is that they be realistic. This requires that they not only take into consideration national policies and aspirations, but also that they are based on the best available information on disease occurrence, intervention effects, logistical considerations and resource availability.

Quantification of the intervention options and their effects This step in the process is often the stumbling block to high-quality and credible decision-support aids due to inadequacies in data. It is necessary to articulate and quantify accurately the components and costs of the different intervention strategies under evaluation, and furthermore, what outcomes they will produce, in both epidemiological and economic terms. Invariably, there are data gaps and key assumptions have to be made but as long as they are reasonable, and are well articulated in the analysis, they need not be an impediment to developing quality decision-support aids.

Secondary data are often available from a broad range of sources, based on the types of information systems or studies described in the preceding section, with considerable variations in accuracy and precision. When the appropriate secondary data are lacking, primary data collection may be necessary to properly capture the specific epidemiological and socioeconomic consequences of the different control options under consideration.

It is often relatively straightforward to measure and quantify the direct impacts of disease control in terms of the market value of changes in production losses and control costs. Identifying and valuing additional indirect or secondary impacts may present much more of a challenge. For example, indirect, longer term impacts of improved reproductive performance may require use of a herd simulation model and a set of assumptions about how herd structures and off-take rates are expected to evolve over time. Off-site impacts, such as those on the environment or on other parts of the marketing chain and beyond (e.g. market prices, employment, human health), may require auxiliary analyses that draw on the economic literature. Still others may be 'intangibles' that are much more difficult to value, but clearly need to be recognized. How, for example, can the value of a control option better targeted to the poor be quantified compared to one targeted to commercial producers?

The challenge is to assemble information from disparate sources in a meaningful decision-making framework, combining both epidemiological and economic information.

Development of clear decision criteria This is an area that is still poorly developed. The commonest criteria used are just biological, based on efficacy. Does the intervention successfully control the disease? However, as has been repeatedly demonstrated in empirical studies, what makes sense technically (the technical optimum) only on occasion makes sense economically. The decision criterion is therefore usually reframed in terms of: does the value of the impacts of disease control justify the effort (cost) invested? Or, if various options are being considered, which yields the best value for the effort or resources (cost) invested? Translating disease control interventions and their impacts into dollars and cents also provides the benefit of a common unit of measurement that can serve as the basis for comparing apples and oranges, e.g. control that improves reproductive performance versus another that improves weight gain in an animal.

If the economic criterion is indeed the one adopted for decision-making, then several different measures of economic worth may be considered. The most popular include the benefit:cost ratio, the net present value and the internal rate of return-all measures from financial investment analysis. As noted above, however, these quantitative measures will often need to be qualified or supplemented by additional criteria for impacts more difficult to quantify or value, such as the degree to which benefits are distributed equitably among different segments of society.

Also affecting the criteria is the level at which the decision is to be made. Decision-makers work at all the different levels — farm, community, local authority, national, regional and international. Although certainly not the rule, as the decision moves from the farm to the national level, the criteria generally change from financial to economic. Financial analysis refers to costs and returns associated with cash transactions at nominal market prices directly incurred by the decision-maker, whereas economic analysis broadens the scope to include non-cash costs and returns valued at their 'true' prices (e.g. without distortions introduced by taxes and subsidies), and evaluates the value of control for society as a whole rather than the individual user. Financial analysis is therefore appropriate when trying to assess whether the control intervention will be feasible from the individual user's perspective — can farmers or others handle its demands on their resources and cash flow, and is it sufficiently attractive in terms of financial benefits to merit their participation? Economic analysis, on the other hand, tends to be used when the decision-maker is interested in the public good, especially if public resources are used to support an intervention.

The choice of criteria, the perspective of the decision-maker, and the particular context of the disease under consideration then inform the specific analytical approach or technique to be used to evaluate the disease control intervention.

***Ex post* evaluation of the decision-making process** It is clearly important that scenarios developed, data used, analyses conducted, and syntheses prepared, are appropriate for the decision-maker in question, in which case the adoption of the results should approach 100 per cent. However, they rarely do. One way to avoid the pitfalls of developing decision aids for farmers or national strategy development that are not used is to learn from each analysis carried out, and conduct an *ex post* impact assessment of the evaluation. This is rarely done, but is an area that needs developing if veterinary epidemiology and economics are to have greater impact on the quality of decision-making in the future.

The special case of eradication

In scenario development, the most common framework compares alternative control options. However, occasionally an eradication scenario is included, and this requires special consideration, for if eradication is to be a viable scenario, certain criteria must be met.[62] These are summarized as follows:

- effective tools for breaking transmission are available;
- epidemiological and population ecological features of the disease make it amenable to eradication (e.g. clinical cases and/or infections are easily detectable, the size of the area affected is not too large and has a relatively stable population);
- the disease is of sufficient socioeconomic priority;
- there is compelling evidence that the cost to benefits of eradication outweigh those of control; and
- adequate managerial, operational and financial resources are available.

With these criteria, the feasibility, advantages and risks of eradication versus control can be carefully evaluated before a decision to embark on an eradication programme is made. The most powerful advantage of achieving eradication is that there may be no subsequent disease control costs, depending on whether eradication is global or local: in the latter case, costs continue to be incurred to avoid reintroduction of the disease. However, if eradication fails, continuing disease control costs remain and previous eradication programme costs (often substantial) are sunk and do not accrue the expected benefits. In addition, eradication programmes may divert funding from other more general public or animal health programmes of benefit.[84]

Evaluating other control strategies

If eradication is not being considered, the next set of decisions revolves around how best to live with the disease. This means whether to treat, to control, or to prevent (which usually infers the use of vaccines, but can also be through chemoprophylaxis and the use of genetic resistance). At the farm level, this decision will be influenced mainly by the type of infectious disease, the impact the disease is having or is likely to have, the (global) availability of different technical options, the local availability of those options, their probability of success, the impact of success, the relative costs of different intervention options, the availability of resources in the context of other constraints and obligations, and any social or regulatory responsibilities of the farmer to follow certain procedures with a given disease. At the national level, decisions will be influenced by the same factors, but the assembly of data and the evaluation of options will be infinitely more complex.

Approaches for financial and economic analysis

A wide range of analytical techniques has been successfully adapted from the economics literature for application to decision-making for animal disease control. Before briefly summarizing some of the most commonly used, it is useful

to note three features of these applications that distinguish the field of animal health economics:

- **The nature of livestock production, particularly for larger animals.** Livestock are often a production asset and at the same time, the product. Control affects both, and its benefits may need to be valued from both perspectives. This requires taking into account the productive lifespan of the animal, and the multiple production cycles associated with off-take of livestock products. For this reason, economic analysis must often integrate some type of herd dynamics model that can describe how the production assets and off-take evolve over time.
- **The nature of infectious disease.** Risk of disease and the impact of control often are not limited to the individual animal; they may depend as well upon the epidemiological dynamics within the host population. Again, economic analysis must try to capture this dimension, and as a result, there have been a number of attempts to integrate epidemiological modelling with economic techniques. This also means that economic costs and benefits of disease control may extend well beyond the individual animal, herd, or farm.
- **The multiple roles of livestock in smallholder farming systems.** Economists like simple production processes where an added input results in an easily identifiable added output. In a commercial livestock production system, inputs and outputs are usually well defined and quantifiable, though complicated to some extent by the preceding two points. In smallholder farming systems, as described in preceding sections, livestock serve a number of different purposes, many unrelated to commercial sale. As a result, the benefits of disease control may need to be valued through their impact on draught power and improved crop production, or through other intangible impacts such as enhanced social status. Similarly, farmers have multiple objectives, so their decision-making may not be a function of the cash costs and benefits alone, which may violate the underlying assumption of many of the standard analytical methods.

Each of these features has considerably complicated the economic assessment of disease control, and the collaboration between veterinarians, economists, and livestock productionists to address these challenges has led to the development of a specialized field of animal health economics.[52]

In **Table 9.14** the principal analytical approaches used for decision-making based on economic criteria are summarized. The first set refers to farm-level decision-making, and are used either to advise the farmer which control option will be most profitable to use by comparing costs and benefits, or to understand and predict what level of control the farmer is likely to adopt by evaluating the trade-offs in terms of overall farm resource allocation. The second set of approaches is better suited for the decision-maker managing larger-scale control interventions, often involving public resources.

Comparing costs and benefits is the basis underlying the majority of financial and economic analyses applied to animal disease control. Most may be broadly termed as 'cost-benefit analysis' in spirit, though we must be careful to avoid confusion since this term is also the name of one of the specific techniques in this class.

Enterprise budgeting and gross margin analysis

These are standard tools for farm management in which a budget is developed for one or more livestock production activities. The budget details all inputs and their costs, together with the outputs and revenues, so that the net profit can be calculated. Enterprise budgets include both variable and fixed costs, whereas gross margin analysis only considers variable costs. For disease-control decision-making, disease control is treated as an integral part of the production process, and so budgets are estimated for production activities based on different control options. Okello-Onen *et al.*,[171] for example, treat three cattle herds under different tick control regimes (no control, once-a-month dipping, twice-a-week dipping) as different production activities and estimate gross margins for each. More typically, these approaches could be expected to be used in cases where the disease control intervention affects use of other inputs or the quality of outputs, thus fundamentally changing the production process, such as comparing the use of trypano-tolerant breeds of cattle versus regular chemotherapy with trypano-susceptible breeds.

Partial budget analysis

This is an abbreviated version of gross margin analysis. Rather than estimate a complete budget for a production activity, it considers only those costs and revenues that change due to the control intervention to determine if, and to what degree, incremental costs associated with the intervention (cost of the intervention plus any returns foregone due to the control) are rewarded by incremental gains (added output value plus any costs savings). The analysis is usually limited to a single production cycle or time period, and provides absolute (net return) and relative (benefit-cost ratio) indicators of investment profitability. With its modest data requirements, it is one of the easiest and most practical approaches. Pegram *et al.*[177] used this approach to compare the value of intensive tick control versus no tick control in traditionally grazed cattle herds in Zambia. Gummow and Mapham[82] provided a well-organized example of partial budget analysis enhanced with a stochastic dimension applied to experimental trial data from South Africa for evaluating a vaccine against *Pasteurella haemolytica* in cattle; while Kamau *et al.*[105] reported a similar analysis to evaluate cypermethrin pour-ons to control trypanosomosis in Kenya.

Table 9.14 Methods for the financial and economic analysis of disease and its control

FARM LEVEL		
METHOD	OBJECTIVE	FEATURES
Gross margin/enterprise budgets	Evaluate financial viability by comparing all costs and revenues for a given production activity incorporating a specific disease control strategy	– Suited to commercial production activities with market-valued inputs and outputs – Fails to incorporate impacts beyond the specific production activity
Partial budgets Cost-benefit analysis (CBA)	Evaluate financial or economic viability by comparing incremental costs incurred and revenues generated by adopting a specific disease control intervention	– Single time period – Least demanding in data requirements – Multiple time periods – Cost and revenue flows discounted to account for time value of money – Indicators: NPV, BCR, IRR
Decision analysis	Compare the expected net returns from a process of sequential decision-making and probability-weighted outcomes	– Suited for analysis of response to epidemic disease occurrence that can be expressed as risk probability, or disease control having multiple potential outcomes – Mimics farmer decision-making – Fails to incorporate secondary impacts
Optimization models	Maximize an objective function (often profits), subject to resource and other constraints	– Useful for estimating optimal level of disease control, regardless of whether control is 'lumpy' or continuous variable – E.g.: linear programming, dynamic programming, econometric agricultural household model
Simulation models	Use a systems model to perform 'what-if' scenario analysis	– Typically based on biological models (esp., herd dynamics model, livestock/crop model)
REGIONAL/NATIONAL LEVEL		
METHOD	OBJECTIVE	FEATURES
Cost-benefit analysis (CBA) Economic surplus	Evaluate financial or economic viability by comparing incremental costs incurred and revenues generated by adopting a specific disease control intervention	– Multiple time periods – Cost and revenue flows discounted to account for time value of money – Indicators: NPV, BCR, IRR – Can be extended to include a wide range of impacts if they can be assigned a market value – Extension of CBA to capture impact on market prices and distributional impacts between producers and consumers – Typically used for evaluating returns to research investments
Decision analysis	Compare the expected net returns from a process of sequential decision-making and probability-weighted outcomes	– Suited for analysis of response to epidemic disease occurrence that can be expressed as risk probability, or disease control having multiple potential outcomes – Mimics veterinary service decision-making – Fails to incorporate secondary impacts
Optimization models	Maximize an objective function (often profits), subject to resource and other constraints	– Useful for estimating optimal level of disease control, regardless of whether control is 'lumpy' or continuous variable – E.g.: linear programming, dynamic programming
Simulation models and systems analysis	Use a systems model to perform 'what-if' scenario analysis	– Can integrate a variety of production and post-production systems

Cost-benefit analysis (CBA)

This is essentially partial budget analysis extended over a longer period of time. Incremental costs and gains are identified for each time period, and discounting is introduced to capture the time value of money. The time value of money refers to the idea that $1 today is worth more than $1 a year from now, recognizing that today's $1 can be invested and expected to earn interest (in a risk-free savings account, for instance), yielding $1.05 next year. In addition to profitability indicators of absolute returns (Net Present

Value, the sum of discounted stream of net returns) and relative returns (Benefit-Cost Ratio), an Internal Rate of Return can be estimated that expresses the average annual interest rate yielded by the investment. This approach is appropriate when the costs or benefits extend beyond the current time period. Gittinger,[79] an authority on CBA, offered an extensive explanation of the method and numerous examples.

Cost-benefit analysis tends to be used mainly for evaluating larger-scale interventions. Penne and D'Haese,[178] for example, conducted a CBA of various ECF control options in Zambia. Benefits are estimated using a 30-year simulation model of traditionally managed cattle herds, and the results indicate that from an economic perspective, immunization makes more sense than chemotherapy or tick control. Stem[231] applied this approach to evaluate a public vaccination campaign for peste des petits ruminants in Niger, and demonstrated the high economic return yielded by the intervention. Similarly, Perry *et al.*[188] evaluated the returns to FMD eradication in Thailand, and found that such a programme would be justified if it led to export trade to high-value markets elsewhere. Considering the case of a country with export trade already established, Berentsen *et al.*[26] used CBA combined with simulation models to evaluate different control strategies if FMD were to be reintroduced into the Netherlands.

Economic surplus models

These are an extension of CBA in which the impact of intervention on supply and demand in markets for livestock and livestock products is captured. This can be important; if a given intervention has broad impact and results in avoiding mortality, the supply of meat will increase, lowering the relevant equilibrium market prices and therefore the estimated value of the benefits from the intervention. Changing prices and amounts marketed also translate into changes in consumer and producer surplus, and these can be quantified in the model to measure the welfare gains and losses to each group. Kristjanson *et al.*[111] used this approach to evaluate the potential impact of a vaccine against trypanosomosis, and predicted that due to lower market prices, consumers will reap 55 per cent of the estimated US$ 1.3 billion in net benefits generated by the vaccine, versus 45 per cent for producers, who gain from lower production costs. The impact of the recently completed Pan-African Rinderpest Campaign was similarly analysed.[234]

Decision analysis

This is particularly useful for disease control interventions that involve diseases with variable risk of occurring, multiple possible outcomes, or a sequence of actions. This technique estimates the expected net returns for a set of actions (decisions) and outcomes by conditioning each associated cost and return by their probability of occurring.

Optimization models

These models use information on a household's or firm's resource base, market prices, and input–output relationships for its production activities to solve for the resource allocation and mix of production activities that will maximize some defined objective, typically farm or firm profits. One class of models is based on mathematical programming techniques (e.g. linear programming, dynamic programming). Building a mathematical programming model is quite demanding in terms of data and research sophistication. For this reason, such models are used primarily for regional or national analyses in which models are developed for representative farms or herds; they are not practical tools for livestock producers themselves. Researchers at Wageningen University in the Netherlands have developed a number of such models for intensive commercial systems to advise on optimal timing for disease-related culling and stock replacement actions.[95, 101] In a particularly innovative policy study looking at farmer incentives to participate in various components of bovine tuberculosis control in New Zealand, Bicknell *et al.*[27] demonstrated how a mathematical epidemiological model can be transformed into a dynamic bio-economic optimization model. Applications for developing country situations are much less common. An exception is a study by Hall *et al.*[85] in which a dynamic programming model was formulated to evaluate optimal ECF control strategies (tank dipping, immunization) in communal herds in Malawi over a five-year period.

The other main class of optimization models is based on econometric approaches, but these have been used little to date in the field of animal health economics. Chilonda and van Huylenbroeck,[40] however, suggest how an agricultural household model[225] might be adapted to examine smallholder farmer management behaviour for livestock health.

Simulation models

These models have been used extensively in animal health economics, but are usually limited to biological processes and applied to measure the impact of different control scenarios as an auxiliary component within an economic analytical technique, such as CBA or optimization modelling. As described above, analysts often include some type of herd population dynamics simulation model in order to capture benefits of disease control over time, and nearly all of the studies noted in the preceding paragraphs contain such a model. In some cases, though, simulation models have directly integrated financial or economic parameters as well. Such models are often packaged as highly specialized management software for intensive commercial production systems. Examples include PorkCHOP,[51] PigORACLE and DairyORACLE,[142] and DairyMAN.[92] Similar models are rare for smallholder livestock production systems in developing countries, which can be attributed to a large degree to their lower commercial orientation and the difficulties of model-

ling the multiple roles of livestock in such systems. Exceptions are Nyangito *et al.*[166] and Nyangito *et al.*,[167] which model the complete farm household economy rather than the livestock production system alone to evaluate the financial and economic impacts of immunization against ECF on smallholder dairy farms in Kenya.

While certainly not exhaustive, this brief review of economic analytical techniques and examples from the animal health economics literature should give the reader an appreciation for the broad range of situations and types of decision-making to which economic analysis can be applied. Many relevant economic tools have yet to be applied or appropriately adapted to animal disease control problems, particularly in the developing world. A number of opportunities and some of the challenges that remain to make economic assessments more meaningful for effective decision-making are discussed in Perry and Randolph.[183]

Estimating the cost of a disease

Not mentioned above are a number of studies that have estimated the economic cost of a given disease, usually at national or regional level. Examples include Mukhebi *et al.*[156] for ECF in eastern and southern Africa, Freyre *et al.*[67] for toxoplasmosis in Uruguay, and Ngategize *et al.*[163] for ovine faciolosis in Ethiopia. As cautioned by Perry and Randolph,[183] and earlier by McInerney,[133, 134] such estimates may provide an indication of the relative importance of a disease, but will be misleading for decision-making about investing in its control unless this preliminary analysis is then supplemented by information and analysis of the costs and realizable benefits of the control itself. The point here is that the economic cost of the disease never equals the potential benefit to control, even in the case of eradication (e.g. does not capture changes in control costs post-eradication), and in any case, the cost of controlling disease also needs to be factored into the analysis. Estimates of the economic cost of a disease should be viewed as only a preliminary step towards a proper assessment of a control intervention.

Reliability of economic analysis

Economic criteria should be critical to responsible decision-making about disease control. But with what degree of confidence can we trust the results of economic analysis? How reliable are they? Schepers and Dijkhuizen[218] describe, for example, the sometimes contradictory conclusions about mastitis control from different published studies. There are two key issues to consider in answering these concerns.

First, the quality of the analytical results is obviously largely a function of the quality of the data used, with key epidemiological parameters playing a particularly important role. 'Garbage in, garbage out' is the oft-quoted adage. Sufficiently high-quality data are often not available, however, and the needed primary data collection is not likely to be feasible due either to resource or time constraints. Well-designed sensitivity analysis therefore is critical to evaluating the robustness of the quantitative results. This may be accomplished either by estimating results for a wide range of potential scenarios — rather than limiting the analysis to only one or two — or by changing values of individual key parameters that tend to drive the analysis. Stochastic models build this type of sensitivity analysis directly into the analytical model, and researchers have found the spreadsheet-based tools of @RISK™ (Palisade Corporation) particularly useful for this purpose since it allows the analyst to simultaneously represent multiple key parameters by different types of probability distributions. These types of sensitivity analyses can be very powerful in estimating notional confidence intervals for quantitative results.

Sensitivity analysis alone, however, does not ensure reliable results. Even if the data are accurate, the conclusions from the economic analysis may be flawed if the scope of the analysis is too narrow. Unless all potential impacts of disease control — both in terms of costs and benefits — have been captured, then the analytical results will be inaccurate. It is essential that the analysis be as comprehensive as possible to consider and quantify where possible the full range of impacts. Studies are usually good at identifying and measuring the obvious direct impacts, but are often less ambitious about addressing those that are indirect or secondary. Thus, while sensitivity analysis can compensate for certain data inadequacies, attention also needs to be paid to the comprehensiveness of the analysis of impacts.

How can control options be best implemented?

Once the impacts of an infectious disease and its potential control have been evaluated and the optimal control strategy decided, what remains is to implement the control measures. Despite improving knowledge of disease dynamics and the availability of better control technologies, the record of disease control has often been disappointing. This section explores a range of issues that need to be considered for effective disease control.

The historical context

In the introduction to this chapter, reference was made to the early efforts to eradicate ECF in southern Africa. At that time, how disease control should be implemented was clear. The priority was to address the threat of particularly contagious diseases and their devastating impact by imposing authoritarian measures on all livestock keepers for the public good. The need to control the major epidemic diseases was in fact the primary impetus for the creation of state veterinary services in Africa.[99] For these diseases, the technical solution proposed by the veterinary services probably coincided fairly well with what was economically justified.

As the threat of epidemic disease was brought increasingly under control, public veterinary services continued

to expand their bureaucracies by extending their mandates to protecting commercial livestock production from the re-introduction of epidemic diseases or the introduction of new exotic diseases, and by beginning to address zoonotic and endemic diseases. With the shift towards endemic diseases, disease control became less a question of ensuring compliance to imposed control interventions and increasingly one of encouraging farmer-adoption of control technologies. The combination of centrist state policies and the shortage of trained human resources meant, however, that disease control decision-making and implementation continued to be concentrated within state veterinary services, often discouraging the development of private sector provision. In this context, successful implementation of disease control was usually measured in terms of cost effectiveness.

The public fiscal crises experienced by African countries beginning in the 1970s led to pressure on state veterinary services to reduce costs and activities. This coincided with growing acceptance of the paradigm that the private sector can provide many types of disease control more efficiently and effectively than the public sector.[94] As a result, efforts were encouraged to privatize livestock health services and shift the responsibility for much of the decision-making and cost for disease control to livestock keepers themselves.[219] Such efforts have been undertaken to varying degrees and with varying levels of commitment in most, if not all, African countries.

The general consensus is that rather than improve the situation, the reduction of state services and privatization have instead reduced capacity to control diseases effectively in Africa.[136, 219] These trends have prompted research and development efforts to better understand how to improve the design and performance of delivery systems for implementing disease control.

Delivery systems

A 'delivery system' is now commonly accepted as a generic term for describing how disease control is implemented, whether it involves a one-time public-managed disease-eradication campaign or the sustainable provision of veterinary drugs for managing endemic diseases. In **Figure 9.12** the basic components of a delivery system are presented.

Disease control involves two main inputs — veterinary products and knowledge — that are delivered or administered by livestock health services. The supply and distribution of the inputs, as well as the provision of services, may be the responsibility of various combinations of actors in the public, private, or civic sectors. A campaign to eradicate an epidemic disease may be predominantly public sector, with vaccine, manufactured by a parastatal agency, being administered free of charge by government veterinarians based on surveillance information and guidelines issued by publicly funded research institutes. Anthelmintics, on the other hand, are more likely to be produced and distributed primarily through commercial channels, although recommendations on their appropriate use may be disseminated through public extension services. The end-user is the livestock keeper, and which livestock keepers are concerned by a given disease control technology will depend not only on disease incidence, but also on the type of livestock production system as well as their perceptions of the disease and the utility of control. The institutional environment is also an integral component of a delivery system by conditioning the infrastructure and rules by which inputs and services can be provided.

For a specific disease control action, the appropriate composition of the generic delivery system described in **Figure 9.12** will depend on the nature of both the disease and the control strategy:

- **The nature of the disease** In **Table 9.4** diseases are classified into six categories according to the type and scope of impacts they engender. The two key characteristics distinguishing these categories are: (1) contagiousness — to what degree the disease is limited to the affected animal or herd or is likely to spread to other animals and beyond; and (2) whether humans are affected. These characteristics have important implications for deciding who has responsibility for deciding the control response and its implementation.
- **The nature of the control strategy** Several different control options may be available for controlling a disease, each having its particular technical requirements and economic characteristics. As an example of a purely technical characteristic, the thermostability of a vaccine determines whether or not the vaccine requires delivery structured around a rigorous cold chain. Technical properties of a control option may also condition its economic characteristics. From the livestock keeper's perspective, incentives for using a control option meant to protect his/her individual animal from a disease may be obvious, but it is less so for a control option that targets protection at the population level. Whether a control strategy is primarily curative rather than preventive is also important.

Due to the various technical and economic characteristics of a disease and control strategy, there is a range of approaches used, as illustrated by the dotted arrows in **Figure 9.12**. At one extreme, state veterinary services may try to impose control, say, to limit an outbreak of a highly contagious disease (a public good) that requires livestock keepers to respect a quarantine order. In this case, successful disease control is associated with ensuring compliance. At the other extreme, a livestock keeper's willingness to pay a private veterinarian to care for an animal is an example in which successful disease control depends on the livestock keeper's demand rather than compliance. Many disease control interventions represent a mix somewhere between these two extremes.

Improving delivery

Productivity remains well below potential in the majority of livestock production systems in the developing world, and disease continues to be a critical constraint despite the availability, in many cases, of appropriate control technologies. Inadequate health management is often attributed to poor delivery of control technologies. A number of research and development efforts have been devoted to identifying opportunities for improving delivery systems and effective use of disease control technologies. Many of these efforts target specific components of delivery systems as presented in **Figure 9.12**.

Better products Certain characteristics of a control technology can constrain its delivery and adoption. The classic example is that of veterinary products that require a cold chain, i.e. that must be kept refrigerated at low temperatures to remain viable. Lack of infrastructure, especially in areas where livestock keepers are widely dispersed or mobile, has made delivery of such products problematic in much of Africa. One way to overcome these constraints is to adapt veterinary products accordingly. An important theme has been the development of thermostable products, particularly vaccines such as those against rinderpest,[141] Newcastle disease,[2, 25, 230] and ECF.[160] Another has been simplifying techniques of administration to make control more accessible or improve coverage, such as coating chicken feed with thermostable Newcastle disease vaccines or administering them orally using a simple eye-dropper.[25] In the case of trypanosomosis control, the development of the Sterile Insect Technique, though very expensive to implement, avoids need for farmer involvement and many of the environmental drawbacks of alternative vector control options.[3]

Veterinary products can also be improved in terms of their economic characteristics, especially their price. Affordability can be enhanced by more efficient production and distribution that lower cost either through technical or institutional improvements. For products that provide significant benefits beyond the farm where they are used, public subsidies can directly reduce the nominal cost borne by end-users and thereby encourage uptake.[57] Increasing the effectiveness, safety, or ease of application of veterinary products also has the effect of reducing economic risk by minimizing productivity losses and wasted investment when the products do not work to satisfaction.

Better information As already noted in previous sections, epidemiological information is critical for evaluating when and how much disease control is appropriate.[189] Improving the availability and quality of epidemiological information enables delivery systems to target their interventions better and hence become more effective and efficient in their provision of control. Sanson and Thornton,[217] for example, estimated that diagnosis (and consequently control) of salmonellosis after its introduction into New Zealand would be delayed by eight months if the contemporary animal health surveillance activities were reduced. Efforts to improve animal health information, whether through more effective information systems and networks,[46, 89, 170] improved diagnostic tools such as user-friendly pen-side tests, or soliciting information directly from farmers[34] can therefore be expected to contribute to more effective implementation of disease control strategies.

Better information is needed not only to know when to implement control, but also how to implement it properly. Kenyan farmers, for example, report that the lack of printed information or advice on using anthelmintics contributes to under- or overdosing, and other inappropriate practices, including the use of substandard or counterfeit products.[250] Both the private and public sectors are involved in generating and providing information using a variety of media, often through advertising or extension services, and targeted at both service providers and the farmers themselves. Research is now needed to explore such information channels and how their effectiveness can be improved.

Better services Initially, improving the service component of disease control was largely concentrated on upgrading human resources through training and, more specifically, graduating adequate numbers of veterinarians. More recently, Kleeman and others have begun examining the opportunities for improving the quality of services through better management, and to this end Kleeman[108] proposes a number of techniques as a 'toolkit' for analysing services and their efficiency.

Providing adequate livestock health services in areas where livestock production is not market-oriented, or production units are relatively few, dispersed or mobile — especially among pastoralists — has presented a particular challenge. Such areas often do not offer sufficient financial incentives to support private veterinary practice, and in the past depended largely on state-provided services. As state veterinary services have declined over the past three decades, a number of research-development efforts have experimented with community-based systems that could provide basic care in the absence of formal veterinary services.[33, 94] These schemes typically involve the training of a local community member as a Community-Based Animal Health Worker to provide limited paraveterinary services paid for by the community. This approach is being tested for example to provide veterinary care to backyard poultry producers.[2]

Under standing demand As implementing disease control shifts from the public to the private sector, delivery systems need increasingly to take into account the particular

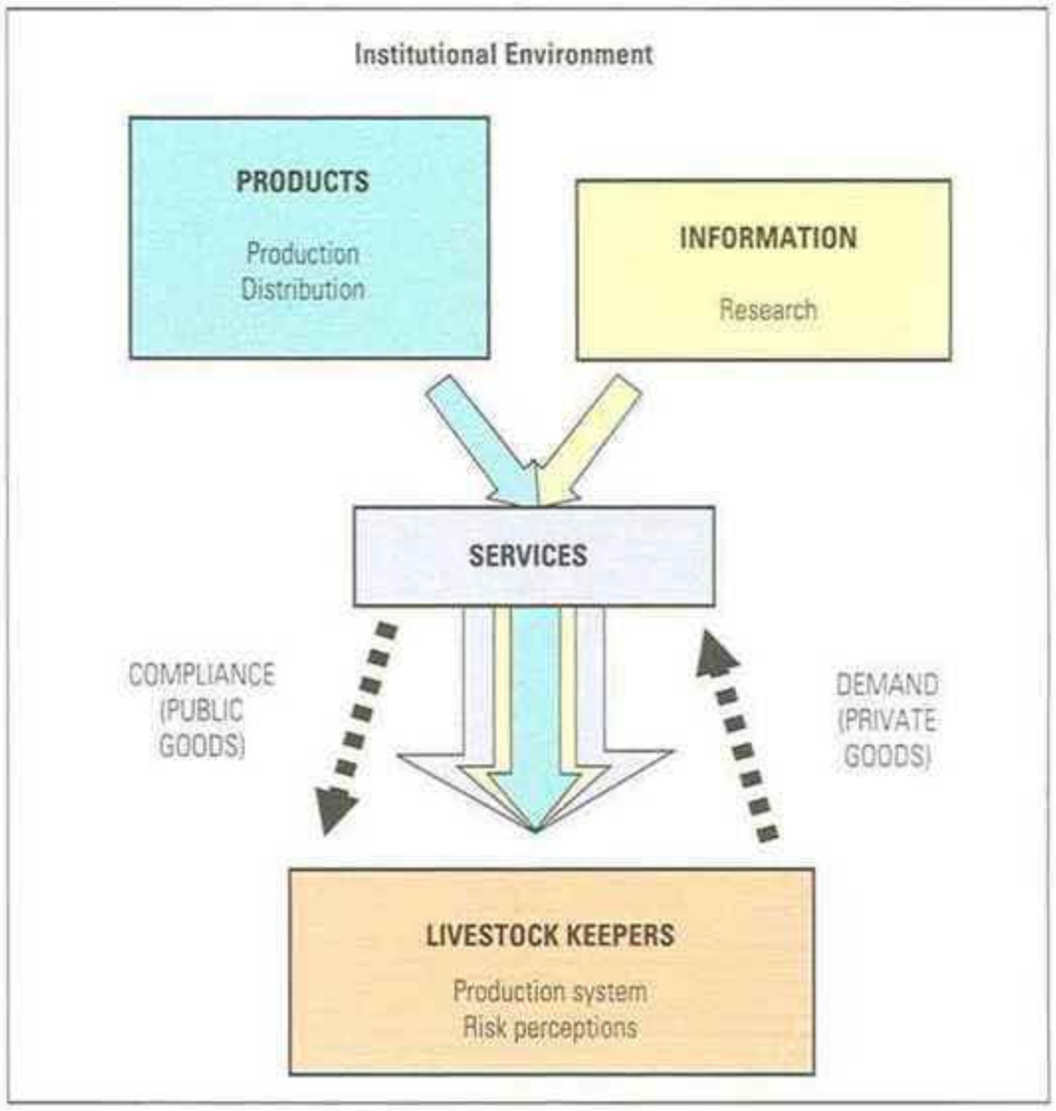


Figure 9.12 A generic delivery system

requirements of livestock keepers and their production systems. This is important because failure to properly evaluate farmer demand risks undermining the financial viability and longer-term sustainability of livestock health services. Animal health information systems provide only one component of farmer demand for control: disease incidence and prevalence. How livestock keepers respond to disease risk will also depend on the role that livestock play in their household economy, their perceptions and understanding of both the disease and the control strategy, their resource levels, and the relative costs and benefits of using control. Evaluating costs and benefits to support decision-making have been discussed above. Various approaches are being developed and used to assess the other various components of demand.

One approach is to solicit needs directly from livestock keepers using participatory techniques. Many of the tools developed to date including diagramming, mapping, scoring, ranking, and interviewing techniques, often used in conjunction with establishing community-based livestock health services have been summarized by Catley.[36]

The other principal approach is based on demand analysis techniques from the field of economics. Several of these techniques and their application to livestock health services in India, including assessing farmers' willingness to pay (WTP) for specific services and products have been described.[1] As pressure has mounted to ensure cost recovery for disease control, it has become evident that better techniques are needed to measure WTP. One of these techniques draws upon WTP methodologies used in market research and contingent valuation adapted to livestock health services.[1] This technique has been used to evaluate the willingness of livestock keepers in Kenya to contribute their labour and money to maintaining tsetse traps to control animal trypanosomosis.[56] Data collection and econometric analyses for other factors such as farmer and community-level characteristics that influence farmer demand have also been reported.[1] In a similar analysis, Swallow *et al.*[233] evaluated demand for pour-ons for trypanosomosis control in Ethiopia. Another technique adapted from marketing research is conjoint analysis used to assess consumer preferences regarding specific attributes of a disease control strategy.[202] Each technique is likely only to capture one dimension of farmer demand, so several may be needed in combination.

Better institutional environment The institutional environment refers to policies that dictate or influence which actors are involved in disease control, the roles they play, and the rules they work under. As noted, the current institutional environment governing veterinary delivery systems in developing countries has been conditioned to a large extent by external macroeconomic trends over the past three decades (especially public fiscal stabilization

programmes) that have reduced capacity of state veterinary services and encouraged privatization and decentralization of livestock health services. These changes have occurred at a variable pace across Africa, and have created considerable confusion regarding public and private sector roles in disease control and the appropriate accompanying adjustments in the regulatory environment. The result has been considerable research devoted to developing a framework for evaluating and improving the institutional environment for delivery systems.

The evolving framework draws upon economic theory, particularly the New Institutional Economics, to characterize the underlying economic characteristics and incentives inherent in disease control products and services. Central to this type of analysis are the concepts of externalities and public goods. An externality occurs when one person's use of goods or a service generates impacts — either negative or positive — on other people. If these impacts are not properly captured in the market cost of the good or service, then the consumer is likely to use either too little or too much from a societal welfare perspective, leading to what economists term as market failure. Market failure occurs when markets are unable to provide certain goods or services in the desired quantity or quality. Public institutions have evolved primarily in response to such market failures, and to take corrective action by providing public goods. For example, smaller livestock keepers are likely to 'under-invest' in vaccination against a contagious disease, and so public veterinary services may be justified in undertaking a vaccination campaign to ensure adequate population coverage. Externalities related to disease control are generally a function of the contagiousness of the disease and its zoonotic impacts. In the absence of externalities, economic theory dictates that markets (i.e. the private sector) are more efficient and cost-effective in providing disease control products and services than the public sector.

Evaluating the degree to which a given disease control action is associated with externalities thereby provides the basis for judging whether it is best left to the private or public sector to deliver. Two criteria are often used: excludability and rivalry (**see definitions in Figure 9.13**). As shown in **Figure 9.13**, veterinary products and services can then be classified as public, toll, common pool, and private goods, each having implications for the appropriate actor to implement, or at least finance, the disease control action.[94] A government supporting a vaccination campaign, for example, may find it more efficient to contract services from private veterinarians rather than setting up and managing its own vaccination teams.

The manner in which this framework could be applied to veterinary services was demonstrated in Kenya[118] and then in Africa more generally.[119] It was also developed as a methodology for classifying individual livestock services.[243] In addition, an excellent overview of progress to date, including a number of examples of how this approach is being used to address specific issues and suggest solutions, has been published.[94] It is not clear yet what impact this research has had on public veterinary policy.

Assessing performance

A general approach for assessing the overall performance of delivery systems for disease control is still lacking. Researchers at Reading University in the UK are in the initial stages of developing an approach based on the following four criteria:[136]

- accessibility, referring to the willingness of providers to supply disease control and time taken to find a service provider or product when needed;
- affordability, the actual cost as well as ability and willingness of livestock keepers to pay;
- acceptability, the form and manner in which the product or service is presented; and
- sustainability, both financial and institutional.

These criteria capture a number of the elements discussed in the preceding paragraphs. The challenge now remains to identify indicators that can be used to evaluate these criteria.

Research at the ILRI is currently exploring two additional techniques for assessing delivery systems.[162] The first draws upon the Structure-Conduct-Performance paradigm from agricultural markets research, which compares delivery systems to what might be expected in a perfectly competitive market, especially in terms of the nominal cost of the final product. Using this technique, delivery systems are examined for evidence, for example, of monopoly power, barriers to entry, or inflated marketing margins that would likely contribute to inefficiency.

The second technique, based on another theme from the New Institutional Economics called Transaction Costs Analysis, shifts the focus to non-price factors that may contribute to poor delivery. Asymmetric information between different actors in a delivery system, for example, gives rise to a number of potential transaction costs. A livestock keeper has difficulty judging whether a veterinarian is treating his/her animal correctly, and therefore faces an added risk — and added cost — of losing the animal plus the value of the treatment. An effective delivery system is one that minimizes such transaction costs to the extent possible.

Institutionalizing impact assessment: the role of epidemiology and economics

The effective and optimal use of epidemiology and economics in impact assessment, national disease control planning and implementation depends very much on how the use of the discipline is 'operationalized'. There has been a fairly wide range of modalities tried in different countries, some of which have been highly successful and have endured, some have struggled, and some have been quite transient. As epidemiologists and economists, it is important to evaluate what has contributed to these different outcomes, and learn

Figure 9.13 Two key economic characteristics of disease control (source: Holden, 1999[94])

RIVALRY (OR SUBTRACTABILITY)*	EXCLUDABILITY*	
	LOW	HIGH
LOW	PUBLIC GOODS – Epidemic or zoonotic disease control (including surveillance, movement control, quarantine services) – Some extension – Some research – Control of food-borne diseases – Drug quality control	TOLL GOODS – Vaccine production – Diagnostic services – Veterinary clinics – Dips
HIGH	COMMON POOL GOODS – Tsetse control on communal land using traps, targets or aerial spraying	PRIVATE GOODS – Endemic disease prevention and control – Sales of drugs and vaccines – Some extension – Some research

* Excludability: the ability of the user to exclude others who have not paid from benefiting from the user's investment in a good or service
Rivalry: consumption of a good or service by one user precludes its use by other users

from the successes and failures in the adoption of our discipline in the past.

Possibly the greatest success in the field of epidemiology and economics has been in research. During the last two decades there has been a steady improvement in the focus, relevance and scientific quality of publications in the field of veterinary epidemiology and economics. This has been particularly important in the development, testing, validation and application of a variety of methodologies, which have very much consolidated the acceptance of veterinary epidemiology and economics as a *bona fide* discipline. It is important to acknowledge the pivotal role that has been played in this process by the journal *Preventive Veterinary Medicine*. This progress has been made by individuals and groups in many countries of the world. During 1998, for example, the distribution of manuscript submissions to this journal was roughly one-third from Europe, one-third from the Americas, and one-third from the rest of the world.[149] But what impact has this scientific success had beyond the research community? In many countries of the developed world, it had a significant impact. There is evidence that it has contributed, for example, to the development of several national disease surveillance programmes, such as the National Animal Health Monitoring System (NAHMS) in the USA, and to the teasing out of health and management constraints at the farm level that has probably had major impacts on national livestock productivity, particularly in the more intensive dairy, feedlot beef, poultry and pig production systems. It has also had an important effect on policy and strategy development for national disease control, for example in the work of Dijkhuizen and his colleagues in the Netherlands on FMD and classical swine fever. In most countries of the developing world, however, the impact has been quite different, and much more modest. There is definitely a raised awareness, and the value of epidemiology units as advisers to directors of veterinary services has been recognized by many donors who have supported training programmes. As a result, there has been a significant development of human capacity. The major obstacles to a more active adoption of the epidemiological and economic tools developed have been a lack of political understanding of the potential returns from their use, and lack of resources to allow the discipline to be effectively institutionalized and practised by its new disciples.

What are the necessary conditions for the effective adoption and use of the research products of veterinary epidemiology and economics, from journals such as *Preventive Veterinary Medicine*, among many others? First, there have to be trained epidemiologists and animal health economists in a given country. In many developing countries, such people, if they exist, do not exceed a handful at most. There is probably a highly skewed geographical distribution of trained epidemiologists, depending in many cases on the previous existence of technical co-operation projects in which capacity building in the field of veterinary epidemiology was a priority. Next, there need to be positions earmarked for epidemiologists and economists in animal health research and development institutions. In most developing countries these would be unlikely to exceed one, if any. Thirdly is the condition that there must be adequate funding for this person to operate effectively, including access to journals, e-mail and Internet. Public sector funding for veterinary epidemiology and economics has had to compete with more operational aspects of veterinary services, and during the structural adjustment process undertaken by many countries, they have suffered badly. As far as access to

electronic media is concerned, we were all made very aware of what was labelled at a recent G8 summit in Okinawa as the 'digital divide'. It is not only the high cost of, and thus poor access to, computers, but also the inadequate telecommunications infrastructures, and the high cost of access to them. The bottom line is that the adoption and impact of scientific developments in our field leaves much to be desired in regions where this impact is needed most. This is particularly the case in Africa.

On the developmental side in which epidemiology and economics function as a component of a national veterinary service, various modalities have been used to integrate the discipline into national disease-control bodies. These include institutionalizing it in departments of veterinary services or in veterinary research institutes (or both), and the development and use of university departments, particularly those with a mandatory 'extension' function. There are a few 'classics' around the world that may serve as role models for some sets of circumstances (but not all), and the Veterinary Epidemiology Department at the Veterinary Laboratories Agency (formerly the Central Veterinary Laboratory) in Weybridge, UK, is certainly one of those national epidemiology units that has endured and gained an international reputation for focus and functionality. In the developing world, epidemiology and economics have been the 'flavour of the moment' in many countries, but while there are several success stories, epidemiology units in many developing countries have generally suffered from a lack of sustainability. Thus, in Africa, where epidemiology has been recognized as playing an important role, given the history of a population, rather than individual animal, approach to disease control, national units have been set up in several countries such as Zambia, Botswana, Nigeria, Zimbabwe, Tanzania, Uganda and Kenya, but their functionality and impact have ebbed and flowed due to their heavy reliance on donor agencies, whose support has varied over the years. However, on the bright side, there has been a considerable development of human capacity during this time, which bodes well for the future. In other parts of the developing world there are some examples of new national indigenous units, supported by funds from their own governments, that appear to be increasing in their impact; these include groups in Thailand, Colombia and Chile.

In all of the developing, as well as much of the developed, world, units or groups have tended to be led by epidemiologists, who have been more comfortable developing the technical dimensions. As a result, there has been a reluctance on the part of both research and development groups to employ economists, so severely limiting potential impact of the discipline. The Veterinary Epidemiology and Economics Research Unit at Reading University in the UK is one of the few academic institutions that truly incorporates both disciplines. Public sector funding of staff positions is in short supply, and economists are considered a lower priority human resource that cannot be afforded.

International organizations also have epidemiology capacity that may play a role in national animal disease control. The FAO has a small epidemiology group within its animal health service in the Rome headquarters, and individual epidemiologists and animal health economists in its many field projects. In addition, the OIE has an epidemiologist at the headquarters in Paris. Somewhat surprisingly for such prominent international organizations, neither has an effective integration of epidemiology and economics, nor do they employ agricultural economists within their animal health staff groupings. The ILRI, an International Agricultural Research Centre of the Consultative Group on International Agricultural Research, has an epidemiology and economics group, the mandate of which is entirely research. At a regional level, some epidemiology and economics capacity exists in the African Union Inter-African Bureau for Animal Resources, and this capacity is currently being enhanced with support from the European Union.

Disappointingly, most of these international and regional units are driven, in the traditional disease-control manner, by technical issues, and the small economics components, if they exist at all, are more of an add-on to provide the necessary figures to justify programmes. Here is a real opportunity for more integration to provide better support to strategic decision-making.

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10

Infectious diseases of animals in sub-Saharan Africa: The wildlife/livestock interface

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Introduction

The critical need for rural development to support the rapidly growing human population in sub-Saharan Africa is increasing the pressure on wildlife in the region. Poverty and lack of food security are root causes of the massive destruction of habitats and animal populations.[25, 58, 59, 96] Wilderness areas are being encroached upon and are often perceived by rural communities as legitimate sources of natural resources from which they are unreasonably precluded access for livestock grazing, collection of firewood, thatching grass and other products, and subsistence hunting. On the other hand, the potential of the rich wildlife heritage of the region for sustainable exploitation and therefore economic development is obvious. It is also important for sub-Saharan Africa to preserve the diversity of its fauna and flora, because nowhere else on Earth is there a comparable resource. It is difficult to reconcile these essentially conflicting imperatives.

The growth in the livestock population of Africa is no longer keeping pace with human population increase, and livestock productivity is not improving in most regions to compensate for the increasing needs.[96] There are a number of reasons for this static livestock economy, the most important being land degradation,[103] declining livestock production and faltering disease control services.[96] There is also a decline in wildlife populations, even within many protected areas, with over 60 per cent of all such areas now being relatively depleted of antelope and large bovids.[48] This resource will no longer provide the supplementary (and free) food enjoyed by past generations. The concomitant increasing need for land on the one hand, and greater awareness of the intrinsic and economic value of wildlife resources and savannah biodiversity on the other, result in the issues at the interface between livestock and wildlife becoming increasingly acute and politicized.

The presence of diseases that occur in both domestic and wild animals and which may be transmitted from one to the other constitutes one of the constraints on land-use options in sub-Saharan Africa. Although the interface results in some disease transmission and economic loss, it should be appreciated that with the massive decline in available habitats for wild herbivores in most regions of Africa, the extent of this interface is actually shrinking on the macro-scale, yet intensifying in other regions. In addition, amongst the wide variety of wild artiodactylids in Africa, only a few species are epidemiologically important with regards to serious economic livestock diseases. These are African buffalo (*Syncerus caffer*), warthog (*Phacochoerus africanus*), bushpig (*Potamochoerus* sp.), greater kudu (*Tragelaphus strepsiceros*), bushbuck (*Tragelaphus scriptus*), and wildebeest (*Connochaetes* spp.), which have all been linked to one or more of the following important diseases: rinderpest (RP), foot-and-mouth disease (FMD), African swine fever (ASF), malignant catarrhal fever (MCF), East Coast fever (ECF), bovine petechial fever, and trypanosomosis. These major infectious diseases of livestock and wildlife are comprehensively presented elsewhere in this book.

With this background, the objective of this chapter is to discuss the key wildlife-related issues that impact on disease persistence and control. These include free-ranging wildlife as disease reservoirs, alien diseases introduced with domestic animals, pastoralism and transhumance, the use of fencing, and compliance with animal health requirements, which are necessary to gain access to international markets and trade. In addition, certain current trends in wildlife management, such as the development of game conservancies and transfrontier parks, and the increasingly popular introduction and relocation of wildlife to privately owned game reserves, which may expand or intensify the interface, are also of concern and are discussed.

Historical perspective

Sub-Saharan Africa has a larger number and diversity of indigenous free-ranging wild mammals than any other comparable land mass. This is especially true for ruminants. As a possible consequence, an equally impressive array of pathogenic micro-organisms and macro-parasites, as well as arthropod vectors of certain micro-organisms, have co-evolved with their hosts for millennia, which has culminated in evolutionary adaptation and tolerance between host and pathogen or parasite, and the development of endemic stability. Infection is usually 'silent' in the definitive wildlife hosts, which serve as reservoirs of specific pathogens. Infection of wildlife species other than the definitive hosts may result in disease, which is generally mild. Infection rates may be high, but clinical expression of disease is generally cryptic in these 'incidental hosts'.

The evolution of the wildlife/livestock interface in sub-Saharan Africa is inseparably linked with the more recent history of *Homo sapiens* on this continent. The best examples of the recent history of human migration and interaction with livestock are those which took place in southern Africa; some of these are used here to illustrate certain major factors that have played or continue to play a role at this interface.

The San (Bushmen) were the first modern humans to occupy southern Africa. They were hunter-gatherers,[19, 97] who left us ample evidence of the distribution and diversity of the resident and migratory wildlife in their numerous rock paintings and engravings. These relatively nomadic and sparsely distributed inhabitants of the sub-continent cultivated no crops and possessed no livestock,[57] and were therefore unaffected by the animal disease agents that were cycling in the wildlife environment.

The next incursion of humans into the sub-continent were the Khoikhoi (Hottentots), who are now regarded as being closely related to the San.[19, 57] They migrated from the central interior of Africa to the grazing lands of the coastal regions of the Western and Eastern Cape provinces of South Africa, in particular. They were pastoralists, herding fat-tailed sheep, cattle and possibly also goats, and were probably the first people to bring livestock into southern Africa.[45] It is possible that their migratory route, which was mainly south-westerly, was influenced by 'belts' of tsetse flies and hence trypanosomosis,[97] and they must have experienced the presence of other serious livestock diseases, such as theileriosis and heartwater, which were prevalent in the east of the sub-continent. This was later followed by southward migrations of different ethnic, but primarily Bantu, groups and evidence of their presence in southern Africa goes back some 2 000 years.[45, 66] They had cattle of the Sanga (Nguni) type as well as sheep and goats. There is, however, evidence of a degree of resistance to certain endemic diseases and parasites in cattle, sheep, goats and dogs that have an historic association with Africa, resulting from their exposure to wildlife and their endemic diseases and parasites, over many centuries.[17, 49, 61] Tick resistance, local trypanotolerance and partial adaptation to certain viral, rickettsial and protozoal diseases were probably the result of natural selection pressures on the livestock of these early migrants.

It was during the colonization of Africa by European nations during the past three-and-a-half centuries that the introduction of imported livestock breeds[17, 104, 105] unmasked the presence of many African endemic diseases that were silently cycling in the free-ranging wildlife. These diseases caused significant morbidity and mortality in the poorly adapted, immunologically naive European breeds, and had a significant influence on the day-to-day lives and activities of the settlers, due to their deleterious effects on draught, transport and production animals. Furthermore, efforts to 'upgrade' the indigenous tribal cattle by cross breeding also probably had negative effects on their disease resistance.

Since the emergence of embryonic government veterinary services in Africa, some ten decades ago, epidemic diseases have received the most attention, and were the most feared because of their devastating effects. Disease control emphasis was accorded to domestic livestock, which were so important for transport, production and trade in the developing colonies. One factor, which has contributed significantly to the persistence of infectious diseases, is the continued dependence on pastoralism as a livelihood for large numbers of the African peoples. For example, over 50 per cent of the Somali community are involved in, or rely on, pastoral cattle and small stock for their livelihood.[58, 59] The large-scale movement of animal populations across borders in some regions, in search of pasture[64] and associated trade, has been the single most important factor in the spread of epidemics such as RP, peste des petits ruminants (PPR), contagious bovine pleuropneumonia (CBPP), and FMD.

The importance of international trade on livestock economics and the impact of disease on this trade must be mentioned. The more developed nations, where much of the trade is centred and a high commercial value for livestock exists, are primarily concerned with food safety and hygiene, whereas food security is the major interest of developing nations.[32] This difference has resulted in a greater emphasis being placed on disease eradication and control in some regions, and international regulations and requirements to support these aims have been implemented. As a consequence the developing countries are frequently severely penalized in economic terms through their inability to trade in these more lucrative markets.[96] In essence, although these poorer nations are highly dependent on livestock for human survival, their livestock is not providing a means of escaping from the poverty trap. This issue has become more prominent with the recent experiences in developed countries of the effects of epidemics of foreign animal diseases such as FMD and ASF on their livestock industries. Recent developments, however, including some associated

with the wildlife/livestock interface in Europe and North America, may result in more flexibility in international trade rules.[5] For example, with regards to bovine tuberculosis, transmissible spongiform encephalopathies,[65, 76] FMD, and classical swine fever, the current control policies are being hotly debated.

The wildlife/livestock disease interface

Many disease-causing agents that cross-infect wildlife and livestock have been identified on most continents.[14] Some of the more important of these in sub-Saharan Africa, where range and habitats are shared or where close contact is possible, are listed in **Table 10.1**. This interface may be linear, as along a dividing fence-line; patchy, reflecting habitat preference of a disease host or vector; or diffuse where range and resources are shared. The sympatric sharing of range with wildlife is typified by the pastoral societies in certain African savannah ecosystems.

Disease, livestock, wildlife and rural subsistence

Wildlife-maintained diseases that infect livestock From an international trade point of view, FMD and ASF are probably the most important wildlife-maintained diseases, and present the greatest constraint to rural agricultural development in sub-Saharan Africa.

In the case of FMD, the pivotal role played by the African buffalo as a sylvatic maintenance host was identified in the late 1960s[39–41] and possible maintenance mechanisms of infection have been discussed.[41, 94, 101] Strict management of the disease around national parks is necessary in some countries, a good example of which is the Kruger National Park in South Africa. This Park is fenced and buffer zones have been created along its South African borders; these

Table 10.1 Diseases of importance at the wildlife/livestock interface

DISEASE AND CAUSATIVE AGENT	TRADITIONAL OR ENDEMIC HOST(S)	MAIN EPIDEMIC HOST(S)
Foot-and-mouth disease *Apthovirus*	Cattle and African buffalo	Most cloven-hoofed livestock and wildlife
Rinderpest *Morbillivirus*	Cattle, wild ruminants?	Cattle and most wild African ruminants and suids
African swine fever *Asfivirus*	Tampans (*Ornithodoros*) Wild African suids	Domestic pigs
Canine distemper *Morbillivirus*	Domestic dogs	Domestic dogs and certain wild carnivores
African horse sickness *Orbivirus*	Zebras and donkeys?	Horses, mules and donkeys
Bluetongue *Orbivirus*	Certain wild ruminants and cattle?	Cattle, sheep and goats
Rift Valley fever *Phlebovirus*	Aedine mosquitoes	Sheep, goats, cattle and certain wild ruminants
Malignant catarrhal fever *Alcelaphine herpesvirus 1* *Ovine herpesvirus 2*	Blue and black wildebeest, sheep	Cattle, cervids and certain captive wild bovids
Rabies *Lyssa virus 1*	Numerous wild canid, felid and viverrid species, domestic dogs	Multiple
Heartwater *Ehrlichia (Cowdria) ruminantium*	Certain wild ruminants, chelonians and gallinaceous birds	Cattle, sheep, goats and some wild ruminants
Bovine petechial fever *Cytoecetes ondiri*	Bushbuck	Cattle
Trypanosomosis *Various Trypanosoma* spp.	Wild bovids, suids, elephant and black rhinoceros	Cattle, horses, pigs, sheep, goats and dogs
Corridor disease African buffalo-derived *Theileria parva*	African buffalo	Cattle
Anthrax *Bacillus anthracis*	Soil	Multiple
Bovine tuberculosis *Mycobacterium bovis*	Cattle	Many wild ruminants, suids, felids and primates
Brucellosis Various species and biotypes of *Brucella* spp.	Cattle, sheep, goats, pigs and marine mammals	African buffalo, eland and waterbuck

together with vaccination of cattle and regular FMD surveillance and testing of animals in the buffer zones have been relatively effective in containing the disease in the only endemically infected area in that country.

With regards to ASF, the elegant studies by Plowright and co-workers[82–85] elucidated the maintenance host and virus amplification role of argasid ticks (*Ornithodoros* spp.) in East Africa. The role played by wild suids in this regard in both East and southern Africa have also been described.[83, 99] The situation in West and Central Africa is, however, different as there is no evidence of a wildlife reservoir. The increasing use of virtually free-ranging pigs in subsistence agriculture in the coastal regions of West Africa and recent outbreaks of ASF experienced there have brought this issue once more to the fore.

However, at the rural, extensive, communal farming level in sub-Saharan Africa, it is most frequently the vector-borne diseases, such as trypanosomosis, heartwater and theileriosis, that are the greatest disease obstacles to local livestock-based agricultural development and prosperity, although certain viral diseases such as ASF, MCF and FMD also play a role in this respect. It is in this area that conflicts between livestock-dependent communities and wildlife most often arise, as the owners of livestock frequently understand the role that wildlife play in the maintenance of these diseases. An example of this is the role of wildlife in the maintenance of tsetse flies (*Glossina* spp.) and trypanosomosis, the resultant restricted access of livestock to infected areas being a major constraint on agricultural development.[70] Conversely, from a conservation point of view, trypanosomosis is held to be a major factor favouring the survival of many of the remaining wildlife populations and their refuges in Africa.

The important role played by wildebeest (*Connochaetes* spp.) in the maintenance and seasonal shedding of alcelaphine herpesvirus 1, a cause of MCF in cattle, has been elucidated.[71, 80, 81] In eastern Africa transmission is most frequent during the wildebeest calving period when pastoral cattle, grazing on shared grassland, come into contact with wildebeest foetal membranes or young calves.[88] The economic impact of this disease on communities is not well defined but is probably limited given the pastoralists' knowledge of this disease. As a preventive measure, livestock are kept off pasture shared with calving wildebeest in Maasailand in eastern Africa until late morning by which time the sun (and time) has rendered infected placentas safe. In southern Africa the increase in wildebeest populations associated with game ranching activities has seen a resurgence of MCF and this is not always associated with direct contact with placentas or young wildebeest calves. The actual mechanism of transmission of infection in this latter situation has not yet been determined.[6]

With regards to certain African infectious tick-borne diseases, wildlife may fill the role of tick amplifiers or carriers of the causative agents. African buffalo, eland (*Taurotragus oryx*), sable antelope (*Hippotragus niger*) and impala (*Aepyceros melampus*) are, for example, the preferred hosts for all three stages in the life cycle of brown ear ticks (*Rhipicephalus appendiculatus* and *R. zambeziensis*).[74] Buffalo, eland and sable antelope have also been shown to be long-term carriers of several *Theileria* spp. parasites, some of which can cause disease in livestock or in certain wildlife hosts. The biology and epidemiology of Corridor disease caused by buffalo-adapted *Theileria parva* in cattle has been well studied. Infection with this organism, which is 'silent' in buffalo, causes high mortality in cattle, making farming of cattle in the presence of infected buffalo and a suitable vector a hazardous undertaking.[72] Cattle are generally 'dead-end' hosts, being unable to infect the tick vectors.

As a further example, giraffe (*Giraffa camelopardalis*) and black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros, African buffalo and warthog are preferred hosts for adult ticks of the genus *Amblyomma*, whereas the nymphs and larvae feed on a variety of large and small mammals, birds and even reptiles.[79] Several of these hosts, including buffalo, gallinaceous birds and tortoises have been shown to be potential carriers of *Ehrlichia (Cowdria) ruminantium*, the cause of heartwater in domestic ruminants.[75]

Diseases that may impact on wildlife It is appropriate that those diseases be considered which may seriously affect wildlife populations directly, or may undermine wildlife management efforts, and ultimately determine the rate of development of the wildlife industry and conservation initiatives in Africa. Diseases that constitute a livestock threat, such as FMD, ASF and theileriosis, are generally managed by the imposition of severe restrictions on wildlife use, translocations and certain management activities.

Foot-and-mouth disease containment measures in several African countries restrict the translocation of wild cloven-hoofed ungulates out of endemically infected areas, thus effectively isolating such populations into 'conservation islands'. South Africa also relies on delineated ASF and Corridor disease control areas to contain their spread, and legislation is in place, which limits the translocation of their natural wildlife hosts out of these areas.

On the other hand, there are certain diseases, such as RP, anthrax, rabies, bovine tuberculosis and canine distemper, which may have significant direct impacts on the population dynamics of certain wildlife species, on various spatial and temporal scales. Some of the agents causing such diseases appear to be historically alien to sub-Saharan Africa, and were probably introduced onto the African continent with the importation of domesticated animal species. Indigenous African free-ranging mammals (within similar taxonomic groupings to the traditional domesticated maintenance host) have little inherent genetic resistance to these foreign agents. Significant morbidity and mortality may therefore be encountered in wildlife where contact with infected domestic animals occurs.

A striking example of a disease with a devastating impact on populations of wildlife is RP, currently still present in Africa in southern Somalia and southern Sudan. The virus is reputed to have been introduced into Eritrea from India by the Italian army in 1887/88, or by a German military expedition that brought infected cattle from Aden and Bombay to the East African coast.[42, 44] Much has been written[63] on the grave social, political and economic repercussions of the massive cattle mortalities caused by this disease as it spread progressively westwards and southwards. Countless wild artiodactylids also perished, with buffalo, tragelaphids (spiral-horned antelope), wild suids and wildebeest being most severely affected, with only relic populations surviving in some areas.[93] The decimation of both cattle and wildlife apparently led to the disappearance of tsetse flies (*Glossina* spp.) from certain areas, and may have contributed to wildlife distribution anomalies, such as the formation of isolated meta-populations of species such as sable antelope and greater kudu.[89]

An interesting progression with regards to RP in wildlife during the twentieth century has been the recognition of various strains with apparent differing pathogenicity and host preferences. The strains that were most commonly associated with high mortality in cattle have been relatively easy to eradicate, but the apparently milder strains for cattle have persisted. Although mild in cattle, the strain currently circulating in East Africa, characterized as part of the lineage 2 group,[8] can be highly pathogenic in African buffalo, lesser kudu (*Tragelaphus imberbis*) and eland, but apparently mild in some other species such as wildebeest and warthog. However, host susceptibility appears not to be consistent, nor in fact is pathogenicity constant with any one strain, species or population of animals, with both temporal and spatial variation occurring. Erratic and low rates of virus shedding occur in cattle. Another complicating factor is the 'atypical' virology of some strains with antigenic epitopes poorly detected by standard test systems, which reduces the sensitivity of surveillance protocols.

The role of wildlife in the persistence of RP has become of interest in the last decade during the final phases of eradication of the virus, with the identification of strains that appear to spread efficiently even in partially immune populations. This may be the key reason for the persistence of the strain in East Africa despite massive vaccination campaigns on the continent between 1960 and 2000. Free-ranging wildlife and pastoral livestock cattle especially in times of drought, may be a key epidemiological factor. Most evidence, however, is that the wildlife is only acting as an indicator and vector of this disease, which is in fact maintained in cattle populations.

Bovine tuberculosis caused by *Mycobacterium bovis* is another foreign animal disease that was most probably introduced into Africa with imported dairy and *Bos taurus*-type beef cattle during the colonial era. This disease has now spread to, and become endemic in, several African buffalo populations in South Africa[10, 15, 28] and Uganda,[38, 107, 108] as well as in a Kafue lechwe (*Kobus leche kafuensis*) population in Zambia.[34] It has also been diagnosed in buffalo and wildebeest in the Serengeti National Park ecosystem in Tanzania.[69] Buffalo and lechwe have become true maintenance hosts of this disease, and sporadic 'spill-over' of infection has been documented in greater kudu,[13, 51, 102] chacma baboon (*Papio ursinus*),[53] lion (*Panthera leo*) and cheetah (*Acinonyx jubatus*),[56] warthog,[107, 108] eland, topi (*Damaliscus lunatus*), giraffe,[69] leopard (*Panthera pardus*), common genet (*Genetta genetta*), spotted hyaena (*Crocuta crocuta*), bushpig (*Potamochoerus larvatus*), and honey badger (*Mellivora capensis*).[52] The long-term effects of this chronic progressive disease on wildlife host populations at sustained high prevalence rates is unknown, but early indications are that it may negatively affect population structure and dynamics in buffalo[86] and lion. Bovine tuberculosis is probably one of the most serious wildlife health issues currently confronting conservationists and veterinary regulatory officials in South Africa, Zambia and Uganda, and possibly some other sub-Saharan countries.

Canine distemper is thought to be another foreign animal disease which entered Africa in the post-colonial era and has demonstrated the potential to impact severely on certain wild carnivore populations. Less robust populations of social carnivores, such as wild dog (*Lycaon pictus*) and Ethiopian wolves (*Canis simensis*) are especially vulnerable. This disease and rabies are considered to be partially responsible for the demise of the wild dog in the Serengeti ecosystem in Tanzania over the past decade.[2] Canine distemper has also demonstrated an ability to cross the 'species barrier' as severe outbreaks with mortality have been reported in Serengeti lions.[87]

Certain endemic multi-species diseases that occur on most continents may also have severe effects on wildlife in the sub-Saharan region.

Anthrax outbreaks have been documented in wildlife populations with no livestock link in the Etosha National Park in Namibia[62] and Kruger National Park in South Africa.[11, 27, 29, 30] Large-scale outbreaks may cross the interface especially where domesticated and non-domesticated species share range and resources, as has occurred in the Luangwa Valley National Park in Zambia,[26] and Serengeti National Park,[68] and more recently in Mago National Park in Ethiopia, where a die-off of many cattle and more than 1 500 lesser kudu was documented.[37, 92]

Rabies is another multi-species disease that is endemic in many areas of sub-Saharan Africa, with sporadic epidemic cycles. It has been diagnosed in 33 carnivorous species and 23 herbivorous species, with a regional variation of dominant epidemiological role-players.[95] In spite of this, by far the largest number of rabies cases reported in the developing world occur in domestic dogs, with 'spill-over' into other domestic livestock, humans and wildlife. Rabies and canine distemper are thought to be partially responsible for

the demise of the wild dog population in the Serengeti ecosystem[35] and is considered a major disease threat to the highly endangered Ethiopian wolf. In Africa, endemic rabies (caused by both viverrid and canid biotypes) is often associated with certain communal burrow-dwelling species, such as the yellow mongoose (*Cynictus penicillata*) and the bat-eared fox (*Otocyon megalotis*), as well as jackals (*Canis* spp.). Rabies outbreaks in certain host species frequently appear to be density dependent. In a unique documented outbreak of rabies in kudu, where non-bite transmission probably occurred as a result of salivary contamination of browse, more than 10 000 kudu died over a four-year period in Namibia.[7] Abnormally high population densities of kudu were cited as being a major contributing factor.

Fencing and wildlife Several of the diseases associated with wildlife that have major epidemic potential and therefore affect international trade in livestock and their products, are highly contagious viral infections. These diseases may have severe adverse effects on livestock-based export economies. It is particularly in this context that the wildlife/livestock interaction is critical. The most significant of these diseases are FMD, RP and ASF for which fencing has been a major tool for control by restriction of animal movement.

If wildlife carriers or vectors of these diseases are allowed to range freely in contact with livestock, creating a diffuse interface, then there is little possibility that exporters will be able to develop trade in live animals or animal products beyond their immediate region.

In certain countries in southern Africa, where there is a well-developed livestock production industry, the means of preventing the maintenance and spread of these diseases in livestock has been based on the separation of wildlife and livestock by strict land use policies and fencing at the interface. Botswana, for example, has a lucrative trade with Europe based on an early and clear understanding of the epidemiology and control measures necessary for containment of FMD.[31] As the economics of wildlife enterprises have proved to be more competitive in marginal, often semi-arid areas, than cattle ranching, there has been a transition of land use from livestock to multi-species or exclusive game management systems.[24] The fencing was already in place in many of these areas, and only game-proofing upgrades were necessary. Game fences are also popular with commercial wildlife ranchers due to the value of individual wild animals and their need to retain them on their properties. The fencing debate has become more contentious as the ecological impact of fencing has become better appreciated. Fences are frequently criticized because wildlife are precluded from following historically important migration routes, which are necessary for survival in ecologically marginal areas where surface water is scarce and grazing availability is seasonal. Detailed studies of this real or perceived problem are scarce and reports that exist on the environmental effects of fencing in Africa[78, 91] show that generalizations are unhelpful. In the Botswana study some fences were successful in the control of diseases such as FMD and CBPP, and were effective in reducing animal movement. The impact of these fences on wildlife was based on population trends, which did not show a consistent picture across all species, the implication being that some species benefit and others do not. In the end the environmental, social and financial costs of some fences may outweigh their benefits but the decision on this will depend on complex analyses, including environmental impact assessments. The recommendations of a study by Scott Wilson in Ngamiland, Botswana,[91] included the removal or realignment of some fences and the endorsement of others as effective and necessary.

There is also the realization amongst ecologists that island communities of wildlife are unlikely to survive intact in the long term.[73] In recent years, this has driven some conservationists to think in the direction of complex conservation systems, including transfrontier areas involving communal, private and public lands with corridors along which wildlife can move, and different land uses without necessarily excluding human activities.[9] This approach is intellectually appealing but highly theoretical, and does not take into account basic humanocentric social and economic realities, and it certainly has no consideration of disease-control implications. Nevertheless, the impact of fencing on migratory populations, such as wildebeest in southern Africa, has provided stark evidence of the deleterious effects certain policies can have on populations.[58, 59] The debate on appropriate conservation policies in Africa will go on and it is definitely not the intention here to criticize or promote any particular approach, but only to highlight animal disease aspects that need to be considered, whilst assessing risks and impact of policy change.

Decisions made by governments pertaining to livestock health are affected by the present global restrictions on trade as set out in the recommendations of the International Animal Health Code of the Office International des Epizooties (OIE). For certain diseases, this Code does support the concept of zonation of infected and non-infected areas, where appropriate control measures are in place at the interface. Thus, without a fencing policy, commercial livestock industries in unrestricted wildlife areas cannot develop in the conventional sense.

The concept of species compartmentalization is also currently being discussed at the OIE. This concept accepts that if an outbreak of a listed disease (all of the OIE list A, and some list B diseases) occurs in a free-ranging wildlife population, but does not involve domestic livestock, then the reporting country should not necessarily be penalized by agricultural trade restrictions. Some southern African countries have taken a vigorous approach to disease control and viable livestock industries have developed.

In eastern Africa the more *laissez-faire* attitude remains a constraint to commercial livestock development, and

governments are under increasing pressure to seek economic solutions to the increasing poverty. The issues of land use, disease control and fencing have not been resolved due to conflicting economic interests involving the expanding and lucrative tourism industry. Ecotourism, based on game viewing of free-ranging wildlife and environmental appreciation, certainly provides significant economic benefits to a region, but frequently, only to a small proportion of the community.[77] Profitable ecotourism and well-marketed environmental appreciation have also made a major contribution to the maintenance of the world heritage of animal life, and indigenous biodiversity. As a result there has been reluctance by governments to tackle the livestock problems, which they believe would impact on the wildlife estate, e.g. through fencing. Ironically it is the very desire to keep the systems open that results in the easy access by livestock to the grazing resources in protected areas, leading not only to intensification of the interface between livestock and wildlife, but also to conflicts between humans and animals, including predation and disease impacts. In practice, with rapidly growing human populations, fencing of wildlife-protected areas is inevitable and beneficial in many aspects. Fences also effectively reduce predation and depredation on livestock and crops of adjoining communities, which are often of a subsistence nature. Some negative ecological effects of fences may have to be accepted in order to maintain viable populations of key African wildlife species.

Transhumance, pastoralism and wildlife

The key constraint to achieving an improved animal health status in much of Africa that would be acceptable to the majority of livestock trading nations, is the current unrestricted trans-boundary movement of people, livestock and wildlife.[64]

The animal health code developed by the OIE,[76] designed to reduce disease risk and facilitate international trade in livestock and livestock products, has become complex and requires a high level of management and investment in veterinary services. Although this code has greatly enhanced the economic potential of the livestock industry, it has also set recommendations and standards with which many African countries cannot comply. Without the benefits of international trade, the value of the livestock sector to these national economies has not improved and this has deterred the governments concerned from maintaining investment in the industry.[96] In addition, as pastoralists are often politically marginalized,[90] there has been little incentive to shift resources in their direction.

In reviewing the wildlife disease impact it is important not to over-emphasize the role of non-domestic artiodactyls because, in pastoral systems, there are frequently adequate populations of free-ranging livestock to enable the maintenance of these infections in the absence of wildlife. Thus, wildlife populations are not always a critical factor, except where they are the only definitive maintenance host for a specific disease. The role of wildlife as reservoirs or vectors of disease are sometimes misunderstood and may be overstated, even among veterinarians.

Many pastoral communities are poorly supported by veterinary services, if at all, so control through vaccination of domestic animals is rarely possible. The issue is whether these free-ranging livestock and wildlife populations can be isolated from commercial ranching to enable a sub-sector of the livestock industry to function and trade. In eastern Africa, for example, without effective movement control, there is little likelihood of FMD ever being controlled (irrespective of wildlife). Nevertheless, wildlife does present a further complicating factor because, even if improved veterinary control such as vaccination were initiated in pastoral livestock, wildlife could still harbour pathogens and so maintain a reservoir of infection.

Pastoralists frequently have extensive ethno-botanical and ethno-veterinary knowledge. They understand many infectious disease processes and often treat animals successfully without sophisticated medicines.[21, 22, 106] Often simply by the ability to move stock rapidly over long distances, they avoid contact with diseased animals at appropriate times, but this can equally assist in spreading infection. This knowledge-base also exists at the wildlife/livestock interface, amply demonstrated in the case of MCF[88] by the response of livestock keepers in avoiding cattle contact with wildebeest particularly during the calving season.

Attempts to improve the delivery of veterinary services to pastoralists will continue in many countries, and a spin-off may be reduced disease challenge to wildlife. This challenge will, however, remain inherently difficult as delivery of expertise and veterinary vaccines and medicines is logistically problematic and economically non-sustainable unless livestock values and markets improve. Therefore, diseases such as FMD, ASF, and CBPP are likely to persist for the foreseeable future. It is only after zonation of land as practised in southern Africa, which identifies problem populations and regions, and the designation of these as disease control areas, that the resultant strict movement control and reduced contact between wildlife and livestock will improve the situation. It is historically interesting that modern human society has seen the decline of the hunter-gatherer communities and most probably will see the next transition, which will be the disappearance of true pastoralism.[58, 59]

Wildlife industry and conservation areas

The three categories of land use involving wildlife are essentially:

- national land set aside for conservation of wildlife, habitats and ecosystem processes;
- private land on which a variety of wildlife-based activities are conducted; and
- communal land with a wildlife-use element.

Formal nature conservation in Africa is only slightly more than 100 years old. The early conservation initiatives were within national programmes, with land set aside exclusively for nature conservation. In broad perspective, the first 50-odd years was a period of consolidation, during which recreational tourism was only a small consideration and a limited income earner. The second half of the twentieth century saw a rapid growth in the tourism industry in formal conservation areas, which coincided with the development of the first private lodges and private nature reserves. Much of this growth was based on foreign visitors and facilitated by improved travelling opportunities, and in both formal and private sectors, facilities and opportunities for tourists increased sharply towards the close of the century. National and international demands for wildlife conservation still continue but as human population growth continues, with increasing hunger for land, conflict arises. As a result there has been a recent trend towards community-based conservation initiatives.[9, 43] Biodiversity conservation with the preservation of local livelihoods[1] is an example of some of the recent thinking. This thinking has evolved as academics believe that previous attempts at conservation through national parks and protected areas have been ecologically inadequate or unable to contribute significantly to human development needs.

In the private sector, differences in land tenure policies between countries in sub-Saharan Africa and the right of private individuals to own land and wildlife has fundamentally affected the way in which wildlife is valued. In some southern African countries where private ownership of land and wildlife is enabled by the country's constitution, exploitation of wildlife resources is gaining momentum and becoming a major economic activity. As a result, borders between enterprises involved in ecotourism and farmland, as well as publicly owned wildlife areas and traditional communal areas, are becoming increasingly sharply demarcated.

East Africa is quite different because the land use system is much less developed, there are fewer fences and considerable mixing of people, livestock and wildlife. The main wildlife use is ecotourism, which is based on a complex of public and private conservation areas, as well as subsistence and some commercial hunting.

In West and Central Africa the situation is more complex with, on paper at least, a multitude of parks as well as hunting reserves. However, non-sustainable exploitation has resulted in a massive decline in numbers of wildlife in these conservation areas. The result is that, unless this trend can be reversed in these regions of Africa, the remaining wildlife will be isolated in small meta-populations sequestered in reserves.

The disease problems associated with the varying approaches to wildlife land use are also different. In the more intensively managed systems, e.g. game farms where animals are in pens or small camps, and are fed artificially throughout the year, the production-related diseases, such as foot rot, verminoses, coccidiosis, and nutrition-related problems, are more prevalent. On small game ranches, where 'free-range' wildlife are often fed supplementary hay in the dry season and during droughts, a common problem that is encountered is relative overstocking, which results in the so-called sick habitat syndrome. This is a multifactorial syndrome related to progressive depletion of palatable grasses and browse, bush encroachment, soil erosion, a progressive build-up of ecto- and endoparasites, and stress and tick toxicosis-related immuno-suppression. The net result is a loss of body condition, anaemia, tick-bite abscesses and mortalities that may be associated with normally benign agents. The relative overstocking due to the inability of the game to move out of the area, also favours the spread of density-dependent infectious diseases.

More extensive systems, such as national parks, game reserves and game conservancies, may vary in size and may be fenced or open. The more natural the ecological system, the less likely that a single disease entity will be problematic, since the relationships between hosts and indigenous parasites should be relatively balanced.[33] In these systems it is predominantly cyclical climatic factors and related animal population densities which lead to expression of disease, for example during and after severe droughts. Under these circumstances, with the clumping of large concentrations of herbivores around dwindling resources, and concomitant nutritional and social stress and immuno-suppression, parasite burdens may increase to pathological levels. Viral and bacterial diseases are also frequently associated with these environmental stress factors, e.g. anthrax, epidemic FMD and possibly RP.[16, 27] However, truly natural ecosystems (defined as having minimal human interference) are now rare, and a natural state in respect of disease ecology is probably the exception.[23]

Looking to the future, disease problems at the wildlife/livestock interface are likely to increase and possibly intensify in some regions of sub-Saharan Africa, as many are related to more recent conservation developments, such as the new community-based wildlife initiatives, large-scale translocation of key wildlife species, enlarging conservancies and transfrontier parks.[36] With regards to transfrontier conservation areas (TFCAs), it is not the intention here to portray these initiatives in a negative light. From an environmental conservation, biodiversity, heterogeneity and ecotourism point of view, the integration of land across international borders as well as the consolidation of state and privately owned land in joint ventures is justifiable, and may have major economic benefits. The message that needs to be conveyed, however, is that all involved parties and role players must approach these initiatives fully aware and forewarned of the potential animal health implications and challenges that may be expected when increasing the geographic range of various pathogens. The possible implications for animal disease control are profound and the potential impact on the economies of the region through loss of life-sustaining livestock and/or trade should be calculated. It is totally predictable that without international boundary

barrier fences and with a 'biological bridge' formed by contiguous wildlife populations, any infectious disease present in any one of the participating countries or areas will eventually spread throughout the entire conservation area unless containment or control measures are established. For example, in the recently created Great Limpopo Transfrontier Park, which incorporates conservation areas in Mozambique (Gaza National Park), South Africa (Kruger National Park) and Zimbabwe (Gonarezhou National Park), and which will eventually constitute one of the largest conservation areas in the world, there are animal disease risks to all three regional participants. Once international barriers have been removed and contiguous wildlife populations have become established, bovine tuberculosis may spread northwards and eastwards from the infected Kruger National Park component, tsetse flies (*Glossina morsitans*) may recolonize the Kruger Park from the north, rabies may enter this park from the north and north-east, new topotypes of FMD virus from Gonarezhou National Park may enter the other two parks, and cattle-adapted *Theileria parva* may move southwards and westwards. With this scenario, proactive disease management strategies need to be discussed at high level by participating countries and agencies.

There is also a trend to create larger ecologically sustainable conservation areas (conservancies) by the private sector. In marginal cattle farming areas, well-managed ecotourism based on wildlife is potentially more profitable than livestock farming. Farmers in these areas are increasingly abandoning traditional cattle ranching for commercial wildlife-based activities, pooling their resources by creating jointly owned reserves enclosed by substantial perimeter fences and with internal fences removed.[4] These conservancies may be very large, and several in South Africa and Zimbabwe exceed 100 000 hectares.[94] This does not, however, preclude the keeping of wildlife and livestock together, which is often done, but currently accepted 'wisdom' has it that productive livestock farming and ecotourism centred on wildlife are incompatible on the same land area as a result of the higher risks of disease and for aesthetic reasons.[30, 77]

This sentiment is probably true for much of East, Central and West Africa, as well as parts of the eastern lowlands of southern Africa where many of the wildlife-associated livestock diseases are endemic. The development of these large conservancies sometimes conflicts with the policies of developing countries, which place a premium on agricultural and industrial development, and has led to the criticism that wildlife is held in higher regard than people.[98]

These ecotourism-based enterprises generally require a wide diversity of game species and adequate populations of them, for easy visitor viewing and frequently necessitate large-scale translocation of wildlife, which in itself may have inherent disease risks. Translocation of any animal is, in fact, the translocation of a 'biological package' consisting of the host together with its attendant macro- and micro-parasites. Furthermore, it is important to realize that once released into a free-ranging system, such animals and their attendant parasites are difficult if not impossible to retrieve. Thus strict quarantine, disease-screening, and ecto- and endoparasite control are essential requirements for the responsible translocation of wildlife.[109] Disease hazard identification in founder populations, followed by risk assessment and risk management strategies, form the backbone of regulatory disease control for wildlife translocations on a national and international level. Endemic diseases and parasites in the ecosystem at the destination of translocated animals may also influence the success of these exercises, and should be taken into consideration.

A current major issue is how to enable increasing commercial exploitation of wildlife resources while at the same time ensuring that the local communities do not become dispossessed. It is a difficult balancing act that is being tilted by Africa's rapidly growing human populations. The Camp Fire Programme in Zimbabwe[67] and related community-based conservation initiatives elsewhere have achieved some limited success in enabling local communities to participate and share in the benefits of ecotourism and commercial hunting. Nevertheless, there is no doubt that more progress on this front needs to be made if the conflicting demands of wildlife conservation, commercial agriculture and deprived rural communities are to be reconciled.[46]

Disease surveillance and control at the interface

The responsibility for disease surveillance generally rests with the veterinary regulatory authorities of a given country, and the surveillance techniques used include passive reporting, farm inspections, problem investigations, abattoir surveys, serological surveys, and dedicated testing for specific disease eradication schemes. Unfortunately, legal frameworks and responsibilities (including financial responsibilities) for wildlife disease surveillance, investigation and reporting are not clearly defined in many African countries and free-ranging pastoral cattle and wildlife do not easily lend themselves to monitoring.

Given the depressed state of veterinary services in many countries in Africa, it is difficult to envisage any systematic collection of surveillance data from livestock in remote areas. The best hope for these areas is community-based approaches to monitoring animal health.[47] On the wildlife side, a growing interest by wildlife managers in wildlife diseases and their potential impact, is improving information flow and should be integrated into conventional epidemiological reporting systems.

The need to improve the control of animal diseases at the interface is a complex evolving issue. Control strategies will increasingly have to accommodate the conflicting requirements of a variety of land users and interest groups, and adaptive policies coupled to pro-active thinking and appropriate planning are necessary to pre-empt the development of serious animal disease events.[100] Innovative possibilities should be examined. For instance, following the repeated failures to develop an effective ASF vaccine, would it not be

more advantageous and appropriate for developing countries that do not export pork to embark on a programme of selection and breeding of pigs that are genetically resistant to ASF in order to address this disease problem in subsistence rural communities? Another innovative strategy is to select or breed wildlife that are free of certain diseases, and to use them for stocking or translocation exercises. The breeding of African buffalo free of FMD, theileriosis, bovine tuberculosis and brucellosis, for example, is becoming an ecological success and a profitable enterprise in southern Africa.[12]

That serious disease events are still occurring is amply demonstrated by the current problems associated with FMD, MCF and bovine tuberculosis control in southern Africa,[3, 28] and the issues of RP (see above), PPR and ASF in eastern and Central Africa.[60]

When dealing with the threat of certain endemic African diseases, such as FMD, ASF and buffalo-derived theileriosis, the containment option has given best results to date. This is usually effected by means of control zones/areas, game-proof fences, physical barriers, cordons and movement controls which effectively separate the wildlife from domestic livestock, thus preventing contact at the interface, and is characteristic of countries with an advanced land-use policy. It is not an option in regions where pastoralism with free-ranging livestock and subsistence agriculture are the norm.

When dealing with endemic arthropod-borne infections, such as trypanosomosis, African horse sickness (AHS), RVF and bluetongue, containment is less likely to succeed, and vaccination and vector control, where possible, may be included to reduce infection and/or transmission.

With regards to alien/exotic diseases that threaten free-ranging wildlife populations, such as RP, canine distemper and bovine tuberculosis, containment and control can best be effected by addressing the disease in the domestic host by mass vaccination and/or test and slaughter respectively. In addition, prevention of contact between infected domestic animals and wildlife is desirable, but unfortunately not always feasible in many countries in sub-Saharan Africa. In these situations, improving the delivery of animal health services (e.g. through vaccination) to domestic livestock sharing ranges with wildlife and/or adjacent to conservation areas would probably be most beneficial if a suitable vaccine is available. Rinderpest control has been based on vaccination and the benefits of eradication are economically significant even without calculating the benefits to wildlife populations. There is the added benefit that resident wild animals may also act as sentinels, providing spatial and temporal (based on age stratification) data to facilitate disease surveillance and identify existing and new foci of infection. Where an alien disease has become established in a conservation area, the situation is serious and control options are limited, and frequently contentious or unpopular.[10] The bovine tuberculosis situation in buffalo in South Africa and Uganda, and lechwe in Zambia are examples.

In general, control of diseases of limited epidemic potential is best addressed at the local level, with the assistance of veterinarians and wildlife experts with detailed knowledge of epidemiological and ecological determinants. For infections capable of causing trans-boundary epidemics, control needs to be co-ordinated at both national and international levels.

While short- and medium-term disease control and long-term eradication goals can be, and have been, attained in domestic livestock using judicious vaccination programmes, vector control and test-and-slaughter policies, these options and techniques are frequently impractical, difficult to execute, and may be culturally or morally unacceptable in free-ranging pastoral livestock and indigenous wildlife populations.

With regard to the multi-species diseases, such as anthrax and rabies, many ecologists will argue that these diseases are also endemic and part of the greater ecosystem, and because they function as natural population regulators, they should not be controlled. However, both these diseases also affect domestic animals, and have significant zoonotic potential. There are now few if any ecosystems that are isolated from humans and domestic animals, and modern societies will no longer accept the impact of disease as natural. This renders the *laissez-faire* argument redundant. For these reasons, control of anthrax outbreaks in wildlife have been attempted, using various techniques including burning/burying of carcasses, strategic veld burning, waterhole disinfection and remote vaccination by means of disposable darts or bio-bullets. These attempts have met with varying success. On the other hand, mass vaccination of foxes against rabies in Europe, using oral bait techniques, has been highly effective and successful.[20] Central to the reduction of livestock losses and human exposures during anthrax or rabies outbreaks in wildlife, are large-scale public awareness campaigns and mass vaccination of livestock.

In the case of multi-species production systems, where livestock and wildlife are farmed together, certain precautionary measures may reduce the possibility of significant disease events occurring; these include:

- avoiding introduction of certain key wildlife species, which are maintenance hosts or 'carriers' of certain indigenous diseases, e.g. FMD, theileriosis, MCF, AHS and ASF; and
- screening all cattle for bovine tuberculosis and brucellosis prior to introduction onto a ranch.

Some positive aspects of such a mixed farming enterprise should also be highlighted; these include:

- the 'vacuum cleaner' effect created by the regular dipping of the cattle component in order to reduce environmental tick burdens;
- the reduction of parasitic ova and larvae when ingested by non-patent species; and
- an appropriate combination of cattle and wild ungulates for a particular veld-type will ensure that a wider diversity of natural plant species will be consumed, which

will optimize the utilization of different strata of the vegetation from ground level to the canopy. This may effectively increase productivity for the ranch.

Conclusion

The long-running conflict between livestock owners and animal health authorities, on one hand, and wildlife conservationists on the other, is largely based on differing attitudes to controlling diseases of livestock associated with wildlife. An attempt is made in this chapter to highlight the key disease issues and to emphasize that these disease problems are frequently bi-directional at the interface. A new dimension currently faced by veterinary regulatory authorities is the spectre of new sylvatic foci of diseases, such as bovine tuberculosis, brucellosis and possibly RP, that threaten to derail national and international eradication schemes which have been implemented and executed with significant success and at great cost.

Conversely, wildlife-based ecotourism in Africa has expanded rapidly over the past few decades, and is the source of much-needed foreign revenue for many African countries. Traditional subsistence farming is still the largest source of dietary sustenance on the continent, and this together with the growth and hunger of historically disadvantaged communities for land, is forcing enterprises and communities with markedly different objectives and land use practices and priorities to operate effectively in close proximity. In sub-Saharan Africa the interface between wildlife and livestock is declining overall as a result of progressive decimation of wild animal populations. Nevertheless, in certain areas there is an intensification of the interface, as shared resources become scarce, and the development of wildlife and livestock enterprises increase the shared use of land. In southern Africa, over recent years, the trend has been contrary to the rest of Africa, with an increase in use of land for wildlife or mixed enterprises. The creation of large wildlife land-holdings (e.g. transfrontier parks and conservancies) accompanied by the introduction of species such as African buffalo, wild suids and wildebeest into certain areas, may have a negative impact on the regional livestock trade as a result of disease risk factors. With the diversion of finance from natural and animal resource sectors to human welfare, health and education (a trend in Africa over recent decades), the risk of losing control of animal diseases is increasing, and this may compound the issue. The increased use of land for mixed wildlife/livestock systems in areas previously subdivided by fences may also increase levels of epidemic disease. The question is whether countries can afford to ignore this problem, however ideal and attractive the theories.

If poverty alleviation is a goal and a necessary step to ensure conservation of resources, a pragmatic approach to the economics of land use may in fact be more important to conservation than recreating 'Eden'. It should also be kept in mind that until relatively recently, many of the developments in wildlife conservation and utilization were initiated and controlled by communities of European origin, and that these may not be sustained with the changing social and political status of countries concerned. Equally, there appears to be an increasing realization among world leaders that globalization is perpetuating the dominance of the developed world and that there is a genuine desire to address the issues of poverty in Africa. This may lead to changes, which will facilitate trade of livestock and their products.

Whatever the attempts at global solutions, the changing land-use patterns in many areas of Africa will require ongoing and focused research to further elucidate the epidemiological determinants of many diseases at the wildlife/livestock interface, in order to develop innovative control strategies that do not limit land use options of communities or a country's ability to market animals and animal products profitably.

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11

Vaccination: An approach to the control of infectious diseases

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Introduction

It must have been observed for centuries that people exposed to a pathogen were often resistant to reinfection by the same or a related pathogen, but the first record of anyone acting on this observation is that of the English physician Edward Jenner.[52] On 14 May 1796 Jenner inoculated James Phipps, a healthy eight-year-old boy who had never been exposed to smallpox, with cowpox virus, and challenged him 48 days later with a virulent dose of smallpox virus. Phipps did not react, and with this momentous medical discovery was born the era of immunology and vaccinology. Since then smallpox has been eradicated by vaccination, and human and animal losses and deaths due to other infectious and parasitic diseases have been dramatically reduced by the development of a range of vaccines. Even so both animals and humans continue to suffer from many common diseases, as well as new and emerging diseases. Among many reasons for this are:

- sub-optimal management conditions;
- exposure to pathogens at a young age before vaccination is recommended;
- unusual or unexpected patterns of the pathogenesis of certain organisms;
- the emergence of new forms of old diseases;
- the emergence of totally new pathogens and diseases (e.g. Nipah virus); and
- the relative ineffectiveness of some existing vaccines.

Despite the current shortcomings of vaccination it seems likely that it is, and will continue to be, a more durable and cost-effective method of combatting bacterial and viral infections than all other therapeutic and prophylactic methods combined. Micro-organisms have survived for millions, even billions, of years in the face of a continuously changing chemical environment, and they are only going to be briefly inconvenienced by the human introduction of new chemicals into their surroundings. The same is true for arthropod vectors of disease. These facts rule out any long-term disease containment strategy based solely on the use of pharmaceuticals, acaricides or insecticides, however successful these may be in the short term. The development of new vaccines is the only long-term strategy that can be foreseen at present for the control of diseases of animals or humans. Mammalian immunological defence mechanisms against infectious agents have also evolved over millions of years, and must therefore be high up on any arbitrary scale of optimality. Finding new ways to specifically stimulate the immune system has to be the most likely way to achieve the lasting control of infectious disease.

Vaccines licensed for use in humans and animals at present are primarily either killed vaccines or attenuated vaccines, produced by one of two conventional methods. In the case of killed vaccines the pathogen is grown in large quantities and then inactivated by some method that prevents its replication but does not significantly alter the antigenic properties. Attenuated vaccines employ a live mutated agent that has been selected *in vitro* for reduced pathogenicity but that is still able to replicate in the animal. Each of these conventional vaccine types has inherent advantages and disadvantages, and there is a need to find new ways to make safer, cheaper and more effective vaccines. Advances in genomics, biotechnology, immunology, and understanding of the pathogenesis of diseases have resulted in the development of many new types of vaccines, such as subunit vaccines, live genetically modified vaccines, and nucleic acid (or 'genetic') vaccines.

Principles of vaccination

The primary mammalian immune response to an invading agent, involving the clonal expansion of small populations of virgin T and B cells, typically takes two weeks to develop, and this is often too slow to prevent serious disease occurring. The

secondary response to any subsequent reintroduction of the same agent, however, involves the stimulation of a population of specific memory cells that were produced during the primary response. This is a larger population than that of the original virgin cells, so the secondary response is faster than the primary response, typically being less than one week.[17, 45, 57, 66] The secondary response will usually clear the pathogen before overt disease occurs. Successful vaccination depends upon exploiting this mechanism by specific stimulation of the primary response with an agent inherently unable to cause disease. During any subsequent exposure to a virulent form of the pathogen the secondary immune response prevents the occurrence of disease. This seems to be a simple concept, but mammalian immune systems involve inordinately complex cascades of molecular signals and cellular responses. For effective immunization we need to know which antigens elicit the appropriate specific protective response, and how best to deliver those antigens, for example, administration of vaccines via the most commonly used routes, either intramuscular or subcutaneous, may induce good systemic immunity but may not induce mucosal immunity, thus failing to protect at the site of entry of many pathogens.[51, 72, 73] If the virulence factor of a bacterium is an extracellular toxin, it will be inappropriate to use whole cell bacterins to protect against this particular infection. Until recently few of the infectious agents encountered in veterinary medicine were characterized at the molecular level, nor had the host immune responses to them been well characterized. The molecular genetic advances made in the last decade have begun to produce significant changes to this situation, and we may expect that the next decade will see many important advances in vaccinology.

Conventional vaccines

The majority of vaccines used today in veterinary medicine are either live attenuated or killed vaccines, produced according to principles first developed by Jenner and Pasteur over 200 and 100 years ago, respectively.[104] Both of these types of vaccines have been effective in reducing or eliminating clinical disease following exposure to virulent field strains of various pathogens. Equally important has been their role in curtailing the spread of pathogens to other animals by reducing the quantity of pathogen shed into the environment. These effects result in what is known as 'herd immunity' to a pathogen[116] and if the transmission is reduced sufficiently the pathogen is eventually eliminated from the herd.[28]

Live attenuated vaccines

Live attenuated vaccines are based on pathogens which have mutated sufficiently not to cause disease, and the two most important considerations are whether the level of attenuation is sufficient to make the vaccine safe and whether the potential exists for reversion to virulence. Attenuation has conventionally been achieved by passaging the agent *in vitro*, either in the presence of mutagens or under specific culture conditions, and then selecting variants that show reduced virulence but which still induce a protective immune response. Over-attenuation may result in such limited replication that the immune response to a virulent challenge is not protective, and under-attenuation will result in the vaccine itself causing clinical disease. Achieving the required fine balance between over- and under-attenuation is, however, purely empirical and there are many practical problems which make this conventional approach both tedious and expensive. Each mutant must be tested *in vivo* under a wide range of circumstances to ensure that it cannot cause disease, while still stimulating effective protection against virulent challenge. Special factors may have to be taken into account, for example, some animals may react adversely to vaccination if they are immunosuppressed due to stress, or pregnant animals may be induced to abort or foetal defects may develop when animals are vaccinated particularly during the first trimester of pregnancy with, for example, the attenuated Rift Valley fever and bluetongue vaccines (**see Chapter 95: Rift Valley fever and Chapter 102: Bluetongue**).[100] Most important, however, is the fact that in the past there has been no information about which genes have been mutated and there has long been a fear that the mutated genes could back-mutate, or recombine in the field with virulent alleles, and cause the pathogen to revert to virulence.[3, 74] Recent advances in the genetic analysis of attenuated vaccine agents have shown that these fears were well founded.[65, 77]

Disadvantages of live vaccines are the possible presence of contaminating extraneous viruses in vaccines grown in cell culture, for example bovine viral diarrhoea (BVD) into vaccines grown in bovine cells,[56, 103, 114] that must replicate the vaccine candidate in order to induce a protective response, and that the presence of passive antibody, such as that from colostrum, will limit this replication. In many instances the level of passive antibody sufficient to interfere with vaccination is below that which will prevent infection with wild types of the pathogen.[57] In such cases vaccination can only be performed after passive antibody has decreased to a predetermined level, which may leave young animals vulnerable to disease for long periods. It may also lead to many animals becoming susceptible at once, at the same time that they are exposed to multiple diseases simultaneously. Animals are therefore often vaccinated with multiple vaccines at the same time, but this provides the opportunity for interference to occur among the responses to different components of the cocktail.[39, 47, 92]

Live vaccines also require appropriate storage conditions between manufacturing and administration, and the maintenance of a cold chain is often a critical factor. This is a serious difficulty in remote areas, particularly in rural Africa, although it is a problem which also affects inactivated vaccines.

Despite all these caveats there are some real advantages to attenuated vaccines as compared to killed vaccines. The live agent replicates in the host, so much less material has to be incorporated into each dose of vaccine than is the case

for inactivated vaccines, making attenuated vaccines less expensive to produce. The immunity engendered by live vaccines is likely to be similar to that induced by natural infection, and of a longer duration, and broader, than that induced by a killed vaccine. This is especially true if the live vaccine is delivered via the mucosal route, which is the natural mode of infection for more than 90 per cent of all pathogens. The resulting mucosal immunity provides better protection at the likely site of entry of the pathogen, and should reduce shedding of the pathogen into the environment. This results in better herd immunity than that provided by killed vaccines, which do not generally induce mucosal immunity.

Inactivated vaccines

Inactivated, or killed, vaccines are produced by inactivating the infectious agent, so that it cannot replicate in the host, while still retaining the immunogenicity of the specific protection-inducing proteins. Most inactivating agents do, however, have an impact on protein immunogenicity and a balance must be sought between inactivation and reduction in immunogenicity.[31, 34] Outbreaks of disease have occurred as a consequence of incomplete inactivation, a problem especially with pathogens that aggregate and thus limit the penetration of the inactivating agent.[20] Better inactivating agents are being developed, but testing the level of inactivation during the quality assurance phase is still vital. Even testing, however, would not have prevented the introduction of scrapie into sheep following immunization with an inactivated looping ill vaccine.[44] Obviously one cannot test for extraneous agents unless one has some information about what might be present.

A problem with inactivated vaccines, especially those produced from bacteria, is that some protective antigens are either not produced *in vitro* at all, or require special culture conditions for their expression. For example, some iron-regulated outer membrane proteins of *Mannheimia* (*Pasteurella*) *haemolytica*, known to stimulate protection *in vivo*, are only produced under restricted iron growth conditions.[26, 27] Bacterial preparations may also lack sufficient concentrations of proteins which are secreted into the growth medium rather than being retained inside the bacterial cells.[95] An example of new ways of overcoming this deficiency will be dealt with under 'subunit vaccines' below.

The main disadvantage of inactivated vaccines is that they are relatively ineffective at generating protective immunity, particularly against intracellular bacteria and protozoa. This deficiency may be mitigated by the use of strong adjuvants[6] (see further on), but in some cases it is the result of fundamental differences between the immune responses against live and killed organisms. For example, vaccination with heat-killed *Listeria monocytogenes* is not protective because it stimulates the production of memory T cells which fail to develop into effector CD8 T cells.[61] This type of problem will be addressed by advances in the understanding of how antigenic epitopes bind to MHC receptors, and the development of algorithms which can predict potentially protective peptide epitopes.[102]

Modern vaccines

Fundamental changes in biology are being brought about by the era of genomic data, and this is bound to impact powerfully on vaccine development. Currently in the public domain are 860 complete viral genomes, and 65 complete and 264 ongoing bacterial genomes. Volumes of genetic data such as these were unimaginable as recently as when the first edition of this book was published, and we will give just one example of the sort of work which is being facilitated by these changes. Bacillus Calmette-Guérin (BCG) is a live attenuated mixture of *Mycobacterium bovis* strains, originally developed at the Institut Pasteur in Lille, around the turn of the twentieth century, by Albert Calmette and Camille Guerin. After extensive testing, beginning in Paris in 1919, Calmette began to use BCG as a vaccine against tuberculosis in France in 1921[22] and it has been, and still is, used for this purpose around the world. The effectiveness of BCG in any area is roughly inversely proportional to the distance of that area from the equator, and this is thought to be because in the tropics there are widespread infections with other, relatively benign, mycobacteria. It is surmised that these generate enough cross-immunity in the exposed human population that BCG does not develop sufficiently to stimulate protective immunity against tuberculosis. In order to unravel the molecular genetic fundamentals of this problem a comparative DNA micro-array expression analysis was performed on 13 strains of BCG, and on virulent strains of *M. bovis* and *M. tuberculosis*.[11] Subsequent genomic sequencing revealed that 91 open reading frames (probably genes) of virulent *M. tuberculosis* were absent from one or more virulent strains of *M. bovis*, and an additional 38 open reading frames that were present in virulent strains of *M. bovis* were absent from some or all of the 13 strains of BCG. This establishes a firm molecular genetic base from which to begin to analyse the reasons for the variable effectiveness of BCG vaccination, and to design better vaccine strains, in a way that Calmette and Guerin would not have begun to conceive.[9, 10]

Subunit vaccines

Subunit vaccines are those which contain one or more pure or semi-pure antigens, and they can only be developed on the basis of an understanding of the mechanisms of pathogenesis and a molecular characterization of the gene products which stimulate protective immunity. Most of the gene products in any pathogen are irrelevant to the induction of protective immunity, and others may be immunosuppressive or even enhance the disease process, so the critical components of the pathogen's complete translation repertoire must be identified.

Subunit vaccines can either be produced by *in vitro*

expression from DNA recombinant constructs or by purifying a specific component from a conventionally produced vaccine, and the products may be used alone as vaccines or may be used to 'spike' conventional vaccines to increase the concentration of a particular protective component. For example, an extracellular leukotoxin of *M. haemolytica* is involved in pathogenesis and causes tissue damage in the lungs of cattle,[95] and immunity to this toxin is critical for inducing protection against the organism. It is possible to isolate and purify the leukotoxin from conventional cultures for incorporation into vaccines, but it is more economical to prepare the leukotoxin by recombinant biotechnology.[47] Subunit vaccines are safer than whole killed vaccines, and may be more effective. There is reduced antigenic competition between the constituents, one may target the vaccine to the site where immunity is required, and there is the potential for differentiating vaccinated from infected animals (**see Marker vaccines, below**).

A novel application of subunit vaccines has been their use to reduce disease transmission by targeting the vaccine against the arthropod vector.[79, 115] When animals are immunized against a specific glycoprotein present in the midgut of ticks, the antibody interacts with the protein in the tick midgut when the tick takes a blood meal. This interferes with the digestive process, thereby reducing both reproductive capacity and the viability of the ticks and therefore disease transmission.

Yeasts, mammalian, plant, and insect cells have all been used for the production of subunit vaccines[13, 69, 70, 105] and of particular interest currently is the possibility that genetic modifications can be made to food plants that can then be fed directly to animals for the induction of protective immunity.[4] Although theoretically attractive much work will need to be done before such a procedure can be demonstrated to be biologically safe and effective.

Live vaccines

The conventional method of producing live attenuated vaccine mutants had no molecular genetic basis. That situation has now completely changed, and it is now possible to pinpoint which genes are involved in virulence and stimulation of protective immunity. The degree of attenuation can therefore be controlled by deleting or mutating the appropriate genes.[33, 35, 80, 84] The deletion of an entire gene makes it unlikely that the pathogen can revert to virulence, although recombination could theoretically occur with a virulent wild strain in the field. If an essential gene is deleted or inactivated from a virus for example, it is necessary to complement the gene function *in vitro* to allow the vaccine to be grown to high titres. After introduction of the vaccine into an animal an infection is initiated and protection is stimulated as the immune system sees almost the full complement of viral proteins. The lack of a replication cycle, however, means that the virus is not shed into the environment.[24, 33, 35, 81] A similar effect can be achieved for bacterial vaccines by modifying the organism to require a specific concentration of a compound to allow it to replicate.[30] This compound can either be introduced with the vaccine or fed to the vaccinated animals to allow the bacterium to replicate for a short time. They will thus be able to stimulate immunity but will not be viable when excreted into the environment.

Genetically engineered vaccines can also be used as vectors of genes expressing protective antigens from other pathogens. One of the first vectored vaccines to be tested was recombinant vaccinia virus expressing rinderpest genes[118] and further developments have led to the development of a recombinant vaccinia virus vaccine that expresses both the fusion and haemagglutinin genes of rinderpest virus under the control of strong synthetic vaccinia virus promoters.[111] This vaccine provides cattle with long-term sterilizing immunity against rinderpest, it is safe, heat-stable, inexpensive, and easily administered, and it allows serological differentiation between vaccinated and naturally infected animals. Another vaccinia-based vaccine, containing the gene which expresses the immunizing glycoprotein of rabies virus, has been incorporated into baits and distributed in areas of Europe where the fox population serves as a reservoir for rabies.[15, 19, 82, 83] The use of this vaccine has resulted in the reduction or elimination of wildlife rabies from large areas of Europe. A number of recombinant vaccines based on avian pox viruses are also being tested or marketed for use in mammals.[91] Since avian pox viruses cannot replicate in mammals these vaccines deliver the required antigens while undergoing an abortive infection cycle *in vivo*. A comprehensive demonstration of the immunostimulatory effectiveness of such vaccines comes from a recent study with an experimental HIV vaccine consisting of a canarypox vector containing *Env*, *Gag*, and *Pro* genes of HIV, which was used in combination with booster injections of recombinant HIV gp120 subunit protein resulted in neutralizing antibodies in 91 per cent of subjects and CD8(+) T cell responses in 62 per cent of subjects.[2]

Another live vaccine vector technique which is being developed is the use of attenuated suicide strains of intracellular bacteria carrying recombinant eukaryotic expression plasmids, or naked DNA vaccine vectors, into host cells. The technique has been most highly studied using *Listeria monocytogenes*, and the vector bacteria have been shown to be lysed after entry into host macrophages and to release the recombinant plasmids.[38, 98, 113] This is an alternative way of delivering DNA vaccine constructs (**see Nucleic acid immunization, below**).

Nucleic acid immunization

Nucleic acid immunization is the title recommended by the World Health Organization for a procedure which has also been called third generation vaccination, genetic immunization, naked DNA immunization, and polynucleotide immunization. The basis of this approach is to vaccinate with recombinant expression plasmids, containing protection stimulating genes, directly into host cells.[29, 50, 117] Various

delivery methods have been used to introduce these plasmids into cells, including injection, particle bombardment ('gene gun') and direct application to mucosal surfaces, all of which result in transfection of cells and expression of the foreign protein, which then induces an immune response.

There are many inherent advantages to nucleic acid vaccines. As a result of the endogenous production of the protein the immune response resembles that observed following a natural viral infection. Typical responses have been shown to include both cellular and humoral immunity, production of memory cells, and protective immunity of long duration.[89, 90] The DNA plasmids naturally contain specific CpG sequences and this helps to create a cytokine microenvironment which enhances the immune response against the novel protein.[25, 54, 59, 60, 99] By delivering the plasmids to mucosal surfaces, it is possible to induce mucosal immune responses and immunological memory.[5, 36, 58, 64] Since a number of plasmids can be introduced simultaneously it should be possible to immunize animals against a variety of diseases at once without the concern of interference that is often observed with conventional vaccines.[18, 47] Also of importance is the fact that any essential post-translational modification of the immunizing protein which may be required to induce specific immunity will occur naturally.

DNA vaccines can induce immunity in neonatal animals, even if they have maternal antibodies to the immunizing agent,[16] and it is even possible to immunize foetuses.[40, 41] If animals were vaccinated at birth they would first be protected by passive antibody acquired from the mother, and as this protection wanes active immunity would develop as a result of vaccination. This will ensure that there is no 'window of opportunity' for the pathogen to establish itself.[33, 107]

Even with these many advantages no nucleic acid vaccines are licensed for any animal species and the main reason for this is the inability to deliver the vaccines effectively. DNA vaccination is very inefficient at present, with as much as 95 per cent of the plasmid being degraded before it enters the cells, and there is unlikely to be any major commercial uptake unless better delivery systems are developed to target the DNA into the nucleus of antigen-presenting cells.

One improved delivery system that is being investigated involves an RNA replicon vector derived from an attenuated strain of Venezuelan equine encephalitis (VEE) virus and packaged into VEE-like particles. The genes of interest were expressed from recombinant replicon RNAs that also encoded the replicase function and were capable of efficient intracellular self-amplification.[46, 49, 85–87] Recent developments in replicon technology, based on the fact that the genes can be delivered in an alphavirus coat, ensures that well over 90 per cent of the plasmids enter cells, and the gene is not only protected by an artificial viral coat/envelope, but the envelope can be engineered in such a way that it targets the antigen-presenting (dendritic) cells.

If the problems of delivery of nucleic acid vaccines are overcome there will still be regulatory issues as potential barriers to the adoption of this technology.[97] There is concern over the use of antibiotic-resistant markers in plasmid selection, but since other selection markers could be used this problem should be easy to overcome. Of greater concern is the possibility of integration of plasmids into the cell nucleus, which could lead to activation of endogenous retroviruses or to deactivation of tumour-suppressor genes.[76] To date, however, the frequency of integration is considered to be less than five plasmids per 150 000 cells,[71, 76] which is two orders of magnitude lower than normal mutations. Another concern is the production of anti-DNA antibodies following immunization with DNA, although experiments suggest that this possibility is almost non-existent. The induction of anti-DNA antibodies against naked DNA is known to be very difficult[42, 43] and it has been observed that milligrams of DNA injected into cattle on numerous occasions over a period of more than a year failed to produce any detectable anti-DNA antibodies.[108] Only when DNA is delivered in Freund's complete adjuvant is it possible to induce anti-DNA antibodies, so regulatory agencies are beginning to accept that this should not be a concern, especially in the case of livestock, where their lifespan is relatively short. There is still a concern that humans consuming animal tissues derived from livestock immunized with nucleic acid vaccines may suffer some untold consequence, and future testing will have to be performed before using such vaccinated animals for food.

Marker vaccines

The ability to differentiate animals that have been vaccinated from those that are potential carriers of an infection is an important requirement, and marker (DIVA – **D**ifferentiate **I**nfected from **V**accinated **A**nimals) vaccines have been developed for this purpose.[109, 110] Efforts are underway to eradicate Aujeszky's disease from the Netherlands by using a DIVA vaccination strategy,[32] and similar vaccines are proposed for the eradication of bovine herpesvirus-1. The concept of DIVA vaccination can be applied to any disease situation and such vaccines are certain to become important in the future.[1, 12, 14, 55, 96] Marker vaccines can also be used to complement quarantine and slaughter-out policies in countries where an exotic disease is introduced. It is possible to vaccinate animals at the periphery of an outbreak area and then to differentiate the vaccinated animals from those that have been exposed to the 'wild' infection. This is a far more economic and humane method of eradicating a disease than the conventional slaughter-out policy.[8, 48, 112]

The basis for DIVA vaccines is the development of a recombinant vaccine from which a gene or genes have been deleted, and then to develop a parallel diagnostic kit which detects and compares the antibodies developed as a result of infection or of immunization. For example, animals infected naturally with Aujeszky's disease or bovine herpesvirus-1 develop antibodies to all of the proteins of the virus. However, if the animal is vaccinated with a single glycoprotein (gD) or with a gE gene-deleted live vaccine, they will not develop antibodies to gE. An

animal with antibodies to gE would therefore be considered to be a latent carrier of the virus.[55] Currently this approach is used to differentiate between animals that have recovered from foot-and-mouth disease (FMD) and may be carriers and those that have merely been vaccinated. Modern FMD vaccines are inactivated and do not contain significant quantities of non-structural proteins of FMD virus, so vaccinated animals do not have antibodies to the non-structural proteins of FMD virus, in contrast to infected animals.[67, 68]

Adjuvants

Both subunit and inactivated vaccines generally require formulation with an adjuvant to enhance immune responses in order to be effective. Purified antigens, however, are generally inherently weaker immunogens than whole killed bacteria, since the latter contain components such as lipopolysacharides and CpG sequences which act as adjuvants. An effective adjuvant is therefore particularly important for successful subunit vaccination. Few experimental adjuvants have been shown to be effective and most of them are expensive and often lead to adverse side reactions and tissue damage. For example, mineral oil-based adjuvants are often effective, but they are not metabolized and remain at the injection site, and for these reasons a number of countries in Europe are threatening to ban them. Similarly, aluminum hydroxide adjuvants are associated with fibrosarcomas in cats.[21, 62] Finally, even if an adjuvant induces good humoral immunity there is frequently very limited, if any, cell-mediated or mucosal immunity. Since these latter types of immune responses are critical for protection against many infections this is a serious concern.

Although the molecular mechanisms by which adjuvants exert their effect are not fully known, it is believed that they help create a micro-environment where the cells of the immune system can interact and create an appropriate cytokine cascade for attracting those cells of the immune system which lead to immunity. Since most adjuvants help retain antigen at the injection site, some believe that maintaining a depot of antigen is critical for the establishment of the required microenvironment for cell and antigen interaction.

Many natural and synthetic immune modulators are used or have been tested as potential adjuvants and it is convenient to classify them into mineral oils, plant, bacterial, or chemical/synthetic adjuvants, although many adjuvants contain a combination of these components.[106] For example, Freund's complete adjuvant is a mixture of a mineral oil and bacterial components, and a new adjuvant is being tested which combines aluminum hydroxide with a synthetic oligonucleotide containing CpG sequences.[25] Adjuvants have to act as delivery vehicles for the antigen, and the vehicles not only affect the adjuvanting properties but may also have adverse effects at injection sites. It has been shown that injection site reactions in cattle resulting in tissue damage cause significant economic losses.[106] As a result efforts are being made to find alternative methods of immunization or to find adjuvants that do not cause adverse tissue reactions.

Readers are directed to specific reviews for a more in-depth treatment on adjuvants.[53, 78, 93]

Passive immunization

The new-born of livestock acquire passive antibody from the colostrum of their dams but this immunity wanes relatively rapidly, leaving the neonate fully susceptible to infection by a variety of infectious agents within a few weeks or months of birth. Neonatal immunity can be extended by hyper-immunization of the mothers, so that higher levels of passive antibody are transferred, a technique which has been used in cattle and pigs to increase the immunity of neonates to diarrhoea caused by *Escherichia coli*, rotavirus, or coronavirus.[7, 75] The dams are immunized prior to parturition and the elevated levels of antibodies in the colostrum and milk bathe the lumen of the neonatal gut, thereby preventing attachment of *E. coli*, rotavirus, or coronavirus to the intestinal villi. It is also possible to feed animals specific antibody for a short period of time when they are most susceptible to disease, an example being the use of monoclonal or polyclonal antibodies to the K99 antigen of *E. coli* to protect calves from *E. coli* induced diarrhoea.[94] Since the infection only occurs in the first week or two of life such an approach is economically feasible.

Passive antibody to a particular infectious agent tends to prevent effective immunization against that agent, especially when using vaccines involving live organisms. Replication of the vaccine agent is inhibited, and a protective immunity fails to develop. In most cases the level of passive antibody which will interfere with vaccination is higher than that which will prevent infection with field strains of the pathogen. We have noted above the closure of this 'window of susceptibility' by using nucleic acid vaccines. Since antibody in animals with passive immunity is not present at mucosal surfaces it is also possible to immunize them in the presence of colostral antibody by vaccine delivery to mucosal surfaces and thereby induce mucosal immunity.[119] Finally, *in utero* vaccination with nucleic acid vaccines has been mentioned, and *in ovo* vaccination is gaining more popularity.[37, 88, 101]

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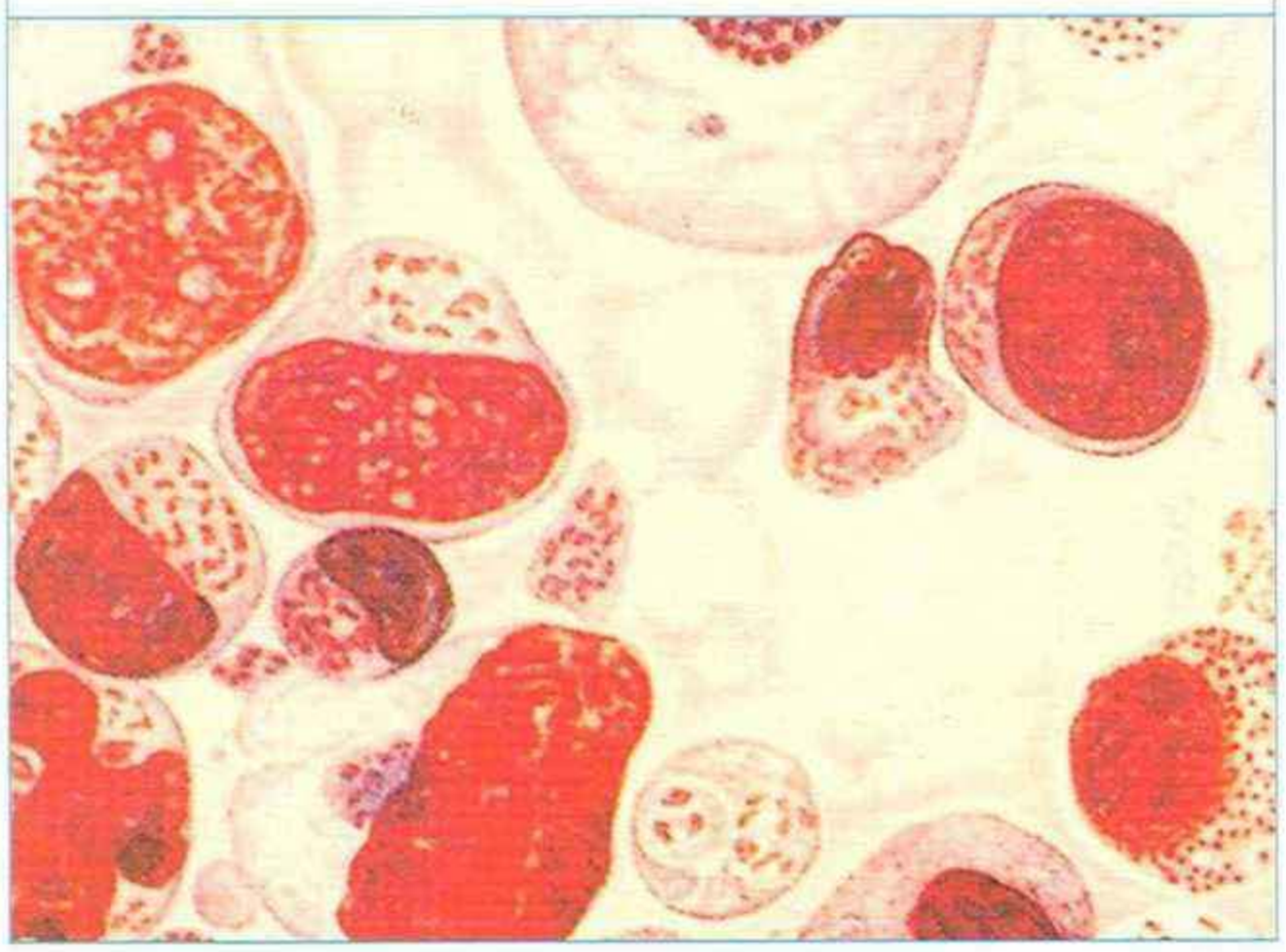
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SECTION TWO

Protozoal Diseases



12

African animal trypanosomoses

Synonyms: Nagana, tsetse fly disease

R J CONNOR AND P VAN DEN BOSSCHE

Introduction

The trypanosomoses are diseases of humans and domestic animals that result from infection with parasitic protozoa of the genus *Trypanosoma*. Trypanosomes parasitize all classes of vertebrates: fish, amphibians, reptiles, birds and mammals. The parasites, with the exception of *Trypanosoma equiperdum*, the cause of dourine, are transmitted from host to host by haematophagous vectors, and usually cause little appreciable harm to either the vector or the vertebrate host. However, several species of trypanosomes which parasitize mammals are less well adapted and commonly cause disease.

The trypanosomoses form a group of diseases, each species of pathogenic trypanosome causing the disease trypanosomosis. The course of a trypanosomal infection varies considerably and depends upon both the species of trypanosome and the host involved. Trypanosomosis is generally characterized by the intermittent presence of parasites in the blood and intermittent fever. Anaemia usually develops in affected animals, and this is followed by loss of body condition, reduced productivity and, often, high mortality.

The first report that associated trypanosomes with disease was made from India by Evans[81] in 1880. He found trypanosomes in the blood of camels and horses which were affected by a disease known locally as 'surra', and which is now known to be caused by *Trypanosoma evansi*. Subsequently, Bruce[35] made the major discovery in Zululand, South Africa, that trypanosomes were the causal organisms of 'nagana', or tsetse fly disease. The early review of trypanosomes and trypanosomoses by Laveran and Mesnil,[155] translated from the French by Nabarro in 1907, was followed by Wenyon's[316] contribution in 1926. Subsequent major reviews of trypanosomes and African trypanosomoses were made by Mulligan and Potts,[204] Ford,[85] Hoare,[111] Jordan,[136] and Stephen.[274] The considerable interest in trypanosomoses arises from their importance and the intriguing biology of the causal organisms.

Two forms of human trypanosomosis exist: Chagas' disease occurs in Central and South America and is transmitted by blood-sucking reduviid bugs, certain small wild animals and dogs harbouring the infection. The second form is human sleeping sickness. This occurs in Africa and is transmitted by blood-sucking flies of the genus *Glossina*, commonly known as 'tsetse flies' or simply as 'tsetse'. The majority of animal diseases caused by trypanosomes occur in the tropics. In Africa, several species of tsetse-transmitted trypanosomes cause African trypanosomoses in domestic animals, which in southern Africa are collectively known as 'nagana', a word derived from the Zulu 'nakane' meaning tsetse fly disease. An important form of trypanosomosis known as dourine, caused by *T. equiperdum*, also occurs in southern Africa, but has no arthropod vector **(see Chapter 13: Dourine)**. 'Surra' is transmitted by biting flies other than tsetse flies and, although it occurs in many parts of the tropics, including northern Africa, it is not present in southern Africa.

Tsetse flies have recently been discovered in Saudi Arabia,[77] but they are only known to be of importance in Africa, south of the Sahara, where the diseases they transmit are responsible for great economic loss **(see Chapter 2: Vectors: Tsetse flies)**.

The large populations of wild animals, which have thrived for millennia in the tsetse-infested tracts of Africa, have evolved with these flies and the trypanosomes they transmit. Hosts and parasites have become mutually adapted and coexist in a balanced relationship. Humans first brought domestic animals into the tsetse fly belts of Africa relatively recently, and although humpless cattle of the *Bos taurus* type, from which the present-day taurine breeds such as the West African Shorthorn, N'Dama, Muturu and Baoule are believed to have descended, were introduced into northern and western Africa from 4 500 BC onwards, the humped Zebu, *Bos indicus* type, arrived some 3 000 years later, and did not reach central and southern Africa until around AD 700.[80] Goats and sheep were introduced at about the same time. Because of this relatively recent introduction, the relationship between tsetse-transmitted trypanosomes and domestic animals has not fully evolved and

infection with these parasites frequently produces disease. However, the West African humpless cattle have had longer to adapt than Zebu cattle and this may explain why they possess the trait of trypanotolerance. They are able to live without drug treatment in tsetse-infested areas where other cattle die. Furthermore, some indigenous breeds of goats and sheep, such as the Dwarf goats and Djallonké sheep of West Africa and the small East African breeds, are more trypanotolerant than are exotic breeds.[211]

The ravages of nagana have long been recognized by the inhabitants of southern Africa, and early attempts to introduce livestock into tsetse-infested areas were unsuccessful as draught animals and other stock succumbed to the disease. The devastation which resulted from the rinderpest pandemic of the 1890s (**see Chapter 49: Rinderpest**) destroyed almost entire populations of wild animals and millions of cattle. Without hosts on which to feed, tsetse disappeared from large areas. With the constraint of tsetse-transmitted trypanosomosis removed, settlement in Zimbabwe was rapid. Some 25 000 cattle had survived the rinderpest catastrophy, and after restocking there were over 2,3 million cattle in the country by 1929.[41] However, at about this time tsetse were dispersing from residual pockets, and trypanosomosis again became a problem for livestock owners. By 1931, tsetse were spreading at a rate of 2 500 km^2 annually,[42] and game elimination to control tsetse began in 1932.[43] Since then, strenuous efforts have been made to contain the tsetse fly (**see Chapter 2: Vectors: Tsetse flies**). In many other parts of southern Africa, livestock owners have also had to live with the tsetse fly and its consequences.

Tsetse infest 10 million square kilometres and affect 37 countries, which makes African animal trypanosomosis a problem of truly continental magnitude (**Figure 12.1**). They live in frost-free areas that have an annual rainfall of 650 mm or more. In arid, marginal habitats, tsetse only exist in the better wooded and better watered strips where the host species concentrate during critical times, such as in the late, hot, dry season. In areas of mixed agriculture, cattle are valued for the milk, draught power and the manure which they provide. They also represent a means of investment. In most rural areas people rely on poultry and small ruminants for meat, cattle only being slaughtered for important social ceremonies or to realize large sums of money. Most of the settled areas of the tsetse fly belts of southern Africa are used for traditional mixed farming, but the presence of tsetse seriously handicaps development.[177] The general improvement of agricultural production depends on the greater use of animal traction, in place of manual tillage, enabling larger areas to be more efficiently cultivated. However, animals alone cannot ensure this development. The availability of credit schemes and supplies of seed, fertilizer and implements are also needed before the potential can be realized. The presence of tsetse is, nevertheless, the largest single obstacle to progress in affected areas.

The distribution of tsetse-transmitted bovine trypanosomosis in southern Africa is largely determined by the distribution of tsetse in three major fly belts. The first fly belt, with *Glossina morsitans morsitans* and *Glossina pallidipes* as the main tsetse species, links tsetse foci in Malawi with the large fly belt common to eastern Zambia, Zimbabwe and Mozambique. The *Glossina morsitans centralis* fly belt covers western Zambia, parts of western Angola, the Kwando River drainage in Namibia's Eastern Caprivi district and the Okavango Delta in Botswana. Finally, a third fly belt with the species *Glossina austeni* and *Glossina brevipalpis* covers parts of Mozambique's southernmost Matutuine district and north-eastern KwaZulu-Natal in South Africa.

Concerted efforts to control tsetse over the past 50 years have resulted in significant changes in the distribution of tsetse and tsetse-transmitted trypanosomosis. Unfortunately, few of these achievements have been sustained. In many countries of southern Africa, the current distribution of tsetse and, hence, tsetse-transmitted trypanosomosis is not much different from the ecological limits of the fly distribution.

The epidemiology of bovine trypanosomosis differs significantly between countries. Generally speaking, three different types of epidemiological situations can be distinguished. First, in Namibia and Zimbabwe, progressive tsetse control operations have gradually pushed the tsetse front to its current position. The interface between tsetse and cattle occurs at the edge of the artificial barrier, preventing tsetse from reinvading cleared areas, with the possibility of cattle entering tsetse-infested country. Second, in Botswana, Malawi and South Africa, interaction between tsetse and cattle occurs at the edge of the ecological limits of tsetse distribution. In principle, cattle are not able to enter tsetse-infested land. Finally, in Angola, Mozambique and Zambia, cattle live in tsetse-infested areas. They are surrounded by tsetse flies and are subjected to continuous challenge.

In Namibia, bovine trypanosomosis is most prevalent along the Kwando River on the western boundary of the Eastern Caprivi.[295] The deployment of odour-baited, insecticide-treated targets along the northern part of the Kwando River has resulted in a significant decline in the incidence of trypanosomosis. Occasionally, cases of nagana are detected in cattle grazing in the vicinity of Katima Mulilo, situated near the border with Zambia. These latter infections are assumed to originate from the neighbouring Sesheke area of Zambia: cattle that graze along the Zambezi River are probably challenged by tsetse that cross the river.

In Zimbabwe, tsetse has been controlled successfully and control efforts have been sustained. This is partly due to the country's favourable position at the edge of the natural limits of the tsetse distribution. Since 1981, approximately 55 000 km^2 of tsetse-infested land has been cleared of tsetse using a variety of methods.[268] A combination of odour-baited targets and insecticide-treated cattle constitute a 'barrier' to reinvasion, confining tsetse to areas less suitable

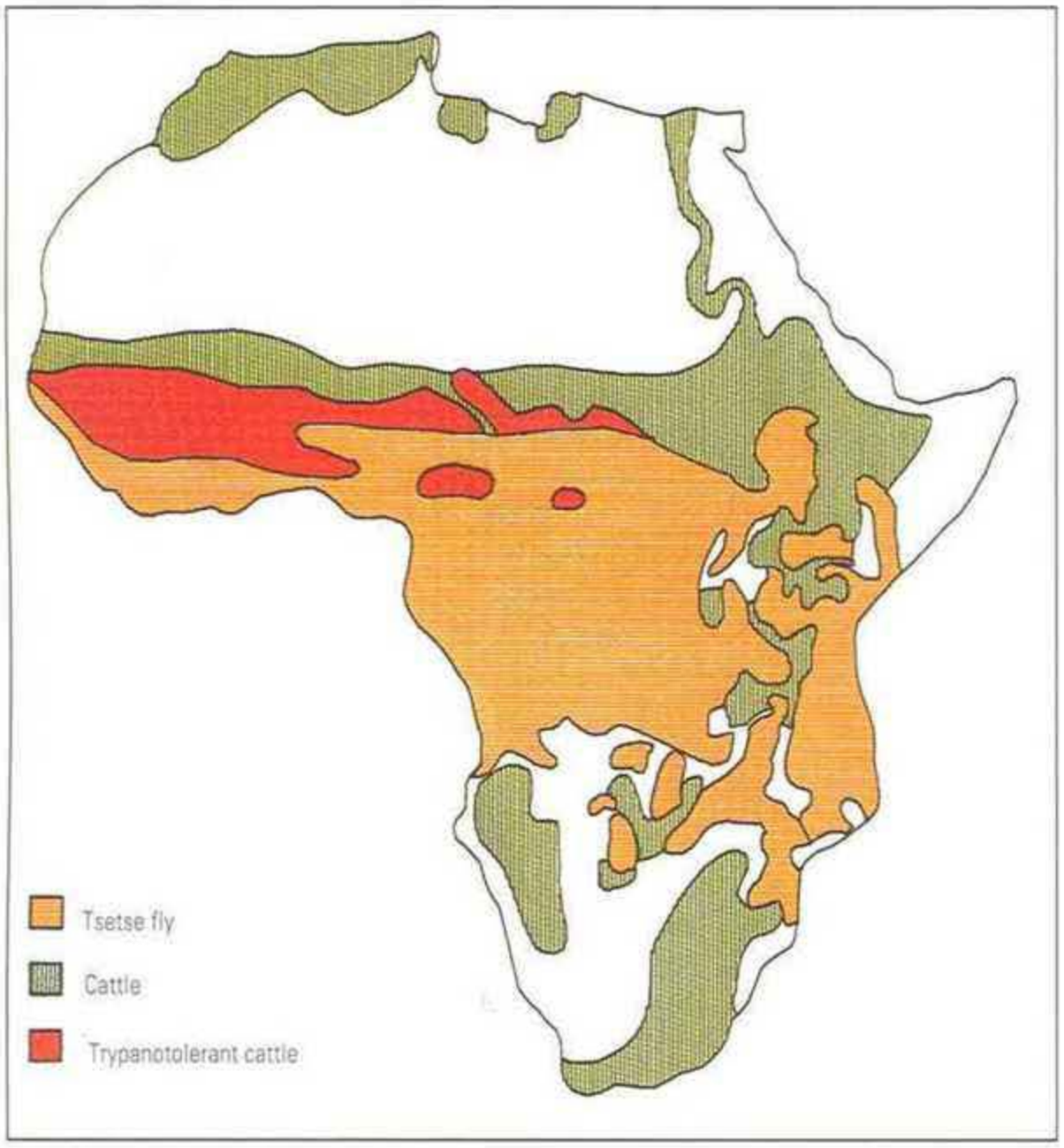


Figure 12.1 General distribution of tsetse flies and cattle in Africa

for agriculture or along international borders, and preventing them from invading previously cleared areas. By the late 1990s, bovine trypanosomosis was prevalent only in the northern and eastern parts of the country. In these areas, cattle contract the disease as they graze within or along the edge of the barrier[294] where they are exposed to tsetse fly. Challenge is generally low and bovine trypanosomosis does not constitute a major constraint to animal health and production. Notwithstanding the favourable disease situation, the trypanosomosis threat is real. A breakdown of the barrier to reinvasion could expose over one million highly susceptible cattle in the commercial and communal farming sectors to the risk of trypanosomosis. Continuous disease surveillance is essential. The speed with which tsetse fly are capable of reinvading cleared areas was demonstrated on an experimental basis when a portion of the target barrier was removed in the northeast of the country.[304] The impact of the breakdown of control on animal health was also clearly shown.

Tsetse flies infest two areas in Botswana, the Kwando–Linyanti–Chobe fly belt and the Okavango fly belt. Most of the tsetse-infested area comprises wildlife zones that are free of cattle. Because cattle–fly contact is low, bovine trypanosomosis is not a major disease in Botswana. The more immediate risk is to the large number of tourists who visit the wildlife areas, as they face the risk of contracting sleeping sickness.

In Malawi, where the human population density is high, the pressure on land and the concomitant clearing of vegetation for cultivation, confines tsetse to game reserves, national parks or forest reserves. Consequently, cases of nagana are restricted to areas surrounding the tsetse-infested zones and areas adjacent to neighbouring Zambia or Mozambique where tsetse is present.[292] The prevalence of infection depends upon the cattle–fly contact and varies between areas and seasons. It decreases with increasing distance from the tsetse infestation. Although bovine trypanosomosis can assume epidemic proportions, it is localized and does not constitute a major threat to livestock production in Malawi. The ever-increasing demand for land will probably further reduce suitable tsetse habitat. However, the expansion of the human population will compel people to live at the edge of tsetse infestations, which is likely to result in an increased incidence of nagana.

In the late 1850s, tsetse flies infested the Limpopo, Mpumalanga and KwaZulu-Natal provinces of South Africa,[110] but the rinderpest pandemic led to the complete disappearance of tsetse from these areas. However, in parts of Zululand, including the present-day game reserves of north-eastern KwaZulu-Natal, pockets of wild animals and tsetse survived, and by 1905 nagana was again threatening livestock in the region. *Glossina pallidipes* dispersed into farming areas causing serious epidemics. The advent of organochlorine insecticides provided a suitable means to remove tsetse, and between 1945 and 1952 *G. pallidipes* was eradicated from Zululand by large-scale aerial spraying operations.[65] Thereafter, the two species found in Zululand, *G. austeni* and *G. brevipalpis*, were responsible for sporadic cases of bovine trypanosomosis.[142] Despite the restricted distribution of tsetse, a widespread outbreak of bovine trypanosomosis occurred in 1990.[13]

In Angola, nagana is a serious constraint. Over a quarter of the country is tsetse-infested[95] and the 3,5 million cattle are kept mainly in the south-western, tsetse-free areas, although there is a small population of trypanotolerant cattle in Cabinda Province. Control measures are currently limited to the use of trypanocidal drugs.

Tsetse fly are a serious threat to livestock development in about two-thirds of Mozambique. More than two decades of civil war reduced the cattle population by more than 80 per cent and made bovine trypanosomosis less prevalent than before. Currently, most of Mozambique's livestock is distributed in areas where tsetse is either absent or present at low levels. Nevertheless, bovine trypanosomosis constitutes a threat to the development of rural areas and could be a serious constraint to restocking programmes.

In Zambia, on the other hand, bovine trypanosomosis is a disease of national importance. Three-fifths of the country provide suitable tsetse habitat. Despite its long history of tsetse control, large-scale operations have met with limited long-term success. This is largely attributable to the absence of natural barriers, which results in a continuous threat of reinvasion of cleared areas by tsetse from adjacent infested zones. In contrast to most other countries in southern Africa, but in common with countries in eastern Africa, cattle are kept in tsetse-infested areas. Under these conditions, disease challenge and prevalence of infection are generally high. Consequently, bovine trypanosomosis constitutes a major constraint to rural development in large areas of western, southern and eastern Zambia.[44]

Early work on trypanosomosis, much of it conducted in southern Africa, concentrated on describing the trypanosomes and studying the natural history of the parasites, their vectors and their hosts. The greatest advances in our knowledge of trypanosomosis over the past two decades have been made in the areas of molecular biology and immunology, rather than at the field level. Although extremely valuable research has been conducted in carefully controlled laboratory experiments, it is often difficult to relate the results to the field situation.

Aetiology and life cycle

Trypanosomes are protozoan parasites of the genus *Trypanosoma*, order Kinetoplastida, and have, as characteristic organelles, a kinetoplast and a flagellum. Typically, trypanosomes are digenetic parasites and thus require two hosts to complete their life cycle: they multiply in the blood, tissues or body fluids of a vertebrate host and, with the exception of *T. equiperdum* which is venereally transmitted, are ingested by a haematophagous invertebrate vector. With a few notable exceptions, a cycle of development and maturation occurs in the vector, after which the parasites are transmitted to another vertebrate host as the vector feeds. Transmission is either by inoculation of trypanosomes with saliva or by contamination of mucosa or broken skin with trypanosomes in the vector's faecal material, voided during the blood meal. The type of development cycle within the vector determines whether or not infective, metacyclic parasites are present in saliva or faeces. On this basis mammalian trypanosomes are classified into the two broad sections of 'salivaria' and 'stercoraria'.[111]

In Africa, the pathogenic trypanosomes that cause sleeping sickness in humans and nagana in domestic animals are salivarian, and cyclical development occurs in tsetse flies (**Figure 12.2**). Transmission of any trypanosome species can take place mechanically without cyclical changes occurring in the vector. In nature, this is effected by biting flies, such as *Tabanus*, *Stomoxys* and *Lyperosia* spp., which feed on more than one animal before repletion. The fly remains infective for only a short time, although in South America and Mauritius (see below) mechanical transmission of *Trypanosoma vivax* by haematophagous flies other than tsetse has enabled the parasite to become established. Experimentally, trypanosomes may be transmitted by 'syringe passage' of infective blood.[270] 'Surra' is a disease that affects a wide range of host animals, and it occurs in North Africa, the Near and Far East, Central and South America, the Philippines and Mauritius. It is caused by *T. evansi*, a dyskinetoplastic form of which — known as *Trypanosoma equinum* — also causes disease in equids in Central and South America, where it is known as 'mal de Caderas' or 'Murrina'. These parasites have adapted to an entirely mechanical, non-cyclical mode of transmission by blood-sucking flies other than tsetse. *Trypanosoma theileri* is a stercorarian parasite of cattle which deserves greater mention. It was first reported by Theiler[281] in the then Transvaal in 1903, and has since been found to occur in cattle throughout the world. It is transmitted by tabanid flies and is widely regarded as being non-pathogenic, but in certain circumstances it has been associated with disease.[111, 280, 315]

The following account of pathogenic trypanosomes

deals only with tsetse-transmitted salivarian parasites, unless otherwise indicated. They are important as they cause severe disease. Human sleeping sickness is caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. Whilst these two subspecies do infect some domestic and wild animals, there are other, more significant pathogens of livestock which are found in three subgenera: *Duttonella*, *Nannomonas* and *Trypanozoon* (**Table 12.1**). The fourth salivarian subgenus, *Pycnomonas*, is represented by only one species, *Trypanosoma suis*, which is of little economic importance.

As a consequence of their pathogenicity and their complex and fascinating biology, the salivarian trypanosomes have been, and will continue to be, the subject of intense research. The remarkable alternate adaptations of these extracellular parasites to mammalian and insect hosts are reflected in morphological changes that are readily detectable by light microscopy. Bloodstream forms are trypomastigotes (**Figure 12.2**); from the posterior portion of an elongated body, some 8 to 35 µm long, arises a flagellum which extends anteriorly, and which is connected to the body by an undulating membrane. Beyond the anterior extremity of some species, the flagellum may extend free of attachment to the undulating membrane. The beating of the flagellum pulls the trypanosome forwards, imparting characteristic motility. Within the cell, in a posterior position and at the base of the flagellum, a kinetoplast is found, and a single nucleus is located almost halfway along the body. In the tsetse fly, trypomastigotes transform to epimastigotes (synonymous with the crithidial stage in the old terminology) in which the kinetoplast has migrated anteriorly, to a position adjacent to the nucleus (**Figure 12.2**). Differences in the morphology of the trypomastigote stages of the various species form the basis for differential diagnosis. The major characteristics are clearly seen in thin blood smears, stained with Giemsa's, Leishman's or other Romanovsky stains. *Trypanosoma congolense* (**Figure 12.3a**) and *T. vivax* (**Figure 12.3b**) are monomorphic parasites, whereas *T. brucei* is polymorphic (**Figure 12.3c**). *Trypanosoma congolense*

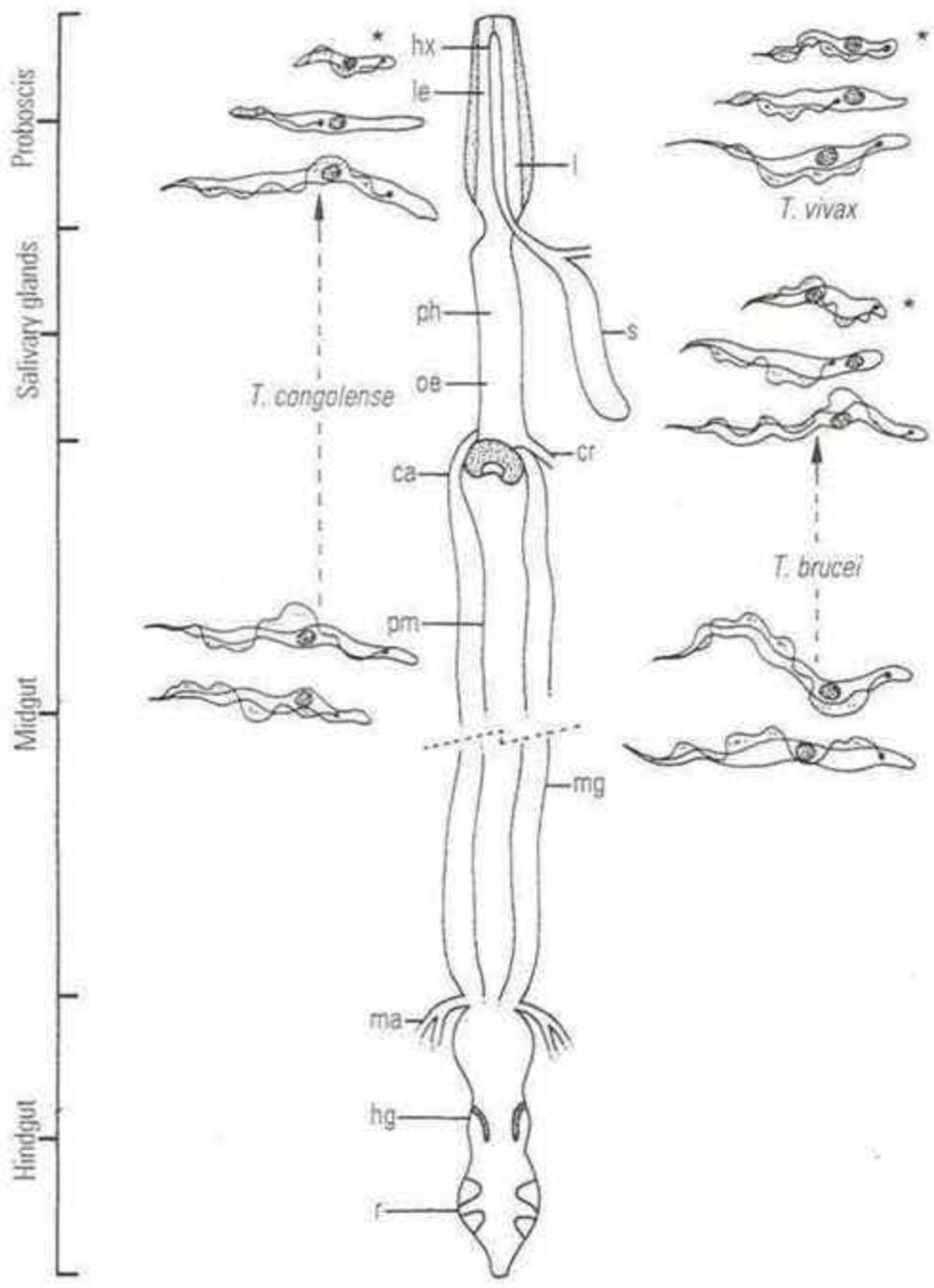


Figure 12.2 Life cycles of African pathogenic trypanosomes

ca = cardia (= proventriculus)
cr = crop duct
hg = hindgut
hx = hypopharynx
l = labium
le = labrum-epipharynx
hx + l + le = proboscis
ma = Malpighian tubes
mg = midgut
oe = oesophagus
ph = pharynx
pm = peritrophic membrane
r = rectum
s = salivary glands
* = infective metatrypanosomes

is the smallest species and is between 8 and 20 μm in length. It has no free flagellum, and the kinetoplast is usually sub-terminal and marginal.[224] *Trypanosoma vivax* is between 20 and 26 μm long, and has a long free flagellum and a large, often terminal kinetoplast. The long slender form of *T. brucei* is characterized by a long free flagellum, the presence of a conspicuous undulating membrane and a sub-terminal kinetoplast. The posterior end is narrow and often pointed, and this form is between 23 and 30 μm long in contrast to the short stumpy forms which range on average between 17 and 22 μm. The short stumpy form has a well-developed undulating membrane and usually has no free flagellum. The lengths of the intermediate forms range from 20 to 25 μm. The kinetoplast of *T. brucei* is smaller than that of either *T. vivax* or *T. congolense*. *Trypanosoma simiae* is between 12 and 24 μm long and displays some polymorphism: most parasites are long stout forms, sometimes with a short free flagellum. Short forms of *T. simiae* resembling *T. congolense* and long slender forms also occur.[111]

The reasons for the cyclical changes from trypomastigote to epimastigote and back to trypomastigote, in the course of the trypanosome's life cycle, have become clearer as a result of ultrastructural and biochemical studies. The morphological changes which are readily seen reflect the trypanosome's adaptation to the different physiological environments encountered in mammalian and insect hosts. Ultrastructural, biochemical and immunological studies[196, 299, 300] have not only revealed the reasons behind the cyclical changes in morphology, but have also elucidated the parasite's survival tactics. Trypanosomes show remarkable adaptation. They survive not only in the turbulent bloodstream, where they face vigorous immunological assault, but they also withstand the digestive enzymes of the tsetse fly's alimentary tract.

Trypanosomes are therefore robust organisms. The cytoskeleton of closely packed, parallel microtubules, strongly interconnected by associated proteins, is very resilient and helps to retain the body's shape. The cytoskeleton supports the surface membrane (**Figures 12.4 and 12.5**), which, in mammal-infective stages, is coated with a surface glycoprotein some 12 to 15 nm thick. Within the cytoplasm of the cell are organelles, which are supported by intermediate filaments.[125] A nucleus, Golgi apparatus, rough and smooth endoplasmic reticulum, glycosomes, lysosomes, vacuoles and single mitochondrion are all present. Microtubules also delimit the entrance to the flagellar pocket, which is an invagination of the cell membrane from which the flagellum emerges (**Figure 12.5**). This pocket is a highly specialized area of the cell membrane at which endocytosis occurs, in a manner similar to that found in mammalian cells.[305] Serum albumin, lipoproteins, iron-bound transferrin and other nutrients are endocytosed from the flagellar pocket by bloodstream trypanosomes. The exchange of the contents of the pocket is assisted by the constant, pump-like beating of the flagellum, which is made up of an axoneme and paraflagellar rod, and which also provides the means of propulsion. It is ensheathed in surface membrane and is attached to the body by a series of junctions and numerous filaments.[262] The beating of the flagellum draws the cytoplasm of the cell up into a series of transient crests which form the undulating membrane.[300] At the base of the flagellum is the basal body which is associated with the kinetoplast. This latter structure consists of bundles of DNA fibrils located in a dilatation of the cell's single mitochondrion.[111, 300]

In the mammalian host, the abundance of glucose as an energy source is associated with repression of the mitochondrion which lacks cristae and is tubular. Energy is derived from the metabolism of glucose to pyruvate.[299]

Table 12.1 Pathogenicity[1] of salivarian trypanosomes to livestock

TRYPANOSOME SUBGENUS	TRYPANOSOME SPECIES	CATTLE	GOATS	SHEEP	PIGS	HORSES	DONKEYS
Trypanozoon	*T. brucei*[2]	+	++	++	+	+++	++
	T. evansi[3]	++	+	+	++	+++	++
	T. equiperdum[4]	–	–	–	–	+++	++
Nannomonas	*T. congolense*	+++	++	++	+	++	++
	T. simiae	–	+	+	+++	–	–
Duttonella	*T. vivax*	+++	++	++	–	++	+
Pycnomonas	*T. suis*[5]	–	–	–	++	–	–

Notes
1 Under usual field conditions, but which is modified by many factors
2 *T. brucei gambiense* and *T. brucei rhodesiense* cause human sleeping sickness in West and East Africa respectively, and have animal reservoirs in which pathogenicity is low. Human sleeping sickness in southern Africa — in the Okavango Swamps, Luangwa and Zambezi valleys — is caused by *T. brucei rhodesiense*. *Trypanosoma brucei brucei* is not infective to humans
3 Mechanical transmission by biting flies other than tsetse
4 Transmission is venereal
5 Rarely encountered

– = not infective/pathogenic + = mildly pathogenic ++ = moderately pathogenic +++ = severely pathogenic

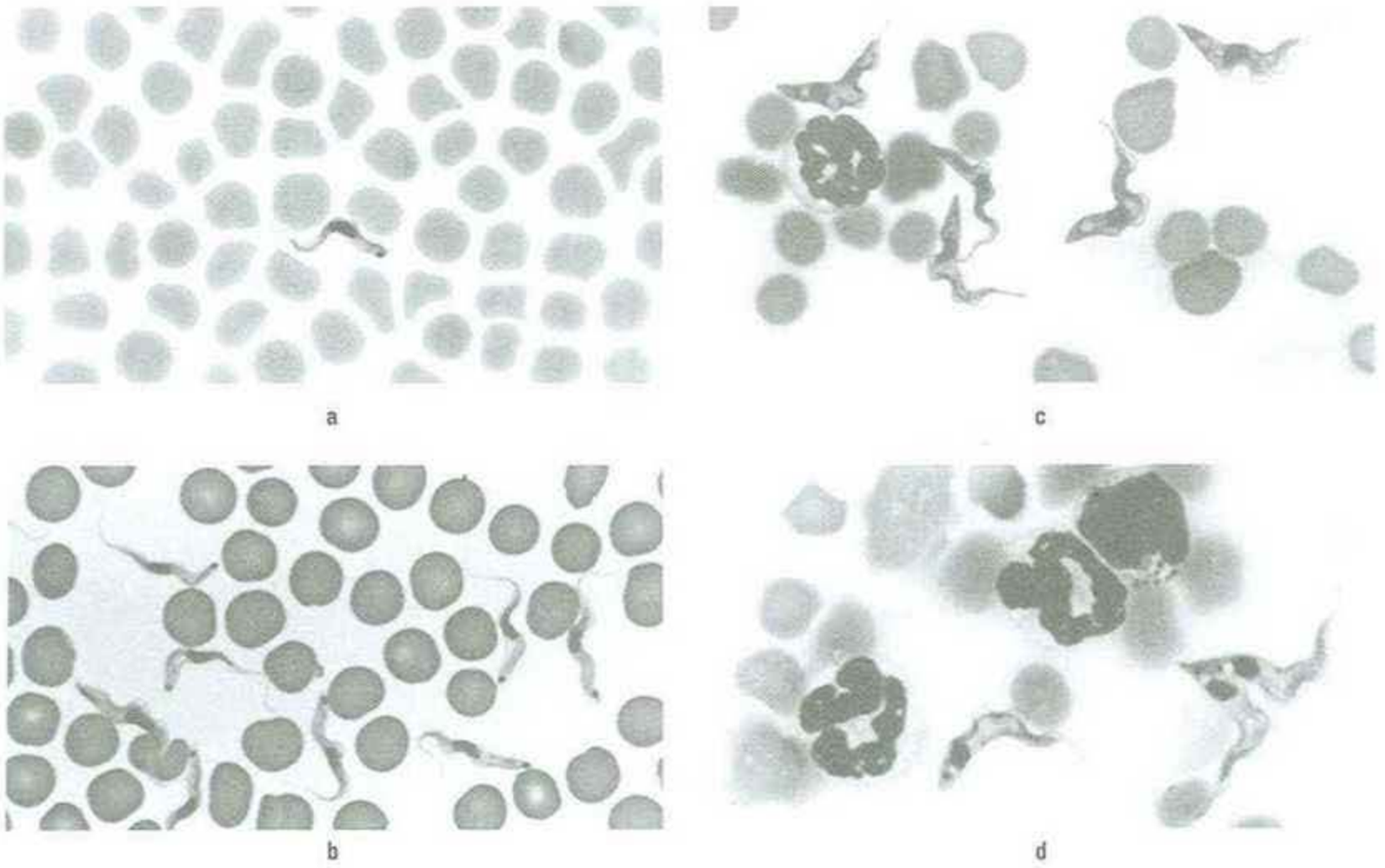


Figure 12.3 Trypanosomes in thin blood smears, x1 000 stained with Diff-Quick. a = *Trypanosoma congolense*: note absence of free flagellum; b = *Trypanosoma vivax*: note long free flagellum and large kinetoplast; c = *Trypanosoma brucei*: note polymorphism, prominent undulating membrane and free flagellum; d = *Trypanosoma brucei* dividing by longitudinal binary fission. (Unpublished photomicrographs by courtesy of Dr L. Logan-Henfrey, International Laboratory for Research on Animal Diseases, PO Box 30709, Nairobi, Kenya)

However, the mitochondrion transforms in the tsetse fly, swelling and acquiring tubular cristae. Enzymes appear which oxidize proline, the main source of energy for both fly and trypanosome.[299] This transformation is evident as the kinetoplast migrates and the trypanosomes assume the epimastigote form in the fly.

Trypanosomes reproduce by longitudinal binary fission, both in the bloodstream (**Figure 12.3d**) and in the fly, although a sexual process can apparently occur in the tsetse fly.[132, 278] Multiplication in each host culminates in the presence of mature trypanosomes, which stop dividing and are pre-adapted to the conditions that they will encounter in the next cyclical host. As a tsetse fly takes its blood meal from an infected host it ingests trypanosomes. Pre-adapted parasites survive in the fly, but trypanosomes that are not metabolically adapted to the new physiological conditions die. The cycle of development of different species of salivarian trypanosomes is very similar, but there are important differences in their localization in the tsetse fly during their development (**Figure 12.2**). *Trypanosoma vivax* has the simplest migratory pattern; development occurs only in the proboscis and the pharynx (cibarium).[111, 130] Bloodstream trypanosomes are taken up through the food canal of the proboscis, and the pre-adapted, late bloodstream forms attach to the labrum wall by means of the flagellum.[88] Parasites that are swept to the midgut do not survive. In the proboscis the attached parasites lose their surface coats, transform to epimastigotes and multiply in the labrum. They then detach and move to the hypopharynx where, after re-attachment, they transform to metacyclics. The complete cycle takes 5 to 13 days, before glycoprotein-coated metatrypanosomes can be inoculated as the fly feeds.[88] The development of members of the subgenus *Nannomonas* and *Trypanozoon* is more complex and goes through an immature or midgut stage. In the midgut of the fly, pre-adapted trypanosomes, or short stumpy bloodstream forms in the case of *T. brucei*, transform to procyclics, elongate and shed the glycoprotein coat. The coat is progressively replaced in a short time by a coat of procyclin, which probably provides protection against proteolytic enzymes of the midgut.[250] The procyclic trypanosomes begin to metabolize proline as a source of energy for which they compete with their host, the tsetse. The transformation of bloodstream trypanosomes into procyclic or midgut forms within the fly's midgut is a crucial first step in the establishment of a trypanosomal infection. Transformation proceeds rapidly in the posterior part of the midgut, the first procyclic forms appearing 11 hours after ingestion.[288] Factors known to influence this process include

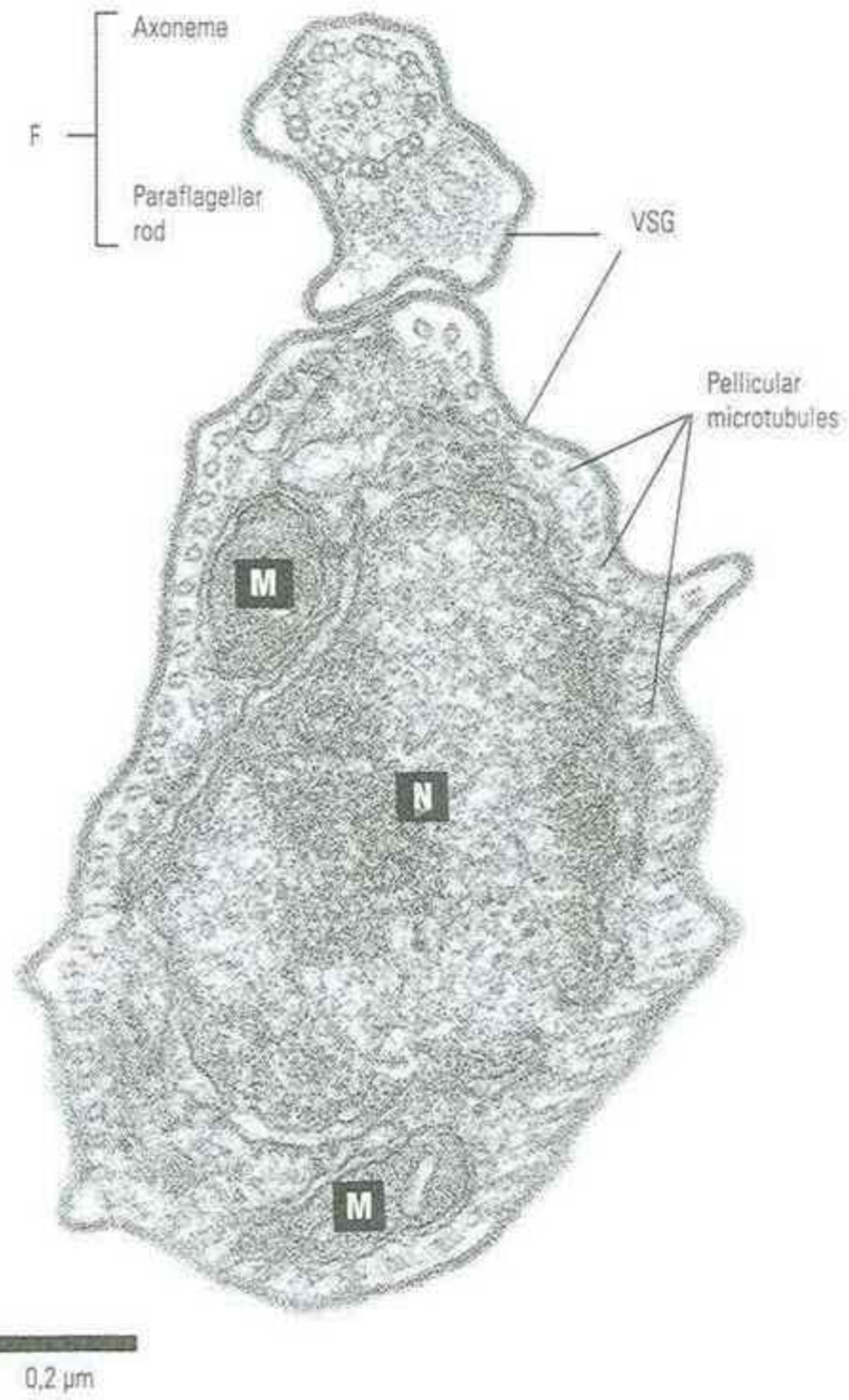


Figure 12.4 Electron microphotograph of *Trypanosoma congolense*: cross-section showing flagellum (F), nucleus (N), mitochondrion (M) and variable surface glycoprotein coat (VSG), x86 000. Bar represents 0,2 µm. (Unpublished electron micrograph by courtesy of Dr P. Webster, Yale University School of Medicine, Department of Cell Biology, New Haven, CT)

trypanolysins and trypsin or trypsin-like molecules in the fly's midgut,[126, 127] the type of host blood at the time of the infective feed,[190, 194, 203, 246] and blood composition.[93, 185, 230] The tsetse fly's immune system also plays an important role. A humoral mechanism involving lectins is implicated in the establishment of trypanosome infections in *Glossina*.[186, 187] In invertebrates, lectins bind to specific carbohydrate groups on cell surfaces and may agglutinate certain cells. This may result in lysis and death of procyclic trypanosomes.[49, 119, 128, 129, 220] In the midgut, the titre of lectins and the degree of inhibition by lectins largely determine the ease with which trypanosomes in an infected blood meal establish a midgut infection.

The mechanism of maturation of a midgut infection is complex and, once established, it does not always progress to maturation. Before the infection is mature, procyclic forms transform into epimastigote and then to metacyclic forms. From the midgut, trypanosomes migrate to the mouthparts or salivary glands. The midgut procyclics are free swimming; they move to the ectoperitrophic space to form an actively dividing population. They lose their glycoprotein coat and move forward to the proventriculus where they stop dividing. The proventricular 'mesocyclic' trypanosomes are longer than their procyclic precursors; they reinvade the endoperitrophic space and, in the case of members of the subgenus *Nannomonas*, move via the oesophagus to the hypopharynx where they attach and complete their development to become coated metacyclics after 7 to 40 days. Members of the subgenus *Trypanozoon* migrate via the oesophagus, mouthparts and salivary ducts to the salivary

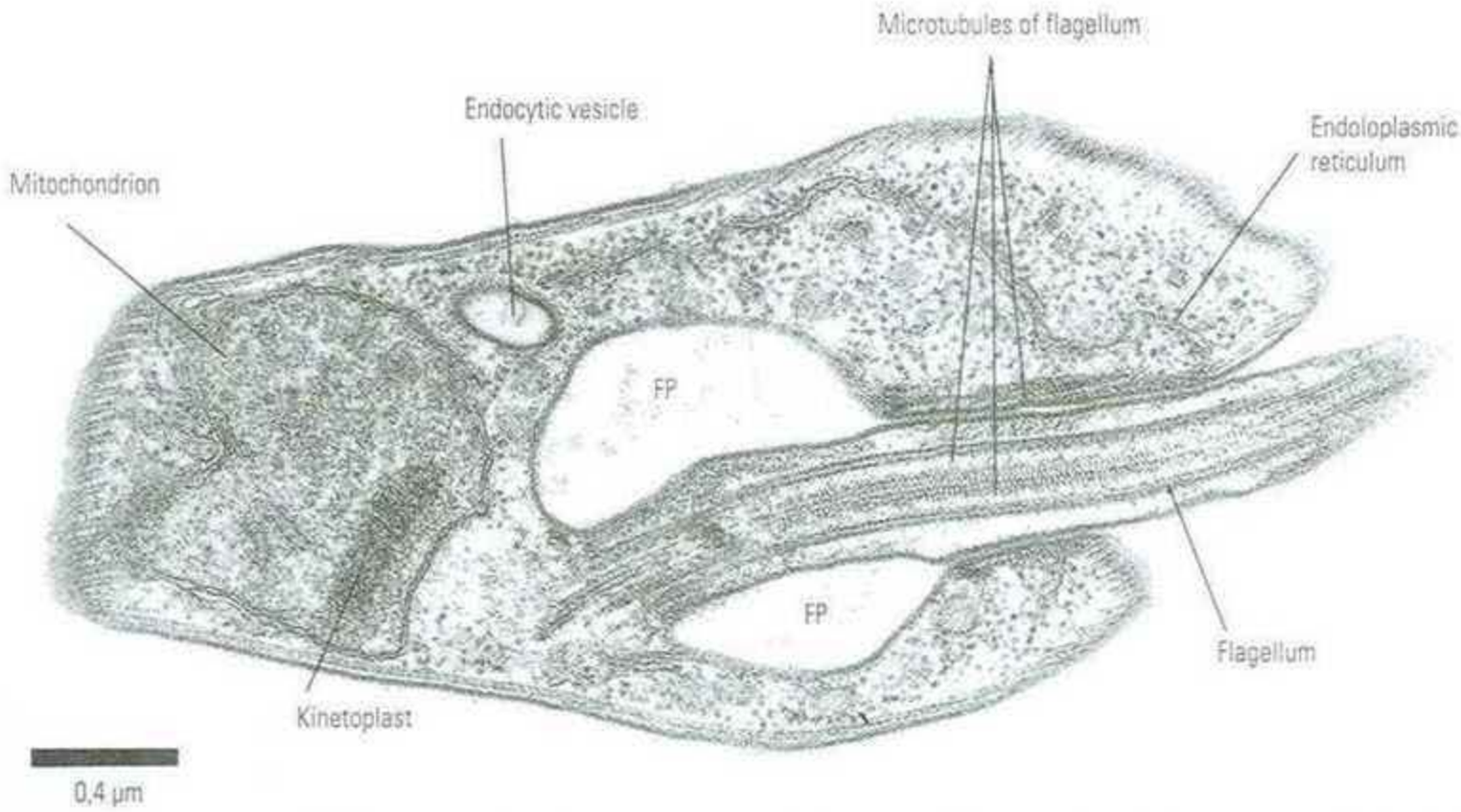


Figure 12.5 Electron micrograph of *Trypanosoma brucei*: section through the flagellar pocket (FP) region of the cell. Microtubules are longitudinally sectioned, x44 000. Bar represents 0,4 µm. (Unpublished electron micrograph by courtesy of Dr P. Webster, Yale University School of Medicine, Department of Cell Biology, New Haven, CT)

glands where the parasites form flagellar attachments to the epithelial cells. In the salivary glands the trypanosomes go through four stages of development. The mature, coated metacyclic trypanosomes undergo morphological and metabolic changes that pre-adapt the parasites for their life in a mammalian host. The development cycle of *T. brucei* takes from 17 to 45 days.[175]

As the infective tsetse fly feeds, metacyclic trypanosomes and saliva pass through the hypopharynx and are inoculated intradermally; it is here that infection is established.[3, 68, 79] Trypanosomes multiply in the skin, and may produce a chancre,[248] which is a local skin reaction that develops into a raised, indurated, hot, painful swelling. The chancre may attain a diameter of 100 mm in 10 to 12 days, but regresses 10 to 15 days later.[3] From the skin, the trypanosomes reach the blood via the draining lymphatics within a few days.[3, 16, 71, 78, 168, 174] Trypanosomes multiply in the bloodstream, and although initially their low numbers make detection difficult, the generation time of only a few hours soon leads to high levels of parasitaemia. Trypanosomes may leave the bloodstream to reach various extravascular sites.

Trypanosoma congolense, for long regarded as a strictly intravascular parasite,[116, 205] recirculates in lymph[4, 71] and has been recovered from the central nervous system.[184] However, this species is more commonly located in capillary beds where parasites attach to the endothelium.[36, 166] *Trypanosoma vivax* shows a greater tendency to invade tissue and has been demonstrated in cerebrospinal fluid, perivascular spaces of the central nervous system, the aqueous humour of the eye, myocardium and in other tissues.[183, 318]

In contrast to *T. congolense* and *T. vivax*, infections with *Trypanozoon* species are characterized by generally low parasitaemias and a marked invasion of tissues.[96, 117]

Because of the greater accessibility of bloodstream parasites to investigators, this stage of the life cycle has been intensively studied. The ability of trypanosomes to establish prolonged infections is attributable to the phenomenon of antigenic variation.[22, 64, 98] Each bloodstream trypanosome is completely clad in a dense surface glycoprotein coat[298] (**Figures 12.4 and 12.5**), which consists of several million tightly packed molecules, each of which is anchored in the parasite's surface membrane.[250] Within a population of trypanosomes originating from a single infection, almost all bear the same glycoprotein coat and are thus of the same antigen type. As parasitaemia rises, a swift antibody response is elicited against the antigen type exposed on the surface of the bloodstream trypanosomes. These specific antibodies attach to the surface glycoprotein and produce complement-mediated lysis of all trypanosomes of that antigen type. However, before antibodies reach trypanolytic levels, some trypanosomes — as few as one in 100 000 — switch off the gene that controls the production of the initial surface glycoprotein and activate a gene that codes for a different protein.[222] Trypanosomes which bear the new surface glycoprotein are of a different antigen type and are not destroyed by antibody against the first antigen type; they survive to produce another parasitaemic wave, which in turn is removed by antibody specific for that antigen type.

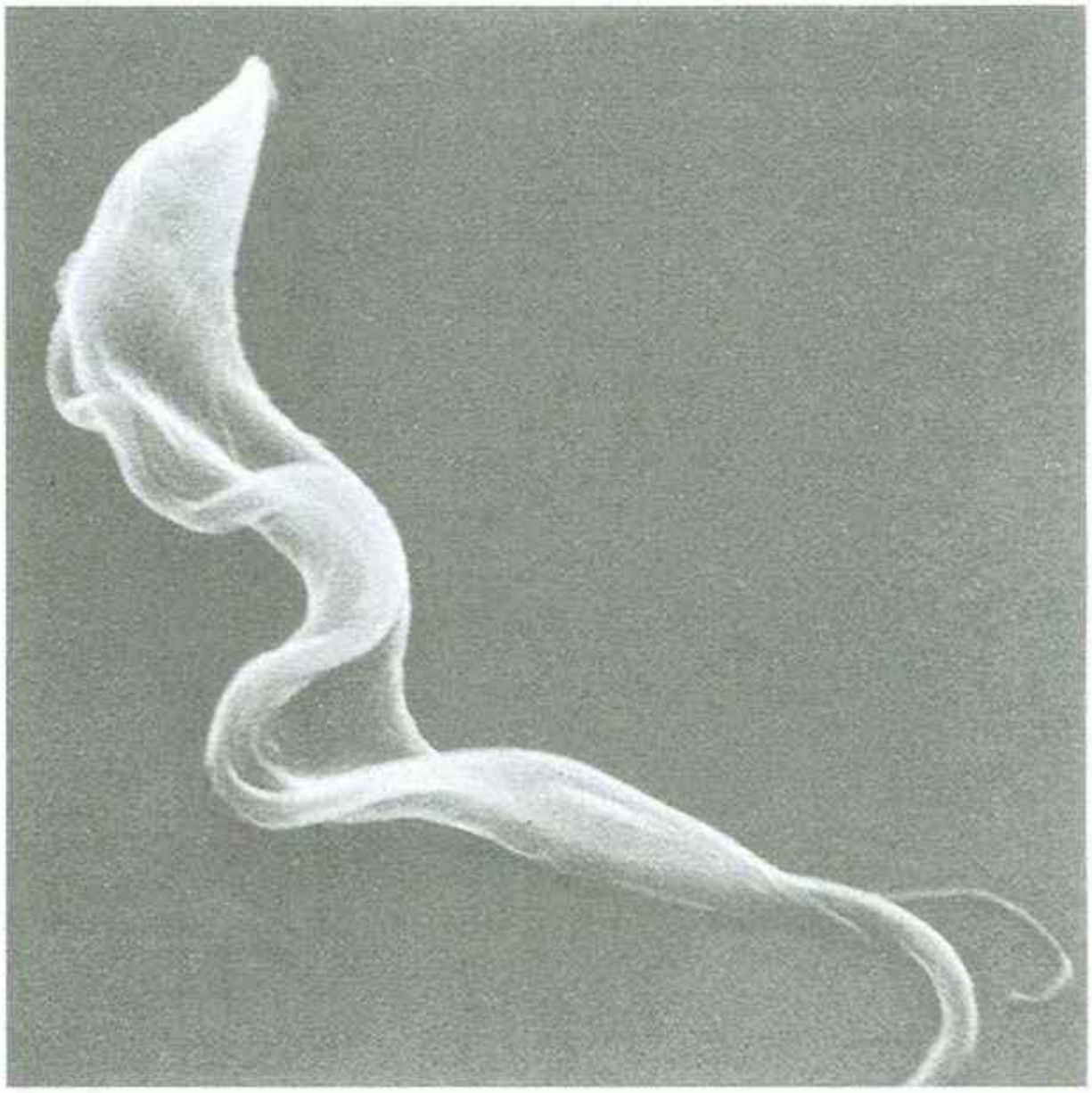


Figure 12.6 Scanning electron micrograph of an intermediate (bloodstream) form of *Trypanosoma brucei* from the blood of a mouse. Note the prominent undulating membrane, pointed posterior end and long, free flagellum. A 'streamer' or filopodium can also be seen. (By courtesy of Dr P. Gardiner and reprinted by kind permission of Vinand Nantulya and *Parasitology Today*)

By this time a third variant has arisen, and, escaping the effect of host antibody, it survives to produce the next parasitaemic peak. This antigenic variation is the result of sequential expression of variable surface glycoproteins (VSGs) which constitute a repertoire of variable antigen types (VATs). Infections arising from a single trypanosome may have a repertoire of more than 100 VATs.[222] Thus shielded from total destruction, trypanosome infections usually run prolonged courses, since each VAT is present for several days before being removed. Although there is a mixture of a small number of VATs within a parasitaemic peak, the sequence of expression of VATs tends to be quite stable in clonally derived trypanosomes.[206] This imparts immunologically distinct characteristics to a strain of trypanosomes, the distinct strain being called a 'serodeme'. In the course of successive parasitaemic waves, some trypanosomes stop dividing and transform to the pre-adapted form able to survive in the tsetse. This is most readily seen in *T. brucei* infections. The long, slender trypanosomes rapidly divide to produce parasitaemic waves, but some differentiate through intermediate forms to become non-dividing, short stumpy parasites. These pre-adapted parasites do not shed their surface coats.[299] Precisely what induces transformation is not known, but it is thought to be related to the host's susceptibility to infection and disease.[24, 198, 263] Similar pre-adaptive changes have been found in *T. vivax*[88] and are believed to be necessary for the cyclical development of *T. congolense*.

The survival of the non-dividing, pre-adapted trypanosomes in the bloodstream may be associated with the ability to shed the VSG coat. Also, the 'flow' of VSG molecules over the surface membrane enables VSG-bound antibody to be endocytosed in the flagellar pocket.[300, 306] In addition to removing potentially harmful antibody and delaying complement-mediated lysis of the trypanosome, this process may also enable VSG to be recycled. The shedding of VSG by stumpy forms of *T. brucei*[198] and the formation of 'streamers' or filopodia (**Figure 12.6**), consisting of coated membrane,[300] may represent a protective mechanism.[290] The stiffer cell membranes of stumpy *T. brucei* parasites may also provide some protection against antibody-mediated lysis.[299]

After ingestion by the tsetse, pre-adapted trypanosomes shed the glycoprotein coat, transform, multiply and finally mature. Infective tsetse then transmit metacyclic trypanosomes to another host. Irrespective of the VAT of the

bloodstream trypanosomes ingested by a fly, the metacyclic VATs of a serodeme are relatively constant. After repeated tsetse-transmission of a single serodeme, the composition of metacyclic VATs is usually very similar. Although the metacyclic VATs of a serodeme are always similar in contrast to the large VAT repertoire of bloodstream trypanosomes, there is nevertheless antigen heterogeneity, even among the metacyclics within a single tsetse fly.[206, 222] Many serodemes occur within a single species, each serodeme having its own VAT repertoire. The antigenic diversity within a species leads to the possibility of animals in a tsetse-infested area being exposed to a large number of antigenically distinct trypanosomes, but although trypanosomes within a species may be antigenically dissimilar, they are morphologically indistinguishable.

The characterization of trypanosomes for a long time relied on comparisons of their morphology, motility, host specificity, tsetse transmissibility and their development within the fly, but more recent characterization methods include isoenzyme typing, analysis of kinetoplast DNA by polyacrilamide gel electrophoresis, pulsed field gradient electrophoresis of chromosomal digests and DNA hybridization.[88, 91] In view of the great diversity which exists within a species, attempts have been made to standardize the nomenclature of distinct populations of trypanosomes.[323] It has been recommended that the use of the term 'strain' should be avoided, but it is still widely used. In the context of veterinary medicine, certain strains of trypanosomes are recognized for their low or high virulence, the ease with which they are transmitted by tsetse, or for other broad characteristics which may not be entirely stable.

The sequel to infection with salivarian trypanosomes is not always disease. The outcome is determined by many factors, frequently related to the susceptibility of the host and the pathogenicity of the trypanosome. In the case of wild animals, a natural cycle of trypanosome transmission occurs which is not associated with disease. Similarly, in some breeds of domestic animals, infection with salivarian trypanosomes is tolerated, and host and parasite reach an equilibrium. Disturbance of the equilibrium may precipitate disease a long time after establishment of the infection. Thus, although the tsetse-transmitted trypanosomes are aetiological agents of African trypanosomosis, infection is not always synonymous with disease.

The occurrence of *T. theileri* in healthy cattle throughout the world[111] exemplifies a well-developed host–parasite relationship. This stercorarian species is the largest trypanosome of cattle, and bloodstream forms reach a length of 100 μm. This stage is characterized by a long, thin, pointed posterior end, a long, free flagellum and a prominent undulating membrane. The large kinetoplast lies far from the posterior extremity, often in a marginal position. The nucleus is found midway along the body. However, these parasites are infrequently seen since infections are characterized by low parasitaemia. Within the bovine, *T. theileri* multiplies by binary fission in the tissues, mainly in the epimastigote form. When multiplying parasites are found in the bloodstream, polymorphic 'immature' forms are present.

When *T. theileri* is ingested by tabanid flies, a cycle of development occurs in the hindgut and infective metatrypanosomes are voided with faecal material. Several species of horseflies have been incriminated in the transmission of *T. theileri* (**see Chapter 4: Vectors: Tabanidae**), which is mainly accomplished as metatrypanosomes penetrate intact oral mucosa.[27] This occurs as cattle use their tongues to fend off biting tabanids and simultaneously ingest their faecal material. The contamination of broken skin with metatrypanosomes of *T. theileri* is a less important route of entry. The prepatent period ranges from four to six days,[27] but detectable parasitaemia is short-lived and is not accompanied by clinical signs although infrequent reports incriminating *T. theileri* as a pathogen have been made.[280] In the latter, concomitant diseases usually existed and probably accounted for clinical signs, reduced host resistance and a consequent rise in *T. theileri* parasitaemia.

Similar events occur in many wild animals which harbour tsetse-transmitted trypanosomes. Infected animals show no clinical signs,[202] but when they are subjected to the stress of capture, for example, their immunity is reduced, parasitaemia flares up and clinical disease may be precipitated. The equilibrium can be restored by reducing the stress factors and by providing trypanocidal treatment. Losses in captured wild animals due to trypanosomosis may be reduced by providing trypanocidal treatment at the time of capture.

Epidemiology

The epidemiology of African trypanosomosis is almost entirely dependent on tsetse flies.[136] African trypanosomes are well-adapted parasites of many species of wild animals, and sylvatic cycles of trypanosome transmission occur throughout the 10 million square kilometres infested by this unique vector. The distribution of tsetse in southern Africa is described in **Chapter 2: Vectors: Tsetse flies**, but within the general ecological limits of distribution the problem of trypanosomosis is not static. The 1990 outbreak of bovine trypanosomosis in the KwaZulu-Natal Province of South Africa[28] testifies to the dynamic nature of the problem.

The natural hosts of salivarian trypanosomes usually show no clinical signs of infection, host and parasites being in equilibrium.[202] The large numbers of naturally infected wild animal hosts constitute a huge reservoir of trypanosomes. Once infected, tsetse remain so for life and thus they too form a reservoir of infection. Consequently, when domestic animals are introduced into areas in which sylvatic cycles of trypanosome transmission occur, trypanosomosis always emerges as a serious disease.[175] Wild animals are the natural hosts of *T. brucei rhodesiense*, the aetiological agent of human sleeping sickness in central,

eastern and southern Africa. Thus, people living and working in tsetse areas are at risk of contracting the disease, but for animal trypanosomosis to occur it is not always necessary for livestock to enter tsetse-infested areas; tsetse also move. The seasonal dispersal of fly, during the single rainy season in southern Africa, frequently results in an increased seasonal disease risk to livestock kept some distance from the primary tsetse habitat.[292]

Changes in land use may also alter the extent of tsetse infestation (**see Chapter 2: Vectors: Tsetse flies**). The abandonment of cultivation, for various reasons, permits the regrowth of vegetation, which may then provide suitable tsetse habitats. Conversely, the intensive settlement and cultivation seen in some areas (throughout Malawi,[55, 292] for example) destroy tsetse habitats. Although removal of tsetse should also remove the problem of trypanosomosis, livestock may have to graze some distance away from cultivated areas, along the edges of forest reserves, and may therefore still be exposed to tsetse fly challenge.

The epidemiology of tsetse-transmitted trypanosomosis is complicated and is well reviewed.[174, 175, 254, 255, 324] Whiteside[321] identified at least 18 major variables in the epidemiology of African animal trypanosomosis, which relate to the interactions of tsetse, wild hosts, livestock and their management, the trypanosomes and climatic conditions. The trypanosomal infection rate in tsetse is of prime importance. The ease with which infections develop in tsetse depends upon the fly's vectorial capacity and specific factors related to the blood of host animals. However, within a particular tsetse population, the prevalence of metacyclic infections can vary significantly. For example, a recent study conducted in Zambia showed that the monthly total prevalence of mature trypanosomal infections in *G. pallidipes* could vary from 3,2 to 16 per cent[330] and is determined by factors such as the age of the tsetse population and the prevalence of trypanosomal infections in the host.

Tsetse can only transmit metacyclic trypanosomes if the flies live longer than the duration of the developmental cycle of a particular trypanosome species. The developmental period of trypanosomes varies and is parasite species-specific although a high degree of variability within a species has been observed.[53] Generally speaking, the duration of the development of trypanosomes in tsetse increases with increasing complexity of the developmental cycle. The relatively simple cycle that *T. vivax* undergoes may last only for five days, compared with the 17 to 45 days required for the completion of *T. brucei's* complicated cycle. Hence, the proportion of *T. vivax* infections in tsetse increases under adverse ecological conditions, when the fly's shortened lifespan prevents the completion of longer cycles. Such adverse conditions often occur at the edge of fly-belts and result in the predominance of *T. vivax* infections in cattle.[85, 159] A high proportion of *T. vivax* infections in cattle is, for example, observed at the edge of the fly-belts in western Zambia[48] and in the Lianshulu/Mamilli area of Namibia's eastern Caprivi.[295]

The prevalence of metacyclic infections in field-caught tsetse increases with the age of flies.[107, 158, 257, 260, 331, 333] In the case of *T. vivax*, which does not pass through the tsetse's midgut, the rate of acquisition of new infections with increasing age is often constant.[332] Up to 100 per cent infection rates with *T. vivax* can occur in tsetse that repeatedly feed on *T. vivax*-infected hosts.[39] For those trypanosome species that go through a procyclic stage in the midgut of tsetse (*T. congolense* and *T. brucei*), the relationship between age and infection is more complex and is affected by the reduced susceptibility of tsetse to infection as they age.

The prevalence of trypanosomal infections in tsetse is also affected by host preference. Two aspects are important in this context: firstly, there is a diversity of host preference among *Glossina* and, secondly, there is a variation among different species of hosts in their susceptibility to infection with the different species of trypanosomes.[111] For example, *G. austeni* in Uganda, which has a preference for feeding on suids, has a low *T. vivax* infection rate, since pigs are refractory to infection with this species of trypanosome. In contrast, *G. palpalis*, which shows a preference for bovid blood meals, has been found to have considerably higher *T. vivax* infection rates.[195] Tsetse species are often grouped according to those that feed mainly on suids, bovids, suids and bovids, and lastly, those that feed on most available hosts, including humans.[307] However, host preference is not a rigid behavioural characteristic of a particular tsetse species.[29, 40, 135, 193, 249] The relative abundance of a host species may contribute to observed host preference. For example, within a country a tsetse species may feed mainly on suids in one locality and on bovids in another. This is the case in the Eastern Province of Zambia: on the plateau *G. m. morsitans* takes 75 per cent of its feeds on cattle,[296] in the adjacent Luangwa Valley the same species takes most of its feeds from suids.[264]

An animal entering a tsetse-infested area risks becoming infected with potentially pathogenic trypanosomes that may produce disease. The degree of risk depends largely on the 'challenge'. Although there is no adequate definition of 'challenge',[255] it is generally regarded as reflecting the number of infective bites that an animal receives in a given time. However, true challenge, or risk, is determined by the interaction of the number of infective tsetse bites, host preference, host susceptibility and the virulence of the parasite. Of particular importance is the relationship between absolute tsetse density and biting rate. A good understanding of this relationship is essential when predicting the impact of tsetse control interventions. Usually the challenge increases with the tsetse population density but even at low densities tsetse can still cause a substantial disease problem. This is partly attributed to the often observed, increased frequency with which flies that have metacyclic infections in their mouthparts probe.[133, 197, 247]

There are various reasons why a particular animal species may be subjected to greater challenge than another. The behaviour of a potential host can influence the ease with which a tsetse can engorge and this may contribute to observed preference. In Zimbabwe, the results of one study showed that the frequency with which blood meals were taken from cattle, sheep and goats by *G. morsitans* and *G. pallidipes* was 500:5:1 respectively. Observations on the relative attractiveness of these host species showed that the ratio of 'probes:successful feeds' was 3,8:1 for cattle, 28,0:1 for sheep, and 63,2:1 for goats respectively.[243] Thus goats are attractive to tsetse but in response to the flies' attempts to feed, they are vigorously defensive; they shake their heads, wrinkle their skin, stamp their feet and even attempt to bite the flies! Nevertheless, goats and sheep do acquire trypanosomal infections under natural conditions.[21, 102, 271] Impala (*Aepyceros melampus*) behave similarly when tsetse attempt to feed. It can be surmised that sick animals, which are less vigorous, are at greater risk of being fed upon by tsetse. This may explain the increased feeding success of *G. pallidipes* on *T. congolense*-infected oxen.[18, 19]

Management practices may also alter the challenge to which livestock are subjected, and in this sense management is central to the epidemiology of trypanosomosis. The different management of calves and adult cattle can significantly reduce the level of challenge to which young animals are subjected.[52] Observations on the grazing ranges of livestock in West Africa showed that while cattle foraged widely in tsetse-infested habitats, sheep, goats and donkeys remained closer to the villages. As a result, small ruminants and equids were less exposed to attack by tsetse than cattle.[267] In southern Africa, the grazing patterns of communal cattle vary from season to season.[261] In the cropping season (November to March) cattle are penned and herded away from the crops. In the early dry season (April to July), after the crops have been harvested, cattle are allowed to roam freely; they feed unattended mainly on crop residues. In the late dry season (August to October), the cattle have to move further afield to find grazing and browse, often entering tsetse-infested areas. Furthermore, the movement of animals from tsetse-free pastures to tsetse-infested watering points can create temporary challenge. Sedentary animals may face a seasonal increase in tsetse challenge as flies disperse during the rainy season. Even within the vicinity of a village, the actual challenge can be quite variable and can lead to differences in herd infection rates within a small area.[302] Consequently, a thorough knowledge of local management practices is important to understand the epidemiology of nagana in a particular area.

Management also determines the general well-being of livestock. If, for example, animals are heat-stressed and their feed intake is therefore reduced, their resistance to trypanosomosis is also reduced, increasing the incidence of the disease.[54] A careful distinction has to be made between an occurrence of this kind and increased challenge. A major influence on the epidemiology of trypanosomosis is the use of trypanocidal drugs. Whilst permitting the use of tsetse-infested land, chemotherapy may alter the prevalence of trypanosome species in the area. In Zimbabwe, it was reported that the use of diminazene increased the prevalence of *T. vivax* in cattle.[32] Furthermore, the repeated use of trypanocides may result in the emergence of drug-resistant strains of trypanosomes, and the epidemiological picture then changes.[259]

Wild animal hosts of tsetse, such as kudu (*Tragelaphus strepsiceros*), warthog (*Phacochoerus aethiopicus*), bushbuck (*Tragelaphus scriptus*), bushpig (*Potamochoerus porcus*), African buffalo (*Syncerus caffer*), African elephant (*Loxodonta africana*) and square-lipped and hooked-lipped rhinoceroses (*Ceratotherium simum* and *Diceros bicornis* respectively), are vital to sylvatic cycles of trypanosome transmission and they form a major reservoir of infection. The increasing popularity of game ranching or farming frequently entails the translocation of these animals from tsetse habitats to tsetse-free areas. As a result, clinical disease may occur in animals normally regarded as 'immune', in the absence of the tsetse vector. There is also the possibility that trypanosomes could be mechanically transmitted from these species to livestock.

Whilst the bite of an infective tsetse clearly represents a trypanosomosis risk, infection does not always become established. Furthermore, even when an infection is established, disease does not always ensue. A variety of host-associated properties modifies the outcome and further complicates the epidemiology of trypanosomosis. Estimates of the efficiency of transmission to susceptible cattle vary widely but field investigations suggest that it is very low (2,5 per cent).[76, 191, 257]

Wild animal hosts of tsetse and certain West African taurine cattle are tolerant of tsetse-transmitted trypanosomes. Carefully controlled experiments have shown that the trypanotolerance of N'Dama cattle and African buffalo is an innate characteristic.[2, 106, 196, 208] This trait also occurs in some breeds of sheep and goats.[102, 123, 217] Trypanotolerance is a much studied phenomenon.[124, 198, 207, 210, 211, 252] Although the underlying mechanisms are incompletely understood, it is generally accepted that innate and acquired resistance, as well as environmental and management factors, affect trypanotolerance. Lower trypanosomal parasitaemias and less severe anaemia occur in trypanotolerant livestock than in trypanosusceptible animals.[66] The epidemiological significance of this trait lies in the lower morbidity and mortality due to trypanosomosis in tolerant breeds. Nevertheless, even within a generally tolerant breed some individuals are less tolerant than others[253] and tolerance may be affected by the degree of challenge.[67] Similarly, within susceptible breeds individuals can be found that are apparently less susceptible to the effects of trypanosomal infection than most other animals.[50, 217, 231] Although trypanosome-infected animals

may effect self-cure, the usual sequel to infection in tolerant animals is the establishment of a balance between host and parasite. If the host is stressed, the equilibrium is disturbed and a clinical episode of variable severity is precipitated. Stress takes many forms. Animals in late pregnancy or that are lactating are more susceptible to trypanosomosis.[175, 208, 233] Overwork also constitutes a stress, which, in infected trypanotolerant stock, may precipitate disease.[175] This has serious consequences for animal traction and for hard-working bulls used in restricted breeding seasons in tsetse-infested areas. In a similar manner, intercurrent disease is stressful; trypanosome-infected animals with helminthosis or other diseases are more severely affected than those with either disease alone.[104, 118, 175, 271]

Adequate nutrition enhances the ability of infected animals to withstand the adverse effects of a trypanosomal infection.[145] Hence, trypanotolerance is greatly reduced by a low plane of nutrition, such as that available during the dry season.[175] Inadequate nutrition results in generally lower average herd haematocrits, at all levels of disease prevalence. In the dry season, animals may also have to trek long distances to watering points. The combined effect of poor nutrition and increased exercise is often associated with an increased incidence of trypanosomosis in the dry season. This is not necessarily due to an increase in trypanosome transmission at this time of the year but could be explained by higher parasitaemias in animals with reduced tolerance. However, dry season grazing areas or watering points are often located in tsetse-infested areas, in which case the actual incidence can rise. Similarly, such a rise of incidence has been observed when cattle graze beyond the boundaries of a tsetse control area during the dry season.[291]

Age also has a significant effect on resistance to trypanosomosis.[211] It is widely recognized that cattle born in an infested area do not immediately succumb to disease, even though they acquire trypanosomal infections when young, whereas cattle brought into the area readily succumb. Three issues arise from this observation: firstly, young animals may be protected by maternally derived antibodies;[175, 211, 312] secondly, the ability of animals to acquire protective immunity is implied; and, lastly, young animals may be less attractive to tsetse and, thus, face a reduced challenge.

Despite the antigenic complexity of trypanosomes, infected animals do mount an immune response which, especially when supported by chemotherapy, can confer specific protection against homologous serodemes.[97, 207, 329] Thus, within a defined area, animals may acquire protective immunity against locally prevalent serodemes. However, the movement of these animals to another area may expose them to different strains, or serodemes, to which they may succumb.[175, 211] This type of tolerance requires continuous challenge and explains the low mortality of cattle in high challenge, endemic areas (such as the Eastern Province of Zambia) where trypanocidal drugs are used to prevent animals from succumbing to nagana. The introduction of tsetse control measures in such areas would result in a loss of immunity that would render the population highly susceptible to infection and disease. If tsetse control measures break down, severe outbreaks of trypanosomosis would ensue, probably with high mortality, as happened following the disruption of tsetse control during Zimbabwe's liberation war.[156]

Of great significance to the epidemiology of the disease is the nature of the parasite. Not all host species are equally susceptible to infection with each species of trypanosome (**Table 12.1**). Whereas *T. vivax* is a serious pathogen of cattle, pigs are highly resistant and, consequently, *T. vivax* infections are not seen in pigs. Conversely, *T. simiae* causes peracute and rapidly fatal disease in pigs, whereas cattle are apparently refractory to infection with this parasite.[111] Within a trypanosome species are strains of differing virulence. Usually, *T. vivax* infections in domestic ruminants are prolonged with long aparasitaemic intervals. Occasionally, however, an acute haemorrhagic syndrome is seen in cattle infected with some strains of *T. vivax*.[33, 179, 310] Infection with a single species of trypanosome can, therefore, produce quite different signs in the same host species.

An interference phenomenon also occurs, which either delays or prevents the establishment of an antigenically distinct trypanosome in animals with an existing trypanosomal infection.[69, 70, 200] The mechanism of this interference remains unknown, but if the phenomenon occurs under natural conditions it would be epidemiologically important, since it could limit the number of infections to which an animal is subjected. However, by testing bovine sera with the highly sensitive polymerase chain reaction (PCR) technique it has been found that mixed infections are far more prevalent than had previously been appreciated.[245]

The occurrence of trypanosomal infections in areas apparently free of tsetse promoted the theory that infections can be maintained in nature by the mechanical transmission of trypanosomes by other haematophagous flies. In Malawi, a firm opinion developed that tsetse-transmitted trypanosomosis only occurred in close proximity to wildlife sanctuaries, where the flies were readily caught. Elsewhere, mechanical transmission alone was believed to occur.[8] It is only relatively recently, with improved trapping methods that use attractant odours, that it has been possible to reveal the presence of *Glossina* at low population densities, which were hitherto undetectable. The recent discovery of *G. pallidipes* by these methods in the areas of Malawi thought to be free of tsetse[55] may explain that part of the epidemiology of the disease in which mechanical transmission was thought to play a role.

Trypanosoma vivax has become established outside Africa, and especially in South America and Mauritius. In South America it is believed to be mechanically transmitted.[315] The cyclical development of *T. vivax* is confined to the mouthparts of tsetse flies and there is limited migration.

The close similarity of this mode of transmission to non-cyclical, mechanical transmission of *T. vivax* on the contaminated mouthparts of other haematophagous flies is widely assumed to have enabled this species of trypanosome to adapt readily to acyclical transmission. However, the possibility that cyclical development might occur in a vector, or vectors, other than *Glossina* spp., has not been completely eliminated. Nevertheless, there is some evidence that mechanical transmission of *T. vivax* can occur in areas adjacent to tsetse-infested areas. Reports of fulminating *T. vivax* infections which spread rapidly in the apparent absence of tsetse, leave little doubt that transmission can be mechanical,[46, 251] and the rapid spread of *T. simiae* within a piggery is believed to be due to mechanical transmission.

A wide variety of factors are involved in the epidemiology of trypanosomosis. It is their interaction which determines the clinical picture in an area, and, even though mathematical models have been developed to quantify the relative importance of these variables,[256] much remains to be clarified and the models have yet to be applied to the field.

Pathogenesis

The precise pathogenesis of the trypanosomoses remains far from clear. Reviews of the subject[164, 165, 274] highlight the complexity of this group of diseases: the various species of hosts involved differ in their susceptibility; there is great diversity among parasites within a species; and it is difficult to extrapolate the results obtained from experimental infections in rodents, ruminants and other animals, to explain the pathogenesis of natural infection.[274] Three features — anaemia, tissue damage, and the suppression of immune responses[205, 289] — dominate the pathology of trypanosomosis, and research aimed at elucidating the pathogenesis of the disease has largely addressed these areas.

Infection becomes established at the site of inoculation of metacyclic trypanosomes in the skin, where a chancre may form. Multiplication of the parasites induces an inflammatory response, the severity of which depends upon the size of the inoculum, the species of parasite and the breed and species of the host. In cattle, *T. congolense* is located in dilated lymphatics of the papillary dermis and hypodermis, in which the parasites form flagellar attachments to the endothelial cells.[3] Dead and dying parasites are also present in the lesion, and various proteins and peptides have been isolated from the chancre. These may originate from either the host or the parasites.[124] The chancre reaches a maximum diameter of some 100 mm, 10 to 14 days after an infective tsetse fly has fed, its development preceding invasion of the bloodstream by trypanosomes, and is accompanied by enlargement of the draining lymph nodes.[3, 72, 79]

Trypanosomes are detectable in the blood 13 to 16 days after an infective tsetse fly has fed.[170] At this time the chancre begins to regress, and the characteristic series of intermittent parasitaemias begins. Parasitaemia rises and is accompanied by a febrile response, which is then followed by an aparasitaemic, afebrile period. The time between parasitaemic peaks varies from four to seven days in goats with *T. vivax* infections,[183] to 12 days in cattle infected with *T. congolense*.[82] In long-standing infections parasites may not be detected for months, and when parasitaemia does occur, it is often low grade and the animal is afebrile.

After an infection has become established, a protracted battle ensues as the parasite provokes an immune response, only to evade its full effect. Tightly clad in the thick, disposable protective glycoprotein coat, the trypanosome population is assured of continued survival in the face of the host's vigorous defence. Anti-VSG antibodies destroy large numbers of trypanosomes, and dead and dying parasites and host cells are found extracellularly. These are removed by phagocytes and presented as antigenic evidence to cells of the lymphoid series. In the course of the contest, many harmful substances are released from both intact and damaged parasites and host cells. The destruction of large numbers of parasites releases lysosomal and other enzymes as well as structural proteins. Some of these enzymes have been identified and are thought to be directly harmful to the host. Many biological mediators, such as the vasoactive amines, are released from activated or damaged host cells. Activated macrophages release powerful cytokines,[23] including interleukin-1 and cachectin (tumour necrosis factor), but their roles in the pathogenesis of trypanosomosis have yet to be investigated fully.

Much work has concentrated on comparisons of the responses of trypanotolerant and trypanosusceptible animals to infection. There is clear evidence that tolerant animals achieve better control of parasitaemia than do susceptible animals, and this ability is correlated with less severe anaemia and less severe disease.[66] Anaemia is a cardinal sign of trypanosomosis in many domestic animals, and the aetiology is probably similar in all species. There is no single cause of the anaemia in trypanosomosis; the pathogenesis is complex and involves a variety of mechanisms, some of which are better understood than others.

Comprehensive reviews of anaemia in trypanosomosis[209, 276] form the basis of the following account.

Trypanosomes release haemolysins and enzymes (proteases, phospholipases and neuraminidases) which directly damage red blood cell membranes. The fragility of erythrocytes is also increased by fever, and affected cells have a shortened lifespan. Erythrocytes are also damaged by trypanosomal antigens, which adhere to the red blood cell surface labelling it for removal. The attachment of antigen-antibody complexes to red blood cell membranes also results in damage. Complement can be directly activated by trypanosomal VSG as well as being activated by antigen-antibody complexes. The attachment of components of complement or complexed complement to erythrocytes produces damage and promotes erythrophagocytosis. Anaemia occurs largely because damaged erythrocytes are

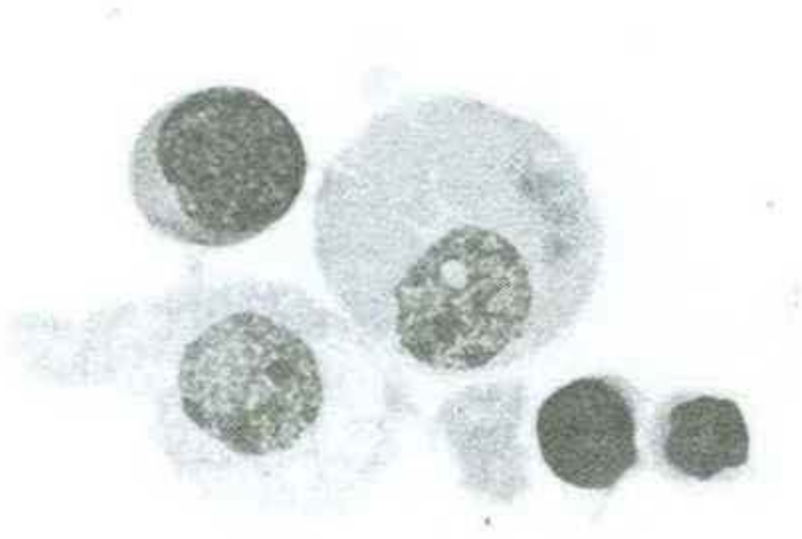


Figure 12.7 Bone marrow impression smear from a calf with an acute *Trypanosoma vivax* infection, showing a macrophage which has phagocytosed several erythrocytes x1 200. Stained with Wrights-Leishman. (Unpublished photomicrograph by courtesy of Dr L. Logan-Henfrey, International Laboratory for Research on Animal Diseases, PO Box 30709, Nairobi, Kenya)

removed from the circulation by cells of the mononuclear phagocytic system (MPS) in the spleen, bone marrow (**Figure 12.7**), lungs and haemal lymph nodes. To deal with the abundance of cellular debris and antigen-antibody complexes the MPS becomes greatly hyperplastic, which further enhances erythrophagocytosis. Intravascular haemolysis is thus not a prominent feature of trypanosomosis. A haemorrhagic syndrome is sometimes seen in acute *T. vivax* infections of cattle, in which sudden and severe thrombocytopenia occurs. This is associated with fulminating parasitaemia and extensive petechial and ecchymotic haemorrhages. The thrombocytopenia and resultant clotting defects, as well as the presence of circulating immune complexes, damage the capillary endothelium, leading to disseminated intravascular coagulation.

The removal of large numbers of red blood cells from the circulation occurs soon after the onset of parasitaemia, and produces a fall in the packed cell volume (PCV) — haematocrit. Packed cell volume is a reliable indicator of anaemia and is correlated with parasitaemia — the higher the parasitaemias, the lower the PCVs.

Accompanying anaemia is leukopenia, which possibly arises from direct inhibition of stem cell differentiation. Although there is an erythropoietic response to anaemia early in the course of infection, this seems to be impaired. Whereas anaemia is largely attributable to an increased rate of erythrophagocytosis early in infection, the anaemia of the late phase of infection has a different pathogenesis.

There are several possible sequelae to the early phase of infection, which depend largely on the nature of the parasite and the susceptibility of the animal. There may be spontaneous recovery or death, but very often there is a chronic phase which is characterized by infrequent, low-grade parasitaemias. Animals lose weight and condition and, as a result of dyshaemopoiesis, remain anaemic. Extensive haemosiderosis occurs as a result of erythrophagocytosis, and the trapping of iron in phagocytes is believed to contribute to the failure of erythropoiesis. Despite the apparent absence of parasites in the circulation, red blood cell destruction continues, and insufficient erythropoietic compensation results in persistent anaemia.

Trypanosomal parasitaemia correlates with complement activity in cattle. In the sera of N'Dama (trypanotolerant) cattle which develop lower parasitaemias than Boran cattle, complement activity is higher than in the sera of Borans infected with the same serodeme.[125] Hypocomplementaemia is a frequent feature of the disease, with levels of component C3 being markedly reduced; this may be associated with reduced resistance to other infections.[277] Various changes have been noted in the composition of leukocyte populations during the course of infection, but beyond the usual occurrence of leukopenia, other reported changes are equivocal.

The suppressive effect of trypanosomal infections on the immune responses of laboratory animals has often been demonstrated experimentally, but its significance in livestock has not been determined. Trypanosome-infected animals do mount protective, antibody-mediated immune responses against the parasites,[198, 290] and both IgG and IgM are produced. The IgM levels are consistently elevated during infection and appear to be directed mainly against VSG antigens. Whilst some IgGs are directed against the common, somatic trypanosomal antigens, others appear to be directed against some of the host's own cells. Anti-erythrocyte antibodies contribute to the anaemia of trypanosomosis and it is possible that a similar mechanism operates against leukocytes, contributing to leukopenia.[209]

Some animals are able to control parasitaemia quite effectively, and this appears to be antibody mediated, at least in part. Trypanotolerant Baoule cattle mount earlier and greater antibody responses to the first parasitaemic peak of *T. congolense*, compared with similarly infected Zebu cattle.[2, 244] This ability to control the first parasitaemic peak is associated with the less severe anaemia occurring in trypanotolerant animals.

The antibody responses of trypanosome-infected cattle, sheep and goats to non-trypanosomal antigens are, nevertheless, depressed.[173] The results of one investigation revealed that *T. congolense*-infected cattle produced poorer anamnestic responses to secondary vaccination with a polyvalent clostridial vaccine than did uninfected control cattle.[112] On the other hand, cattle experimentally infected with *T. congolense* and vaccinated against foot-and-mouth disease were considered to have developed protective antibody titres.[266] However, the effect of trypanosome-induced immunosuppression superimposed on the stresses of malnutrition, trekking, pregnancy or lactation may explain why affected animals frequently succumb to other disorders. The

precise aetiology of immunosuppression in trypanosomosis is obscure. It may involve B-cell mitogens, factors which block the release of antibody from plasma cells, reduced T-helper cell function, and depletion of lymphoid elements in the spleen and lymph nodes.[290] Particles from dead and dying trypanosomes stimulate the MPS, and the cytokines released by cells of the MPS may contribute to the immunosuppression associated with the disease, as may products released by disintegrating trypanosomes.[12, 290]

There is a growing appreciation of the close functional relationship between the immunological apparatus and the endocrine system of the hypothalamo–pituitary–adrenal axis.[17] Both systems are characterized by delicately balanced regulatory mechanisms, mediated by potent cytokines which have short half-lives. Interleukin-1 is a polypeptide released by macrophages and has a wide range of effects on many tissues. As well as stimulating T cells to produce interleukin-2, it is a potent pyrogen, stimulating the hypothalamus, the pituitary and the adrenal glands. Locally, interleukin-1 increases vascular permeability and is involved in the acute phase changes of the inflammatory process. The effects of trypanosomosis on the endocrine system have recently been demonstrated in cattle and goats.[94] Trypanosome-infected animals show abnormalities of the thyroid gland, ovaries, testes, adrenal glands and pituitary. To what extent the changes are mediated by the direct action of the parasite, the host's immune response or by imbalances in the endocrine system, remains to be determined precisely.

A parasite peptidase has been demonstrated in the plasma of *T. congolense*-infected heifers.[151] The possibility exists that this enzyme might inactivate certain hormones or interfere with the host's enzymes that are responsible for endocrine regulation. Such disturbance of the homeostatic mechanism would have serious consequences. In infected cattle, although levels of adreno-corticotrophic hormone are similar to those in uninfected controls, cortisol levels are lower, and this might indicate adrenal dysfunction or a decreased half-life of cortisol.[125] The interpretation of research findings from laboratory-housed animals should always be made bearing in mind that chronic infections in stressed animals are commonly encountered in the field.

The pathogenesis of tissue lesions varies with the species of trypanosome. *Trypanosoma congolense* and *T. vivax* are mainly intravascular parasites: they induce changes in the endothelium of capillaries, and so indirectly cause damage to adjacent tissues. *Trypanosoma brucei*, on the other hand, has an affinity for tissues. Its presence in the extravascular compartment is associated with marked lesions in parasitized tissues.

The severity of endothelial injury also depends on the interaction of host and parasite. *Trypanosoma congolense* often attaches to erythrocytes and capillary endothelium. Damage to endothelial cells by parasite products, immune complexes, vasoactive amines and cytokines increases vascular permeability. In *T. congolense* infections a generalized dilatation of capillary beds, which alters the haemodynamics, is observed.[164] The concomitant anaemia and more sluggish tissue perfusion affect the exchange of metabolites and are associated with intracellular oedema of capillary endothelial cells.[221] Fibrinous microthrombi form in response to endothelial damage. These changes can be prominent in *T. vivax* infections, with which disseminated intravascular coagulation is more commonly associated.

Alterations to the microcirculation produce secondary degenerative changes in tissues. As capillary permeability increases, phagocytes and products of the parasite extravasate more readily and are responsible, in part, for some of the tissue lesions. Cytokines and parasite-derived substances, such as proteases, may directly injure host cells, an effect that is exacerbated by the formation of immune complexes in tissues. In the case of *T. brucei*, parasites localized in tissues cause mechanical disruption of host cells and probably also have some direct toxic effect on host cells, possibly mediated by their enzymes. The cellular response provoked by parasites within the tissues causes further damage, and auto-antibodies are also thought to play a role in inducing lesions.

The influence of all of these changes on the course of the disease depends upon their severity and upon the degree of impairment of the affected organs. An important feature of the pathogenesis of trypanosomosis is the effect on lymphoid tissue. As the disease progresses, the hyperplastic MPS grossly increases the volume of tissue in the spleen, lymph nodes and bone marrow. This hyperplasia of reticuloendothelial cells reduces lymphoid cell density, and eventual lymphoid depletion can occur.[297]

Chronic disease is associated with progressive emaciation and eventually cachexia. This is usually accompanied by low levels of parasitaemia, but the pathogenesis is poorly understood. The reduced lifespan of red blood cells, the increased catabolism of many proteins,[277] and the associated negative nitrogen balance of clinically affected animals would appear to be largely responsible for the pitifully thin condition of animals suffering from chronic trypanosomosis.

Clinical signs, pathology and diagnosis in cattle

African trypanosomosis is transmitted by tsetse flies and is primarily caused in cattle by *T. congolense* and *T. vivax*. In endemic areas, the disease causes economic losses, but it can also occur in areas remote from tsetse infestations, as a result of the movement of previously infected animals which, when stressed, show clinical signs of trypanosomosis. Additionally, small numbers of infected tsetse flies, carried long distances by vehicles, can transmit trypanosomes in areas otherwise free of trypanosomosis. Recognition of the wide range of clinical signs associated with the disease is thus important, both within and outside endemic areas.

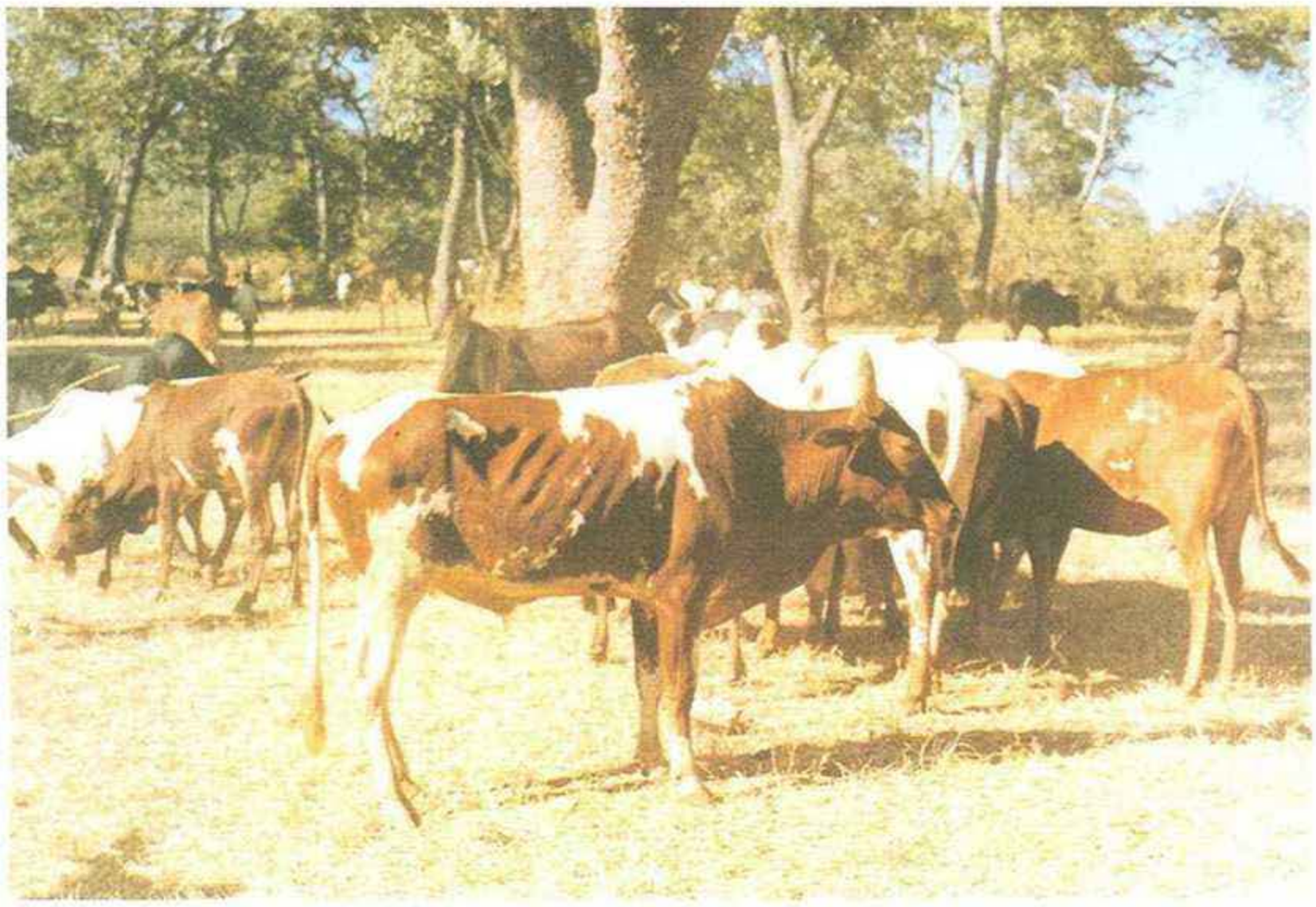


Figure 12.8 Subacute trypanosomosis in an ox: the animal has lost weight and condition and it is dejected, with drooping ears and flaccid tail. (Reprinted by kind permission of FGU Consulting and Engineering GmbH, Königstein, Germany, and the Regional Coordinator, RTTCP, Harare, Zimbabwe)

Cattle living in tsetse-infested areas frequently have mixed trypanosomal infections which cause more severe disease than infections with a single species of trypanosome. Generally, in eastern and southern Africa, *T. congolense* is more pathogenic to cattle than *T. vivax*, and produces serious disease, whereas *T. brucei* is widely regarded as being of minor clinical significance.

Clinical signs

The course of disease due to infection with salivarian trypanosomes is variable and there are no clinical signs specific to bovine trypanosomosis. The manifestations of disease depend upon the degree of damage to specific organs and upon the degree of anaemia. Trypanosomosis in cattle may be acute, subacute or chronic; acute disease may be fatal after brief illness lasting two to six weeks, but chronic disease lasting many months or even years is more common.

Chancres are rarely seen in cattle with naturally acquired infections. The first signs of disease are due to the fever which accompanies the onset of parasitaemia. As parasitaemia falls, so does fever; the course of infection is therefore characterized by fluctuating parasitaemia and parallel fluctuations in body temperature. Intermittent parasitaemia and malaise are followed by increasingly severe clinical signs.

Acute trypanosomosis causes sudden reduction of milk yields and also abortion.[116] Oxen, bulls and young stock with acute infections are reported to be suddenly 'off-colour'. They are generally in good bodily condition but are dejected, their ears droop and they may walk stiffly. If animals are febrile at the time of examination, rectal temperatures may reach 39 °C in *T. congolense* infections, whilst in acute *T. vivax* infections they may be as high as 41 °C. Pulse and respiratory rates are raised in febrile animals and there may be piloerection. Conjunctival mucosae may be congested early in the acute phase, but later, as anaemia develops, pallor of the mucous membranes is evident.

Acutely affected animals quickly lose weight and body condition, although they continue to eat. They become weak, dejected and lethargic, often standing alone, away from the rest of the herd, not seeking shade. Excessive lachrymation is sometimes seen at this stage. After a short illness some acutely affected cattle become recumbent for a few days before death occurs. Corneal opacity of varying severity has been reported in some *T. brucei* and *T. vivax* infections.[274] A proportion of acutely affected cattle gradually improve to enter the chronic phase of the disease. Occasionally, acute *T. vivax* infections induce a haemorrhagic syndrome,[88] when animals bleed spontaneously from the ears, nose, mouth,

anus and skin. Petechiae and ecchymoses may also be evident in the conjunctival mucosa.[33, 179, 310]

In contrast with the acute disease, subacute trypanosomosis is more common and runs a more prolonged course, with many animals apparently making a spontaneous recovery. However, they remain infected and frequently suffer from chronic trypanosomosis. In subacute cases (**Figure 12.8**), animals are intermittently 'off-colour', becoming weak and dejected, with drooping ears and flaccid tail. Within a few weeks they lose weight and condition, the coat becomes dull and a marked jugular pulse develops. On closer examination, although intermittent fever occurs, it is rarely marked; mucous membranes are pale, and pulse and respiratory rates are slightly raised. Superficial lymph nodes and haemal lymph nodes are frequently enlarged and are readily visible. Gradually, the frequency and intensity of parasitaemia decrease and fever subsides. Dependent oedema, particularly in the submandibular region, may develop. Thin, rough-coated, anaemic, lethargic cattle with generalized lymph node enlargement are said to have a 'fly-struck' appearance. Death may occur between four and six months after the onset of disease, but many animals make a gradual recovery, which is assisted by a good plane of nutrition.[87]

Chronic bovine trypanosomosis is by far the most common form of the disease in endemic areas. It may arise either as a result of partial recovery from acute or subacute disease, or from an initial subclinical or mild infection. Severely affected cattle may be extremely emaciated, having lost a large proportion of their muscle mass; they are often just 'skin and bone'. Their general condition is very poor — the hair is sparse and the coat is rough, dull and staring. The skin is dry and scaly, and in long-standing infections the hair of the tail switch may be completely absent. The precrural lymph nodes and haemal lymph nodes are usually visible from a distance, and there is a marked jugular pulse. Although chronically affected animals continue to eat, they are weak and lethargic and are unable to keep up with the rest of the herd. Geophagia has been associated with bovine trypanosomosis, mainly in West Africa,[108] but careful anamnesis often reveals that cattle with chronic trypanosomosis in other parts of Africa also have a tendency to eat sand or lick anthills. The chronic syndrome is characterized by infrequent, scanty parasitaemias, which are not accompanied by fever. Anaemia persists and is usually severe. Mucous membranes are very pale, the pulse is rapid, and breathing is laboured; in resisting restraint, the animal may collapse in respiratory distress after brief exertion. The combined effect of anaemia, circulatory disturbance and myocardial damage frequently produces acute cardiac decompensation which leads to sudden death from congestive heart failure.

Although *T. brucei* normally produces mild or subclinical disease in cattle, signs of cerebral trypanosomosis arising from natural *T. brucei* infections have been described.[313] One affected animal was described as a 'loner' — it would stray from the herd and become lost. This weak, emaciated animal displayed periodic generalized tremor, whereas another similarly affected animal in good condition showed hyperaesthesia and agitation.

Whilst for descriptive purposes it is convenient to classify trypanosomosis as being acute, subacute or chronic, the course that an infection actually follows is modified by many factors. A subclinically affected animal may, when stressed, suffer an acute clinical episode, while acutely affected animals may make a gradual recovery only to remain chronically affected. Yet other animals may effect an apparent self-cure, whilst continuing to harbour trypanosomes. The occurrence of concomitant disease frequently masks the underlying trypanosomal infection, but on occasions may exacerbate the clinical signs of trypanosomosis. The immunosuppression induced by trypanosomosis renders infected stock more susceptible to other diseases. In many cases, the underlying cause of disease or death may be concealed by the presence of the more readily detectable, secondary infection, such as anaplasmosis, helminthosis or lumpy skin disease. The consequence of this is that the true extent of trypanosomosis is often underestimated.

In endemic areas, trypanosomosis in cattle is a herd problem. The general condition of the herd is poor, although some individual animals are in surprisingly good condition. Trypanosomosis causes serious reduction in productivity, and careful analysis of records reveals its impact on fertility, which is reduced in both males and females.[121, 131, 236] In addition to their being generally weak, the semen quality of affected bulls is reduced.[265] Infected cows lose weight and their oestrus cycles become irregular and may cease altogether.[162] This may result in a significant reduction in calving rates. In the Eastern Province of Zambia, for example, the calving rate in a trypanosomosis endemic area is 44,1 per cent compared to 60,4 per cent in an adjacent tsetse-free area.[61] Trypanosomal infection during pregnancy may induce abortion,[116, 131, 236] but when pregnancy is carried to term, the birth weights of calves are low.[63, 234] Trypanosome-infected young stock grow poorly; they show signs of ill-thrift and remain stunted. The compounded losses from lower fertility and milk yields, increased mortality and reduced growth rates are reflected in low herd performance and often in decreasing herd size. A composite productivity index incorporating these parameters has been found useful when assessing the impact of bovine trypanosomosis and its control.[182]

The clinical signs of trypanosomosis tend to be more severe in exotic breeds of cattle than in the local breeds which, in part, reflects the adaptation of local breeds to their environment. Additionally, exotic breeds are often under greater production stress, which further exacerbates the disease.

Trypanosoma theileri is not considered to be a pathogen,[274] although high parasitaemias can occur in cattle with intercurrent disease.[280] There are several reports associating *T. theileri* infection with such clinical signs as anaemia,

diarrhoea or reduced milk yields, but conclusive evidence of the role of this species of trypanosome in the pathogenesis of these disorders is lacking.

Pathology

The PCV begins to fall with the onset of parasitaemia and fever. From an initial value of 0.32 ℓ/ℓ it may be reduced to 0.20 ℓ/ℓ or less in a matter of a few weeks. The early erythropoietic response to anaemia[209] is usually normochromic and normocytic, but an early macrocytic response may occur. In chronic disease there is a microcytic anaemia, associated with hypoferraemia and dyshaemopoiesis due to iron trapping. Initial leukopenia also occurs and may be followed by leukocytosis. Thrombocytopenia is a constant feature of trypanosomosis and may account for the clotting defect that occurs in the *T. vivax*-haemorrhagic syndrome.[179, 209] Hypocomplementaemia is also a constant finding in trypanosomosis,[277] which is correlated with the level of parasitaemia:[125] complement levels are higher in cattle that have lower trypanosomal parasitaemias. The levels of other serum proteins also alter in the course of trypanosomosis. Albumin is decreased and globulin levels are frequently raised.[86, 277] Other changes also occur in blood chemistry — blood chloride levels rise and the calcium-to-phosphorus ratio in infected adult cattle is significantly reduced.[82] More recently, attention has been directed to the effect of trypanosomosis on hormone levels. Results indicate that although plasma cortisol levels of infected cattle are reduced, parasitaemia appears not to alter levels of plasma adrenocorticotrophic hormone.[125]

The gross pathology of affected animals varies with the duration and severity of the disease, and many organs and tissues are affected. Acute infections may end with the death of an animal still in good bodily condition. In such cases the carcass is well-fleshed, although the mucous membranes may be pale. The overall appearance of the carcass is one of paleness, and petechial and ecchymotic haemorrhages are frequently present, especially on serosal surfaces. In acute *T. vivax* infections, there are extensive haemorrhages, resembling those seen in septicaemic conditions: large, epi- and endocardial haemorrhages are present, in addition to those on serosal surfaces.

Lymph nodes are enlarged and, when incised, are oedematous and often have a dark pigmented medullary area. Typically, the spleen is greatly enlarged and dark red. Excessive peritoneal fluid is present which is often blood-tinged. Acute *T. congolense* infections can produce extensive oedema which is most noticeable in the perirenal and mesenteric fat, and the abomasal mucosa.[164] *Trypanosoma brucei* may cause keratitis, although this is rare in cattle.

The gross pathology of chronic trypanosomosis is characterized by cachexia and anaemia. The coat is dry and dull, the skin is scaly and inelastic, the eyes are sunken, and the skeletal muscles are atrophied. There is hydrothorax, hydropericardium and ascites, and the carcass, in general, appears oedematous. Residual fat around the heart and kidneys is gelatinous. Lymph nodes, haemal nodes and spleen are generally enlarged and have an appearance similar to that seen in acute trypanosomosis, but after prolonged disease there may be splenic atrophy. Haemorrhages are not a feature of chronic trypanosomosis. The heart is enlarged and flabby, the liver swollen and pale. Whereas red marrow is present in the long bones in acute trypanosomosis, all the bone marrow is yellow and gelatinous in the chronic disease.

Gross changes are not always obvious, but microscopic lesions are extensive. These are found particularly in the cardiovascular system and lymphoid tissues, and there is widespread infiltration of many organs by mononuclear inflammatory cells. Dilation of the microvasculature and oedema and structural changes in vessel walls are usually seen, especially with *T. congolense* infections. Trypanosomes are commonly present in the lumens of blood vessels, and, in the case of *T. vivax*, parasites may be associated with fibrinous thrombi in the larger blood vessels. In some acute cases, there is disseminated intravascular coagulation, when microthrombi may be associated with haemorrhage and ischaemic necrosis. A constant feature of trypanosomosis is initial hyperplasia of the lymphoid tissues and of the mononuclear phagocytic system. In the cortical area of lymph nodes and white pulp of the spleen, germinal centres form early in the disease, although in chronic infections lymphoid tissue becomes depleted.[205] The expansion of the mononuclear phagocytic system leads to increased erythrophagocytosis and there is widespread haemosiderosis.

Monocytes and lymphocytes aggregate in the microvasculature together with trypanosomes. Invasion of tissues by the parasites leads to infiltration by mononuclear cells, which is mainly perivascular. In the late stage of acute trypanosomosis, severe anaemia and extensive vascular damage produce degenerative changes in the tissues, but these are less serious than are the changes that arise from cellular infiltration. Since *T. brucei* is more invasive, it provokes considerable cellular infiltration. The heart is commonly affected, and macrophages, lymphocytes and plasma cells infiltrate the myocardium. Degenerative changes and focal necrosis of myocytes and, in long-standing infections, fibrosis are evident in the heart. Infiltrations also occur frequently in other organs such as the pituitary, thyroids, adrenals, kidneys and gonads. Congestion and interstitial oedema also occur, and, when the substantia propria is affected, corneal opacity then develops. Severe degenerative changes occur in the thyroids[82] and a reduction in erythroid progenitor cells of the bone marrow has been reported.[209]

Diagnosis

The specific diagnosis of trypanosomosis is notoriously difficult. Not only are there no specific clinical signs, but the

intermittent and frequently low parasitaemias make detection of the parasites difficult. Furthermore, infection is not synonymous with disease; many subclinically infected animals live in delicate balance with potentially pathogenic trypanosomes. An element of clinical judgement is therefore necessary when making a diagnosis of trypanosomosis. Several authors[51, 148, 223] have compared the different methods for the parasitological diagnosis of animal trypanosomosis. Important considerations are that the number of detectable parasites is not necessarily related to the severity of the disease and, in endemic areas, the disease is a herd problem. The detection of infection in a few clinically affected cattle warrants careful examination of the entire herd.

Economic principles, the availability of expertise and the diagnostic requirements will guide the choice of a particular diagnostic test. The diagnostic method will differ between situations, depending, for example, upon whether species-specific diagnosis is required or whether surveys are conducted simply to determine the presence or absence of the disease. Often a combination of diagnostic tests is needed to obtain the required results.

Although in many tsetse-infested areas diagnostic facilities are not readily available, clinical signs of trypanosomosis are well recognized. Farmers and veterinary personnel commonly resort to treatment of sick animals, and use the response to therapy for retrospective diagnosis. In such areas, a history of the presence of tsetse flies and the use of trypanocidal drugs, when considered with presenting clinical signs, are sufficient to make a tentative diagnosis. However, the presence of concurrent disease may mask trypanosomosis and complicate the clinical picture. Thus, the only way to confirm diagnosis in clinically infected animals is to demonstrate and identify the parasites in body fluids.

The parasite detection methods are highly specific but their diagnostic sensitivity (the proportion of infections that the methods detect) is relatively low. This is especially the case when results are considered for an individual animal rather than in a herd. As a result, the apparent prevalence of trypanosomosis determined by parasitological diagnostic tests is an underestimate of the true parasitological prevalence. This is a problem in areas where the disease is present at low prevalence or is seasonal, or when attempting to confirm the absence of the disease in a particular area.

The body fluid most commonly examined is blood, either capillary blood from the tip of the tail or venous blood from an ear vein or from the jugular vein. Lymph, aspirated from a punctured superficial lymph node (usually the prescapular), provides useful supplementary diagnostic material. Whereas cerebrospinal fluid is routinely examined for the presence of trypanosomes in the diagnosis of human sleeping sickness, this has little practical use in veterinary medicine.[314]

Wet blood smears are prepared by placing a drop of blood (about 2 µl), taken directly from a punctured ear vein or the tip of the tail, onto a clean, dry, grease-free slide. This is immediately covered with a cover slip and examined microscopically as a fresh, wet preparation with a ×25 or ×40 objective lens. Approximately 50 to 100 fields are examined. Live, motile trypanosomes may be seen as they bore their way between blood cells (the name 'trypanosome' is derived from the Greek 'trypanon', a borer, and 'soma', meaning body). The wet blood smear method is simple, inexpensive and gives immediate results. It can be used to examine a large number of animals. Depending on the trypanosome's size and movements a presumptive identification can be made of the trypanosome species involved. However, the diagnostic sensitivity of the method is low and depends to a large extent on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the red blood cells before examination using a powerful haemolytic agent such as sodium dodecyl sulphate (SDS).[228]

More commonly, for routine diagnosis in veterinary practice, thick and thin smears of blood are prepared. A drop of blood taken directly from a punctured blood vessel in the ear or tail tip is placed on a glass slide; a thick and a thin blood smear can then be made on a single slide. Lymph smears are prepared in a similar manner. The smears are kept dry and protected from dust, heat, flies and other insects. Alternatively, under dusty field conditions, venous blood can be taken into tubes containing an anticoagulant, such as heparin or EDTA, and blood smears can be made subsequently. The dry smears are stained with Giemsa's stain, thin smears after having been fixed, and examined under a ×50 or ×100 oil-immersion objective lens. Although it may be necessary to examine up to 700 fields to find a single trypanosome,[51] the unfixed de-haemoglobinized thick smear allows approximately 120 times more blood to be scanned than a thin smear[148] and, thus, has higher diagnostic sensitivity than the thin smears. Trypanosomes are easily recognized by their general morphology but may be damaged during the staining process. This makes it difficult to make a species-specific identification on thick smears. The thin smear, on the other hand, permits accurate speciation of the parasites. However, due to the staining process, results are delayed.

The probability of detecting trypanosomal infections in a sample of infected animals can be improved by increasing the volume of blood to be examined and concentrating the trypanosomes. This is achieved by collecting fresh blood (about 70 µl) in a heparinized capillary tube and separating the different components of the blood by centrifugation, according to their specific gravity. Trypanosomes are concentrated in the plasma/white blood cell interface or buffy coat. The buffy coat is examined microscopically whilst slowly rotating the capillary tube (microhaematocrit centrifugation technique or Woo-method).[330] Trypanosome movement can first be detected using the ×10 objective lens with reduced condenser aperture; the trypanosomes can be seen more clearly using a ×40 objective lens, preferably of a long working distance (allowing considerable depth of focus

through the capillary tube). The microhaematocrit centrifugation technique is more sensitive than the direct examination techniques but identification of trypanosome species is difficult. Alternatively, the buffy coat and the uppermost layer of red blood cells can be extruded onto a clean microscope slide and covered with a cover slip (buffy coat technique or Murray method[212]). Approximately 200 fields of the preparation are examined microscopically for the presence of motile trypanosomes with a dark-ground/phase-contrast illumination and a ×40 objective lens. Trypanosome species can be identified by reference to the following criteria:

- *T. vivax*: Large, extremely active, traverses the whole field very quickly, pausing occasionally.
- *T. brucei*: Various sizes, rapid movement in confined areas.
- *T. congolense*: Small, sluggish, adheres to red blood cells by anterior end.
- *T. theileri*: More than twice the size of pathogenic trypanosomes.

Since the specific gravity of *T. congolense* is similar to that of red blood cells, these parasites are often found below the buffy coat within the uppermost layer of red blood cells. The two concentration methods are the most sensitive for detecting *T. congolense* and *T. vivax* infections.[238] The sensitivity of the concentration methods can be further improved by using the buffy coat double centrifugation technique.[153] A total amount of 1 500 to 2 000 µl of blood is centrifuged after which the buffy coat is aspirated into a microhaematocrit capillary tube and centrifuged again. The buffy coat is then examined as previously described. A modification of the microhaematocrit centrifugation technique is the Quantitative Buffy Coat (QBC) method.[14] This method is based on expanding the buffy coat by inserting a cylindrical float in a capillary tube that is precoated with acridine orange. The method has been used to diagnose *T. b. gambiense* infections but is too expensive for routine use in the diagnosis of animal trypanosomosis.

The microhaematocrit centrifugation and buffy coat techniques give immediate results and can be used for screening large numbers of animals. They require, however, specialized equipment and an electricity supply which makes the tests more expensive compared to the examination of the wet blood film, but this is compensated for by the increased diagnostic sensitivity; furthermore, haematocrit readings may be obtained (see below). Both parasite concentration techniques rely on the detection of live, motile trypanosomes. Since trypanosomes are heat sensitive, samples collected in capillary tubes should be kept cool; overheating of the microhaematocrit centrifuge should be avoided. Compared to the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate species identification and for retention as a permanent record.

The microhaematocrit centrifugation and buffy coat techniques are particularly useful in that the haematocrit or PCV can be assessed after centrifugation, by placing the microhaematocrit capillary tube in a haematocrit reader. The PCV of venous blood is a useful indicator of anaemia, which is one of the most typical signs of trypanosomosis in domestic animals.[209] The PCV of individual animals and the average PCV of herds can be determined. In individual animals, haematocrit readings supplement parasitological diagnosis. In trypanosomosis-endemic areas, parasitologically negative animals that have low haematocrits are regarded as having trypanosomal infections. In surveys to determine the presence or absence of trypanosomosis, samples collected from animals with low haematocrits can be investigated more thoroughly to increase the probability of detecting a trypanosomal infection. In parasitologically positive animals, the haematocrit indicates the severity of disease. The decision of the cattle owner to administer a trypanocidal drug is more often based on the effect of anaemia on the animal's performance rather than the confirmed presence of infection. At the herd level, the haematocrit profile or the herd average PCV is a useful indicator of infection and herd health. However, other factors such as nutrition and fasciolosis may also cause anaemia on a herd basis. Therefore, it is important to establish the haematocrit profile of trypanosomosis-negative herds before relying on it as an indicator of trypanosomosis in a herd. Because of the low sensitivity of parasitological diagnostic tests, baseline PCV-profiles are best obtained from herds of animals that do not have anti-trypanosomal antibodies (**Figure 12.9**). In the absence of PCV-profiles of negative herds, comparisons of herd average PCVs at various distances from the source of infection — along a transect — can give useful information on the variation in disease challenge (**Figure 12.10**). In trypanosomosis endemic areas, herd average PCV is significantly correlated with parasitological prevalence (**Figure 12.11**). The slope of this relationship is determined by the effect of disease prevalence on herd average PCV and may be used as an indicator of the impact of trypanosomosis on herd health.

The subinoculation of blood into rodents, usually mice or rats, allows a greater proportion of *T. brucei* infections to be detected than by direct examination of the buffy coat. For practical reasons, subinoculation of blood into laboratory or other animals is not used as a routine diagnostic procedure. The method is expensive and diagnosis is not immediate. Furthermore, since rodents are refractory to *T. vivax* and not all *T. congolense* and *T. brucei* infections become established in the new host, even this method has serious limitations. Mixed trypanosomal infections may also remain undetected.

A procedure for the *in vitro* cultivation of *T. brucei* from the blood of infected animals has been described but success has been variable. Moreover, the method needs sophisticated equipment, yields results only after a considerable

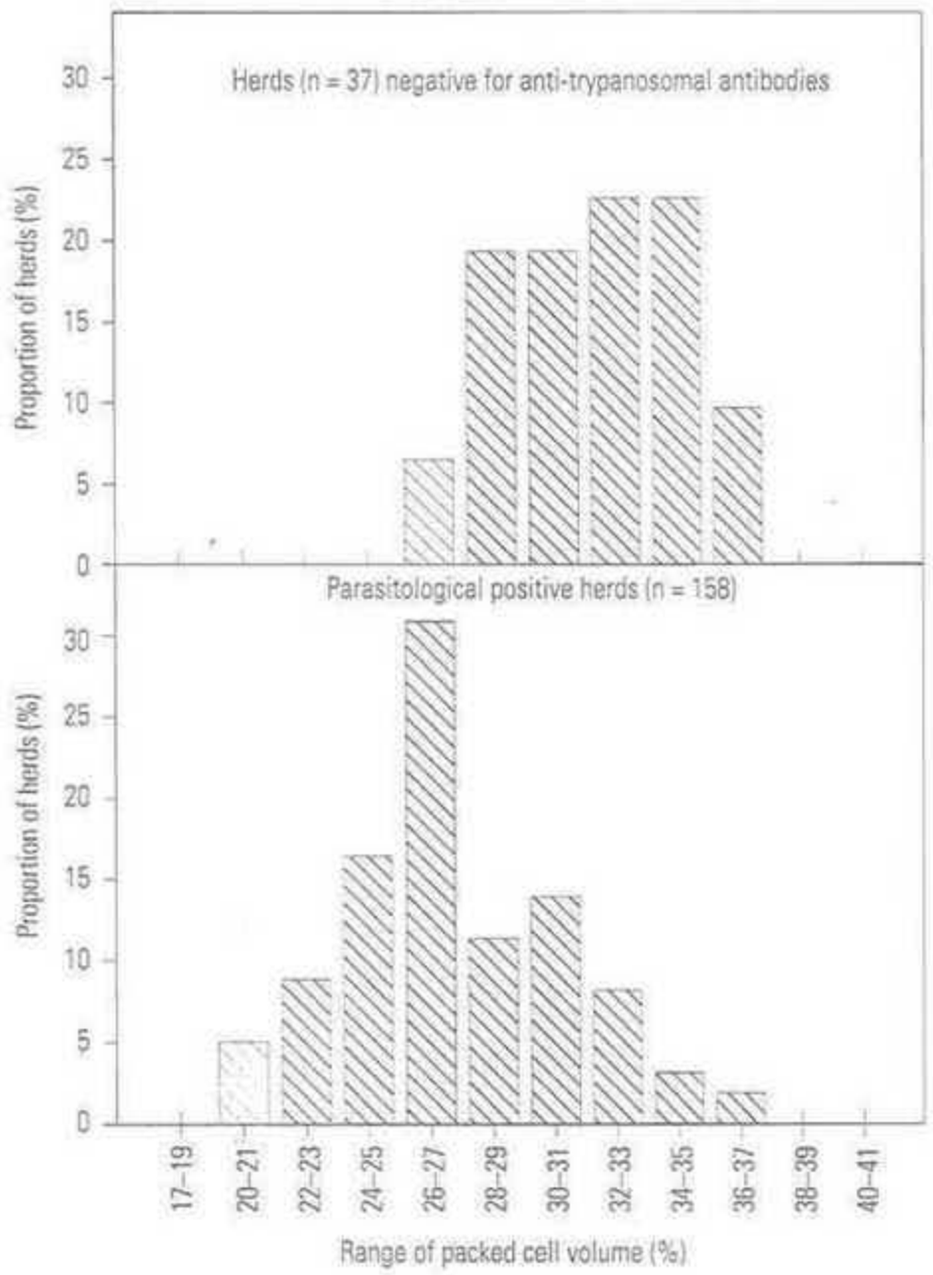


Figure 12.9 Herd average haematocrit profiles of anti-trypanosomal antibody negative herds and parasitological positive herds (Eastern Province, Zambia)

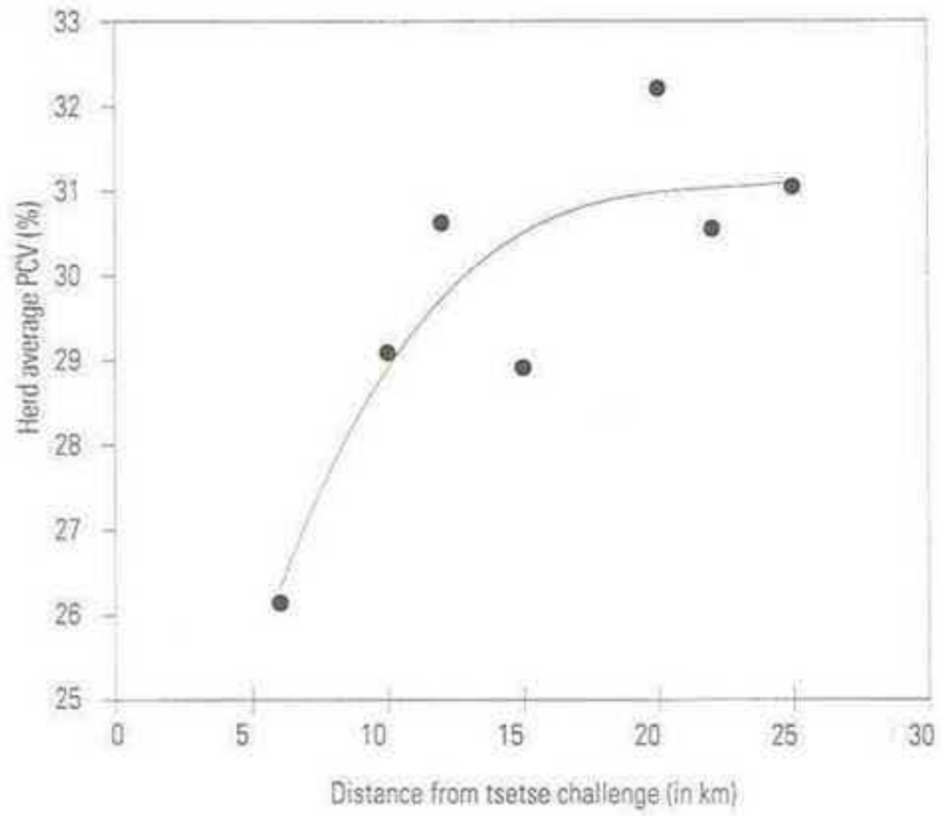


Figure 12.10 Herd average packed cell volume at increasing distance (in km) from the tsetse front in Eastern Zimbabwe

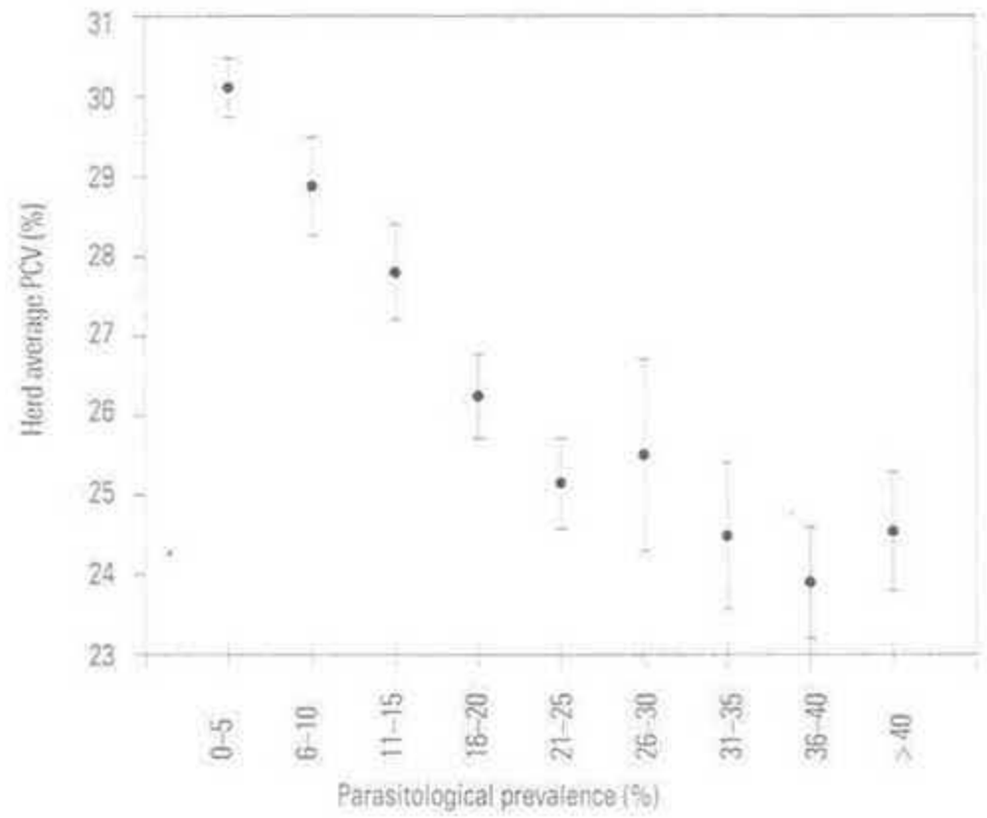


Figure 12.11 Relationship between herd average packed cell volume and parasitological prevalence of bovine trypanosomosis (%) in a trypanosomosis endemic area in the Eastern Province of Zambia

delay and is certainly not suitable for widespread use. A recently described kit for *in vitro* isolation (KIVI) of trypanosomes has proved promising for isolating and amplifying *T. b. gambiense* in humans, domestic and game animals.[287] The test's value in isolating *T. congolense* and *T. vivax* is still unknown. Since it is based on the cultivation of procyclic forms of trypanosomes, species differentiation is not possible.[152]

A miniature anion-exchange technique has been described for field use in the diagnosis of human trypanosomosis,[154, 171] but is too cumbersome for routine use in veterinary practice. The use of a minicentrifuge with buffy coat technique has been advocated.[147] It is not satisfactory in bovine practice where large numbers of samples often have to be examined.

In the diagnosis of human trypanosomosis the cerebrospinal fluid (CSF) is examined for the presence of inflammatory cells, raised protein levels and parasites. For parasitology, the CSF is examined after centrifugation; the sediment obtained from 2 to 5 ml CSF is placed under a cover slip and examined microscopically for motile trypanosomes.[311]

The non-specific diagnostic tests to detect raised protein levels in the serum of animals suspected to have trypanosomal infections[148] have no role in diagnosis today. The development of anti-trypanosomal antibody detection techniques has been a major improvement in the serodiagnosis of trypanosomosis. The indirect immunofluorescent antibody test (IFAT)[327] has been and still is used widely to diagnose trypanosomosis. The test has undergone several modifications so that it can differentiate, to a limited extent, between trypanosome species in ruminants.[146] The serodiagnosis of trypanosomosis has greatly benefited from the introduction of enzyme immunoassays. The enzyme-linked immunosorbent assay (ELISA) was first used to detect antibodies against *T. b. rhodesiense* in humans.[301] It was further developed for use in animal trypanosomosis[167] and was recently modified for large-scale use in trypanosomosis surveys.[115] Antigens can be prepared using bloodstream forms or procyclic trypanosomes.[101] Purified antigens are used preferably to ensure that tests are standardized and optical density cut-off values do not vary greatly between batches of antigen.

The ELISA compares favourably with the IFAT[169] and has been found to give results that correlate with the local history of trypanocide usage.[45] However, even if a trypanosomal infection has been cured, anti-trypanosomal antibodies persist for several months[25] and antibody detection tests do not distinguish between current and past infections. They can only provide a presumptive diagnosis. Although the prevalence of anti-trypanosomal antibodies often increases with increasing prevalence of trypanosomal infections in a herd, antibody detection methods are not suitable for monitoring disease challenge in trypanosomosis endemic areas. Nevertheless, antibody detection tests, especially the antibody-detection ELISA, have been very useful tools for determining the distribution of trypanosomosis.[292, 295] This is especially the case in areas where disease prevalence is low and where trypanocidal drugs are used frequently. Moreover, the prevalence of anti-trypanosomal antibodies is a sensitive indicator of the impact of tsetse control operations on disease challenge and for confirming the disease-free status of animals in areas from which tsetse has been eradicated.

Another alternative to the parasitological diagnosis of nagana is the use of assays to detect trypanosome-specific antigen, or species-specific or subspecies-specific DNA. An antigen detection ELISA for trypanosomosis has been described[225, 226, 227] but field evaluations of the test have given inconsistent results. Additional work is needed to develop the test for routine diagnosis of trypanosomosis. A polymerase chain reaction (PCR) method has been developed for the diagnosis of infections with African trypanosomes in humans, animals and tsetse flies.[92] Specific repetitive nuclear DNA sequences can be amplified for *T. vivax* and each of the three *T. congolense* subgroups.[57, 201] A common primer set is available for detection of the three *T. brucei* subspecies. The test requires specialized equipment and highly trained personnel, consequently it is not suitable for use in most laboratories. Sample collection has been simplified by adapting the test using blood spotted on filter papers[143] so that a large number of samples can be processed at one time. This makes the test potentially suitable for large-scale surveys. However, the cost of PCR analyses prohibits its routine use in veterinary investigation. The PCR technique's ability to detect latent or mixed trypanosomal infections in different hosts renders it suitable for research into the complex relationships between trypanosomes and their vectors and hosts.[245]

Despite the development of more sensitive, more sophisticated and expensive diagnostic methods, the clinician will, for the foreseeable future, have to rely upon examination of blood smears, buffy coat preparations and findings at necropsy to confirm diagnoses of trypanosomosis. Accurate diagnosis is crucial to subsequent interventions to control nagana, and to monitor their success. At the herd level, the confidence placed in the results will largely depend on the size of the sample. In some cases, however, repeated sampling of an individual animal may be necessary before a diagnosis can be confirmed.

Differential diagnosis

In its various stages trypanosomosis resembles a number of other disease conditions, and it frequently occurs at the same time as other infections. In the acute febrile stage, trypanosomosis must be differentiated from redwater (babesiosis), anaplasmosis and East Coast fever. The haemorrhagic syndrome in acute *T. vivax* infection can be distinguished from anthrax and haemorrhagic septicaemia (*Pasteurella multocida* infection) by examination of Giemsa-stained blood smears, when large numbers of trypanosomes are found.

Acute trypanosomosis rarely produces icterus and is not accompanied by haemoglobinuria. These features and parasitological findings differentiate trypanosomosis from redwater. The two infections, however, may be concurrent. Similarly, anaplasmosis can occur in trypanosome-infected cattle. On its own, anaplasmosis often produces icterus, as well as fever and anaemia; there may also be enlargement of lymph glands. Differentiation relies upon detection of the parasites in blood smears. Although lymph node enlargement and fever occur in East Coast fever, animals often cough and have diarrhoea. The presence of schizonts in stained smears of lymph node material and of piroplasms in blood smears confirms East Coast fever. At necropsy, the abomasal ulceration and kidney lesions characteristic of East Coast fever are not seen in trypanosomosis. Carcasses of animals which have succumbed to trypanosomosis are often oedematous and anaemic.

Chronic trypanosomosis is an afebrile disease, in which anaemia, emaciation and lymph node enlargement are prominent findings. It is important to differentiate this disease from malnutrition and helminthosis. In neither of these two conditions is lymph node enlargement found. The detection of helminth eggs in faeces is a useful adjunct to the diagnosis of helminthosis, but the absence of eggs in faecal samples taken from cattle with chronic fasciolosis, and the occurrence of anaemia and subcutaneous oedema can make differential diagnosis difficult. In cases of malnutrition, the degree of anaemia is rarely as extensive or severe on a herd basis as it is with trypanosomosis, although marked individual variation occurs. The late stage of enzootic bovine leukosis is characterized by weight loss and lymph node enlargement, but, unlike animals suffering from chronic trypanosomosis, appetite is diminished and anaemia is rarely severe.

When *T. theileri* is found in blood smears it is easily distinguished from other trypanosomes by its large size. Although *T. theileri* may occur in cattle infected with another species of trypanosome, the careful examination of thin Giemsa-stained blood smears is more likely to reveal the presence of mixed infections of tsetse-transmitted trypanosomes.

Clinical signs, pathology and diagnosis in goats and sheep

Trypanosomosis of goats and sheep has been stated to be of little significance because natural infections are seldom detected.[273] However, trypanosomosis of small ruminants is a major constraint on their health and productivity,[102] and trypanosomal infections in goats and sheep are known to be prevalent in parts of southern Africa, occurring in Mozambique[271] and Zambia.[21] Small ruminants have a much shorter generation time than cattle, and it is likely that in tsetse areas this factor has produced greater natural selection for trypanotolerance in these species than has occurred with cattle. This may explain the fact that goats and sheep are found apparently thriving in tsetse-infested areas in which cattle cannot be kept without the use of drugs. Compared with exotic breeds, the local breeds of goats and sheep in the tsetse belts of East Africa are more tolerant of experimental trypanosomal infections,[103] but trypanosomosis does nonetheless cause significant production losses even among local breeds.[20, 21, 141]

Of the salivarian trypanosomes, *T. vivax* is apparently the least pathogenic to these hosts, often producing mild infections. *Trypanosoma congolense* is an important pathogen and usually produces subacute or chronic disease whilst *T. brucei* infections may follow an acute, rapidly fatal course, especially in experimental infections. Frequently, however, naturally acquired *T. brucei* infections are chronic.[273] Under natural conditions mixed trypanosomal infections are probably very common, although on parasitological examination one species usually predominates. *Trypanosoma simiae* also infects sheep and goats, but reports of clinical infections are rare.[137, 172]

Clinical signs

The general clinical signs of trypanosomosis of goats and sheep are similar to those in cattle. Acute, subacute and chronic disease syndromes occur naturally in goats and sheep in East Africa,[103] and have been observed in goats in Zambia.[21]

Acute disease is characterized by intermittent and increasing dullness. During bouts of fever, sick animals walk stiffly, or stand with head held low, ears drooping and tail flaccid. Early-morning rectal temperatures may exceed 41 °C in the febrile periods, when the pulse is rapid and the breathing shallow and fast. Anaemia develops rapidly and the PCV falls from 0,35 ℓ/ℓ to 0,18 ℓ/ℓ or less in two to four weeks, when mucous membranes become pale. Affected animals rapidly lose weight and condition, and may die within a month of the onset of detectable parasitaemia. Although corneal opacity occurs in experimental infections with *T. vivax*[318] and *T. brucei*,[122] this lesion is not common in natural cases.

The signs of subacute trypanosomosis are less marked, and the course of the infection resembles that in cattle. Anaemia develops quite rapidly, lymph nodes become enlarged (**Figure 12.12**) and, in goats, a marked jugular pulse can be seen. Although death occurs some 10 to 12 weeks after the onset of parasitaemia, the condition of many animals does stabilize and gradually improves. The improvement may be apparently complete; PCV, live weight and general condition may return to normal, and fever and parasitaemia abate. Other animals show slight improvement, but they remain anaemic, emaciated, weak and chronically affected. Chronic infections are very common and may develop without marked clinical signs. Parasites are rarely found in the blood and animals are afebrile. Clinically, it is difficult to distinguish such animals from those with helminthosis. Furthermore, the immunosuppression induced



Figure 12.12 A goat with a naturally acquired *Trypanosoma vivax* infection: prescapular lymph nodes are greatly enlarged

by trypanosomosis in goats[105] and sheep[173] permits the establishment of large helminth burdens, which thus exacerbate the clinical signs in affected animals.[104]

As with cattle, trypanosomosis of small ruminants is a herd or flock disease which reduces productivity. Fertility is markedly reduced.[121] The birth rate of goats in a tsetse-infested area of Zambia has been improved by regular chemoprophylaxis, even though few trypanosomal parasitaemias were detected in untreated control goats.[21] Results from eastern Africa also indicate the importance of subclinical trypanosomosis on goat productivity.[109]

Pathology

The pathology of trypanosomosis in goats and sheep does not differ greatly from that in cattle.

Because of their smaller size and lower maintenance costs, small ruminants are popular experimental animals, and there are many reports on the pathology of experimental trypanosomal infections in these species. Recently, great attention has been focused on the effects of trypanosomosis on endocrine functions of these animals. Tsetse-transmitted *T. congolense* infections induce weight loss and irregular oestrous cycles in goats,[161] whilst the inoculation of goats with bloodstream trypanosomes alters plasma levels of several hormones. Levels of thyroxine,[219] testosterone,[303] and plasma cortisol[215] are depressed while plasma prostaglandin F_2 levels rise during experimentally induced trypanosomosis in goats.[218]

Functional abnormalities have also been demonstrated experimentally: semen quality of infected rams is reduced[1, 5] and ovarian dysfunction occurs in infected goats.[216] Marked testicular lesions have been reported in experimental infections. In *T. brucei*-infected sheep, initial oedema of the scrotum was followed by a hardening of the scrotal wall and atrophy of the testes.[120] Histologically, the most severe inflammatory reaction affected the tunica vaginalis and was characterized by a thick fibrinous exudate on the surfaces which led to adhesions and organization. Diffuse atrophy of the seminiferous tubules was associated with intertubular oedema, mononuclear cell infiltration and fibrosis. Gross testicular atrophy also occurred in goats infected with *T. congolense*,[139] while, histologically, degenerative lesions were evident. Seminiferous tubules were atrophied and their lumina were devoid of spermatids. Whilst these findings concur with field observations of reduced fertility, the same is not true for the severe central nervous system pathology of experimental infections of goats.[319] The oedema and inflammation of the choroid plexus observed in experimental *T. vivax* infections may occur in natural infections, but the under-diagnosis of trypanosomosis and the common practice of slaughtering sick animals generally precludes full evaluation of the pathology of natural infections.

Diagnosis

The diagnosis of trypanosomosis in small ruminants is no different from that already discussed for cattle, but it is much more difficult because of the larger proportion of subclinical infections. In these cases very low parasitaemias occur in the absence of obvious clinical signs. It is undoubtedly these factors which are responsible for the serious underestimation of trypanosomosis in small ruminants.

Differential diagnosis

The main disease entity which resembles trypanosomosis in goats and sheep is helminthosis, especially haemonchosis. Anaemia, ill-thrift, weight loss, submandibular oedema and high mortality rates are common to the two disease complexes, although diarrhoea sometimes accompanies helminthosis. Parasitological techniques have to be used for differential diagnosis. Frequently the two problems are concurrent, and the final diagnosis and treatment must depend upon clinical judgement.

Clinical signs, pathology and diagnosis in pigs

In many parts of southern Africa, indigenous breeds of domestic pigs survive in trypanosomosis-endemic areas. Pigs are refractory to infection with *T. vivax*, but are mildly affected by *T. brucei*, *T. congolense* and *T. suis*. In contrast, *T. simiae* is highly pathogenic to improved breeds of pigs, but local breeds seem to escape the ravages of this lethal trypanosome species. Of zoonotic significance is the role of the domestic pig in harbouring the human parasites *T. brucei gambiense*[181] and probably *T. brucei rhodesiense*.[11, 144] However, this latter association has not been demonstrated in southern Africa.

Trypanosomosis of pigs in Africa has been comprehensively reviewed,[121, 274] and only an outline of the problem is presented here.

Clinical signs

Trypanosoma brucei and *T. congolense* infections in pigs generally produce only mild disease.[237] Occasionally anaemia occurs, which is accompanied by loss of condition and progressive weakness leading to incoordination. *Trypanosoma suis* infections are of minor clinical importance and have only rarely been recorded; infections run a chronic course, but may kill young pigs in less than two months.

Trypanosoma simiae infections in pigs eclipse in importance all other trypanosomal infections in this host. This parasite may kill pigs after an incubation period of only four to six days, and infected pigs may collapse and die within 24 hours of the first signs of disease. The introduction of the parasite to a piggery is usually by an infected tsetse fly, after which spread is believed to be by mechanical transmission involving biting flies, often found in large numbers in pig pens.

The course of infection is so short that pigs die before losing condition, although some animals may live for as long as eight to ten days. Affected pigs are dull and inappetant or completely anorexic. They have a stiff, unsteady gait and eventually become prostrate. Dyspnoea is evident and

cyanosis may be seen. Some pigs froth at the mouth and there may be diarrhoea. Rectal temperatures reach 41 °C but the extremities are usually cold.

The course of the disease within a herd is dramatic. After one or two early deaths, several more occur within a week, and after a short time there may be only a few survivors.

Pathology

The necropsy findings vary considerably, and there are no pathognomonic changes. The blood is usually cyanotic, clots slowly, and chicken fat clots may be found in the heart. Haemorrhages affect the epi- and endocardium, kidneys and serosal surfaces. The spleen is usually enlarged and the pulp soft with a 'strawberry jam' appearance; there is excessive serous or sero-sanguineous fluid in the pericardial sac and pleural and peritoneal cavities; the trachea may be filled with froth when there is also pulmonary oedema. Thoracic and abdominal lymph nodes are usually enlarged and oedematous, and may be haemorrhagic.

Diagnosis and differential diagnosis

A history of sudden death in a number of pigs, combined with clinical signs as described, is suggestive of *T. simiae* in areas adjacent to tsetse infestations. Although sudden death may arise from African swine fever, anthrax, and other infectious and non-infectious diseases, the presence of fulminating parasitaemias, readily detected in Giemsa-stained blood smears, confirms the diagnosis.

Chronic trypanosomosis should be differentiated from helminthosis and malnutrition.

Clinical signs, pathology and diagnosis in horses and donkeys

With the advent of motorized transport and mechanized cultivation, the role of the horse has diminished in many parts of the world, including southern Africa. Furthermore, the peoples living in or near tsetse-infested habitats in this part of Africa have no tradition of keeping horses, but in some areas donkeys are important. Horses do not survive long in the presence of infected tsetse flies, whereas donkeys are more tolerant of tsetse-transmitted trypanosomes and frequently appear to thrive in lightly infested habitats. Nevertheless, *T. brucei* causes acute disease in donkeys, and is also a most serious pathogen of horses. *Trypanosoma congolense* produces a more chronic, wasting condition, whilst *T. vivax*, although usually producing chronic and more mild disease in equids,[174] has been known to cause acute infection in the horse.[56, 275]

Clinical signs

The general clinical signs resemble those seen in ruminants. Horses with acute trypanosomosis are very dejected. Intermittent parasitaemia occurs and is accompanied by fever; rectal temperatures may reach 40.5 °C and there is tachycardia. Oedema of the lower limbs occurs early in the course of disease, as affected horses are less active than normal. Subcutaneous oedema then affects the ventral thorax and abdomen. Oedematous plaques may form on the flanks, but dependent oedema is more pronounced. Weakness sets in rapidly. Initially, mucous membranes may be congested and icteric but they become pale as the disease progresses. Ophthalmitis is not a constant finding; iritis may occur and up to 20 per cent of horses clinically affected by *T. brucei* may develop keratitis and corneal opacity.[117] Although lymph nodes become enlarged, they are not easily palpable. The condition of the animal deteriorates rapidly and weakness may progress to paraplegia. Oedema becomes increasingly severe. Animals die within two to four weeks from the onset of clinical signs.

Chronic trypanosomosis commonly occurs in horses and donkeys. Animals lose condition and weight (**Figure 12.13**) and the coat becomes harsh and dry. The development of anaemia follows a course similar to that in cattle. Animals become extremely weak and show signs of ataxia, but usually continue to eat. Subcutaneous oedema, initially affecting the limbs and ventral abdomen, may extend to the sheath, scrotum, perineum and occasionally the head. The condition is exacerbated by strongylosis and is fatal, often after several months' duration. In a survey in the Sebungwe area of Zimbabwe, a high proportion of donkeys was found to harbour *T. brucei* and *T. congolense* infections.[34] Such mixed infections in horses give rise to more severe disease.

Pathology

The general pathology of equine trypanosomosis resembles that occurring in cattle. A very full account of the histopathology of experimental *T. brucei* infections in horses was given by McCully and Neitz.[189]

The histological lesions in the central nervous system of horses with experimental chronic *T. brucei* infections were characterized by a severe pleocytosis of the meninges and an extensive subpial gliosis. There was also segmented demyelination of optic tracts and some other areas of white matter as well as grey matter. Extensive perivascular cuffing with lymphocytes, plasmocytes, large mononuclear and Mott cells also occurred. Histological changes in the visceral and other organs were not uniform, but included reactive lymph nodes, interstitial myocarditis, haemosiderosis and focal endophlebitic lesions in veins of the testes.

Diagnosis and differential diagnosis

The diagnostic methods for equids are essentially the same as those for cattle. It is often necessary to repeatedly test those individuals suspected to be suffering from trypanosomosis in order to confirm diagnosis.

Acute disease must be distinguished from African horse sickness, anthrax and babesiosis, in which high fever,



Figure 12.13 A horse with a chronic *Trypanosoma congolense* infection showing emaciation and ventral oedema

sudden onset of signs, congestion of mucous membranes and petechiation occur. Similarly, in chronic trypanosomosis the oedema has to be differentiated from that occurring in African horse sickness, and the anaemia from that of equine infectious anaemia. Chronic babesiosis may also produce signs similar to chronic trypanosomosis.

The signs of weight loss, emaciation and oedema caused by dourine (*T. equiperdum* infection), strongylosis, malnutrition and dental disorders should be differentiated from those caused by salivarian trypanosomes.

Clinical signs, pathology and diagnosis in dogs

Dogs are of greater cultural than economic importance in Africa. The typical village mongrel survives in many tsetse-infested areas with little or no attention from its owner. These are usually small animals, used for hunting and herding, and as watch-dogs. The exotic breeds, selected for similar purposes, quickly succumb to trypanosomosis when they are exposed to tsetse challenge. Exposure of susceptible breeds may also occur as they are transported through tsetse-infested areas, or as small numbers of infected tsetse are carried to otherwise tsetse-free areas by vehicles.

Dogs have been incriminated as reservoir hosts of *T. brucei gambiense*, the cause of West African human sleeping sickness, but such infections are subclinical in dogs.[180] On the other hand, *T. brucei brucei* is the most pathogenic trypanosome in dogs, producing more acute disease than *T. congolense*, although strain differences occur, some strains being highly virulent. Dogs are refractory to infection with *T. vivax*.

Clinical signs

The general clinical signs of trypanosomosis in dogs resemble those in livestock, but the course and severity of the disease are modified by the virulence of the parasite and the susceptibility of the host. In acute disease the dog suddenly becomes dejected and inappetant. Pulse rate, respiratory rate and rectal temperature are raised. Anaemia develops rapidly; mucous membranes become pale and the dog shows signs of weakness and depression. There is progressive weight loss; as the condition deteriorates, the coat becomes dry and subcutaneous oedema, particularly of the head and limbs, may develop.

Ocular lesions characterize both *T. brucei* and *T. congolense* infections but are more severe in the former case. *Trypanosoma brucei* invades all tissues of the eye, producing blepharitis, conjunctivitis, keratitis and uveitis. There may be transient glaucoma,[199] and excessive lachrymation is common. Additionally, *T. brucei* invades many other

tissues, and by entering the cerebrospinal fluid causes ataxia and partial paralysis. Death ensues within 4 to 10 weeks of the onset of signs.

Infections with *T. congolense* generally follow a more protracted course. In chronic cases, dogs become severely emaciated, and ulceration of the oral and gastrointestinal mucosa may develop, leading to haemorrhage and melaena.[110] Inappetance may only be intermittent, with animals being periodically 'off colour'. In exotic breeds death occurs after only a few months, but indigenous dogs may remain infected for long periods.

Pathology

Lesions of trypanosomosis in dogs are broadly similar to those found in livestock. Anaemia is the most readily detectable change early in the course of infection, and the PCV drops markedly. Whilst *T. congolense* infections produce extensive interstitial oedema and ulceration of gastrointestinal mucosa, tissue lesions induced by *T. brucei* are much more severe. There is marked cellular infiltration and cellular degeneration and necrosis.[199] The heart, choroid plexus and eyes are consistently and severely affected. There are also marked changes in lymph nodes due to the initial proliferation of lymphoid cells, although these may subsequently be depleted.

Diagnosis and differential diagnosis

Standard diagnostic methods are used to detect trypanosomes in the blood. However, after the initial phase of high parasitaemia, repeated sampling over several days may be necessary before trypanosomes can be found.

The anaemia caused by trypanosomosis must be differentiated from that arising from infection with *Ancylostoma caninum*, *Babesia canis* or *Ehrlichia canis*. Hookworm infection may be concurrent with trypanosomosis and can be confirmed by faecal examination. In young dogs, babesiosis (or biliary fever) is usually acute; rectal temperature may reach 41 °C and intraerythrocytic piroplasms are usually readily detectable in Giemsa-stained blood smears. The diagnosis of ehrlichiosis (Nairobi bleeding disease, tropical canine pancytopenia or tick fever) in an endemic area relies on the presence of such clinical signs as a moderate fever, crusty mucopurulent nasal and ocular discharges, foetid breath, stained teeth and pale mucous membranes.

Bilateral corneal opacity produced by trypanosomosis must be distinguished from that seen in canine hepatitis (adenovirus-2 infection), in which vomition and acute abdominal pain are presenting signs.

Control with trypanocides

Under light tsetse challenge a therapeutic approach to control can be successful and economical. Vigilance is necessary to ensure that infections are treated at an early stage, before disease has progressed too far and caused production losses. In these circumstances the need for supportive therapy is greatly reduced. However, the cost of constant monitoring has to be borne in mind. There are numerous well-documented examples of susceptible breeds of livestock being successfully reared in many tsetse-infested areas of Africa as a result of chemotherapy or chemoprophylaxis.[113, 163, 182, 286] Considerable success in maintaining cattle, in the face of heavy trypanosome challenge, has been achieved in Zimbabwe[30] and in Mozambique.[279] To control trypanosomosis successfully by chemoprophylaxis, it is necessary to treat a high proportion of cattle at risk at regular intervals. This is not always possible, especially if owners have to pay for treatment, and consequently a therapeutic approach to control is widely used. There is considerable evidence that such therapeutic control assists the acquisition of protective immunity to locally prevalent trypanosome serodemes,[113, 321] whereas with chemoprophylaxis immunity to trypanosomes does not seem to develop.[114]

Treatment

The satisfactory treatment of trypanosomosis requires more than a correctly administered trypanocidal drug, and the speed of recovery is largely determined by the plane of nutrition, the amount of exercise during convalescence and the duration of the disease. Well-rested and well-fed animals recover more rapidly after trypanocidal therapy than do undernourished animals which have to trek long distances to pasture and water. However, chronic trypanosomosis often fails to respond to therapy; the ferrokinetic disturbances and accompanying dyshaemopoiesis appear to be irreversible, and affected animals may remain thin and anaemic despite trypanocidal treatment.

History of trypanocidal drugs

Not all compounds active against trypanosomes are directly lethal to the parasites, and consequently these agents should be referred to as antitrypanosomal drugs. However, it is usual to refer to this category of medicines as trypanocidal drugs, or trypanocides. This latter term is used in the following account.

The small but important group of chemotherapeutic and chemoprophylactic compounds has been frequently reviewed.[83, 157, 239, 325, 326] Trypanocides have a history which dates back to the early part of the twentieth century, when hundreds of substances were tested for antimicrobial activity and for activity against the then recently discovered trypanosomes, the first trypanocidal substances to be found being several dyes. Since then arsenical and antimonial compounds have been shown to be active against trypanosomes, and sodium antimony tartrate (better known as tartar emetic) became widely used throughout Africa, trypanosome-infected cattle receiving an intravenous course of the drug. Although this treatment was first used in Zimbabwe (then Southern Rhodesia) with great success, it was claimed that tartar emetic was used in Zambia (then

Northern Rhodesia) on a scale unrivalled elsewhere.[7]

In 1921, suramin was marketed to treat *T. evansi* infections and human sleeping sickness. The drug is still widely used, nearly 80 years later, and is the drug of choice for the treatment of early cases of sleeping sickness. Research which began in the 1930s on phenanthridinium compounds led to the discovery of dimidium bromide. By the early 1950s this compound was used extensively both for therapeutic and for prophylactic purposes. Dimidium is relatively toxic; it produces reactions at the site of inoculation, and treated animals may become photosensitive. Despite this, heavy reliance was placed on the drug, and in the Lower River district of Malawi (then Nyasaland) cattle were successfully maintained as long as they received regular treatments of dimidium. However, in Malawi,[188] as elsewhere, drug resistance to dimidium bromide emerged in the mid-1950s, and this drug was superseded by quinapyramine, which became available in the early 1950s. Serious drug resistance also emerged to this compound and by 1977 it had been withdrawn from the market. In 1955, homidium was marketed, but once again resistant strains of trypanosomes emerged and became widespread. At almost the same time, diminazene became available and was used to overcome quinapyramine-resistance. Being babesicidal as well as trypanocidal, diminazene has the advantage that it may also be used for treating animals suffering from redwater. Pyrithidium bromide then became available; it was marketed in 1956, but, again due to the emergence of drug resistance, it was withdrawn from the market in 1985.

When isometamidium was marketed in 1961 the active search for trypanocides diminished. This coincided with the period when many African countries gained independence from colonial rule and increasingly stringent requirements for the registration of drugs were demanded worldwide. Furthermore, comparable investments in pharmaceutical areas other than trypanocides are potentially much more profitable, so at the end of a century in which great scientific advances have been made, Africa remains with only four compounds (**Table 12.2**) with which to treat and prevent one of the continent's biggest scourges.

Principles of trypanocide usage

The detailed pharmacokinetics and modes of action of the trypanocides are incompletely understood, but these compounds are regarded as having either therapeutic or prophylactic properties.[149, 214, 240, 282] Diminazene and homidium are therapeutic trypanocides, although homidium does provide prophylaxis for several weeks.[59] Isometamidium, whilst having a curative effect, is widely regarded as being the only trypanocide worthy of being used for prophylactic purposes.[150] The duration of chemoprophylaxis is influenced by several factors,[232] which largely relate to the rate of excretion of the drugs.[113] Diminazene is more rapidly excreted than isometamidium. After injection, initial high drug levels fall rapidly, although in cattle residual protection may last for up to 12 days after administration at a dosage rate of 7.0 mg/kg.[309] The duration of protection conferred by isometamidium is dose-dependent,[242] a higher dose providing longer protection. Experimentally, the prophylactic effect of isometamidium has been increased significantly by incorporating the drug in polymers in order to produce a sustained release device which is implanted subcutaneously.[58, 89, 90] However, results from experiments with laboratory-adapted strains of parasites in relatively stress-free experimental animals, may bear little relation to events in the field. There are numerous reports that the higher the tsetse challenge, the shorter the duration of prophylaxis. Additionally, stressed animals on low planes of nutrition or with intercurrent disease do not always receive the same level of protection as do better managed animals. The most important factor determining the duration of prophylaxis is the susceptibility of the trypanosome population to the drug.[241]

Trypanocides are toxic compounds and have narrow therapeutic indices, so therefore the dosage rates cannot be greatly increased to obtain greater trypanocidal efficacy. Furthermore, they are not equally effective in the treatment of all species of trypanosomes. A single injection of diminazene cures *T. congolense* and *T. vivax* infections at a dosage rate of 3,5 mg/kg but a dosage rate of 7,0 mg/kg is necessary to treat *T. brucei* infections. The efficacy of the phenanthridine compounds, homidium and isometamidium against *T. congolense* and *T. vivax* is more marked than against *T. brucei* at recommended dosage rates. Dosage rates of isometamidium in excess of 2.0 mg/kg are toxic to cattle, and even below this level, the drug is irritant and has marked local effects.

The prophylactic effect of isometamidium depends upon the slow release of the drug from the depot created at the intramuscular injection site. Diminazene is rapidly excreted and has a negligible prophylactic effect. Homidium is absorbed more slowly than diminazene from an intramuscular injection site, but more rapidly than isometamidium. Isometamidium is irritant, and, at the site of injection, it induces an intense inflammatory response and causes necrosis of muscle fibres. This lesion becomes encapsulated and is later organized by fibrous connective tissue. From this depot, the drug is slowly released. Attempts to overcome this irritant side-effect by reformulation of the drug have been unsuccessful to date. Two important practical points are related to the injection site reaction. Firstly, the repeated inoculation of cattle kept under tsetse challenge induces extensive muscular scarring, and, to reduce carcass damage and subsequent losses from condemnation, the drug is usually administered into the less economically valuable neck muscles, often with untoward side-effects. Secondly, from the viewpoint of human consumption of treated animals, the question of drug residues in the animals' tissues arises. It is thought that because of the poor absorption of the drug from the gastrointestinal tract,[150] and the lack of untoward reports of toxicity in humans, the risks posed to human health by residues in meat and milk are minimal.

Table 12.2 Generic and trade names of trypanocides for the treatment of African animal trypanosomosis

COMPOUND		MANUFACTURER	ACTION	RANGE OF DOSAGE RATES MG/KG	ROUTE OF ADMINIS-TRATION	REMARKS
GENERIC NAME	TRADE NAME					
Diminazene aceturate	Berenil	Hoechst A.G., Germany	T	3,5–7,0	i/m s/c	Also babesicidal. Toxic to horses, donkeys, dogs and camels
Homidium bromide	Ethidium	CAMCO Animal Health, U.K.	T(P)	1,0	i/m	
Homidium chloride	Novidium	Rhone-Merieux, France	T(P)	1,0	i/m	
Isometa-midium	Samorin	Rhone-Merieux, France	P/T	0,25–1,0	i/m, (i/v)***	Toxic above 2,0 mg/kg. Highly irritant. Avoid subcutaneous administration
	Trypamidium	Rhone-Poulenc Sante, France	P/T	0,25–1,0	i/m, (i/v)***	
Quinapyramine sulphate*	Trypacide	Rhone-Merieux, France	T	3,0–5,0	s/c	Rest animals before and after treatment
Quinapyramine prosalt	Trypacide prosalt**	Rhone-Merieux, France	P/T	3,0–5,0	s/c	Dosage calculated as sulphate

* Reintroduced in 1985 to treat mainly *T. evansi* infections
** Prosalt is a mixture of sulphate and chloride salts of quinapyramine
*** Source: reference [63]
i/m = intramuscular
s/c = subcutaneous
i/v = intravenous
T = Therapeutic action
P = Prophylactic action
(P) = Short prophylactic activity

Trypanocide use in cattle, goats and sheep

These animals tolerate diminazene, homidium and isometamidium at recommended dosage rates (**Table 12.2**), but quinapyramine is no longer used in these species. Trypanocides are dissolved in water immediately before use, and normal aseptic precautions are necessary in order to reduce reactions at the injection site. Work oxen and dairy animals should receive deep intramuscular injections into the gluteal muscles instead of the neck muscles. Calves, goats and sheep receive deep intramuscular injections in the thigh, care being taken to avoid injury to the sciatic nerve. The intravenous administration of isometamidium as a 1 per cent w/v solution has been successfully employed to treat bovine trypanosomosis,[63] but careful injection is essential to avoid systemic and local reactions.[15]

The practice of using different trypanocides alternately to overcome drug resistance warrants mention. Diminazene should be administered not less than one month after isometamidium treatment,[9] and should be followed by an interval of at least one week before further isometamidium treatment to avoid lethal synergistic toxic effects when normal dosage rates of the two drugs are administered a short time apart. Affected cattle lose condition rapidly, becoming dehydrated and emaciated. Animals may recover after a long time, but up to 6 per cent die. Necropsy findings usually include severe fatty changes in the liver, with enteritis being more variable.

Trypanocide use in pigs

Chemotherapy of *T. simiae* has limited success because by the time signs of disease are seen it is usually too late to save

the animal's life by treatment. Even when early cases are treated, few drugs are effective. Simultaneous treatment with quinapyramine sulphate at 5.0 mg/kg and diminazene at 10.0 mg/kg can effect a cure. Other dosage rates of these drugs have also been used, but relapses after treatment are common and may be due to the presence of parasites in the cerebrospinal fluid.[334] Constant vigilance and retreatment are therefore necessary. In the face of an outbreak it is usual to treat all pigs at risk prophylactically in order to halt the spread of infection, and for this purpose quinapyramine prosalt has been used at a dosage rate of 11.7 mg/kg (calculated as the sulphate) with variable success. Isometamidium given as a 4 per cent w/v solution by deep intramuscular injection at dosage rates of between 15.0 and 25.0 mg/kg are effective in halting the spread of disease, but large, crater-like ulcers develop at the sites of injection.[84]

Prophylaxis has been attempted with a suramin-quinapyramine complex at 40 mg/kg and with quinapyramine chloride at 50 mg/kg, but neither of these formulations is commercially available.

Trypanocide use in equine infections

Diminazene is poorly tolerated by donkeys[31] and, on occasion, horses. The use of the higher dosage rate (7.0 mg/kg) required to treat *T. brucei* infections is often accompanied by nervous signs. Isometamidium may be used to treat and prevent equine *T. congolense* and *T. vivax* infections, but careful aseptic precautions and attention to the intramuscular injection technique are needed to reduce local tissue reactions. Separate needles should be used for filling the syringe and giving the injection. To avoid depositing the irritant solution subcutaneously, a sufficiently long needle should be used; leak-back should be reduced by using a small-gauge needle, applying firm pressure as the needle is withdrawn and massaging the injection site well to disperse the solution in the muscle. Quinapyramine sulphate may be used to treat *T. brucei*, *T. congolense* and *T. vivax* infections, but animals must be well rested beforehand, since side-effects usually occur, which usually pass within one to two hours. These are transient and include restlessness, salivation, sweating, fasciculations, diarrhoea and abdominal discomfort, which may cause the animal to back away from the handler or to become recumbent and roll. In weakened animals the dose should be divided in two; the second half should be administered by subcutaneous injection four to six hours after the first.

Horses and donkeys can be protected by quinapyramine prosalt given at a dosage rate of 3.0 mg/kg. Since the prosalt is insoluble, it is administered as a suspension by subcutaneous injection. The injection site should be massaged well to minimize the local reaction, but a depot of drug is formed in the tissues at the injection site, which induces an inflammatory and fibroblastic response. The nodule may ulcerate and lead to discharge of the drug to the exterior.

Trypanocide use in dogs

Diminazene is toxic to dogs at doses higher than 3.5 mg/kg and its use is best avoided in the treatment of trypanosomosis, especially of *T. brucei* infections which require treatment at 7.0 mg/kg. However, simultaneous administration of difluoromethylornithrine (DFMO) and diminazene has resulted in the successful treatment of *T. brucei* infections.[6] Homidium, at a dosage rate of 1.0 mg/kg, or isometamidium, at a dosage rate of between 0.5 mg/kg and 1.0 mg/kg, may be used to treat *T. congolense* infections. However, the intramuscular injection of these drugs often produces hot, painful inflammatory lesions at the injection site, which results in lameness of varying severity. Treated dogs should be well rested until the inflammation has subsided.

Quinapyramine sulphate has been found to be suitable for the protection of dogs against trypanosomosis,[10] and can also be used for treatment. Animals must be cool and rested before and for at least two hours after subcutaneous injection of quinapyramine sulphate; a dosage rate of 5.0 mg/kg is used, and may be repeated at monthly intervals for prophylaxis.

Failure of trypanocides

When trypanocides fail to provide the expected cure or protection there is a tendency to assume that drug resistance has arisen. Whilst this may be true, there are several other reasons which can contribute to their failure (**Table 12.3**). Considerable logistical constraints exist throughout Africa in the provision of veterinary services to rural areas, and farmers often resort to the treatment of their own animals. A survey conducted in the Eastern Province of Zambia, for example, revealed that 67 per cent of the trypanocides were administered by the cattle owners.[61] Trypanocides are relatively inexpensive drugs. They are sold in granular, tabletted or powder form and have to be dissolved in water to obtain a solution for injection. Factors which contribute to their widespread underdosage are incorrect dilution and the inaccurate estimation of the live weights of animals. The use of unsterilized syringes, needles and the water in which the drugs are administered may induce the formation of abscesses which, if they burst to the exterior, results in the loss of the drug. Consequently, treatment quite often fails to produce the expected results.

The use of trypanocides at subtherapeutic levels is believed to promote drug resistance in trypanosomes.[89, 322] Since diminazene is rapidly excreted, the risk of trypanosomes being exposed to sublethal levels of the drug is less than is the case with isometamidium. Prophylactic levels of isometamidium wane over much longer periods. Even when cattle receive isometamidium at the correct dosage rate, it may be administered at the wrong time. Tsetse challenge may increase at a time when drug levels have declined and are not protective. Furthermore, it appears that the duration of the protection is reduced in those cattle which are in poor

Table 12.3 Causes of apparent drug resistance in trypanosomes: failure of trypanocides

Underdosage
underestimated body weight
overdiluted solution of trypanocide
incorrectly calculated dose volume
deliberate underdosage to enable treatment of more animals
incorrect injection technique
— short needles
— non-sterility/abscessation
— early withdrawal of needle
— elastic recoil of tissue produces excessive 'leak-back'
Incorrect strategy
irregular treatment
prolonged intervals between treatments (challenge when drug levels have waned)
lack of knowledge of seasonality
Stress-induced metabolic changes
malnutrition
lactation
intercurrent disease
breed-associated stress
work and trekking
Increase in challenge
seasonal
changed grazing area
absolute increase in tsetse numbers
Relapsing infections
True drug resistance

general health or are on a low plane of nutrition, and it is thought likely that trypanocides require an active immune response by the host for them to be fully effective.[113]

A serious cause for the failure of trypanocides is the phenomenon of relapsing infections.[113] Trypanosomes in extravascular sites may not be exposed to lethal concentrations of trypanocides, and they may then emerge at a time when drug levels in the blood have declined. *Trypanosoma brucei* in the cerebrospinal fluid is not exposed to trypanocidal levels of diminazene, which does not readily cross the blood–brain barrier.[317] Although trypanosomes are cleared from the blood after treatment, the infection may relapse as parasites reinvade the bloodstream from drug-inaccessible sites. A similar event occurs with *T. vivax*[178] which is known to invade the aqueous humour of the eye and the cerebrospinal fluid of goats.[318] The extent of the occurrence of relapsing infections under natural conditions is not known, as it is difficult to distinguish between true drug resistance and a relapse in the field, and the occurrence of a relapsed infection may often simply be ascribed to 'resistance'.

True resistance to trypanocides is a spectre which looms large. The development of resistance has led to the withdrawal of many trypanocides in the past, and resistance to the few remaining currently used trypanocides is documented.[89, 239] The degree of resistance in trypanosomes is not clear cut; a spectrum of drug sensitivity occurs, and resistance to low levels of a drug may be overcome by increasing the dosage rate.[113] Cross-resistance between different trypanocidal compounds can also occur.[325] Multiple resistance of a strain of *T. vivax* to normal therapeutic dosage rates of isometamidium, diminazene, quinapyramine and homidium has been confirmed,[258] but fortunately such cross-resistance is rare. Resistance to quinapyramine developed after only six months in some areas of Kenya[321] — a sanative treatment of either homidium or diminazene was administered to control this. Cattle thus treated and removed from the challenge area were subsequently found to be free of infection and also grew well.[321] Nevertheless, induction of resistance to quinapyramine in *T. congolense* has been shown to result in cross-resistance to homidium, isometamidium and diminazene.[229] Hence, quinapyramine should not be used in cattle. Diminazene and isometamidium are regarded as a sanative pair, since cross-resistance between the two is very rarely seen in the field[113] and, in fact, the multiple resistance in *T. vivax* mentioned above[258] was overcome with diminazene at 7.0 mg/kg.

In southern Africa, the only well-substantiated report of resistance to isometamidium was made from Zimbabwe in 1974.[160] Infection of two non-immune cattle with the suspected isometamidium-resistant *T. congolense* strain was not cured by isometamidium treatment at 1.0 mg/kg, although treatment at 2.0 mg/kg did effect a cure. The problem in the field was overcome by herd sanative treatments with diminazene. More recently, diminazene aceturate resistant stocks of *T. congolense* have been isolated from cattle in the Zambezi Valley of Zimbabwe[138] and diminazene and isometamidium resistance has been reported in Zambia.[37] In cases of serious drug resistance, cattle have to be withdrawn from the tsetse area, but resistant trypanosomes are cyclically transmitted by tsetse, and they may then enter the wild host reservoir. Transmission of the strain will then continue, albeit much diluted, between wild hosts.[99, 100, 134] The reintroduction of livestock into such areas may quickly lead to the re-emergence of drug resistance.

Methods to prove easily that certain strains of parasites are resistant are not available. Currently, three techniques are commonly used to detect drug resistance. Firstly, tests in ruminants provide direct and accurate information on the dose required to cure a trypanosomal infection.[269] The frequently used mouse inoculation tests can, at best, only broadly indicate the sensitivity of a strain.[269] Secondly, attempts to conduct *in vitro* bioassays with culture-adapted trypanosomes are being made, but not all field strains will adapt to culture systems and, of those that do, some may acquire an altered pattern of drug sensitivity.[140] Thirdly, as an alternative to bioassays for determining the sensitivity of

trypanosome strains, is the use of a trypanocidal drug-ELISA in conjunction with parasite detection tests. A competitive ELISA capable of detecting small amounts of isometamidium in the serum of cattle has been developed[74, 75, 319] and has been validated under field conditions.[73]

Strategic use of trypanocides

The single rainy season in southern Africa influences tsetse dispersal and leads to a seasonal peak in the incidence of trypanosomosis.[44] The determination of the precise trypanosome challenge is a complicated matter,[255] but from the practical point of view it is more important to establish the disease incidence or risk. This tends to be seasonal, with many new infections being contracted soon after the beginning of the rainy season. This is the time at which isometamidium chemoprophylaxis should be used. Repeated treatments may be needed during the rainy season, and cattle can be maintained by chemotherapy with diminazene during the lower risk period. Evidence pointing to the potential value of this strategy has been obtained with sentinel cattle in southern Tanzania, which also has unimodal rainfall.[47]

To apply this or any other strategy it is necessary to meet all of the logistical requirements of drug administration and disease surveillance. It is frequently the logistical bottlenecks which reduce the efficacy of trypanocides rather than the inherent inactivity of the drugs.[113] The cattle owner's attitude towards his animals and the management of disease in his animals is an important factor contributing to the success or failure of drug use. A survey of trypanocide use in several countries of southern Africa[61] showed that, despite the availability of diminazene and isometamidium, cattle owners preferred to treat animals that were clinically affected using a curative rather than a prophylactic approach. Moreover, preference was given to the treatment of the more productive animals, such as oxen and cows. Although this treatment regime does not improve the reproductive performance of cows, it significantly reduces mortality due to nagana and improves the condition of oxen. This may, from a cattle owner's point of view, be the most effective way of dealing with trypanosomosis. It is not evident that other strategies will be readily accepted.

The warning has been sounded: 'Cattle cannot be kept alive indefinitely on the end of a needle',[176] and this must be recognized at the outset of any control programme. The use of trypanocides can be highly effective when there is close veterinary supervision, but it is only palliative. Attempts must be made to reduce tsetse numbers and trypanosome challenge. In the short term, the benefits which accrue from the control of trypanosomosis with trypanocides amply justify the costs. In the long term this approach is not sustainable.

Trypanosomosis control strategies

For more than 60 years, governments in southern Africa have made concerted efforts to control tsetse in their battle against trypanosomosis. The ultimate goal, and the basis of many control strategies, was the eradication of the tsetse fly. The Tsetse and Trypanosomiasis Control Branch of Zimbabwe's Department of Veterinary Services has achieved notable success in controlling the disease by progressively removing the vector. Different strategies have been used, and within each strategy different methods have been applied as they have become available. A brief consideration of trypanosomosis control in Zimbabwe is thus very instructive.

Vector control began in the 1930s when wild hosts of tsetse were eliminated by large-scale hunting operations. This method of control was abandoned in 1960. Tsetse control then relied initially on bush clearing and the application of residual insecticides to tsetse resting sites by ground-based spraying teams. However, these methods on their own did not result in satisfactory control of nagana, and in 1964 selective hunting began in fenced corridors that delimited tsetse-infested regions. To limit the extent of the disease, livestock were excluded from many tsetse areas by well-maintained cattle fences. As a result of progressive operations, tsetse were well under control by the early 1970s, and a system of compulsory monthly inspection of all livestock, with the treatment of sick animals, effectively contained the disease. These gains were reversed during the liberation war years of the 1970s. In the mid-1970s an estimated half a million cattle were at risk of trypanosomosis. Every year some 140 000 blood smears were taken from cattle in risk areas, and approximately 100 000 treatments were given annually.[44] From 1980, large-scale aerial spraying of insecticides was used, which was aimed at reclaiming areas invaded by tsetse during the war. By 1989, trypanosomosis was again well under control. In the year to September 1989, two million cattle were inspected and, of 67 563 blood samples examined, only 596 cases were detected. From 1991 onwards, odour-baited, insecticide-treated cloth targets became the method of choice of the Zimbabwean Tsetse and Trypanosomiasis Control Branch and almost completely replaced aerial and ground spraying. By 1999, approximately 60 000 targets were deployed as an effective barrier against tsetse reinvasion from neighbouring countries and the tsetse-infested areas in the north of the country. The barrier protects more than 1 000 000 head of cattle, mainly in communal lands, from the threat of tsetse.[62]

Despite considerable gains in other countries, few achievements have been sustained because of the prohibitive cost of preventing tsetse from reinvading previously cleared areas in the absence of natural barriers. Nagana, therefore, remains a serious constraint to development throughout much of Africa and new approaches towards its control have been devised.

The new control strategy aims at intervening in areas where achievements are likely to be sustained by the beneficiaries. Consequently, in most cases, trypanosomosis will be

controlled on a smaller, localized scale in areas that have to be selected carefully. Socio-economic and technical criteria are the major determinants in the selection process.

Control interventions are more likely to be sustained where the beneficiaries perceive trypanosomosis as a serious constraint to their development and where its control will result in significant economic gains. To identify such areas, accurate information is required on the distribution and socio-economic importance of the disease, and on farmers' perceptions of the impact of the disease and its control.

The impact of nagana on rural development is often unknown and control operations have frequently been planned and implemented based on spurious or incomplete information and invalid assumptions. The socio-economic impact of bovine trypanosomosis varies widely and is influenced by factors such as the disease challenge, the degree of tolerance to infection and the application of control measures.

The long-term success of an intervention to control nagana (and many other diseases) does not depend entirely on its effect on livestock production. The livestock owner's perception of its impact is of utmost importance. This perception will vary from area to area depending on the farming or livestock management practices and may vary in time, depending on other factors constraining development. For example, in areas where cattle are mainly used for draught power, the effect of trypanosomosis on fertility is likely to be perceived as a minor problem. In such areas, cattle owners are unlikely to sustain control measures that greatly improve reproductive performance. The opposite may be true in areas where the sale or accumulation of cattle is of prime importance. Even if nagana is perceived as a constraint, control interventions will not always result in financial and economic gains. This will normally be the case when either the potential for herd expansion is limited or where there is limited opportunity to increase the area under cultivation. These considerations apply, for example, to many parts of Malawi. Under such conditions, interventions to improve agricultural and livestock production may have minimal impact and are unlikely to be sustained by the livestock owner.

Several methods are available to control trypanosomosis. They may be directed against the vector or the parasite, or towards the livestock and modified management. In choosing a method, technical and economic aspects have to be considered and the most viable option selected.

Some of the tsetse control techniques that have been developed with large-scale eradication in mind are not well suited for use in localized, small-scale control operations. Currently, tsetse control utilizes odour-baited, insecticide-treated cloth targets and insecticide-treatments of cattle (**see Chapter 2: Vectors: Tsetse flies**). Odour-baited, insecticide-treated targets have been very effective in Zimbabwe and have been employed in other African countries to control various species of tsetse under different ecological conditions. The concept of using insecticide-treated cattle to control tsetse has been revived since the development of synthetic pyrethroids for fly and tick control. This method has been used successfully and, at first sight, its simplicity and its effect on ticks and biting flies have prompted demands for its more widespread use wherever cattle are exposed to tsetse.[38, 60, 192] However, caution is necessary for several important reasons. The first relates to cost since pyrethroids are relatively expensive. For a commercial farm, changing from its usual acaricide to a pyrethroid could increase costs significantly. In areas where traditional farming methods are practised, tick control measures are not always routinely used, and the regular use of pyrethroids to treat cattle would be a high additional expense. In view of the high operational costs, the duration of treatment should be kept to a minimum. Therefore, before embarking on a control operation with this method, there should be evidence that it will be effective. Several important questions have to be asked: Do tsetse in the area take a high proportion of feeds from cattle? Can tsetse reinvasion of the area be successfully controlled?[304] How many animals should be treated and at what treatment interval? For how long must this measure be enforced? To continue to treat cattle with deltamethrin indefinitely to combat constant tsetse reinvasion raises not only the question of its cost, but also of the effectiveness of its insecticidal and acaricidal properties. The emergence of ticks that are resistant to deltamethrin would be serious, although this may take several years to develop.

The control of ticks in hitherto untreated herds may lead to instability in the relationships between cattle and tick-borne disease agents. The endemic stability prevalent in many traditionally owned herds (**see Chapter 25: Bovine babesiosis**) is likely to be disturbed by rigorous pyrethroid treatment.[294] To prevent losses, vigilant veterinary staff would need to be available to treat sick animals, and there are implications for the vaccination of cattle against tick-borne diseases, which may rely upon moderate sustained challenge for continued immunity. Equally, with the cessation of treatment and increasing tick burdens, the intensity of transmission of *Anaplasma* spp., *Babesia* spp., *Theileria* spp. and *Ehrlichia* (*Cowdria*) *ruminantium* might then increase, leading to increased mortality rates. Despite these negative considerations, the method has much to commend it. It can have a rapid impact on tsetse numbers, but it should be used for the shortest time possible, in conjunction with other control measures. The management of such insecticide-treated cattle in an active, offensive way is also possible. Animals could be treated and moved deliberately into a tsetse focus in an attempt to reduce fly numbers quickly. Protection of these cattle against trypanosomosis would be necessary, because tsetse could still feed and transmit trypanosomes before being killed.[293]

Despite the proven effectiveness of insecticide-treated cattle and odour-baited targets, the success of a tsetse control operation will depend largely on its planning and implementation. Government-controlled interventions are normally carefully planned and implemented by highly

skilled technical personnel. Although community-driven tsetse control operations may still benefit from skilled personnel in the planning phase, the responsibility for the long-term implementation is likely to be in the hands of the beneficiaries. The degree of co-ordination and synchronization of activities between the community members will determine the effectiveness of such tsetse control operations. Synchronizing and co-ordinating the tsetse control activities of a heterogeneous group of community members, with diverse objectives, may be difficult. Therefore, where tsetse eradication cannot be achieved it is unlikely that tsetse control measures will be the only tool to control nagana, and a combination of methods is likely to be used. Depending on the perception of the disease impact, preference will usually be given to a particular method. Where endemic human sleeping sickness occurs, such as in parts of Uganda, there is evidence that communities will sustain the use of traps to control tsetse numbers.

Trypanocidal drugs will continue to play an important role in the integrated control of trypanosomosis. They appeal to communal cattle owners because they provide a means of protecting private goods and may achieve impressive results at low costs. Although the low-level usage of these drugs may reduce mortality rates, such usage generally has low impact on animal production. This will be the case particularly in high challenge areas and when farmers use trypanocides therapeutically rather than prophylactically. The development of resistance in trypanosomes to trypanocides is a continuous threat to their sustainable use in the control of nagana. Even in areas where resistance to trypanocides has not yet been demonstrated, the probability of its development should influence the selection of an appropriate control strategy.

Millions of domestic animals are kept in tsetse-infested areas, but the degree to which they are exposed to tsetse is frequently determined by management practices. The avoidance of heavily infested watering points or grazing reserves reduces challenge, and thus controls trypanosomosis to some extent, but the availability of alternative water sources and supplies of feed are then necessary. Prevention of livestock movement into tsetse habitats also limits the disease, but the enforcement of livestock movement control in Africa is not easy, because of communal grazing practices, seasonal migrations and local political pressures usurping the law.

If animals are exposed to tsetse fly, the severity of the subsequent trypanosomal infections is modified by the animals' general well-being. Well-fed animals are better able to resist the effects of trypanosomal infections, and many animals attain an equilibrium with the parasites. The provision of supplementary feed, especially during the dry season, is an important factor in mitigating disease. Lactating animals, draught oxen and selected breeding bulls benefit from a high plane of nutrition; they are then better able to withstand the effects of trypanosomosis. The general principles of good management also require the prevention and treatment of other diseases, and regular, tactical tick control, anthelmintic treatment and vaccination are useful adjuncts to trypanosomosis control. Sadly, it is often the case that many livestock owners prefer a simple cure to the investment of their time, energy and resources in improved management. The role of good husbandry in disease control is frequently underestimated. Improved breeds of livestock not adapted to tropical conditions generally fare poorly in hot climates, where pastures are often of low quality and tick challenge is frequently encountered. They also readily succumb to trypanosomosis.

Immunological control

The antigenic complexity of trypanosomes has thwarted attempts to develop a vaccine.[207, 222] Although potential immunological targets within the parasite have been identified, no vaccine will be commercially available in the near future, and the greatest hope for the immunological control of animal trypanosomosis lies in the exploitation of trypanotolerant breeds of livestock.[213] Trypanotolerance is a general breed characteristic and, as such, some individuals are more tolerant than others.[253] Furthermore, the trait is complex; it is related to the ability to resist anaemia, the ability to control parasitaemia and, possibly, the ability to mount a more effective immune response to the trypanosome.[213] It also appears that while these processes are under genetic control, they are not directly linked to each other. The search for genetic markers has concentrated on the major histocompatibility complex and on leukocyte antigens.[285]

In the meantime, the correlation of packed cell volume and productivity parameters of N'Dama cattle[284] forms the basis of a field test to select young trypanotolerant animals.[283] It is intended, through a process of selective breeding of trypanotolerant animals, to reduce the problem of trypanosomosis and to improve livestock productivity. However, this approach offers little hope for southern Africa today: inheritance is complex and the number of trypanotolerant animals is relatively small.[213] On the other hand, the fact that some Zebu cattle survive in tsetse-infested areas provides a basis for attempting to select the more tolerant of these animals for breeding purposes. To do so would hasten the process of natural selection that is already occurring.

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13

Dourine

Synonym: Slapsiekte (Afrik.)

A G LUCKINS, P R BARROWMAN, W H STOLTSZ AND J J VAN DER LUGT

Introduction

Dourine is a chronic contagious disease of horses, mules and donkeys. It is characterized by swelling of the external genitalia and subcutaneous tissues of the ventral abdomen, cachexia and, in some cases, involvement of the nervous system. The disease is caused by the venereally transmitted protozoan parasite *Trypanosoma equiperdum*.

Trypanosoma equiperdum is essentially a tissue parasite which has developed the ability to invade and survive in the genital tract, thus enabling direct transmission from one host to another. The disease consequently differs from other African trypanosomoses in that it does not require an arthropod vector for transmission.

Dourine was described as early as AD 400 by Chiron, a Byzantine veterinarian,[60] and is probably one of the oldest recognized diseases of horses. The disease was known to the Arabs and horsemen of North Africa for centuries before it was reported in Europe in the late eighteenth century.[30] The dissemination of Arab horses for breeding purposes may have introduced dourine to Europe, from where it spread to many parts of the world. Rouget, in 1894, was the first to demonstrate a trypanosome in the blood of an infected horse,[35] while the first experimental transmission of the parasite occurred five years later.[56] The organism was named *Trypanosoma equiperdum* by Doflein in 1901.[18]

Control measures based on clinical identification,[35] and later by serological testing,[66] have eliminated the disease from many countries. Intensive campaigns led to its eradication in Canada[66] and the final pockets of infection in the USA were eliminated in 1949.[36] By 1960 most European countries were free of the disease.[19] Outside southern Africa dourine still occurs in certain parts of North Africa, Russia, the Middle East, and Burma.[19] Recent outbreaks have occurred in Italy.[9]

Dourine was known to farmers in Griqualand West in South Africa for several years before it was officially recorded for the first time in 1914.[64] During the same year the disease was reported from Namibia by Maag as cited by Schulz.[60] A shipment of horses from Germany to Namibia may have brought the disease to the subcontinent.[33] Schulz believed that dourine was brought into South Africa by donkey jacks imported from Canada or by transport horses returning from Namibia during the Herero wars.[58] Dourine has a scattered distribution in Namibia, Botswana, Swaziland, Lesotho and South Africa.[8, 19, 20, 68]

Although the disease is not of great economic importance in South Africa, its continued presence necessitates strict surveillance of horses intended for breeding and export purposes.

Aetiology

Trypanosoma equiperdum is a salivarian trypanosome of the subgenus *Trypanozoon*, which includes *Trypanosoma evansi* and the *Trypanosoma brucei* complex.[17] The mammalian blood forms of the three species have similar morphology and share common somatic antigens, which prevent their distinction by most serological tests.[52, 70] *Trypanosoma equiperdum* is closely related to *T. evansi*, the causative organism of surra, which is endemic in North Africa,[28] and it is possible that they are derived from a common ancestor descended from *T. brucei*.[12] Evidence that they might belong to the same species comes from isoenzyme characterization that has shown isolates of *T. equiperdum* and *T. evansi* from different geographical regions form a homogeneous group,[39] and analysis of kinetoplast DNA, nuclear DNA and molecular karyotyping has confirmed the closeness of their phylogenetic relationships.[39, 40, 41, 71] A major difference between the two species is in the presence of maxicircles in the DNA of *T. equiperdum* and their absence in *T. evansi*,[51] so it is unlikely that *T. equiperdum* could have originated from *T. evansi*, as suggested by Hoare,[28] as this would have required the reacquisition of the maxicircles.

Trypanosoma equiperdum is monomorphic and typically occurs as slender forms 15,5 to 36 µm long, with a distinct free flagellum and a subterminal kinetoplast.

Occasional stumpy forms are seen.[29] The organism is only found naturally in the definitive host and multiplies asexually by binary fission. *Trypanosoma equiperdum* strains have been maintained for experimental purposes in the laboratory in animals such as mice, rats and guinea pigs for many generations.[49, 66] These strains have enhanced virulence and produce acute disease when used to infect horses.[49] They have a tendency to produce dyskinetoplastic strains, particularly in rodents.[30]

Although *T. equiperdum* is essentially a tissue parasite, parasitaemia does occur 21 to 23 days after natural infection and may persist for three to four months. Thereafter it is difficult to demonstrate the parasite in the blood of infected horses.[6, 7]

For many years, *T. equiperdum* could only be maintained by serial passage in laboratory animals or developing chick embryos.[44] Recently an *in vitro* culture system has been developed in which a laboratory strain of *T. equiperdum* can be continuously maintained in a semi-defined medium, whilst still retaining its morphological and biochemical characteristics.[5, 47] These *in vitro* systems have been utilized in studies on the efficacy of trypanocidal drugs[11, 72] and could be used also in the study of the physiology, biochemistry and antigenic variation of the parasite.

Epidemiology

Dourine occurs in horses, mules and donkeys. Improved breeds of horses such as Thoroughbreds tend to be more susceptible to the disease than native horses, donkeys and mules, which are often subclinically or only mildly affected.[66] It is a true venereal disease. Natural transmission of the organism occurs during genital contact between animals, and infection may occur from stallion to mare or *vice versa*. Biological transmission of *T. equiperdum* by tsetse flies has not been reported but the disease can be transmitted by subinoculating large volumes of blood from infected horses to susceptible horses and donkeys.[6, 49] The disease is not limited to certain environmental conditions,[62] as is usually the case with the tsetse fly-transmitted trypanosomoses.

The presence of dourine in a herd can usually be traced to an infected animal which showed no clinical signs at the time of introduction. Due to the insidious nature of the disease, it may take several months before clinical signs appear and by this time the disease may already be widespread in a herd. Many infected animals are subclinical carriers[26, 58] and they can only be reliably detected by serological methods. Sexual activity is impeded only in cases showing extreme emaciation or severe oedema of the genitalia.

The disease is more likely to spread in herds or areas where indiscriminate breeding of horses and mules occurs. Donkeys used for the breeding of mules may be particularly important in this respect. Mule foals may become infected in the same way as horse and donkey foals (see below).

Transmission of the disease is usually limited to the breeding season when the parasite is transmitted from an infected stallion to a number of mares.[58] Trypanosomes may be numerous in the vaginal discharges of affected mares or in the urethras of stallions, particularly during the early stages of the disease. Although transmission is erratic and will not occur at every mating, it is more likely to occur when a recently infected animal is involved.[6, 49] In South Africa, the disease is seldom seen in Thoroughbred horses, as breeding of these animals is usually done under strict supervision. Great care is taken to test breeding animals before or during the breeding season.

Occasionally foals become infected — possibly during parturition (from vaginal discharges) or by drinking the milk of an infected dam.[58] These foals remain serologically positive and may transmit the disease when sexually mature.[54] Non-infected foals may also become serologically positive by ingesting colostrum of seropositive dams, but they lose the antibodies within five to six months and are not infective.[15]

Pathogenesis

Most pathological effects of *T. equiperdum* infection in the host are characteristic of trypanosome infections in general, particularly those of the *T. brucei* complex **(see Chapter 12: African animal trypanosomoses)**. Natural transmission of the disease occurs when the parasite is deposited on the mucous membranes of the genitalia during coitus. Like the other members of the *T. brucei* complex, *T. equiperdum* has the ability to invade intact mucous membranes and lymphatic vessels but also has a predilection for connective tissue.[22]

Experimental infection of laboratory animals has shown that the major route taken by a laboratory strain of *T. equiperdum* from an intradermal inoculation site to other locations was via the peripheral lymphatic vessels. It appears that organisms reach the regional lymph node via the afferent lymphatic vessels, from where they reach the bloodstream. Only after some time do they leave the node via the efferent lymphatic vessels.[63]

Trypanosoma equiperdum multiplies predominantly in extracellular tissue spaces. It is seldom found in peripheral blood and probably only uses the bloodstream as a means of transport from one site to another. York, as cited by Goodwin,[22] reported that the nutrient content of extracellular fluid may favour the growth of the parasites. To some extent this site may also aid their evasion of the action of antibodies present in circulating blood.

As in other trypanosomoses, *T. equiperdum* elicits a marked immune response in the host.[7] Antigen-antibody reactions are accompanied by the release of a number of physiologically active substances including histamine and kinin. The latter may be responsible for the vascular changes and oedema after the initial histamine release. The exact cause of the often observed anaemia has not yet been determined. A combination of the resultant local hypoxia and the

proteolytic and lysosomal enzymes released (in the host's bid to contain the infection) probably contribute largely to the extensive damage caused to connective tissues.[22]

Although during the course of infection trypanosomes are constantly exposed to the immune responses of the host, they are able to evade the deleterious consequences of the immune mechanisms by a process known as antigenic variation. Trypanosomes are covered by a surface coat consisting of a single protein known as the variable surface glycoprotein (VSG). This is a powerful immunogen and elicits the production of protective antibodies, but as the host responds to one VSG, trypanosomes expressing another VSG become dominant. The different VSGs are expressed in a general order, with certain VSGs appearing early in infection and some later (**see also Chapter 12: African animal trypanosomoses**).[4, 55]

Immunodepression is a characteristic feature of infections with pathogenic trypanosomes in both domesticated livestock and laboratory hosts,[59] and it may well be an important mechanism in the induction of disease. Infection results in reduced primary immune responses to non-trypanosomal antigens and diminished T cell proliferation and cytokine production.[14, 59] Complement components are reduced, inappropriate antibody responses are made and there are defects in isotype switching and impaired B cell memory responses.[2, 67] In experimental infections with *T. equiperdum*, a rheumatoid factor-like substance destroying IgG antibodies and an unidentified soluble substance causing functional suppression of T and B lymphocytes have been identified.[32, 33] These factors probably predispose the host to secondary infections.[23, 53]

The onset of the nervous form of the disease appears to coincide with the presence of parasites in the cerebrospinal fluid (CSF). The CSF may represent a favourable environment for the parasite as the immunogobulin levels are much lower than in plasma, inflammatory cells are fewer and glucose levels similar.[2] The neuropathology of dourine differs from that described for other trypanosomoses, including *T. brucei* infection in horses.[43] The primary changes in *T. equiperdum* infections comprise cellular infiltrations of spinal nerves and some major nerve trunks, but without much involvement of the central nervous system. A possible explanation for the site of these lesions is the drainage of CSF which moves along the sheaths of the spinal nerves, i.e. antigens of trypanosomes present in CSF may elicit a host response at these sites where nerve tissue is no longer protected by the blood–brain barrier.[6, 7]

Passive protection against *T. equiperdum* infection by transfer of immune serum to susceptible animals has been demonstrated,[34] and passive transfer of immunity from infected dams to their offspring may thus occur via colostrum. The efficacy of this protection is, however, limited by the ability of the parasite to rapidly change antigenically during the course of infection. Resistance to reinfection is limited only to homologous strains.[61]

Clinical signs

Dourine in southern Africa[6, 26, 58, 64] is similar to but usually milder than the disease in Europe[31, 35] and North America.[66] The course of infection and clinical signs vary considerably, depending on the virulence of the strain and the susceptibility of the host. After an incubation period ranging from one week to a few months,[26, 30, 58, 62] the disease may assume one of three forms, namely an asymptomatic, interstitial or oedematous, or nervous form. The nervous form occurs as a sequel to the interstitial or oedematous form and is invariably fatal.

South African strains of *T. equiperdum* tend to induce asymptomatic or latent infections, particularly in donkeys.[26] These animals remain seropositive for several years and are capable of transmitting the infection. Clinical signs may be precipitated in latent cases by stress such as that caused by unfavourable weather, physical exhaustion and malnutrition.[26, 49, 58] A moderate anaemia is often observed in animals which show no apparent clinical signs, as well as in those with chronic oedematous lesions or nervous signs.[6, 62, 66]

The interstitial or oedematous form is usually a manifestation of chronic infection, lasting several months to several years. A common clinical sign in both stallions and mares is progressive weight loss, with the animal eventually becoming severely emaciated (**Figure 13.1**). These debilitated animals are prone to infection by other pathogens and parasites, and many die from secondary causes.[53] The interstitial form of the disease may or may not be associated with oedematous swellings in the early stages. Stallions show varying degrees of oedematous swelling of the sheath, glans penis and scrotum (**Figure 13.2**) and, in severe cases, the oedema may extend to the perineum and ventral abdomen and thorax (**Figure 13.1**). Such swellings are neither hot nor painful and often disappear after a few days, only to recur more severely within two to three weeks. The oedema may persist for up to 18 months after which the swellings often become permanent due to their organization by fibrous connective tissue.[6] The penis may protrude and occasionally there is prolapse of the urethra.[26] In mares, the vulva and perineum and occasionally the ventral abdomen and udder are swollen. In some cases, a viscous vaginal discharge is seen which may be copious. Hyperaemia of the mucous membranes of the vulva, vagina and clitoris is sometimes accompanied by a few nodules and ulcers which heal leaving white scars. Some mares show frequent micturition and behave as if continually in oestrus. In other countries, signs such as ulceration of the penis, frequent erections, and ocular manifestations such as photophobia, keratitis and corneal opacity have been reported, but these have not been recorded in southern Africa. Raised urticarial skin plaques, 40 to 50 mm in diameter, referred to as 'silver dollar' plaques, have only rarely been recorded in animals suffering from dourine in southern Africa.[6, 58, 64] Early



Figure 13.1 Horse showing emaciation, ventral oedema and swollen prepuce

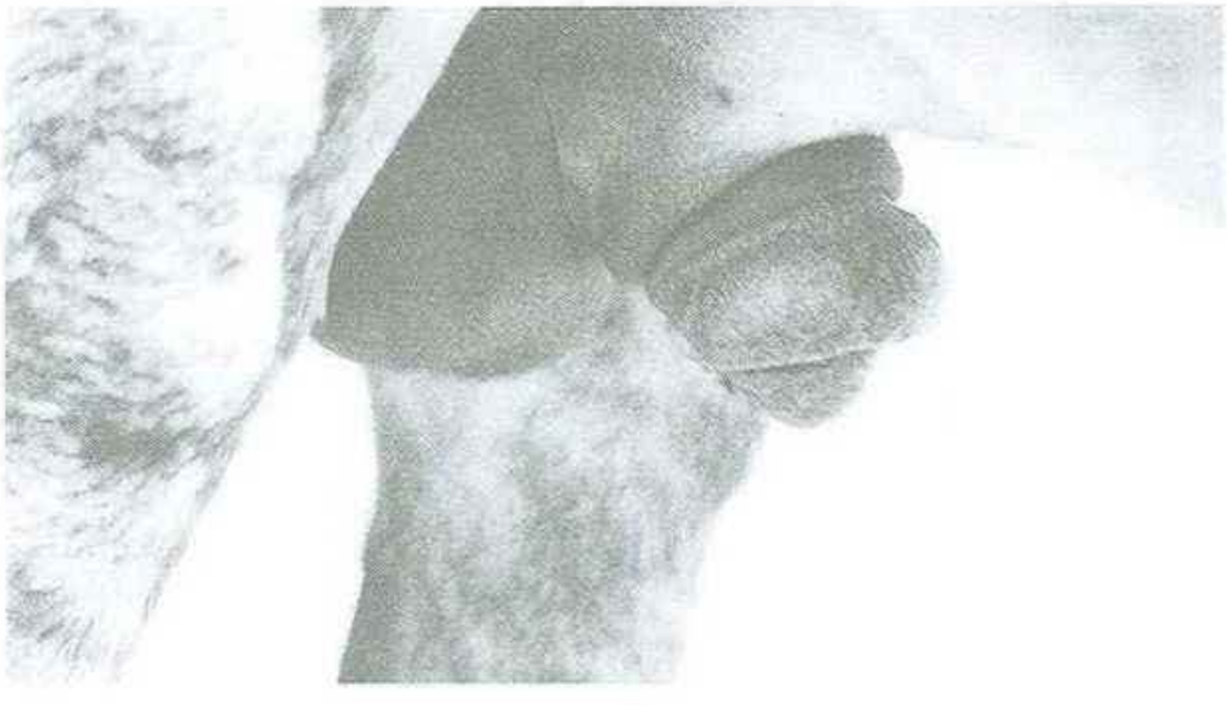


Figure 13.2 Horse showing scrotal and preputial oedema, and phimosis

reports from Europe, North Africa,[50] and India described these plaques as being of common occurrence.[25] Although not frequently seen in Canada, Watson[66] considered them to be pathognomonic.

Nervous disorders occur in some cases and may set in soon after the emaciation or the oedema appear or, alternatively, some time may elapse before their onset. Initially there is acute generalized or localized hyperaesthesia of the skin.[31] Later, sensitivity decreases and is followed by paralysis of motor nerves. The motor involvement originates in the perineum and hindlegs and may eventually extend to affect the forelegs. Affected animals become restless and move uneasily from one hindleg to the other. There may be stiffness, weakness or lameness in one or both hindlegs and the animal may have a swaying or straddling gait. The hindlegs are often set wide apart and the feet are frequently dragged along the ground with knuckling over of the fetlocks. Animals may have difficulty in rising and lying down. Movement becomes increasingly difficult and affected animals are therefore reluctant to do so. Eventually they go down in sternal or lateral recumbency. Although some cases do show evidence of apparent improvement, the condition of animals suffering from the nervous form deteriorates progressively and they invariably die.[26]

Early reports from Canada and Europe describe facial or recurrent nerve paralysis affecting the lips, nostrils, ears and throat.[31, 66] These findings are far less frequent in southern Africa but they have been reported from the Cape provinces.[58]

Pregnant mares may very rarely abort[64] but usually carry their foals to full term.[15, 58]

Pathology

Animals that die from dourine are usually severely emaciated and anaemic, and changes similar to those found in other wasting diseases are seen. There is subcutaneous oedema, particularly of the perineum, male and female genitalia, and ventral parts of the abdomen and chest. In longstanding chronic cases the oedema is often accompanied by fibrosis of the scrotum, prepuce and tunica vaginalis. In mares vaginitis, metritis and cystitis may be present.[58] Numerous cysts occur in the uterus of some mares, and the ovaries are enlarged and show cystic degeneration.[58] Most of the internal lymph nodes, but especially those draining the inguinal and pelvic regions, are enlarged, oedematous and firm on palpation. On cut surfaces the affected nodes are pale and there is little differentiation between cortex and medulla.[7]

In horses that exhibit neurological disturbances, the perineural connective tissues of the larger nerve trunks, particularly those leading to the extremities, are oedematous,[7, 31, 58] or the nerves are transformed into fibrous cords fused with the surrounding fascia.[31] There may be a serous infiltration around the lumbar and sacral portions of the spinal cord[6, 45, 57] and the cord may be softened.[26, 66]

Microscopically, variable numbers of lymphocytes, plasma cells and macrophages infiltrate the dorsal and ventral spinal nerve roots, spinal nerves, ganglia and meninges of the lumbar and sacral regions, and in more advanced cases, the larger peripheral nerves, notably the sciatic and obturator nerves.[6, 7, 31, 45, 46, 66] In cases of longer duration, spinal nerve roots in the thoracic region and the brachial nerves are also affected, although to a lesser extent.[6, 7] Earlier workers referred to 'degenerative changes', 'atrophy' and demyelination of nerve fibres in the spinal and peripheral nerves and the spinal cord,[21, 31, 45, 46, 57, 61] but Barrowman[6] stated that the lesions in dourine in southern Africa are largely confined to the peripheral nervous system. Marek[42] described inflammatory changes in the facial and trigeminal nerves.

Microscopic changes in affected lymph nodes include extensive lymphoid hyperplasia, reticuloendothelial proliferation and varying degrees of lymph stasis, as well as areas of fibrosis in the more chronic cases.[7]

Diagnosis

The diagnosis of dourine is usually made indirectly, due to difficulty in demonstrating organisms in either the blood or tissues or in discharges from the genitalia. It is generally futile to search for trypanosomes in blood smears because their presence here is transient, although organisms may be found in recently infected animals. If an attempt to demonstrate them is made, a large quantity of citrated blood should be collected and either be centrifuged or allowed to sediment out. The lowest part of the plasma layer is then examined for parasites.[6, 49, 64]

The clinical signs of dourine are not specific and, as many cases in southern Africa are asymptomatic,[6, 7, 49] serology remains the only reliable and practical method of diagnosis. The complement fixation (CF) test has been used for the diagnosis of dourine since 1915 and is still the most commonly used serological technique.[27] However, it is only appropriate for making a specific diagnosis in countries in which other members of the *T. brucei* complex are not present, since antigenic cross-reactions within the group occur. The sera of infected animals give positive CF tests from 20 to 30 days after infection[6] and remain positive, with fluctuating titre, for up to 10 years.[66] Sera from donkeys and mules are more frequently anticomplementary than are those of horses, which are rarely so.[68]

Other serological tests for the diagnosis of dourine have been applied experimentally.[13] These are the enzyme-linked immunosorbent assay (ELISA), and the radio immunoassay, immuno-electro-osmophoresis, and fluorescent antibody tests. The results obtained show that the CF test is still the most reliable although the ELISA has a satisfactory concordance ratio with the CF test. Discrepancies encountered with the other tests are attributed to many causes, including the subjectivity of test interpretation and differences in antibody populations detected by the different tests. Caporale *et al.*,[13] however, consider that the ELISA is the method of choice as the antigen is used in minute quantities and appears to be more stable than the CF test antigen. This was supported by the work of Wassall and his co-workers,[65] who concluded that the CF test was less sensitive than either the indirect fluorescent antibody test or the ELISA. They considered that ELISA was the test most likely to lend itself to standardization, allow considerable automation and could be adopted eventually as an internationally approved test for equine health certification for freedom from dourine. ELISAs based on *Trypanozoon* group-specific antigen have also shown promise for detecting antibodies to pathogenic trypanosomes, including *T. equiperdum*,[10, 38] and tests based on defined antigens could more readily enable standardization than tests based on the use of crude, sonicated antigen preparations. Enzyme-linked immunosorbent assays for antigen detection (Ag-ELISA) have also shown promise experimentally[10] and both Ab- and Ag-ELISA tests have been used to investigate the prevalence of *T. equiperdum* in horses in Ethiopia.[1] A commercial card agglutination test for trypanosomosis, using formalin-fixed *T. evansi*, is available[3] and might be useful in the diagnosis of equine trypanosomosis. The test has not been evaluated for use in the diagnosis of *T. equiperdum*, but a similar test, based on *T. brucei gambiense* antigen, has been shown to detect antibodies in horses that show clinical signs of dourine.[53]

Diagnosis by subinoculation of blood into experimental animals is not practical as the southern African strains of *T. equiperdum* do not transfer readily to dogs, rabbits, rats or other laboratory animals.[6, 26, 64] Infection of these

laboratory animals would occur more readily after passage in equine animals, but this method is rarely used for the diagnosis of the disease.[25, 49]

Differential diagnosis

In southern Africa, oedema of the genitalia in horses may occur in other conditions, e.g. nagana (caused by *T. brucei*); equine viral arteritis, which was recently diagnosed in South Africa; coital exanthema; purpura haemorrhagica, where, in addition, extensive petechial and ecchymotic haemorrhages may occur on the skin and mucous membranes; and trauma of the genitalia.

Conditions leading to chronic weight loss and emaciation, such as malnutrition, verminosis, dental pathology, other chronic infections (e.g. trypanosomosis) and chronic liver disease (e.g. that occurring in seneciosis) can be confused with, or mask, the signs of dourine. The nervous signs associated with dourine should be differentiated from similar signs recognized in a variety of infectious and non-infectious causes, such as equid herpesvirus-1 encephalomyelitis, cervical stenotic myelopathy ('wobbler syndrome'), injuries to the back and pelvis, and iliac thrombosis.[16]

Control

Several chemotherapeutic drugs, particularly homidium, suramin, quinapyramine and some melaminyl antimony compounds may have some beneficial effect in clinically affected animals, but none have been proven effective in the sterilization of the infection.[7, 70] The efficacy of most trypanocidal drugs is limited by their inability to cross the blood–brain barrier. The use of chemotherapy for the control of dourine is thus not recommended as treated animals may show clinical improvement but remain carriers of the parasite.

In most countries dourine is a scheduled disease and control measures are enforced through legislation. In countries free of the disease, the issue of import permits for domestic equids is subject to certification by an official laboratory of negative serological results (usually the CF test) for dourine.

There is no legislation restricting the movement of horses within the borders of South Africa and Namibia as far as dourine is concerned. In these countries all outbreaks of the disease are notifiable by law, but compulsory testing of equine animals (all stallions and maiden and barren mares under the jurisdiction of the Jockey Club of South Africa must be tested for dourine prior to the commencement of each breeding season) to control or eradicate the disease is not enforced. Serological testing of animals is performed at the request of the owners and is subject to them signing an indemnity form which requires them to have all positive animals destroyed, castrated or ovariectomized. Neutering may lead to clinical improvement but animals remain infected.[58] Castration of infected stallions is not always effective in preventing them from transmitting the disease, as they may still serve mares for some time.

The presence of fences may help to control the spread of the disease, although stallions have been reported to serve mares over fences, even those constructed of barbed wire.

Due to the presence of variable surface antigens on *T. equiperdum*, the development of an effective vaccine has not been achieved.[24]

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14

Trichomonosis

P C IRONS, A P SCHUTTE, S HERR* AND J P KITCHING

Introduction

Trichomonosis is a venereal disease of cattle caused by the protozoan *Tritrichomonas foetus* which parasitizes the genital tract. Clinically, it is characterized by aberrant oestrus cycles, infertility, a low percentage of early abortions, and pyometra.

According to Morgan[85] *T. foetus* was first identified by Kunstles in France in 1888 but Mazanti is generally credited with the discovery of the parasite in Italy in 1900.[93]

Trichomonosis occurs in virtually every country of the world where cattle are raised. It was first recorded in Pennsylvania in 1932 and has since been diagnosed throughout the USA.[1, 6, 13, 119, 145] The disease is also reported to be present in Europe,[63, 149] Australia,[32, 33] Africa,[23, 103, 123] India,[88, 98] South and Central America,[10, 145, 194] and Canada,[24, 40, 120] and should be considered endemic in extensive cattle-raising regions.[130]

The disease in cattle was first recorded in South Africa by Robinson[122] in 1937 and has since been diagnosed in herds throughout the country.[15, 78, 84, 102, 103, 142, 143] At first it was not considered to be of importance in cattle breeding. Later reports, however, identified it as a major cause of infertility in beef cattle in southern Africa.[78, 113, 137]

Trichomonosis occurs in beef and dairy breeds. At present it seems to be more frequent in beef herds probably due to the extensive use of artificial insemination in dairy cattle, which effectively reduces the chances of transmission.[113, 121]

Economic losses associated with the disease can be ascribed to the increased number of days that cows are open, decreased calving percentage, decreased growing time of calves born later in the season, cost of culling and replacing animals, and the costs of treatment.[55, 90, 109, 110] Modelling of the extent of economic losses indicates that the losses increase exponentially as the prevalence of infection in a herd increases, with an expected loss of revenue of 35 per cent for each cow confined with an infected bull in a herd with a disease prevalence of 40 per cent.[110] Economic losses in an infected dairy herd were estimated at US$ 665 per infected cow.[55]

Aetiology

The causative organism, *Tritrichomonas foetus*, has been variously named, the more common names being *Trichomonas foetus*, *Trichomonas bovis*, *Trichomonas genitalis* and *Trichomonas bovinus*.[94, 119] It is an obligatory inhabitant of the bovine reproductive tract and has only a trophozoite form. The similarities between *Tritrichomonas suis* and *T. foetus* have prompted suggestions of reclassifying these two species, with recent studies of the haemagglutination and adherence properties, neuraminidase activity and sequencing of the 5.8S rRNA gene supporting this view.[46, 96] Molecular analysis has gone one step further by indicating that *Tritrichomonas mobilensis* may also belong to the same species.[96]

The parasite can be identified by the presence of three anterior flagellae and a characteristic undulating membrane (**Figure 14.1**) which is visible because of its wave-like motion on one side of the organism when viewed under phase-contrast or dark-field microscopy.[16, 85] The undulating membrane is typically somewhat shorter than the body, and its external margin is supported by a recurrent flagellum which continues beyond the posterior end as a free posterior flagellum.[119]

The flagellated protozoan tends to be pyriform and elongated when a fresh specimen obtained from an infected animal is examined (**Figure 14.2**), but on culture it does show pleomorphism and a tendency to become spherical.[94] In fixed and stained preparations its approximate size is 9–25 × 3–15 µm, and is thus slightly longer and slimmer than polymorphonuclear cells.[144] In wet preparations its jerky, rolling motion at magnifications of 100x or 250x under phase-contrast and dark-field microscopy is characteristic. However, ancillary structures should be confirmed at higher magnifica-

* Deceased

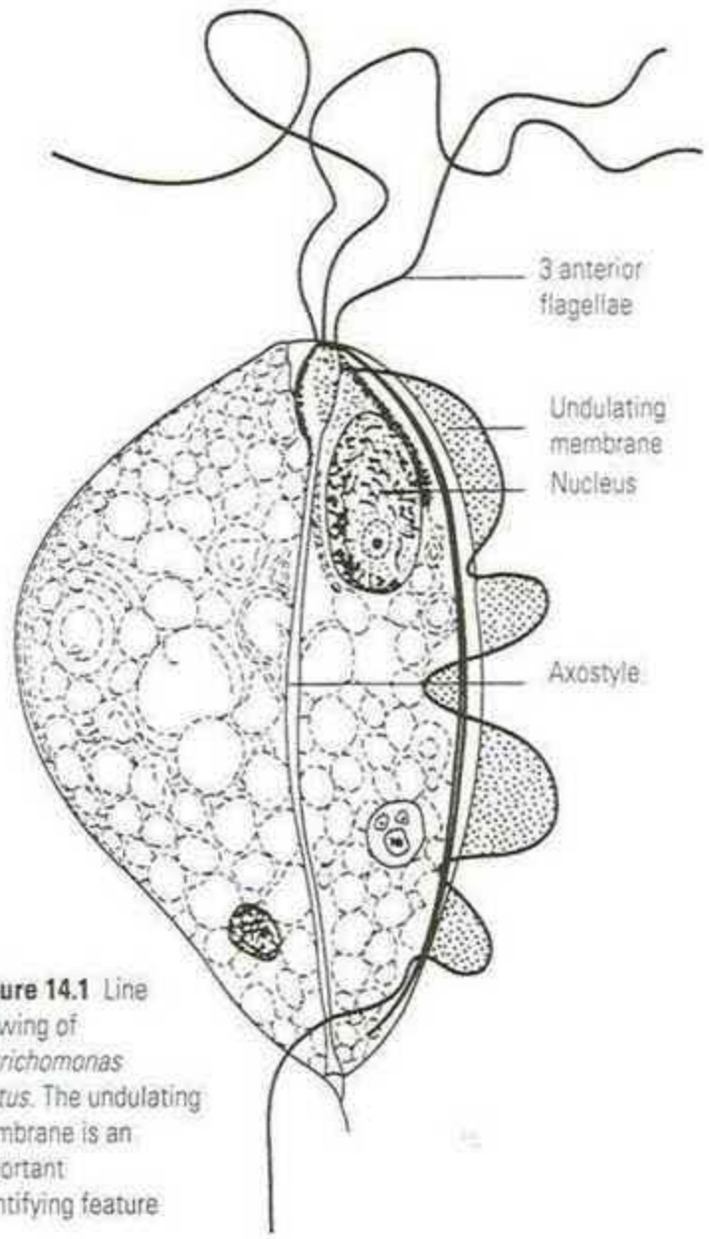


Figure 14.1 Line drawing of *Tritrichomonas foetus*. The undulating membrane is an important identifying feature

tions. Protozoon parasites with similar motion, morphological features and size can be recovered from the preputial cavity of bulls.[20]

The existence of different serotypes was first recorded by Kerr and Robertson,[74] and was subsequently confirmed and elaborated on by others.[4, 22, 47, 58, 72, 107, 119] Three serotypes, namely Brisbane, Belfast and Manley, are recognized.[42] The pathogenicity of the serotypes *in vivo* appears to be similar.[47] The antigenic characteristics of *T. foetus* have been reviewed.[41] Analysis of the total protein and antigen profiles of different field isolates showed strong similarities of the protein profiles and strong antigenic cross-reactivity, indicating its relative antigenic homogeneity.[41, 65] Polymerase chain reaction (PCR) studies have shown both conserved genetic loci as well as polymorphic loci in different isolates.[120] No attempt has been made to establish the antigenic relationship between South African isolates and those from other parts of the world. Using an indirect immunofluorescence technique, all the South African isolates tested were shown to have common antigens.[126]

Epidemiology

Persistently infected bulls are the primary source of infection. Infection rates in bulls are typically between 1 and 40 per cent.[2, 3, 13, 34, 43, 45, 57, 88, 89, 90, 104, 148] Prevalence data for various regions in southern Africa concur with these figures, with various laboratories reporting infection rates ranging from 0 to 25 per cent of samples and 0 to 46 per cent of herds.[15, 44, 78, 84, 102, 103, 118] The population dynamics of herd bulls generally keeps the prevalence within a herd to below 50 per cent.[110]

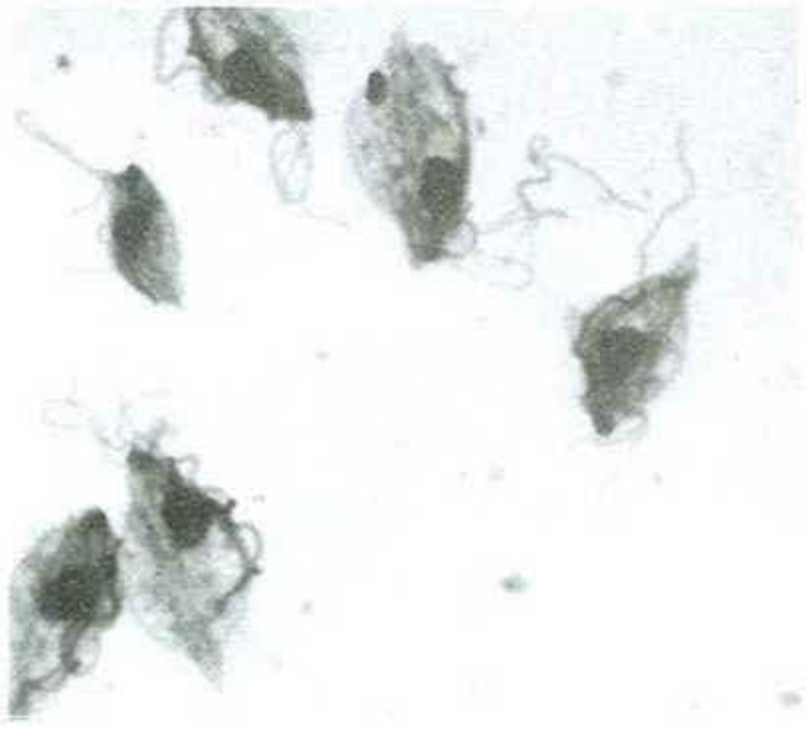


Figure 14.2 Typical morphology of *Tritrichomonas foetus* in a specimen from an infected animal

Specific risk factors associated with an increased prevalence in bulls are:

- increased age.[18, 90, 103, 104, 110, 133] Bulls of up to three years of age are relatively resistant to infection.[16, 31, 34] However, some young bulls do harbour the organism and are significant in the perpetuation of infection in a herd.[18, 49, 133]
- beef herds,
- sexual rest,[104]
- commingled grazing,[54] and
- exposure to a greater number of other herds.[54]

Bulls of most breeds are susceptible to infection, with some variations in actual infection rates which may reflect differences in breed susceptibility, breeding activity,[104] or management.[11, 18, 44, 110, 113, 130]

Once infected, bulls remain life-long carriers and perpetuate the infection from season to season,[17, 93] although spontaneous recovery can occur.[39] Concurrent herd infection with *Campylobacter foetus* subsp. *venerealis* is common,[91, 103, 104] although there is no evidence to suggest that infection with one agent affects the chances of being infected with the other.[26, 90]

Trichomonosis is usually contracted during coitus. However, *T. foetus* can also be disseminated by artificial breeding as it can survive the standard processing methods for semen used in artificial insemination.[13, 70, 92] Despite the low frequency of infection, bulls in artificial insemination units in

the USA continue to be screened.[92] Mechanical transfer of infection by young bulls from infected to susceptible females has only been demonstrated in one case in which two services occurred within a short period of less than 30 minutes.[34]

Transmission of *T. foetus* between bulls when a susceptible bull mounts another which has recently been contaminated by an infected bull is possible but documented evidence is lacking.[1, 75, 121, 130] Iatrogenic transfer may occur if basic sterile techniques are overlooked when collecting preputial material for diagnostic purposes or when performing vaginal examinations on cows.[55] Transmission of the disease between females by contact or mechanically by flies is unlikely.[34]

Sexually active females of all ages are equally susceptible, with spontaneous clearance of infection within seven months, providing that normal oestrus cycles are occurring.[1, 47, 55, 121, 131] If pyometra develops after infection, the oestrus cycle is interrupted and animals remain infected and capable of disseminating the organisms for much longer periods. Shedding of large numbers of organisms occurs following abortion. Individual females may also retain the infection throughout a normal gestation and only become free of the infection when two or three oestrus cycles have occurred after parturition.[1, 129, 141] A partial immunity develops in infected females, which does not prevent reinfection but enables the animal to overcome the infection in a shorter period of time.[35, 141] No immunity has been recorded in bulls at any age.

The number of cows in a herd that become infected by a carrier bull varies. In one survey 42 per cent of the 73 cows served during the first year became infected, while all those which showed resistance to the initial exposure became infected during the second breeding season.[35] Bartlett[13] recorded that 28 natural services produced infection in 24 susceptible females. In another study, 23 of 26 heifers became infected after natural service which resulted in the low pregnancy rate of 45 per cent.[141]

Pathogenesis and clinical signs

If a bull is closely observed following infection, a swelling of the prepuce with accumulation of a mucopurulent exudate within the preputial cavity may be seen; this does not, however, last beyond two weeks.[62, 119] In older bulls, *T. foetus* readily colonizes the preputial and penile mucosa and, less commonly, the urethral orifice.[100] The organisms are confined mainly to the preputial cavity and its secretions, and do not invade the mucosa.[16, 100, 117] It has, however, also been isolated from other parts of the male genital tract, such as the seminal vesicles.[61, 62, 100, 121, 141] Older bulls remain chronically infected although spontaneous recovery has been recorded.[39] The development of deeper epithelial crypts in the mucosa of the prepuce in older animals has been put forward as a reason for increased susceptibility.[16]

In heifers and cows, the vagina is initially colonized by *T. foetus*. From there organisms migrate through the cervix to the uterus and Fallopian tubes and during the process they produce a cervicitis and, in some cases, endometritis and salpingitis. Endometritis is only seen from approximately 60 days after infection.[101] *Tritrichomonas foetus* may be isolated from exudates collected from these sites, depending on the stage of infection.[13, 60, 121, 141] While clinical evidence of vaginitis, cervicitis or endometritis may be noted, generally there are no observable signs at this stage of the disease.[1, 17]

If salpingitis develops, the normal process of fertilization may be disturbed. However, conception is generally not interrupted.[101] Endometritis may cause death of the embryo or foetus due to interference with its nutrition or nidation. The occurrence of foetal death is closely associated with the appearance of histological evidence of endometritis.[101] The exact mechanism of foetal death is not known, although several hypotheses have been formulated.[8, 17] Embryonal and foetal death have been reported from 14 to 18 days up until five months of gestation, with occasional later abortions.[13, 16, 33] Where embryonal death occurs after recognition of pregnancy (day 17), irregular oestrus cycles occur. Generally, up to 5 per cent or more of the foetuses that die during the early stage of pregnancy are retained.[91] These cows subsequently develop pyometra which may not resolve for many months.[16] With the exception of animals which develop pyometra or abort later, the majority of infections will spontaneously resolve, reported clearance times being 95 days and two to four months.[1, 17, 33, 47, 55, 101] Clearance from the vagina, cervix and uterus is simultaneous.[53, 131] Infection does, however, persist for longer in some cows, resulting in a so-called carrier status which can perpetuate the infection in a herd despite commonly applied control measures.[55, 87, 131] Rarely, cows may carry the infection throughout pregnancy and calve at term.[55] This was demonstrated in two out of 40 infected cows in one study, the two cows remaining infected for six and nine weeks postpartum, respectively.[129] Repeated challenge leads to transient infection in some animals, which is cleared within three weeks.[131] This partial immunity is of sufficient duration to carry over from one breeding season to another, reducing the rate of pregnancy loss in the subsequent season.[35] However, cows appear to be fully susceptible again after 15 months.[35] Normal fertility usually is restored two to six months after the initial infection.[1] Infertility following the development of a chronic endometritis or pyometra is rarely seen.[119]

Host–parasite interaction is the subject of recent reviews.[41, 130] Evasive properties of the organism include its non-specific immunoglobulin-binding properties and its production of extracellular proteinases.[139] *Tritrichomonas foetus* organisms bind to and cause extensive damage to bovine vaginal epithelial cells *in vitro* in a species-specific fashion suggestive of a receptor-ligand interaction.[128] This

binding is enhanced by exposure of the vaginal cells to progesterone. Only IgG and complement-mediated defences are known to kill trichomonads.[41] Bulls show only a weak circulating antibody response, but antibodies are secreted into the preputial secretions following challenge, suggesting local processing of antigens.[27, 117, 135] In contrast, heifers mount a significant systemic antibody response by 11 weeks after infection.[131, 135] IgA and IgG1 are secreted into vaginal mucus from the fifth week post-infection, remaining high up to at least week 24 post-infection, and responding to challenge.[53, 69, 131, 135] IgG2 and IgM responses in the genital tract are transient and inconsistent.[131] Uterine responses were found to be slower and of shorter duration but qualitatively similar to those of the cervix and vagina.[131] The local immune response in the uterus and uterine tubes is thought to be as a result of antigenic stimulation acting on lymphoid follicles which act as inductive sites.[8] Anti-*T. foetus* antibodies have been demonstrated in some unexposed heifers in serum and genital tract secretions, the occurrence of which is thought to be natural.[131]

Typical calving percentages in chronically infected herds are between 70 and 85 per cent, while much lower percentages can be expected in susceptible herds.[11, 35] Staggering of the gestational ages of pregnancies following the breeding season is often the first indication of the disease.[17] The disease tends to be more insidious in dairy than in beef herds.[130] While pyometra is generally cited as an uncommon occurrence, it often presents in 5 to 10 per cent of cows in a recently infected herd and was reported in 21.3 per cent of exposed animals in one herd.[17, 91]

Pathology

Gross lesions may be evident in bulls infected with *T. foetus* during the first two weeks of infection and are manifested by a mild swelling of the prepuce and a slight mucopurulent preputial discharge.[33] Histologically, a mixed cellular infiltration characterizes the inflammatory reaction. Debris accumulates in the crypts of the preputial mucosa, but the causative organism is not demonstrable.[71, 100]

In the female, few lesions are observed within 50 days of infection.[101] Varying degrees of vaginitis, cervicitis, endometritis and salpingitis occur in most cases between 50 and 100 days of infection.[8, 101] *Tritrichomonas foetus* may be demonstrated on the surface and in the lumen of endometrial glands using a silver stain. A slight opacity of the normal clear oestral mucus in infected females has been ascribed to the infection,[71] but this phenomenon is often a normal occurrence brought about by the presence of migrating leukocytes at the end of oestrus.[95]

The uterine content in pyometra following *T. foetus* infection is usually voluminous, fluid, odourless, greyish-white and contains trichomonads in great numbers.[16, 119] Following experimental infection, a single heifer in a group of 17 manifested pyometra characterized by the presence of several litres of exudate which persisted in the uterus for eight months after infection.[137]

Placentas from aborted foetuses are oedematous but otherwise unremarkable.[115] Aborted foetuses may be fresh or autolysed.[114, 115] Gross changes were absent in most foetuses.[115] Foetuses aborted in late gestation show pyogranulomatous bronchopneumonia and necrotizing enteritis characterized by thrombotic lesions with tissue invasion by trichomonads.[114] Placental lesions consist of stromal oedema, mixed inflammatory cell infiltration, and focal necrosis of the chorionic epithelium.[115] Organisms may be found randomly scattered in chorionic stroma and in necrotic exudate adherent to the villi.[115, 116]

Diagnosis

The clinical picture of an infected herd is usually one of moderate calving percentages, prolonged intercalving periods, heifers failing to conceive, sporadic abortions, aberrant oestrus cycles, and the presence of post-coital pyometra in some animals.[113, 130]

A positive diagnosis of trichomonosis depends on the demonstration of live *T. foetus* organisms in specimens obtained from the genital tract of females, preputial material of bulls, or aborted foetal and placental tissues.

Bulls are the best animals to select for testing, unless females suffering from pyometra or recent abortion, or aborted material are available. The number of organisms in the preputial secretions of infected bulls fluctuates, reaching a peak every 5 to 10 days.[60] Samples should preferably not be collected from bulls during the breeding season, as active breeding appears to diminish the number of organisms in preputial secretions.[35] Suitable times to collect preputial material from bulls is therefore at least two weeks after the end of the breeding season and shortly before the onset of a breeding season. The moderate sensitivity of currently available tests and the fluctuation in numbers of organisms necessitates the collection of samples on three occasions a week or more apart in order to reliably confirm that a bull is free of infection.[99, 106, 133]

Preputial material may be collected by sheath wash or sheath scraping, the two methods not differing significantly in sensitivity.[125] Both methods have been well described.[11, 21, 127, 138] Rinses taken from artificial vagina liners immediately after use have proved to be almost as reliable as sheath wash samples for the detection of infection.[56] Whichever method is used, measures must be implemented to exclude the potential of pathogen transmission between animals.

Sheath washes are collected by means of infusing 50 ml of sterile phosphate-buffered saline into the preputial cavity via a funnel and flexible tube, massaging the preputium vigorously, and retrieving the fluid into a clear glass bottle for inspection. A suitable sample is opaque and contains visible flecks of cellular debris. Urine contamination of the sample does not kill the organisms.[100]

Sheath scraping is performed with a rigid artificial insemination pipette connected to a hypodermic syringe. This is introduced into the caudal reaches of the preputial cavity and then, under guidance of a hand massaging the preputial skin, is manipulated vigorously, scraping against the preputial lining and glans penis while negative pressure is placed on the syringe plunger. On withdrawal of the apparatus a small amount of thick, opaque material should be present in the pipette. The presence of blood does not constitute a problem and is considered by some to indicate adequate depth of sampling.[11] The material is then flushed into a fluid medium in a container by repeatedly aspirating the medium up into the pipette and then ejecting it into the container. Placing a drinking straw into the preputial opening facilitates repeated passage of the pipette in an effort to obtain a good sample, while roughening the tip of the pipette may also enhance the quality of the sample. Custom-made instruments for the collection of preputial material have not shown any advantage over the foregoing method.[99]

Cows selected for collection of genital tract fluids should include those which have aborted recently and those with pyometra.[50] The detection of infection in clinically normal cows which have not been recently infected is less reliable. Five successive negative cultures are suggested to confirm the negative status of female animals, although two samples may be collected at each sampling opportunity.[53] Vaginal, cervical or uterine samples may be obtained from females using a pipette attached to a syringe or by a swab, douche or scraping methods.[13, 21, 42, 87, 93] Pipettes or swabs may be protected with a sanitary sleeve to reduce contamination of the sample. The pipette is passed to the relevant section of the genital tract and suction applied to the plunger. Infusion of small volume of Ringer's solution facilitates sample collection in cows with very thick secretions.[50] Samples collected in plastic pipettes may be chilled and transported in the pipette with the ends sealed.

Suitable samples from aborted material include foetal abomasal contents and foetal fluids. Foetal and placental tissues should also be collected for histopathological examination.

Vials or pouches of transport or culture media may be inoculated at the crushside immediately after collection (**Figure 14.3**).[82] Refrigerated buffered saline containing foetal bovine serum, lactated Ringer's solution, Kupferberg medium, and milk-based media maintain viability of the organisms for 48 to 96 hours.[25, 112, 130, 136] Alternatively, samples should be kept cool and transported to the laboratory as soon as possible. While organisms survive in buffered saline for 24 hours, samples kept for this time in transport media do suffer from a decreased viability,[76, 133] and transport to the laboratory should not be delayed.

Samples may be subjected to direct examination or cultured and then examined. Dark field, phase contrast or differential interference contrast microscopy are favoured for demonstration of the motile organisms. However, normal bright field microscopy with the correct condenser settings can also be used. Care must be taken to positively identify the parasites as other protozoa closely resembling *T. foetus* have been isolated from preputial samples from virgin bulls.[20] Direct examination of sheath wash samples is reported to have a sensitivity of 20 per cent,[118] while direct examination of vaginal and cervical secretions from recently infected heifers was found to be 36 and 40 per cent sensitive respectively.[132] In contrast, it has been found that direct examination of vaginal mucus is 73,9 per cent sensitive.[118] Direct examination and culture of uterine secretions from clinically normal animals is less sensitive, and the cervix appears to be the best area for the isolation of organisms.[132] Centrifugation should be applied to concentrate samples when necessary.

Various nutrient media have been used for the culture of



Figure 14.3 Inoculation of a commercially available transport and culture kit with preputial material collected by preputial scraping

T. foetus. Most consist of peptones, yeast extract, serum, maltose or dextrose, buffers and antibiotics. Diamond's medium, which consists of trypticase-yeast extract-maltose or TYM, is the most commonly used in the USA, and has a reported sensitivity of 81,6 to 93,2 per cent.[76, 99, 125, 133] The sensitivity of a commercially available modified Diamond's medium for diagnosis of *Trichomonas vaginalis* in humans was found to be poor in isolating field strains of *T. foetus*.[9] Organisms are present in greatest concentrations at the bottom of culture vessels. Positive cultures are usually identified by 48 hours, but examination should continue until seven days.

Various transport media are commercially available. The most widely used consists of a protease peptone medium in a clear pouch (In-Pouch TF, Biomed Diagnostics).[21] This product has various advantages facilitating sample transport, incubation and examination in situations where sophisticated laboratory facilities are not available.[21, 138] However, recovery of trichomonads other than *T. foetus* has been demonstrated.[20] Overnight shipping of samples prior to incubation reduces the sensitivity of this test by 10 per cent.[79] Another combined transport and culture system (Trichtube, Vrede Veterinary Laboratory) as well as a transport medium suitable for both trichomonads and *C. foetus* (Steve's TM, Vrede Veterinary Laboratory) are commercially available in South Africa, but these have yet to be extensively tested.[102]

Most investigations have found Diamond's, Claussen's and the In-Pouch media to have comparable sensitivities for preputial samples, generally in the 70 to 95 per cent range.[9, 54, 99, 104, 106, 113, 138] Some investigators slightly favour the In-Pouch technique, which also has the advantage of identifying positive cultures more rapidly than is the case with Diamond's medium.[21, 79, 138] Culture of cervicovaginal mucus was 56 per cent sensitive in naturally infected cows,[55] while greater sensitivity has been found after experimental infection.[101, 132]

Trichomonads are often visible in haematoxylin and eosin-stained tissue smears, but special staining may be necessary to differentiate them from leukocytes, and identification of the parasite is often not possible.[15, 116] Immunohistochemistry has proven successful in demonstrating organisms using poly- and monoclonal antibodies.[28, 114, 116, 117] This technique can be used for the examination of foetal lung, gastrointestinal tract and placental tissues, as well as vaginal mucus smears.[28, 114] Marked autolysis of tissues makes identification of organisms more difficult.[116]

Vaginal mucus agglutination tests have been well documented,[62, 72, 73, 93, 107, 108, 119] but lack sufficient specificity for accurate diagnosis.[111, 119] An ELISA assay to the protective surface antigen TF1.17 has been shown to detect long-lasting IgA responses in vaginal mucous starting six weeks after infection.[69] Preliminary reports of a serological assay with an apparent sensitivity and specificity of 85 and 95 per cent respectively for cows and heifers are encouraging.[17] This test does not identify infected bulls. A haemolytic test on blood samples has also been used.[29] An intradermal test has been proposed as a screening test in female animals but further research is required.[135]

While an early DNA probe was found to lack sensitivity and specificity,[9] the use of PCR amplification technology has enabled the development of highly sensitive and specific tests for diagnostic and research purposes.[46, 64, 120] While these techniques are not yet commercially available in southern Africa, they are under development.

Differential diagnosis

Early abortions and infertility may be caused by a number of factors, not all of which are infectious. The syndrome of infertility, irregular oestrus cycles and occasional abortion resulting from *T. foetus* infection is indistinguishable from that caused by *C. foetus* subsp. *venerealis*. Therefore, laboratory tests are essential for distinguishing the one from the other.

In beef herds where vaccination against campylobacteriosis has been practised for some years it may be possible to make a diagnosis of trichomonosis based on the clinical manifestations alone.

Control

There is no universally successful and easy treatment for *T. foetus*-infected bulls although curative treatment has been achieved by a number of workers. Early methods included administration of potassium iodide orally or sodium iodide intravenously, and topical application of nitrofurazone, iodine, hydrogen peroxide, acriflavine, diminazene, or an ointment containing 0,5 per cent diaminomethylacridine and 0,5 per cent bismethyl-aminochinolylcarbamide hydrochloride (Bovoflavin).[48, 62, 63, 83, 121, 140, 141] A topical high-pressure treatment of 3 per cent hydrogen peroxide with a wetting agent has also been described.[63] Topical treatment methods have the disadvantage of being cumbersome and time-consuming.[1]

The above methods have been largely superseded by the use of modern antiprotozoal agents. Metronidazole, ipronidazole and dimetridazole have all been used to good effect.[5, 12, 51, 76, 97, 133, 134, 147] Successful use of these agents in bulls requires simultaneous intrapreputial or systemic antibiotic therapy to reduce the resident bacterial flora in the preputium which metabolize the imidazole compounds.[113] Due to the unavailability of ipronidazole and the cost and residue concerns of metronidazole, dimetridazole is the most commonly used compound although its use in cattle constitutes extralabel use. It is available in South Africa in a premix and a soluble form, whereas the parenteral route is used elsewhere.[26] Practical problems associated with the use of these compounds include intractability of bulls due to discomfort caused by the treatment, indigestion, inappetance, abscesses at the injection sites when injectable formulations are used,

and the rapid development of resistance by the organism.[11, 16, 134, 147] In addition, not all bulls are cured and testing must be repeated following a course of treatment. A delay of eight weeks between treatment and retesting is recommended due to the greater number of false negative tests which occur during this period.[133] The negative status of bulls must be confirmed before they are used for service.

A commonly used regime for the use of dimetridazole is oral dosing with 50 mg/kg once daily for five days, either by drenching or using capsules.[11] This is preferred to feed medication due to the difficulty of ensuring adequate intake using that method and the likelihood of treatment failure or the development of resistance if the full dose is not consumed.[130] Dimetridazole is also effective in the treatment of campylobacteriosis in bulls.[26]

Due to the practical problems outlined above, treatment of infected bulls is seldom applied; they are generally culled.

Treatment of cows by the systemic administration of ipronidazole has been suggested.[16] The efficacy of such treatment is difficult to evaluate due to the poor sensitivity of diagnostic methods in the chronically infected cows. Treatment of cows suffering from pyometra with prostaglandin F_2 leads to evacuation of the uterine content and return to cyclicity but infection can persist, leading to recurrence of the pyometra or reinfection of the bulls in the herd.[49] Furthermore, cows treated in this way have a high incidence of permanent infertility.[7]

Artificial insemination is the best means of preventing transmission of the disease. However, the semen must originate from disease-free bulls and suitable hygiene measures must be applied during insemination to avoid perpetuating the infection.[49] Unfortunately the management, logistical and physical requirements of artificial insemination preclude its use in many herds.

Many managemental strategies have been used to control trichomonosis. All are based on a sound understanding of the epidemiology. These strategies have been reviewed.[11] Control measures are often dictated by the practicalities and economics of the situation and will differ from herd to herd. Control of the disease in an infected herd depends on the removal of infected bulls by treatment or culling and retesting prior to the next breeding season, reduction of the infection rate or elimination of the infection from the female herd, increasing herd immunity by vaccination, and prevention of reintroduction of the infection.

Sexual rest before rebreeding allows for spontaneous cure to occur in most clinically normal heifers and cows and a return to acceptable levels of fertility, as infertility may persist for two to six months following infection.[1, 8, 42, 47, 55] A period of three months is generally considered to be adequate, providing that the animals are cycling regularly during this period. However, some clinically normal animals do remain infected for up to 10 months, necessitating the use of additional control measures.[87, 131] A similar period of sexual rest following normal calving is necessary to allow for elimination of infection from pregnant carrier cows.[129]

One strategy for elimination of the infection from a herd involves the establishment of a clean herd, consisting of virgin heifers and other previously unexposed animals, on which only young, unexposed bulls are used.[11] This herd is then built up as the previously exposed herd is gradually reduced by culling. This strategy requires absolutely strict segregation of the clean and previously exposed herds, making excellent fencing and management mandatory.

Bulls can be ensured to be free of infection by various means. Young bulls which have never bred are assumed to be safe. Unfortunately, even young bulls can act as sources of infection and when their negative status cannot be guaranteed, testing should be performed.[18] Older bulls should be sourced from a certified negative herd and should be tested three times before use. Previously exposed animals must test negative in three successive tests at least six weeks after withdrawal from breeding. Annual replacement of all bulls over four years of age with young bulls has been applied with success,[38] but may be ineffective or impractical due to significant numbers of infected bulls remaining and the marked shortening of the productive lifespan of the bulls.[90] The inability to detect and eliminate clinically normal carrier cows may also render a control programme focused solely on ensuring safe bulls ineffective.[59]

Wherever possible, the maintenance of a closed herd is fundamental to the effective prevention of the introduction of *T. foetus* into a herd. This entails good maintenance of perimeter fences, limited introduction of stock from other sources, and the testing and quarantine of all animals which are brought into the herd. Virgin heifers and bulls, as prescribed above, may be introduced into the herd. Older cows can only be assumed negative if they are from a known negative source or if they have calved normally and have undergone regular cycles for three months without breeding. Where avoidance of commingling is not possible, limiting the number of herds in contact with each other during the breeding season has been recommended as a control measure although it is not expected to eliminate the disease completely.[54]

The reduction of the duration of the breeding season has been advocated for the control of this disease.[1, 16, 43] Shorter, more intense breeding is likely to reduce the transmission rates by infected bulls.[11] In addition, a short breeding season allows for accurate identification of the potentially infected females and segregation or culling of these animals. These high-risk animals include any which are open five months after the end of the breeding season, as most pregnancy loss occurs before this time.[11, 32] Cows with pyometra should be culled. Some authors advocate the culling of all females which are not pregnant five months after the breeding season and those which fail to calve.[59] The disadvantage of short breeding seasons is the likely reduction of the pregnancy percentage in infected herds.[11]

A membrane protein vaccine administered to bulls gave

some protection against infection and a significant rise in antibody titre of serum and seminal plasma but not of preputial washings.[27, 36, 37, 134] In contrast, the current commercial whole cell vaccine (TrichGuard, Fort Dodge) has no protective effect for bulls and vaccination of these animals is not recommended.

Vaccines containing killed *T. foetus* organisms or membrane proteins do not offer complete protection but do reduce the percentage of animals which become infected and the duration of the infection.[30, 53, 66, 67, 81, 124] Furthermore, a significant improvement in first service conception rate and services per conception and a reduction in pregnancy loss has also been shown (62,1 per cent calved compared to 31,5 per cent for controls).[59, 66, 81] It is suggested that the more rapid elimination of infection avoids the occurrence of foetal death, which usually occurs between 60 and 120 days of gestation.[8, 53, 81] Vaccination has the added benefit of reducing or eliminating the carrier state from the cow herd.[80]

Some progress toward the production of subunit vaccines has been made. Surface proteins which elicit a local immune response in infected animals have been identified, and have been shown to be well conserved.[52] Vaccination with purified TF 1,15/1,17 antigen also enhanced the antibody response, induced significant protection against challenge and reduced the duration of infection and lesions found in infected animals.[8, 19, 41] Furthermore, this antigen is well conserved between different strains of *T. foetus*.[68]

The immune response following both whole cell and subunit vaccination is characterized by predominantly IgG production systemically and a significant increase in IgA in cervicovaginal mucus.[41, 53]

Vaccination of heifers and cows, in combination with other control measures, is recommended for control of the disease in infected or high-risk herds.[41, 105] Two doses of inactivated whole cell vaccine (TrichGuard, Fort Dodge) should be administered to heifers and cows with a two- to four-week interval, the second dose being given four weeks before the onset of the breeding season. Annual revaccination with a single dose is required. The high cost of the vaccine has limited adoption in southern Africa. In Nevada, USA, it was found that 62 per cent of ranchers were potential users of the vaccine, these being mainly larger herds which made use of the services of extension officers and veterinarians.[14]

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15

Amoebic infections

J J VAN DER LUGT

Introduction

Infections caused by the parasitic amoeba *Entamoeba histolytica* and free-living amoebae of the genera *Naegleria*, *Acanthamoeba* and *Balamuthia* occur principally in humans and are rare in domestic animals. In humans, *E. histolytica* is the cause of amoebiosis, which is chiefly characterized by the development of ulcers in the colon and, in some cases, abscesses in organs such as the liver, while the brain is specifically involved in disease caused by *Naegleria*, *Acanthamoeba* and *Balamuthia* spp. Few cases of the diseases have been diagnosed in livestock in southern Africa.

Aetiology, life cycle and epidemiology

The amoebae are protozoa belonging to the subphylum Sarcodina, class Lobosea, and they characteristically move with the aid of pseudopodia.[11, 15]

Entamoeba histolytica, although usually a commensal inhabitant of the crypts of the large intestine, is a pathogen of humans and a variety of animal species including dogs, pigs and cattle.[2, 4] *Entomoeba histolytica* occurs worldwide; infections in humans are more generally associated with poor hygiene in tropical and subtropical countries.[10]

The trophozoites have an irregular shape, are 15 to 50 µm in diameter, and have a vesicular nucleus containing a small, central karyosome, and peripherally arranged chromatin granules. The fine granular endoplasm often contains glycogen and food vacuoles harbouring red blood cells and cellular debris. Cystic forms of the organism occur in the intestinal lumen but not in solid tissues.[2, 5]

Naegleria and *Acanthamoeba* spp. are free-living, ubiquitous protozoa of soil and water.[5, 9, 11] In tissue sections, the trophozoites of *Acanthamoeba* spp., which are 15 to 50 µm in diameter, have a granular cytoplasm and a single nucleus which contains a large, centrally located karyosome surrounded by a halo. Cystic forms of the organism, which occur in animal tissues, are 10 to 25 µm in diameter, spherical and uninucleated. They contain a smooth or wrinkled outer ectocyst wall and a polyhedral or stellate inner endocyst wall. The trophozoites of *Naegleria* spp. are slightly smaller (10 to 35 µm) but the cystic stage has not been observed in tissue sections.[5, 7] Recently, a new species of free-living amoeba, *Balamuthia mandrillaris*, has been described, but has yet to be isolated from nature. Both trophic stages and cysts are formed in tissues. The trophozoites measure 12 to 60 µm and are usually uninucleate with a large vesicular nucleus and central nucleolus. Cysts are generally round, sometimes oval, 6 to 30 µm, uninucleate, with a layer of granules below the cyst wall.[19]

Pathogenesis, clinical signs and pathology

In most species, the clinical signs of amoebiosis vary according to the location and severity of the lesions. In the intestinal disease a catarrhal or haemorrhagic diarrhoea or dysentery may be present.[5, 10]

Humans infected with *E. histolytica* are often the source of infection for animals. After the ingestion of infective cysts, trophozoites invade the mucosa of the large intestine where they may be responsible for the development of numerous, pinpoint to large, confluent ulcers which are often flask-shaped and covered with a greyish exudate.[2, 4, 9] The trophozoites of *E. histolytica* commonly occur in small clusters in necrotic debris or in the adjacent viable tissue.[4, 9] Amoebic abscesses, especially in the liver and lungs, occur relatively commonly in humans, but are rare in domestic animals.[4, 5, 10]

Naegleria fowleri is the cause of primary amoebic meningoencephalitis in humans and is almost invariably associated with a history of recent swimming, diving or engaging in other water sports in fresh water lakes and ponds. It is believed that the amoebae invade the cranial cavity via the nasal mucosa and cribiform plate. Natural infection resulting in meningoencephalitis has been reported in a South American tapir (*Tapiris* sp.),[12] while sheep are susceptible to experimental infection with *N. fowleri* following intranasal inoculation of the organism.[17]

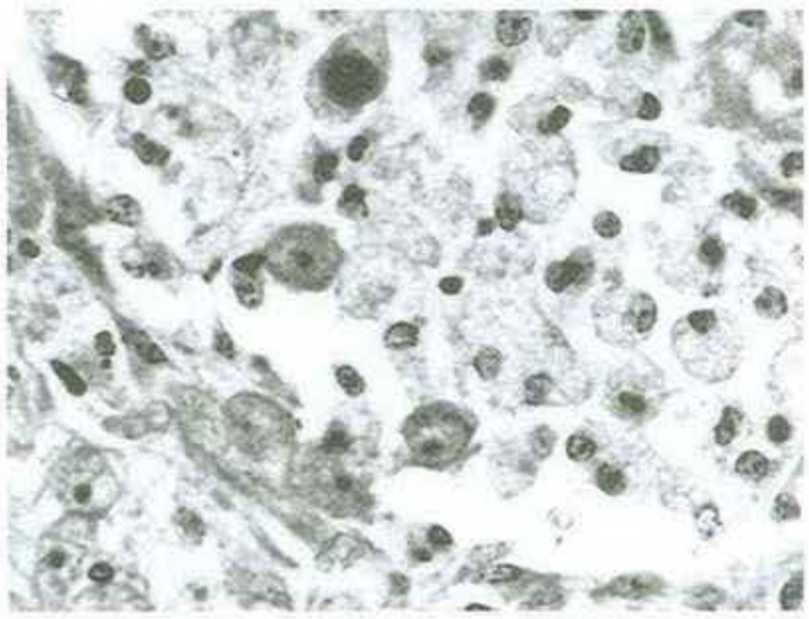


Figure 15.1 Cerebrum of a sheep: encephalomalacia showing Gitter cells, and trophozoites and cysts resembling those of *Acanthamoeba* spp.

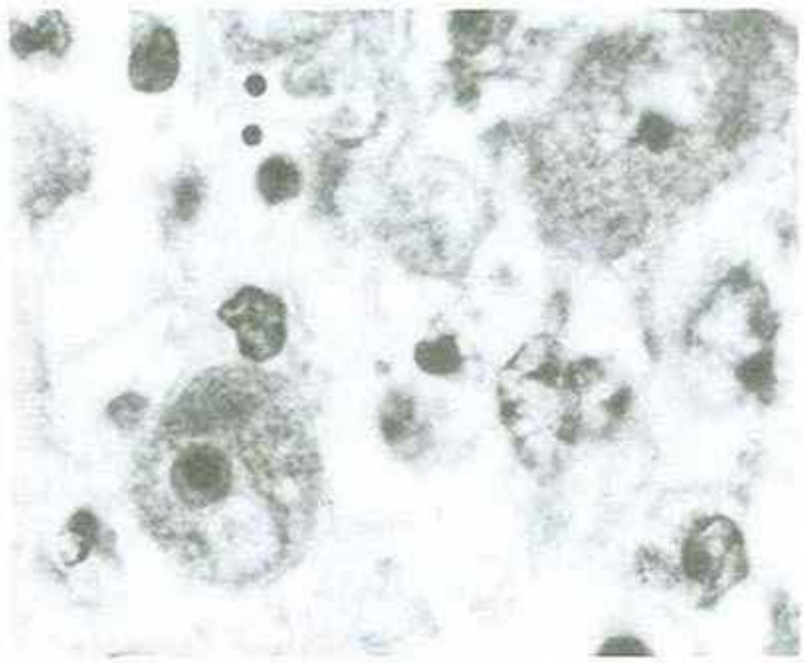


Figure 15.3 Higher magnification of a cyst

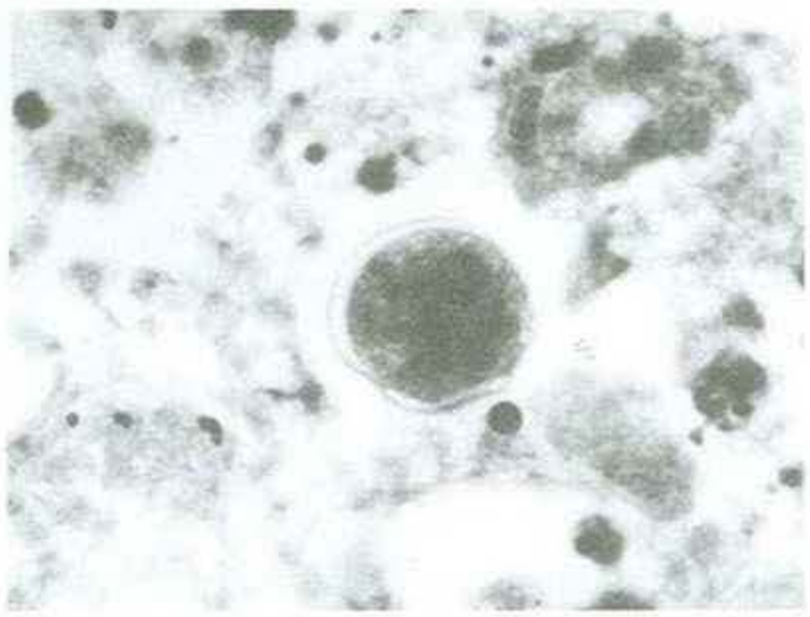


Figure 15.2 Higher magnification of a trophozoite

In humans, *Acanthamoeba* spp., in addition to causing a granulomatous amoebic encephalitis in immunosuppressed or chronically ill people, can give rise to keratitis, uveitis and granulomatous lesions in various organs.[5, 6, 7, 13] *Acanthamoeba* spp. rarely cause pneumonia in cattle[1, 14] and necrohaemorrhagic lesions in various organs of dogs.[3, 16] A multifocal necrogranulomatous meningoencephalitis and cerebral vasculitis associated with trophozoites and cysts morphologically resembling *Acanthamoeba* spp. have been reported in a sheep in South Africa (**Figures 15.1 to 15.3**).[18]

Balamuthia infections have been diagnosed in a sheep, lowland gorilla (*Gorilla gorilla*), orang-utan (*Pongo pygmaeus*), and a mandrill (*Mandrillus sphinx*) as a cause of meningoencephalitis.[6, 19, 8]

Diagnosis

The diagnosis of amoebiosis is based on demonstration of the parasite in faeces, or in biopsy and necropsy specimens, and on positive serological tests, for example agar gel diffusion, cellulose acetate diffusion, and indirect fluorescent antibody tests.[5, 10]

Control

Metronidazole is the drug of choice in amoebic dysentery. Control measures in humans consist of improving environmental and food sanitation.[5, 10]

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16

Coccidiosis

Synonym: Koksidiose (Afrik.)

C G STEWART AND B L PENZHORN

Introduction

Coccidiosis is a protozoal disease which occurs in many mammalian and all domestic livestock species. It is caused by infection with species of the genera *Eimeria* or *Isospora* and is characterized clinically in livestock by enteritis, following the invasion of mucosal cells of the gastrointestinal tract. Subclinical infections are frequent. The disease is relatively common in sheep, goats and cattle.

Coccidiosis occurs universally, but is most prevalent where animals are subjected to overcrowding, unhygienic environments, or where other stress factors are present. *Eimeria* spp. are important causes of the disease in cattle, sheep and goats. Coccidiosis is rare in horses[34] and in Europe and North America is an important disease of pigs.[68] Although a number of *Eimeria* spp. are commonly found in pig faeces, clinical disease is caused only by infection with *Isospora suis*. Coccidiosis due to *I. suis* has not yet been described in southern African countries, but it is associated with clinical disease of very young piglets in many other parts of the world.

Coccidia are generally host-specific, and, in the gut, specific to a particular location. Host specificity is more stringent in *Eimeria* than in *Isospora* infections.

Economic losses from coccidiosis due to mortality, poor performance, and the cost of treatment and prevention may be considerable, especially on stud farms and in intensive calf or lamb rearing systems.

Aetiology and life cycle

Coccidia are classified as protozoa in the class Sporozoasida, subclass Coccidiasina, and suborder Eimeriorina.

Both morphological and biological characteristics are used to identify the species of coccidia.[34] These criteria require subjective assessment and are therefore not always completely reliable. For more accurate identification computer-assisted image analyses have been used in cattle[62] and pigs.[54] Random amplified polymerase DNA (RAPD) tests have also been used in cattle, sheep and rodents.[38] The developmental cycle of the parasite occurs intracellularly within the host (see below), while the final infective form, the mature oocyst, is passed out to the external environment. Oocysts of sheep and cattle can be distinguished by their shape and size, the appearance of their surface (particularly the colour and texture), and the thickness of the wall.[37] Variation of oocyst morphology occurs within species, however, and there is a tendency for 'bizarre forms' to occur in severely infected sheep.[7] In a typical sporulated oocyst of the genus *Eimeria* (**Figure 16.1**), the oocyst wall is composed of one or two layers and may have a micropyle which in turn may be covered by a micropyle cap. An oocyst or sporocyst residium, consisting of material remaining after the formation of these structures, may be present. A sporocyst may have a knob, the Stieda body, at one end.[30, 34] At sporulation oocysts of the genus *Eimeria* develop into four sporocysts, each containing two sporozoites, while those of the genus *Isospora* have two sporocysts, each of which contains four sporozoites.

Coccidia of livestock are primarily intracellular parasites of the gastrointestinal tract, but in some animal species they may be found in other organs such as the liver or kidneys,[34] or even the uterus.[41] Development in the coccidial species mentioned in this chapter occurs by means of an initial asexual cycle, followed by a sexual one. Both take place in the same host.

There are three stages in the life cycle: merogony (the asexual cycle), gametogony (the sexual cycle) and sporogony (**Figure 16.2**). The first two occur within host cells, while sporogony occurs outside the host.[34] Following ingestion of infective sporulated oocysts by a susceptible host animal, the sporozoites are released through the action of the digestive juices, and, depending on the parasite species, enter epithelial or other cells of the mucosa of the gut. Here they become meronts and multiply by merogony (schizogony) to produce merozoites, the number depending on the species. These enter nearby epithelial cells in the gut and again multiply by merogony. The number of asexual

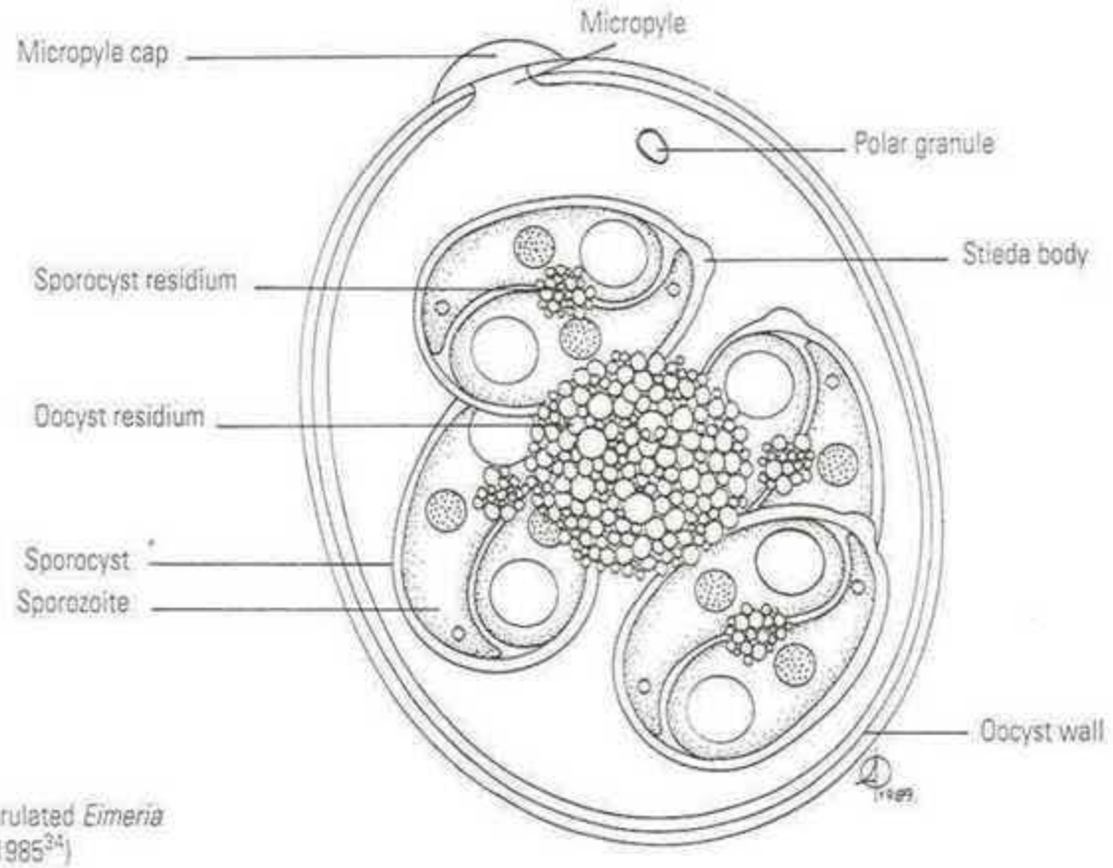


Figure 16.1 Structure of a sporulated *Eimeria* oocyst. (Adapted from Levine, 1985[34])

generations which occur remains constant for each species. Merozoites of the last generation infect new host cells and become gamonts, thus initiating the sexual stage of the life cycle. Some of the gamonts develop into macrogametes without further multiplication, while others divide by multiple fission (asexual) to form a large number of flagellated microgametes. These microgametes are released and penetrate the macrogametes to form zygotes. A thick encompassing wall is formed around each zygote, which is then known as an oocyst. When the cell is ruptured, the oocysts are released into the lumen of the gut, and are excreted in the faeces in an unsporulated state. If environmental conditions of temperature, oxygen supply and humidity are suitable, oocysts sporulate and sporocysts containing infective sporozoites are formed. This process takes between two and five days under optimal external conditions, which include a temperature of 24 to 30 °C.[10]

Coccidia are haploid throughout their life cycle, except in the zygote stage. The first sporogonous division is meiotic, re-establishing the haploid state. Oocysts administered to aberrant hosts will excyst and begin merogony, but they disappear after 48 hours.[40]

Sheep

The most important species that cause clinical disease in sheep are *Eimeria ahsata*, *E. ovinoidalis* and *E. bakuensis*.[33] Other species (**Table 16.1**) are less pathogenic but may play an augmentative role as mixed coccidial infections are the rule in field outbreaks.[55]

The characteristic features of the sporulated oocysts of the most common coccidia occurring in sheep are shown in **Figure 16.3** and **Table 16.1**. Oocysts of *E. weybridgensis* are similar in size to *E. crandallis*, but the sporozoites of *E. weybridgensis* are more elongate and lie lengthwise in the sporocyst, while those of *E. crandallis* are more ovoid and lie at either end of the sporocyst.[37]

The meronts of the non-pathogenic *E. gilruthi* are visible to the naked eye during necropsy as whitish nodules (300 to 900 µm in diameter) in the mucosa of the abomasum of sheep and goats.[34]

Goats

Most of the coccidia species which infect goats are, in spite of being morphologically similar, distinct from those of sheep.[49] The more important species to cause disease in goats can be identified by their close similarity to their counterparts in sheep (**Figure 16.3**). These, with their ovine counterparts given in brackets, are: *E. arloingi* (*E. bakuensis*), *E. christenseni* (*E. ahsata*), *E. hirci* (*E. crandallis*), *E. jolchijevi* (*E. granulosa*), *E. alijevi* (*E. parva*), *E. kocharli* (*E. intricata*), *E. apsheronica* (*E. faurei*), *E. ninakohlyakimovae* (*E. ovinoidalis*), *E. caprina* (no counterpart), and *E. caprovina*, which occurs in both sheep and goats.[25] *Eimeria caprina* oocysts are 27–40 × 19–26 µm in size, have a micropyle cap and can be distinguished from the other caprine coccidia which possess a micropyle cap, by their very much larger size. *Eimeria apsheronica* differs from *E. caprina* in that it is ovoid and has a small micropyle.[36] The most pathogenic species are *E. ninakohlyakimovae*, *E. christenseni* and *E. arloingi*.[29]

Cattle

At present there are between 19 and 22 species of *Eimeria* which have been identified in cattle. Most of these are relatively non-pathogenic, and field outbreaks of coccidiosis are generally caused by mixed infections of *E. zuernii*, playing

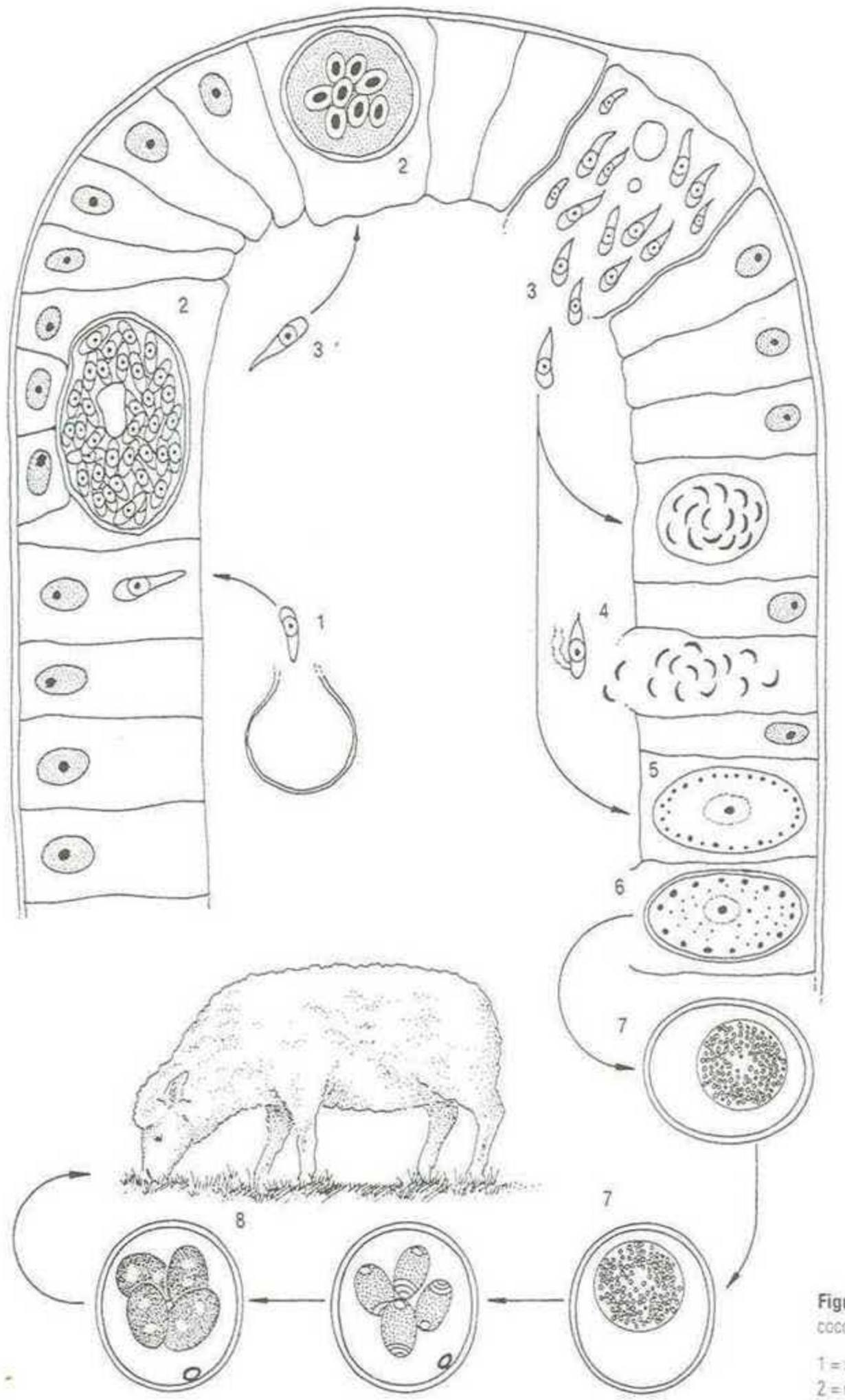


Figure 16.2 Life cycle of a coccidium in sheep

1 = sporozoite
2 = meront
3 = merozoite
4 = microgamete
5 = macrogamete
6 = zygote
7 = unsporulated oocyst
8 = sporulated oocyst

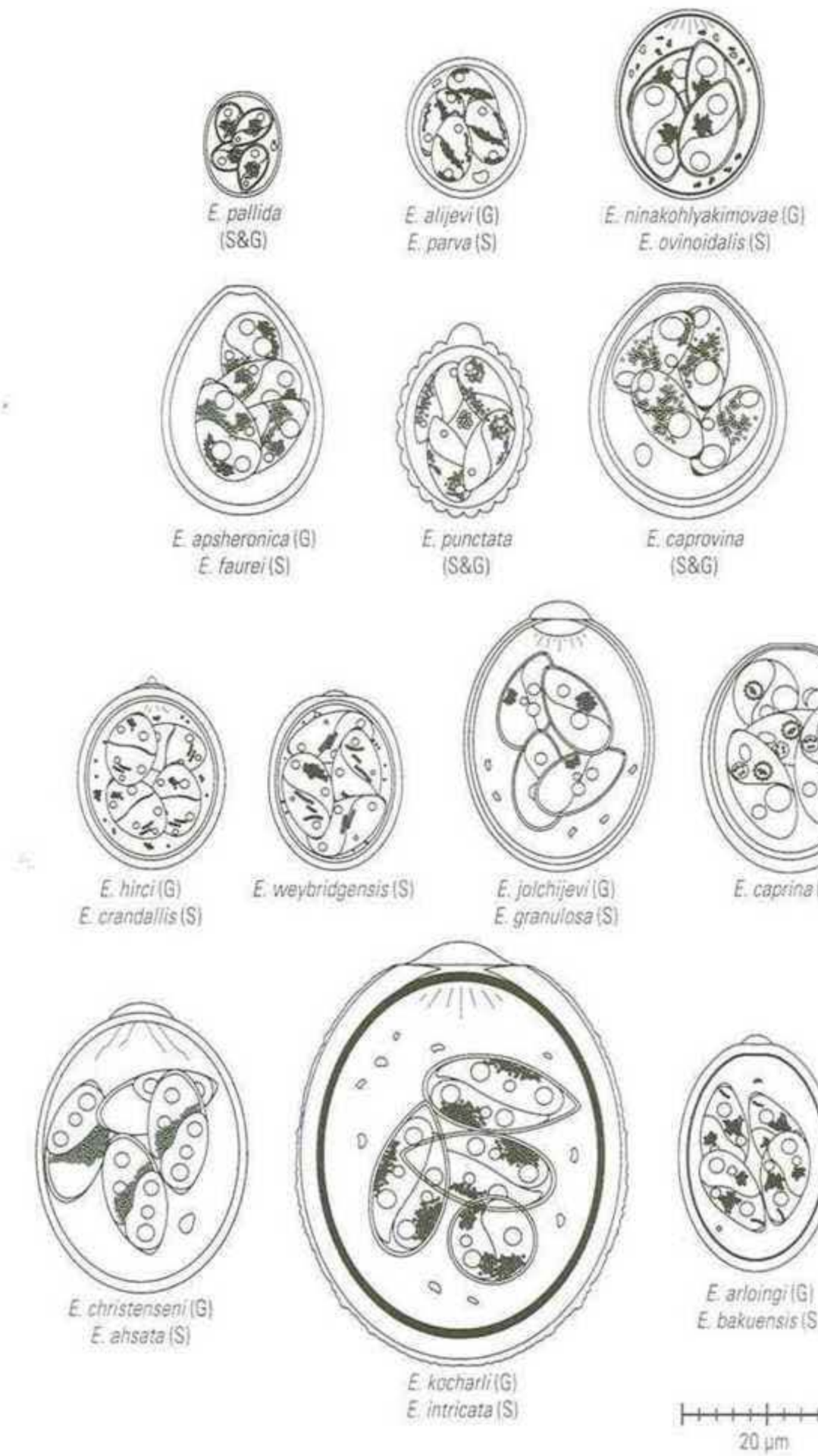


Figure 16.3 The sporulated oocysts of coccidia from goats (G) and sheep (S). (Adapted from Levine, 1985[34])

the major pathogenic role, and *E. bovis* playing a lesser one. *Eimeria alabamensis* has been reported as a cause of coccidiosis in northern Europe. *Eimeria auburnensis* and *E. ellipsoidolis* have produced diarrhoea in experiments.[12]

The oocysts of *E. zuernii* (**Figure 16.4a**) are subspherical to ovoid, measure 12–29 × 10–21 µm and have no micropyle or residium[34] (**Figure 16.3**). Large, first-generation meronts (115–225 × 78–202 µm in size), containing about 120 000 merozoites,[12] occur in the last part of the small intestine of the lamina propria near the muscularis mucosae. The second generation of meronts (13–18 × 13–18 µm in size, each containing a mean of 32 merozoites) and gamonts develop in epithelial cells of the caecum and colon.[65]

Oocysts of *E. bovis* (**Figure 16.4b**) are ovoid (23–24 × 17–23 µm) and have an inconspicuous micropyle but no polar granule or residium. The sporocysts have an

Table 16.1 Characteristics of sporulated oocysts occurring in sheep. (Adapted from Levine, 1985[34])

EIMERIA SPP.	OOCYST SIZE (µm)	PRESENCE OF MICROPYLE	MICROPYLAR CAP	OOCYST RESIDIUM	POLAR GRANULE/S	STIEDA BODY	SPOROCYST RESIDIUM
E. ahsata	23–48 x 17–30	+	+	–	+	+	+
E. crandallis	18–28 x 15–20	+	+	–	+	–	+
E. faurei	25–37 x 18–28	+	–	–	+	–	+
E. granulosa	22–37 x 17–26	+	+	–	+	+	+
E. intricata	39–59 x 27–47	+	+	–	+	–	+
E. bakuensis	23–36 x 15–24	+	+	–	–	–	+
E. ovinoidalis	16–30 x 13–22	+	–	–	+	+	+
E. pallida	12–20 x 8–15	–	–	–	+	–	+
E. parva	12–23 x 10–19	–	–	–	+	+	+
E. punctata	18–28 x 16–21	+	+	+	+	+	+
E. weybridgensis	17–31 x 14–19	+	+	–	+	–	+

inconspicuous Stieda body and a residium. There are two asexual generations. First-generation meronts develop in endothelial cells of the lacteals of the villi in the caudal part of the small intestine. They reach a size of 207–435 × 134–367 µm, contain 55 000 to 170 000 merozoites and are macroscopically visible in the mucosa as whitish spots. Second-generation meronts measure about 10 × 9 µm and contain 30 to 36 merozoites. They, and the gamonts, occur in epithelial cells of the caecum and colon.[34]

Pigs

Isospora suis occurs in the small intestine and sometimes in the colon of pigs and is the causative agent of porcine neonatal coccidiosis.[35] Oocysts are spherical to subspherical, 17–25 × 16–21 µm in size, and lack a micropyle, Stieda body, polar granule and residium[34] (**Figure 16.5**). Excretion of oocysts in the faeces starts four to five days after infection and continues for one to three weeks.[35]

Eimeria spp. which commonly occur in pigs are *E. debliecki, E. neodebliecki, E. porci* and *E. suis* (which are all smooth-walled), and *E. scrabra, E. polita, E. perminuta* and *E. spinosa* (which are rough-walled).[68] All *Eimeria* spp. of pigs are considered to be either non- or only mildly pathogenic and are not significant causes of disease in pigs.[35]

Horses

Eimeria leuckarti, which is occasionally encountered in horses, may be pathogenic[2] but is usually an incidental finding. The microgamonts are 300 × 170 µm in size and so are visible to the naked eye. They occur in the small intestine.[34]

Epidemiology

Coccidiosis is essentially a disease that is prevalent in young animals under intensive management systems. Older animals are occasionally clinically affected. Disease usually occurs when the resistance of the host is lowered after stress such as that resulting from overcrowding, weaning and transportation or when animals are maintained under unsanitary conditions. In well-managed animals there is a balance between the host and the parasite.

Young animals become infected after the ingestion of sporulated oocysts and they in turn excrete oocysts, often in large numbers, in their faeces. Hot, humid, unhygienic conditions, such as those that frequently prevail in overstocked feedlots, pens containing straw bedding for raising lambs, kraals and irrigated pastures, favour the survival of oocysts and result in higher infection rates than is the case under extensive farming systems. Infection may spread to animals housed in separate pens by the airborne transfer of oocysts. Most animals are subclinically infected and as their immunity is not absolute and continual reinfection occurs, they shed small numbers of oocysts in their faeces for long periods. Sporulated oocysts are very resistant to adverse environmental conditions and may 'overwinter' on pastures which then act as a source of infection when favourable climatic conditions return.[16]

Sheep and goats

The disease is most severe in lambs two to eight weeks of age[24] and in lambs two to three weeks after weaning. Sheep entering feedlots, or after experiencing severe stress such as that caused by transport or inclement weather are prone to developing severe disease.[16] The most important species are *E. ovinoidalis, E. crandallis* and *E. ahsata.*[3]

Most sheep and goats pick up oocysts as young animals either from contaminated pastures or from faecal contamination of the teats and udder of their dams. The parasite population is increased enormously during the first passage in susceptible lambs putting later-born lambs at greater risk.[9] Sheep and goats usually become infected with a number of *Eimeria* spp. and the patency of oocyst production extends over several months. Mixed infections prolong the patency and increase the number of oocysts produced.[8] Faecal oocyst counts may become high in apparently healthy lambs.[47]

The presence of the more pathogenic species, such as

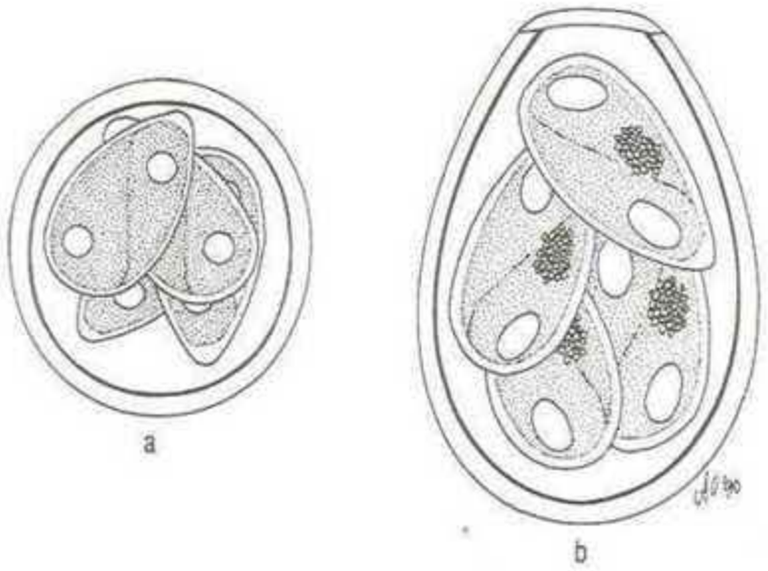


Figure 16.4 *Eimeria zuernii* (a) and *Eimeria bovis* (b) oocysts from cattle

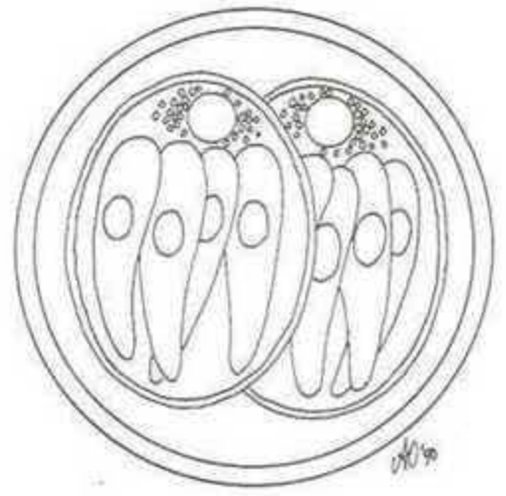


Figure 16.5 *Isospora suis* oocysts from a pig

E. ahsata and *E. ovinoidalis*, plays an important role in the production of overt disease in sheep.[33]

Coccidiosis is often a serious problem in feedlots. Species of coccidia to which animals in the feedlots have not previously been exposed may be introduced together with newly purchased animals. Conversely, non-immune animals may be placed in a pen containing infected animals or which has been contaminated with oocysts. Clinical signs may develop 10 to 20 days after a new lot of lambs are introduced. Mortalities in feedlots may be high and are often associated with sudden outbreaks of the disease. Once a pen has become infected, the oocysts may remain there for a considerable period, creating a hazard for any new introductions as well as for animals already in the pens. Feed troughs may become contaminated with faecal pellets which are inadvertently consumed and, in this way, the production of massive infections or reinfections is facilitated.

High concentrations of oocysts in the pens may result when animals are penned only at night.

Very wet summers are known to result in a high prevalence of coccidiosis because of the survival of larger numbers of oocysts and stressing of the animals caused by the wet conditions.

Cattle

Clinical coccidiosis is primarily a disease of calves between three weeks and six months of age, although older animals may show signs of infection. Overt disease occurs when large numbers of oocysts are ingested while the ingestion of smaller numbers of oocysts leads to subclinical infection followed by the development of immunity. In the summer rainfall areas of South Africa the practice of removing calves from their dams during the winter months of June and July and placing them in pens to facilitate weaning commonly results in outbreaks of coccidiosis.[64] Outbreaks occur despite the fact that under the dry winter conditions oocyst contamination is likely to be fairly low. Outbreaks of coccidiosis in winter have also been reported in Canada.[48] It would appear that stress, such as that produced by weaning or cold weather, may be important in initiating outbreaks.[52] The disease may occur in young cattle kept under extensive farming conditions when high oocyst contamination occurs around watering points.

Eimeria alabamensis is reported to be a problem in calves in Sweden and Germany[69, 71] four to seven days after being placed on contaminated pastures. The source of infection is from oocysts that have overwintered on pasture[70] or contaminated hay.[72]

The presence of subclinical coccidiosis may interfere with the response to vaccination against other diseases.[51]

Pigs

Unsporulated oocysts of *I. suis* sporulate very rapidly (within 16 hours at 30 °C) under suitable moisture and temperature conditions, whereas *Eimeria* spp. take from 8 to 13 days to sporulate.[68] Day-old piglets are more susceptible than month-old piglets maintained free of *I. suis* infection.[68] Good immunity develops after infection. Adult sows do not appear to shed *I. suis*, so the source of infection for piglets is unknown.[68] The most likely source of infection is contaminated farrowing crates.[35]

Pathogenesis

Factors which play an important role in the pathogenesis of the disease include the pathogenicity of the coccidia species concerned, environmental conditions, size of the infective dose, age of the host, number of host cells destroyed, location of the parasite in the tissues and the presence or absence of acquired immunity.[14]

Some species of coccidia are pathogenic whereas others are not. The most important pathogenic *Eimeria* and *Isospora* spp. of livestock infect epithelial and other cells of the mucosa of the gastrointestinal tract, the site along the length of the gut varying with the species. As the number of asexual generations occurring is constant for each species, infection is self-limiting provided that reinfection does not occur.[34] Many of the

affected cells are destroyed or their function impaired,[34] meronts probably being responsible for most of the cellular destruction. In severe infections there is an acute inflammatory reaction. Capillaries in the denuded lamina propria may haemorrhage, leading to hypoproteinaemia and anaemia. Secondary bacterial infections may exacerbate the lesion. All these changes stimulate an increased rate of peristalsis and result in diarrhoea. The reduced absorption capacity of the parasitized mucosa results in loss of fluids via the intestine and decreased absorption of electrolytes such as sodium and potassium. Compensatory increase of absorption in normal portions of the gut may redeem this loss.[11] Dehydration and acidosis due to the loss of bicarbonate ions in the faeces, as well as anaemia, hypoproteinaemia, and shock combined with secondary bacterial infection, are the main causes of death.

The effects of coccidiosis due to *E. zuernii* and *E. bovis* are mainly the result of damage to the mucosa of the large intestine caused by the second-generation meronts and sexual stages.[59] Second-generation meronts and gamonts of *E. zuernii* are simultaneously present in the caecum, colon and rectum, and it is therefore difficult to determine which is the more pathogenic. Maximal destruction of the epithelium of the large intestine occurs in cattle just before the peak output of oocysts.[66] This results in a reduction in the absorption of water and sodium and chlorine ions with a consequent rapid loss in body weight. Animals dying at this stage probably do so from dehydration. There are marked decreases in packed cell volume, haemoglobin levels and erythrocyte counts in severe cases of *E. zuernii* infection. This is in contrast to *E. bovis* infection where there is an increase in packed cell volume and haemoglobin.[66]

Immunity develops after infection, but the determinants of protective immunity have yet to be elucidated.[60] The cellular immunity which usually follows the development of the meront stage is most important as far as protection of the host is concerned, while the sexual stages are poorly immunogenic.[60] Subclinical infection at a young age results in the development of specific immunity early in life. This immunity may, however, break down under conditions of stress such as during transportation, exposure to climatic extremes, changes of diet and overcrowding, or when massive infections occur.[13] Drugs may also interfere with the development of immunity. Lambs exposed to an aureomycin-sulphamezathine supplement did not develop immunity when fed 500 mg/lamb/day for 35 days, but were able to develop resistance when fed a lower dose of 100 mg/lamb/day.[1] Intermittent or continual reinfection enhances the level of immunity. The immunity that develops in response to an *Eimeria* infection is species-specific.[60] Protection against infection has been obtained by immunization of lambs with *E. crandallis* and *E. ovinoidalis*[9, 21] and in calves with *E. alabamensis*.[71] A 'trickle-infection' appears to be the most effective means of producing immunity.

Clinical signs

Sheep and goats

The onset of diarrhoea is usually the first sign of illness. Affected animals pass soft, unpelleted faeces and later a watery diarrhoea. Blood may be present in the faeces but it is less common in sheep and goats than in cattle. The perineum and hind limbs become soiled and this may precipitate fly strike. Anorexia and dehydration develop a few days after the commencement of the diarrhoea, with resultant loss of body mass. These are followed in severe cases by depression, recumbency and death.

The morbidity ranges from 10 to 50 per cent and the mortality may be up to 10 per cent. In Angora goat flocks in the Eastern Cape Province of South Africa morbidity can reach 100 per cent with mortality as high as 50 per cent.[75] In the sandy, arid eastern parts of Namibia a disease known as 'boksiekte' (goat disease) is due to a combined effect of coccidiosis and infestation with the worm *Strongyloides papillosus*.[75] The course varies from one to two weeks and the disease is manifested by the clinical signs of ataxia, opisthotonos and convulsions, in conjunction with various degrees of enteritis.

Cattle

The incubation period is usually about three weeks, but may vary between a week and more than a month.[26]

The predominant clinical sign is diarrhoea, which may be poorly developed at first, but becomes more watery and finally haemorrhagic. As the disease progresses there is an increase in the amount of fresh blood in the faeces, and blood clots may be present. After a few days the amount of blood in the faeces may diminish, being replaced by mucus and muco-fibrinous exudate. Later the discharges become blackish-red to blackish, or greenish-black and foul-smelling; shreds of mucosa may be present. The diarrhoea, which may be profuse and foetid, is often accompanied by tenesmus and pronounced borborygmi. The animal may constantly grind its teeth. Abdominal pain is usually present and, if the tenesmus is intense, prolapse of the rectum may occur. There is often a fever, an increase in the respiratory rate and loss of appetite.[26]

During the course of the disease, severely affected animals become extremely emaciated and anaemic. After about 8 to 12 days, they either show signs of improvement, or deteriorate and die.[26]

Pigs

Coccidiosis usually occurs in 5- to 10-day-old piglets, and is characterized by the development of diarrhoea, dehydration and loss of condition. Faeces are yellow to grey and do not contain visible blood.[35] Vomiting may occur. On some farms all litters may be infected, whereas in others only a few litters become infected. The mortality is usually less than 20 per cent.[68]

Figure 16.6 Flat to dome-shaped, white foci (consisting of coccidial parasites) in the mucosa of the small intestine of a sheep

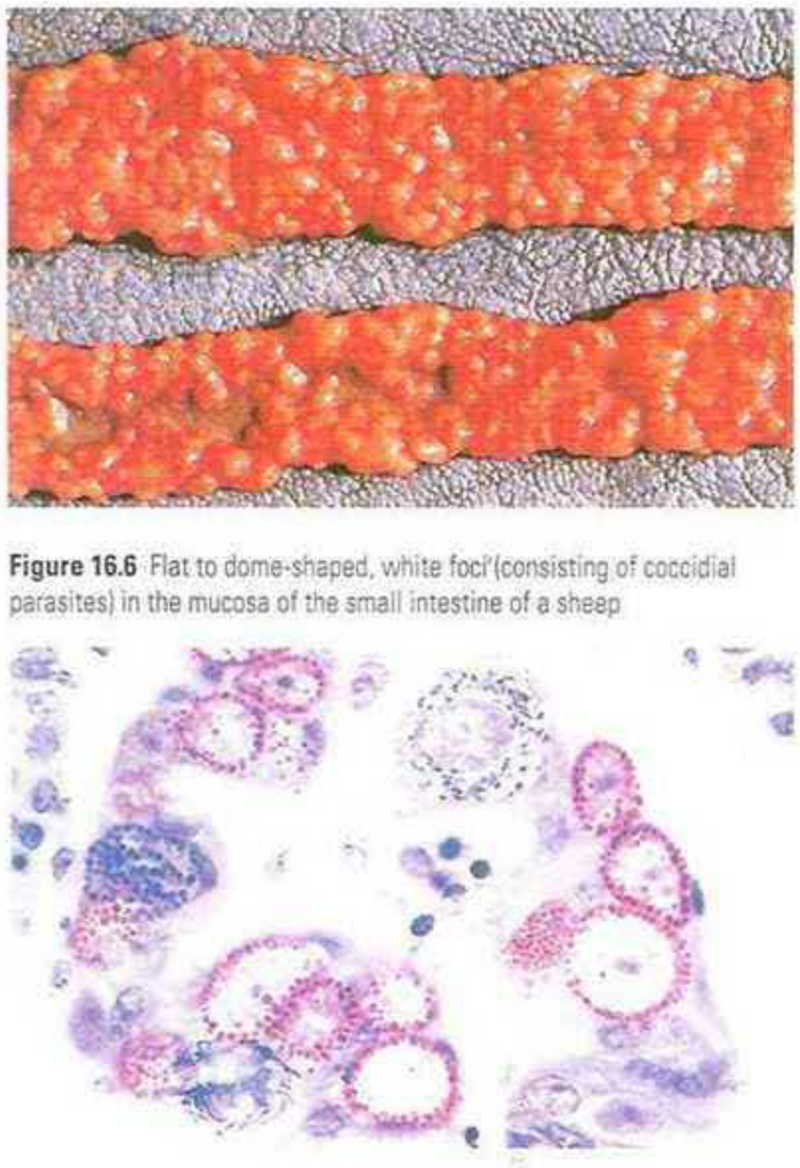


Figure 16.7 Various stages of coccidia in epithelial cells of the small intestine



Figure 16.8 Necrotizing colitis in a calf



Figure 16.9 Haemorrhagic proctitis in a calf

Pathology

Sheep and goats

Decreases in the levels of serum albumin and plasma alkaline phosphatase coincide with the onset of diarrhoea.[5] The animal may be cachectic or even anaemic.

The location of the lesions in the gut, and to some extent their appearance, varies with the *Eimeria* spp. concerned, but most infections are mixed.[8]

In most natural coccidial infections small, flat to dome-shaped, white foci, 1 to 2 mm in diameter, each consisting of an area where there is severe infection of epithelial cells, occur in the mucosa of the small intestine (**Figure 16.6**). These foci, which have a diameter of up to 300 µm, may also be discernible from the serosal surface of the gut[20] and are due to the schizont stage of *E. ovinoidalis* and *E. ahsata* in sheep and *E. ninakohlyakinovae* and *E. christenseni* in goats.[29] Macroscopically visible, discrete opaque patches may also be seen in the mucosa in *E. bakuensis* infections because of the presence of groups of heavily parasitized intestinal villi. These may appear as raised patches or even develop into polyps.[22] Affected areas of the intestine of severely affected animals are oedematous and thickened, and there may be focal or diffuse congestion or haemorrhage in the mucosa.

Sloughing of the intestinal mucosa may occur, sometimes with ulceration. Haemorrhagic enteritis of the caudal part of the small intestine occurs in the case of *E. ovinoidalis* in sheep and *E. ninakohlyakinovae* in goats, and blood may be present in the faeces.[29]

Microscopically there is disorientation of the villous architecture, a reduction in epithelial cell height, and a decrease in the width of the brush border.[57] The villi may be short or absent and may contain proliferative epithelia, with various stages of coccidia being present (**Figure 16.7**). Malabsorption may occur if these lesions are widespread.[29]

Cattle

The sodium and chloride ion levels in the blood decrease about 18 days after experimental infection and reach their lowest levels seven days later.[66]

The meronts of *E. bovis* may be seen as small white spots in the mucosa of the ileum, but those of *E. zuernii* are not visible macroscopically.[12]

In severe cases, fibrino-haemorrhagic typhlocolitis is typically present as is, in many cases, proctitis (**Figures 16.8 and 16.9**). The terminal ileum may be affected.[29] The content of the caecum, colon and rectum becomes semi-fluid and bloody in the early stages of infection. During the later

stages sloughed mucosa and copious amounts of blood and fibrin may form fibrino-necrotic pseudomembranes. The content of the large intestines has an unpleasant smell. The mucosa may contain erosions, and both it and the submucosa are oedematous and congested and contain petechiae. The mesenteric lymph nodes are often enlarged and oedematous.

Microscopically, the mucosa of the caudal part of the small intestine is invaded by first-generation meronts and may be infiltrated by a few lymphocytes and eosinophils, but, in general, the lesions are mild.[12] The lesions caused by second-generation meronts and gamonts, on the other hand, are characterized by extensive changes.[12] In the case of *E. zuernii* infections these are present in the large intestine. In severe infections almost all the epithelial cells of the affected part contain parasites in various stages of development. Many affected cells lose their integrity and become detached, while the crypts become distended by oocysts and cellular debris and the lamina propria is congested and infiltrated with plasma cells, lymphocytes, neutrophils and eosinophils. Extensive haemorrhage occurs in the tissues. Capillaries in the lamina propria denuded of epithelium may be exposed to the intestinal content because of the sloughing of the epithelial cells.

Pigs

Fibrinous or fibrino-necrotic enteritis in the caudal part of the small intestine is present in some fatal cases. Microscopically, severe destruction of the villous epithelium of the jejunum and ileum, and marked atrophy of the villi are evident. Endogenous stages of *I. suis* are apparent in the villous epithelium.[68]

Diagnosis

The history of the case, clinical signs and necropsy findings, as well as the demonstration of the parasite, are all relevant when diagnosing coccidiosis.

In order to visualize oocysts, a small quantity of faeces from an affected animal is mixed with water and examined microscopically, but, in some cases, concentration of the oocysts is required before they can be demonstrated. This is achieved by flotation of the oocysts in a fluid with a specific gravity between 1,1 and 1,3. The most commonly used is either a 40 per cent sugar or a saturated sodium chloride solution, the former being preferred as it is less detrimental to the oocysts. Approximately 2 to 3 g of faeces are first mixed with about 10 ml of water. The mixture is then passed through a sieve to remove gross debris, placed in a test tube and centrifuged. The supernatant fluid is decanted and the sediment mixed with about 10 ml of the flotation solution, and again centrifuged. The oocysts 'float' to the surface and many of them can be removed in a droplet of fluid obtained by touching the surface of the supernatant fluid with a flat object such as the end of a glass rod. The droplet is placed on a glass microscope slide, covered with a cover slip, and examined under a microscope.

Species identification of the coccidia requires some experience, but by comparison with textbook drawings (**Figures 16.3 to 16.5**), some of the more common oocysts of sheep and cattle can be distinguished.[4] Oocysts may be more readily differentiated after sporulation has occurred. Sporulation can be induced by mixing a small sample of faeces with a 2 per cent aqueous potassium dichromate solution and allowing it to stand at room temperature for three to four days.[44] Accurate identification is a task for a parasitologist.

Estimates of the number of oocysts in faeces can be carried out by the modified McMaster method.[44] However, it may be difficult to interpret the results as high counts can occur in apparently healthy animals. Low counts may be present in diseased animals as the majority of the parasites may not have progressed to the oocyst stage at the time of the examination.[56] In these cases, smears of faeces should be made and stained with Giemsa to demonstrate the presence of merozoites.[34] Sheep tend to have higher oocyst counts than goats.[30] At necropsy, either scrapings from lesions or tissue sections of the intestine may be examined microscopically for the presence of meronts, gamonts or oocysts.[34] Mixed infections usually occur.

Healthy lambs between four and eight weeks of age may have very high faecal oocyst counts (of the order of 500 000/g or higher), but after this age there is a progressive reduction in oocyst numbers so that by five months, oocyst numbers are similar to those in adult sheep. This makes the interpretation of oocyst counts in relation to their clinical significance very difficult.[56]

Coccidiosis should be suspected in young cattle whose major presenting sign is one of a haemorrhagic diarrhoea. As most field outbreaks of coccidiosis are due to *E. zuernii*,[12] the oocyst should be identified (**Figure 16.4**). In some cases diarrhoea may begin a day or so before oocysts can be detected in the faeces. Microscopic examination of the faeces of these animals for oocysts should be repeated. It is sometimes possible to detect merozoites in Giemsa-stained smears of faeces in these and other cases.

In piglets the demonstration of oocysts of *I. suis* in the faeces (**Figure 16.5**) may be difficult as they may not be present until about three days after the onset of diarrhoea.[74] Death may occur before this stage, in which case it is necessary to perform histological examinations of sections of the intestinal tract in order to detect the organisms. In some animals, microscopic examination of smears made from the gut contents may reveal merozoites and meronts.

Differential diagnosis

A number of conditions of sheep, including colibacillosis, lamb dysentery, salmonellosis, cryptosporidiosis and rotavirus infection, which usually occur in young lambs under

four weeks of age,[45] should be considered in the differential diagnosis of ovine coccidiosis. Mixed infections may, however, occur. Helminth infestations, either on their own or in conjunction with coccidia infections, may result in diarrhoea, but the eggs of helminths are much larger than oocysts and can readily be differentiated.

Clinically, bovine coccidiosis should be differentiated from conditions in which diarrhoea is a major presenting sign. Foremost among these are infectious diseases, such as salmonellosis, colibacillosis, rotavirus and coronavirus infections, bovine viral diarrhoea-mucosal disease, and cryptosporidiosis; intoxications caused by the ingestion of plants, such as the chincherinchee (*Ornithogalum* spp.), tulp (certain of the *Homeria* and *Moraea* spp.)'and slangkop (*Ornithoglossum* and *Urginea* spp.),[31] and helminth infestations.

Isospora suis infection should be differentiated from other infectious causes of enteritis and diarrhoea in young piglets, such as colibacillosis, and rotavirus and coronavirus infections. Concurrent infections may occur.

Control

Application of the principles of management, hygiene and chemotherapy play a role in the control of coccidiosis.[34] Once clinical signs are apparent, the disease is advanced and considerable contamination of the environment has occurred. Although clinical cases may be successfully treated, treatment may be expensive and the subsequent productivity of the recovered animal is often poor.[34] For these reasons prophylaxis is the most effective approach to control.

Management and hygiene

If the intake of sporulated oocysts by young animals is kept at a low level through good management practices, infections may become established at levels which stimulate immunity without causing clinical disease.[21] Young, susceptible animals should be kept clean and dry, and feed and water troughs should be constructed and maintained in such a manner that as little feed as possible falls to the ground, leakages are prevented, and faecal contamination is minimized. Proper drainage of paddocks, kraals and feedlot pens is also important in order to prevent the accumulation of large numbers of oocysts and to create an adverse environment for their development. The faeces of animals should, if possible, be removed from pens before oocysts have sporulated. Stress associated with such factors as weaning, sudden changes of diet and transportation should be minimized.

Older animals, although partially immune, may continuously shed oocysts in their faeces and so contaminate pastures. Susceptible animals, when placed on these apparently safe pastures, may suffer severe consequences. This occurs especially when animals are transported from the dry extensive farming areas of southern Africa and are placed in intensive production systems or wet environments. Whenever possible, clinically affected animals should be isolated.

In a heavily infected environment, sterilization of bedding and soil with aqueous solutions of 1,25 per cent sodium hypochlorite, 0,5 per cent cresol or phenol, or by fumigation with formaldehyde,[63] may be achieved but this is not a practical control measure. Exposure to sunlight (for at least eight hours), and desiccation (humidity less than 25 per cent) are the most cost-effective methods of killing oocysts.[23]

Chemotherapy

Clinical coccidiosis is difficult to treat, and success is normally limited. By the time clinical signs are evident, the infection has usually progressed to the sexual stage of the life cycle and oocysts are appearing in the faeces. Extensive destruction of intestinal epithelium will have taken place and recovery will be slow. In addition, the parasites may have passed through the stages against which the anticoccidial drugs are effective. Treatment of clinically affected animals should therefore include supportive therapy to aid in the repair of damaged gut and to limit secondary infection.[53]

Supportive treatment includes the administration of antimicrobials, antidiarrhoeal compounds and fluids, and, when necessary, haematinics and blood transfusions.[12] Specific treatment of concurrent infestations with helminths or other infections may be indicated.[6]

Where animals are penned, those showing clinical signs should be removed and not returned until at least two weeks after the cessation of clinical signs, as oocyst shedding may persist for some time. Other animals in the pen should be treated prophylactically, as they may harbour early developmental stages that are susceptible to treatment.

The development of anticoccidial agents for use in ruminants and pigs has largely resulted from an extension of development of these compounds for use in the poultry industry. Relatively few of the compounds registered for use in poultry have been tested and found to be effective in ruminants and pigs.

Anticoccidial compounds are used either prophylactically or therapeutically. The most important anticoccidial drugs for use in cattle, sheep, goats and pigs are listed in **Table 16.2**.

Amprolium administered in feed or water for four days or longer results in clinical recovery and reduction of oocyst output.[27]

Sulphonamides are commonly used for treatment of coccidiosis in ruminants but are only partially effective. Their concurrent antibacterial activity may contribute to their efficacy by controlling secondary bacterial invaders. Potentiated sulphonamide mixtures have been found to have synergistic activity against coccidia in poultry, but have not been evaluated in ruminants. Gut-active sulphonamides (e.g. succinylsulphathiazole and phthalylsulphathiazole) should not be used in treating coccidiosis as they become active only in the large intestine.

Table 16.2 Recommended use and dosage of anticoccidial drugs used in cattle, sheep, goats and pigs

DRUG	USE	ANIMAL	DOSAGE
Amprolium	Therapeutic	Cattle	10 mg/kg daily for 5 days
		Sheep	50 mg/kg daily for 5 days
		Goats	100 mg/kg daily for 5 days
		Pigs	25–65 mg/kg once or twice daily for 3–4 days
	Prophylactic	Cattle	5 mg/kg daily in feed for 21 days
Sulphonamides	Therapeutic	Cattle, sheep and goats	50–100 mg/kg daily for 4 days
Sulphadimidine	Therapeutic	Cattle, sheep and goats	50–100 mg/kg daily for 4 days
Sulphaquinoxaline	Prophylactic	Sheep	0.5–3 g/lamb/day for 20 days
Sulphaguanidine		Pigs	1 g/15 kg daily for 7–10 days
Ionophores	Prophylactic	Cattle, sheep and goats	1 mg/kg daily in feed for 30 days
Monensin			
Lasalocid	Prophylactic	Cattle, sheep and goats	0.5–1 mg/kg per day in feed for up to 6 weeks
Nitrofurazone	Therapeutic	Cattle, sheep, goats and pigs	10–20 mg/kg daily for 5 days
Decoquinate	Prophylactic	Cattle and goats	0.5 mg/kg in feed for at least 28 days
Toltrazuril	Therapeutic	Sheep	20 mg/kg (single treatment)
Diclazuril	Therapeutic	Sheep and goats	1 mg/kg orally

The carboxylic ionophores have broad-spectrum activity, are coccidiocidal in action and have a narrow margin of safety.[42] They act by facilitating the movement if inorganic cations across cell membranes, thus upsetting the balance of physiologically important ions such as sodium and potassium. Activity is most marked against the sporozoites, but merozoites are also affected. They are therefore best used prophylactically.

Oral treatment with nitrofurazone (10 mg/kg per day for five days) is effective against clinical coccidiosis,[57] but nitrofurazone is no longer available for use in food-producing animals in many countries due to persistent residues.

Decoquinate completely controlled *E. bovis* in calves when given in daily doses for three to four weeks, with no relapse after withdrawal of medication.[43]

Toltrazuril and diclazuril are symmetric triazinetriones, a novel chemical class of anticoccidials. A single dose given to lambs 7 to 10 days after being put to pasture was highly efficacious in preventing clinical coccidiosis. It reduced oocyst output to low levels, prevented the development of diarrhoea and improved weight gain during the four to five weeks after treatment.[19]

The level of drug required to be mixed in feed varies according to the amount of feed consumed by the animal each day. Distinction must also be made between full rations, creep feed and supplementary feed when calculating the inclusion rate. The ionophores have a relatively narrow margin of safety, and errors in dosage or feed mixing procedures may result in toxicity.[47, 61]

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17

Cryptosporidiosis

Synonym: Kriptosporidiose (Afrik.)

C G STEWART AND B L PENZHORN

Introduction

Cryptosporidiosis, caused by the genus *Cryptosporidium*, is usually an inapparent infection of a variety of mammals, birds, fishes, and reptiles, but it may assume clinical proportions characterized by lesions in the gastrointestinal tract followed by the development of diarrhoea. This is particularly so in neonatal animals or immunocompromised hosts.

Cryptosporidia are very small coccidia which were first described by Tyzzer in 1907[41] from the intestine of normal mice. Until 1955, when they were incriminated as a cause of disease in turkeys, they were thought to be commensals,[37] but since then, diarrhoea associated with *Cryptosporidium* spp. has been reported in lambs,[7] kids,[45] piglets,[46] immunologically deficient foals,[38] and humans.[33] Bovine intestinal cryptosporidiosis was first described in 1971 in the USA,[35] and subsequent reports from the UK,[44] Canada,[31] Australia,[22] and Germany[19] indicate a wide geographical distribution of the parasite. In South Africa, *Cryptosporidium* infection has been associated with diarrhoea in calves,[21] and a high prevalence of the parasite has been demonstrated in the faeces of healthy calves[28] and in children with diarrhoea.[9, 17]

Aetiology and life cycle

Cryptosporidium spp. are protozoa ascribed to the class Sporozoasida, subclass Coccidiasina, and suborder Eimeriorina of the family Cryptosporidiidae.[24] The parasites complete their life cycle in gastrointestinal epithelial cells.[43] The life cycle of *Cryptosporidium* spp. resembles that of other intestinal coccidia but differs in that the parasites develop just under the surface plasmalemma of the host cell.[23] In young animals the life cycle is completed in three to four days, which is shorter than the life cycle of most other coccidia.

Cryptosporidium spp. infect a broad range of vertebrate hosts, including fish, reptiles, birds, mammals, and humans.[15] *Cryptosporidium parvum* infects the intestinal tract and is associated with disease in mammals, while *C. muris* is found in the cells of the peptic glands of the stomach or abomasum and it has not been associated with disease.[2] *Cryptosporidium meleagrides* and *C. baileyi* infect birds,[32] and *C. nasorum* and *C. serpentis* fish and reptiles, respectively.[48] *Cryptosporidium parvum* generally parasitizes intestinal epithelial cells,[2] and its oocysts can be differentiated from those of *C. muris* by their smaller size: *C. parvum* oocysts measure 4,5–5,4 × 4,2–5 µm and those of *C. muris* 6,6–7,9 × 5,3–6,5 µm.[47]

Following ingestion of oocysts by the host, sporozoites are released into the intestine where they penetrate the brush borders of the epithelial cells to develop into meronts. Although organisms appear to be attached superficially to cells, all stages are intracellular and are contained within a parasitophorous vacuole which is situated between the cytoplasm and outer cell membrane.[15] The parasite is surrounded by the microvillous membrane which fuses with the epithelial plasma membrane to form a thickened adhesion zone at the point of attachment to the host cell (**Figure 17.1**). There is also an adjacent comb-like structure formed by a folding of the parasite pellicle.[34] These specialized structures are thought to facilitate the intake of nutrients by the parasite from the host cell. The meronts are 2 to 5 µm in diameter and contain eight merozoites which are released into the lumen of the gut without destroying the host cell. Second-generation meronts may occur, and these will contain four merozoites when mature. These merozoites invade new host cells where they undergo gamogony by forming macro- and microgamonts. The microgamont has sperm-like microgametes which, when released, fertilize the macrogamonts which then develop into oocysts. Oocysts are round or ellipsoidal, and contain four sporozoites. It is generally accepted that sporocysts do not occur.[24] Sporulation takes place within the host cell and is followed by the development of a resistant thick wall around some of the oocysts, which are shed in the faeces.[15] Other oocysts, however, do not form a thick wall and the single unit membrane surrounding the sporozoites ruptures when these oocysts are

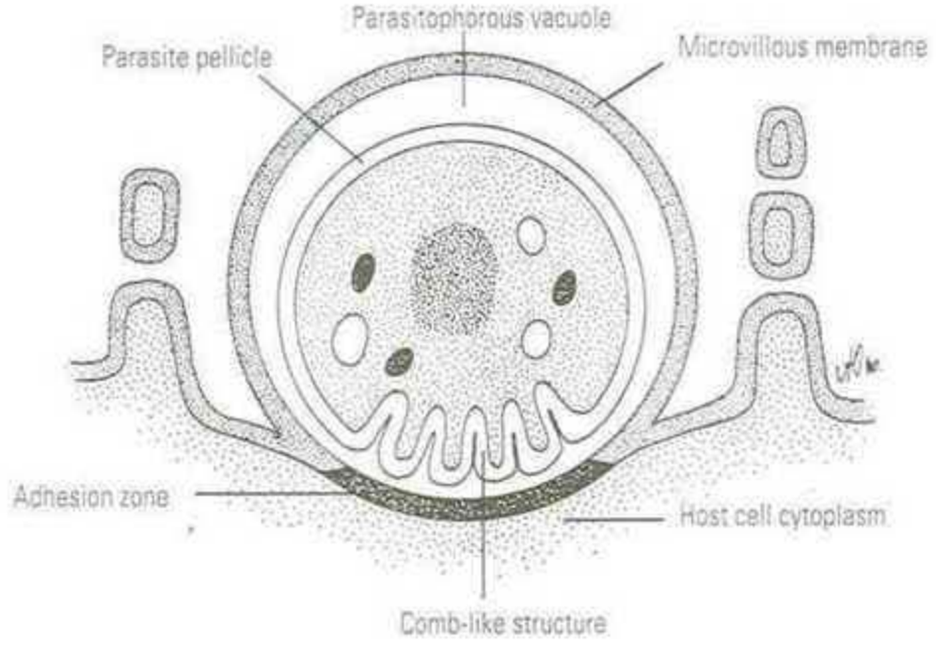


Figure 17.1 Line drawing of *Cryptosporidium* in a host epithelial cell

released from the host cell. These forms are invasive and can infect epithelial cells and so reinitiate the development cycle, resulting in auto-infection.[14]

Epidemiology

In livestock, clinical cryptosporidiosis occurs most frequently in calves, but it has also been recognized in pigs, lambs, kids and immunocompromised foals.[5, 29, 36, 38, 45, 46]

Outbreaks of clinical disease are most common in animals one to four weeks of age. Diarrhoeic calves may shed as many as 10 million oocysts per gram of faeces, resulting in severe contamination of the environment.[4] Cryptosporidia may also be found in the faeces of subclinically infected cattle of all ages.

Oocysts are resistant to most environmental factors, but the time of survival outside the host is unknown. Chlorination of water has little effect on viability of the organisms, hence water-borne outbreaks of the disease are an important public health problem.[49] Under experimental conditions infectivity is lost after storage for six months at 4 °C.[42]

Transmission is by ingestion of oocysts in faecal contaminated food or water, while aerosol transmission may also occur in both humans and animals.[20, 42] Infections of the respiratory tract have been reported, which may indicate aerosol transmission.[42] Calves that consume adequate colostrum and have high serum gammaglobulin concentrations are less likely to become infected.[27] Recovery usually occurs within 30 days.[1] There are reports where *Cryptosporidium* has been incriminated as the only cause of diarrhoea, but crytosporidia infections often occur together with other enteric pathogens, such as rotavirus, coronavirus, *Salmonella* serovars and enterotoxogenic *Escherichia coli*. These multiple infections act synergistically and increase the mortality rate. Severe stress, such as exposure to cold temperatures, may also increase mortality. Immunodeficiency may be important in the development of clinical disease. Severe infections have been reported in Arabian foals suffering from the combined immunodeficiency syndrome[38] and in humans with acquired immune deficiency syndrome (AIDS). In foals the disease is more prevalent in the first three to four weeks of life but can occur in animals up to one year of age.[13] Persistent infection in athymic mice and AIDS patients demonstrated that T cells are necessary for immunity to develop.[49] In calves, a strong cell-mediated immunity develops after infection with an increase in CD8+ T cells.[1] As oocysts are produced in the sporulated state, auto-infection is particularly important in immunologically compromised hosts, resulting in persistent infection.[43]

In humans, cryptosporidiosis may be acquired by contact with infected individuals or from animal sources, and it is a common cause of travellers' diarrhoea.[39] The low host specificity, excretion of large numbers of oocysts and low numbers of oocysts required to initiate infection in susceptible hosts imply that animal species can be potential reservoirs for human infections.[10]

Cryptosporidium parvum can be divided into two distinct populations based on different antigenic and molecular markers and have been referred to as 'animal' and 'human' types. Human infection may be due to either type with anthroponotic (human to human) transmission occurring with the 'human' type and zoonotic transmission occurring with the 'animal' type. It is not clear if the 'human' type can infect animals.[6]

Pathogenesis and clinical signs

Cryptosporidium spp. have a marked effect on membrane-bound digestive enzymes, resulting in poor digestion. Malabsorption may occur as a result of villous atrophy.[43]

Except when it occurs in very young animals, infection is inapparent or, at most, produces only mild disease.[3, 23] The incubation period varies between two and seven days.[30] Diarrhoea occurs at between one and four weeks of age and lasts for one to two weeks. Although the morbidity rate may be high the mortality rate is generally very low.[42] The onset of overt disease is usually sudden and clinical signs are very similar in all affected livestock species. Depression and anorexia are followed by a profuse yellow watery diarrhoea with associated dehydration. Tenesmus may be present. After a few days the diarrhoea becomes intermittent and the faeces pasty for up to 10 days.[23] Loss of body weight may be quite marked. Relapses after apparent recovery may occur.[42] Vomiting may occur in pigs.[46] Most affected animals recover spontaneously unless complicated by infection with other enteropathogens. Severe chronic infections may occur in immunocompromised hosts.[2]

Pathology

Generally, the extent of the mucosal injury in the gastrointestinal tract and the severity of the disease are directly correlated.[23] Enteric lesions increase in severity towards the terminal portion of the ileum, and the caecum and colon may also be infected.[42] The mucosa of affected parts of the intestine, which often contains watery yellow material, is intensely hyperaemic. The carcass may be emaciated and dehydrated.

Stunting, fusion and cross-bridging of adjacent intestinal villi are seen histologically.[4] In affected parts of the intestine, mucosal epithelial cells are often low columnar, cuboidal or even squamous and, in calves, there may be focal necrosis of epithelial cells in some areas.[15, 18] The very small basophilic organisms, generally appearing as small dots, although ring- or crescent-shaped forms may also be identified, are found embedded in the microvilli or just inside the distal cell membrane of epithelial cells (**Figure 17.2**). They are more easily demonstrated in tissue sections that are stained with Giemsa.[5, 40] Extra-intestinal forms in the gall-bladder, mesenteric lymph nodes, trachea, lungs and uterus have been identified in pigs and sheep.[16]

Diagnosis and differential diagnosis

Demonstration of numerous oocysts in the faeces of young animals manifesting clinical signs of enteritis, or in preparations of intestinal tissue, are the most reliable means of diagnosis. However, the finding of *Cryptosporidium* does not necessarily mean that the organism is involved in the disease process, and it is therefore important to associate the relevant clinical signs and histological lesions to the pathogen or other pathogens isolated before making a diagnosis.

In formalin-fixed tissue sections, endogenous stages can be demonstrated in the brush border of intestinal epithelial cells.[42] Care must be taken to distinguish organisms in tissue sections from small droplets of mucus adhering to the surface of epithelial cells.[12]

Cryptosporidium spp. oocysts are very small but can be recognized in faeces smears fixed in methanol and stained with modified Ziehl-Neelsen[4] or safranin. The latter stain may be the most sensitive.[8] Oocysts appear as red spheres against a dark background and need to be differentiated from yeasts, which do not stain red. High sensitivity has been reported using immunofluorescent staining techniques.[13]



Figure 17.2 Cryptosporidia in epithelial cells of the small intestine

Techniques for concentrating oocysts in faecal specimens, such as those used for the diagnosis of coccidiosis, may facilitate identification. The final preparation can be examined directly, or stained by a safranine-methylene blue method.[17, 28]

Clinically, cryptosporidiosis should be differentiated from other enteric conditions, such as colibacillosis, salmonellosis, rotavirus or coronavirus infections and coccidiosis (see the relevant chapters). Under field conditions, however, diarrhoea is often associated with a simultaneous infection with two or more micro-organisms,[23] and this, together with the tendency for the infection to be subclinical, makes diagnosis difficult.

Control

Chemotherapy does not appear to be effective,[26] but, in most cases, infection is self-limiting. Administration of fluid therapy and other symptomatic treatment is important in the prevention of dehydration and the correction of the electrolyte imbalance caused by the diarrhoea. If managed correctly, most animals will recover unless the disease is complicated by infection with other enteropathogens.

Prevention should be aimed at a reduction of the ingestion of oocysts by raising young animals in a clean and dry environment. Faecal material should be removed from pens on a regular basis, and animals suffering from diarrhoea should be kept in isolation. Although oocysts are resistant to many disinfectants and most prevailing environmental conditions, they may be destroyed by exposure to a 5 per cent ammonia solution or 10 per cent formalin.[11]

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18

Toxoplasmosis

Synonym: Toksoplasmose (Afrik.)

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Introduction

The protozoan parasite *Toxoplasma gondii* is a common parasite of many animal species and humans and is distributed throughout the world. Although most of the infections of livestock caused by the parasite are subclinical, toxoplasmosis is a major cause of abortion in sheep in several countries.[3, 30, 46, 56, 57, 79] It is also known to cause reproductive losses in goats.[17, 38, 49] In southern Africa there is a high prevalence of serologically positive animals, but reports of toxoplasmosis in domestic animals are rare.[74, 75] In South Africa natural infections in ferrets kept at the Onderstepoort Veterinary Institute for experimental purposes[6] and losses in chinchillas in the Western Cape Province have been reported.[48] Because toxoplasmosis can affect virtually every warm-blooded animal it is impossible to summarize information for each animal species in this chapter, but the disease in animals up until 1987 has been reviewed.[30] More recent information is included in this chapter.

Aetiology and life cycle

Toxoplasma gondii is a coccidian parasite of domestic cats and other members of the Felidae, with other warm-blooded animal species, comprising essentially all mammals and birds, as intermediate hosts (**Figure 18.1**). It is classified in the phylum Apicomplexa Levine, 1970, class Coccidea Leuckart, 1879, and order Eimeriida Leger, 1911. Opinions differ regarding the placement of *T. gondii* into a family and subfamily. Various authorities, however, have placed it in one or the other of the families Eimeriidae, Michin, 1903, Sarcocystidae, Poche, 1913, or Toxoplasmatidae, Biocca, 1956. The name *Toxoplasma* (toxon = arc, plasma = form) is derived from the crescent shape of the tachyzoite stage. There are three infectious stages of *T. gondii*: the tachyzoites (in groups), the bradyzoites (in tissue cysts), and the sporozoites (in oocysts).[30, 51]

The tachyzoite is often crescent shaped and is approximately 2 × 6 µm in size in tissue smears (**Figure 18.2A**) and globular to oval in sections (**Figure 18.2B**). Its anterior (conoidal) end is pointed and its posterior end is round. It has a pellicle (outer covering), a conoid, rhoptries, micronemes, a well-defined nucleus in the central area of the cell (**Figure 18.3**), and other organelles found in coccidian merozoites.[15, 24]

The tachyzoite enters a host cell in a variety of organs and tissues by active penetration of the cell membrane. After entering the cell the tachyzoite becomes surrounded by a parasitophorous vacuole (**Figure 18.3**). Numerous intravacuolar tubules connect the parasitophorous vacuolar membrane to the parasite pellicle (**Figure 18.3**). The tachyzoite multiplies asexually within the host cell by repeated endodyogeny. Endodyogeny (endo = inside, dyo = two, geny = progeny) is a specialized form of reproduction in which two progeny form within the parent parasite, thereby consuming it (**Figure 18.3**). Tachyzoites continue to divide by endodyogeny until the host cell is filled with parasites,[24] which is the stage associated with acute toxoplasmosis in animals and humans.

After a few divisions, tachyzoites induce the formation of a cyst wall in a host cell and transform into bradyzoites contained within the wall (**Figures 18.2, 4 and 5**). Tissue cysts grow and remain intracellular (**Figures 18.2 and 4**) as the bradyzoites (encysted zoites) slowly divide by endodyogeny. Tissue cysts vary in size. Young tissue cysts may be as small as 5 µm in diameter and contain only two to four bradyzoites (**Figure 18.4**), while older ones may contain hundreds of cresent-shaped slender organisms (**Figures 18.2 and 5**). The tissue cyst wall is elastic, thin (< 0.5 µm) and argyrophilic. Cysts in the brain are round and up to 60 µm in diameter, whereas intramuscular cysts are elongated and may be 100 µm long; live tissue cysts in unstained squash preparations vary in size depending on the pressure applied to the coverslip. Although they may develop in visceral organs, including lungs, liver, and kidneys, they are more prevalent in the brain, skeletal muscles and myocardium. Tissue cyst formation can occur as early as one to two weeks after infection and cysts are probably innocuous for their host and can persist for the length of its life.

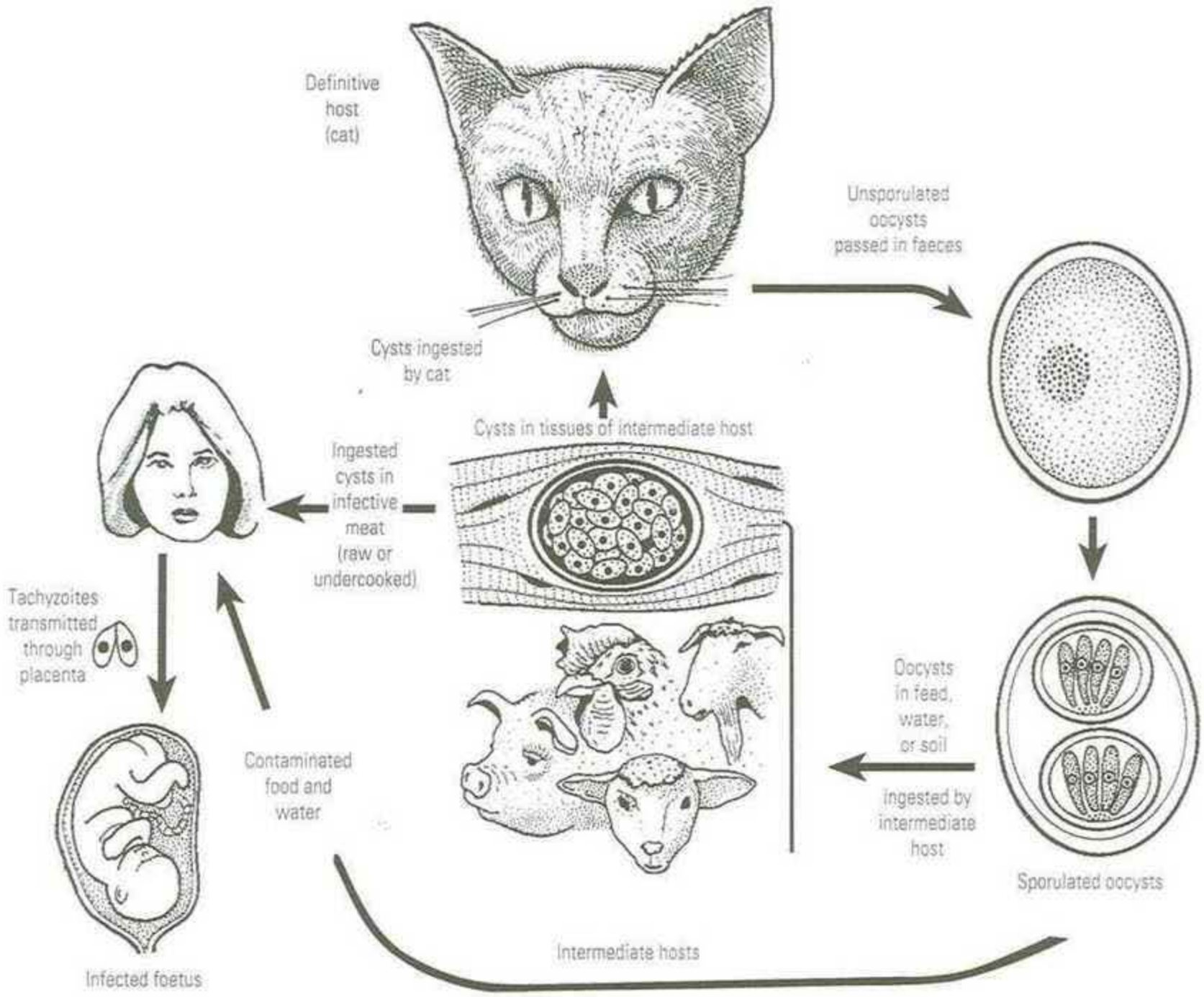


Figure 18.1 Life cycle of *Toxoplasma gondii*

The bradyzoites are approximately 7 × 1.5 µm. Bradyzoites differ structurally only slightly from tachyzoites. The former have a nucleus situated towards the posterior end (**Figure 18.5B**), whereas the nucleus in tachyzoites is more centrally located (**Figure 18.3**). The contents of rhoptries in bradyzoites in older tissue cysts are electron-dense (**Figure 18.5B**).[24] Bradyzoites contain several amylopectin granules which stain red with periodic acid-Schiff (PAS). These particles are absent in tachyzoites.

Cats shed oocysts after ingesting any of the three infectious stages of *T. gondii*, i.e. tachyzoites, bradyzoites, and sporozoites.[33, 34, 37] Prepatent periods (time to the shedding of oocysts after initial infection) and frequency of oocyst shedding vary according to the stage of *T. gondii* ingested. Prepatent periods are 3 to 10 days after ingesting tissue cysts and generally 19 days or more after ingesting tachyzoites or oocysts.[29, 34, 52] Less than 50 per cent of cats shed oocysts after ingesting tachyzoites or oocysts whereas nearly all cats shed oocysts after ingesting tissue cysts. Oocysts are less infectious for the definitive host (cat) than to non-feline hosts.[27, 45]

After the ingestion of tissue cysts by cats, the cyst wall is dissolved by the proteolytic enzymes in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and initiate development of numerous generations of *T. gondii*.[33] Five morphologically distinct types (A to E) of *T. gondii* develop in intestinal epithelial cells before gametogony begins. Types A to E divide asexually by endodyogeny, endopolygeny or schizogony (division into more than two organisms). The origin of gamonts has not been determined, but it is probably the merozoites released from schizonts of types D and E which initiate gamete formation. Gamonts occur throughout the small intestine but most commonly in the ileum (**Figure 18.6**). Gamonts and schizonts are located in surface epithelial cells, usually

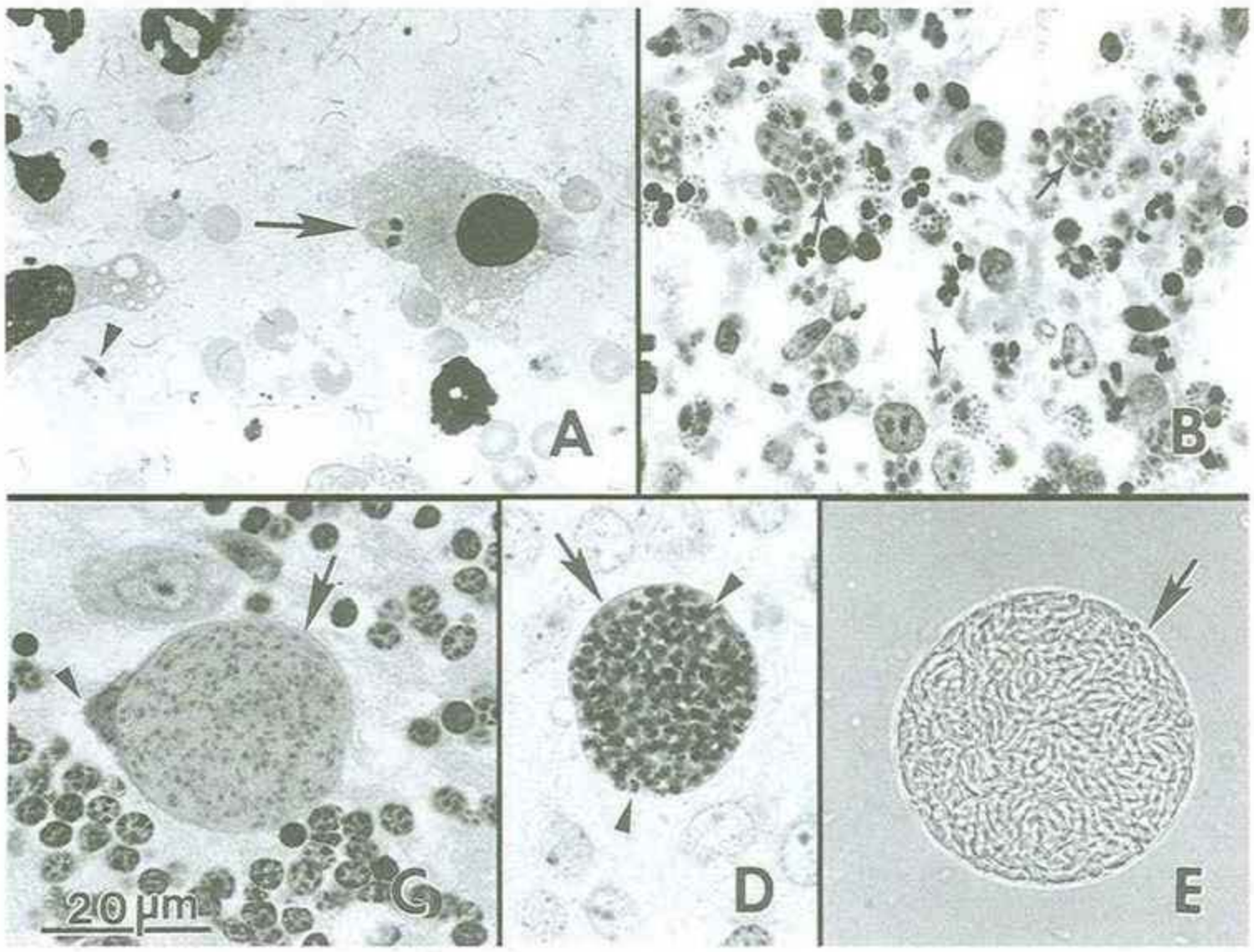


Figure 18.2 Tachyzoites and tissue cysts of *Toxoplasma gondii*. Bar = 20 µm and applies to all photographs in the composite figure

A Extracellular single (arrowhead) and paired tachyzoites (arrow) in a macrophage. Impression smear of lung. Giemsa stain
B Tachyzoites in a section of a lymph node. Note numerous oval-shaped tachyzoites (arrows). Haematoxylin and eosin stain
C Intracellular tissue cyst in a neural cell in section of cerebellum. The host cell nucleus is indented (arrowhead). Note thin cyst wall (arrow) enclosing numerous bradyzoites
D Tissue cyst in section of brain. Note darkly stained bradyzoites (arrowheads) and unstained tissue cyst wall (arrow). Periodic acid-Schiff haematoxylin
E Tissue cyst freed from mouse brain. Note thin cyst wall (arrow). Unstained

above the host cell nucleus (**Figure 18.6**). The female gamete is subspherical and contains a single centrally located nucleus (**Figure 18.6**). Mature male gamonts are ovoid to ellipsoidal in shape and contain 10 to 21 nuclei (**Figure 18.6**). Each microgamete has two flagella. The microgametes swim to and penetrate a mature macrogamete. After penetration, oocyst wall formation begins around the fertilized gamete. When they are mature, the oocysts are discharged into the intestinal lumen following rupture of the infected intestinal epithelial cells (**Figure 18.7**).[33]

Unsporulated oocysts are subspherical to spherical and are 10 × 12 µm in diameter (**Figure 18.7**). Sporulation occurs outside the cat within one to five days depending upon aeration and temperature. Sporulated oocysts are subspherical to ellipsoidal and each sporulated oocyst contains two ellipsoidal sporocysts, each with four sporozoites.

As the enteroepithelial cycle progresses, bradyzoites penetrate the lamina propria of the feline intestine and multiply as tachyzoites.[33] Within a few hours after infection of cats, *T. gondii* may disseminate to extraintestinal tissues. *Toxoplasma gondii* persists in intestinal and extraintestinal tissues of cats for at least several months, if not for the life of the cat. Thus, a cat is both the intermediate and the definitive host.

Intermediate hosts become infected after the ingestion of sporulated oocysts or tissue cysts containing bradyzoites. In the lumen of the small intestine, sporozoites released from oocysts and bradyzoites liberated from tissue cysts penetrate the mucosa to reach the submucosa and regional lymph nodes where they first multiply (**Figure 18.8**) and are then disseminated via the blood and lymph streams[28, 45] to infect a variety of cell types in which they multiply by endodyogeny to form tissue cysts. Sporozoites and bradyzoites of *T. gondii* can be transmitted to the foetus via the placenta. This occurs in humans, sheep, goats and several higher mammals when infection occurs during pregnancy.[24, 30, 31] However, in mice, infection can be transmitted to the foetus from the dam infected before pregnancy and for many generations thereafter.[2]

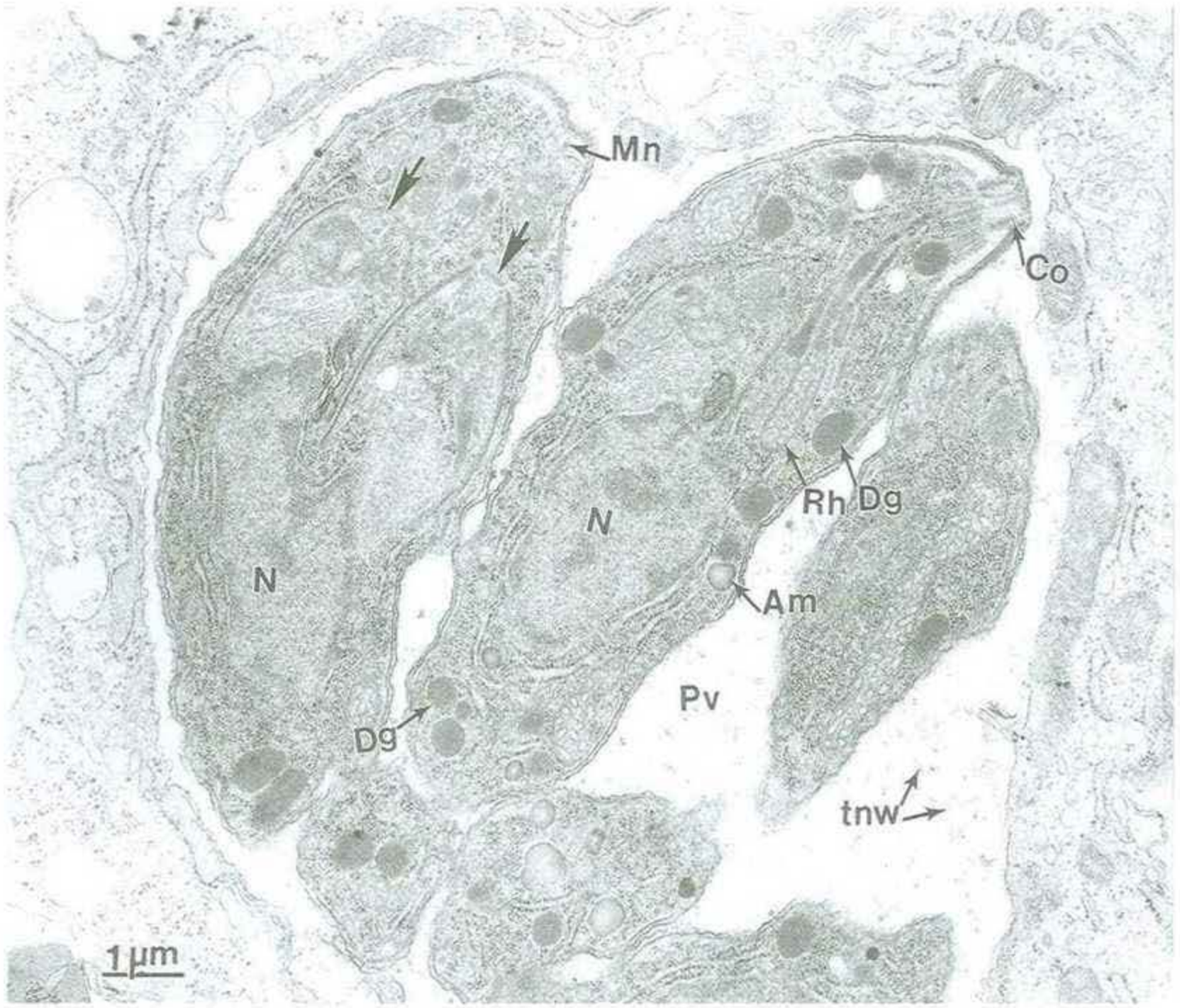


Figure 18.3 Transmission electron micrograph of tachyzoites of *Toxoplasma gondii* in a parasitophorous vacuole (Pv) of host cytoplasm in the peritoneal exudate of a mouse. Note conoid (Co), electron-lucent rhoptries (Rh), dense granules (Dg), amylopectin (Am), a centrally located nucleus (N), and the intravacuolar tubular network (tnw) in Pv. One tachyzoite is dividing into two by endodyogeny. Note conoids of the daughter organisms (arrows) are present above the parent nucleus. (Prof C.A. Speer, College of Agricultural Sciences and Natural Resources, University of Tennessee, Knoxville, and Drs D. Fritz, K. Kuehl and D. Austing, USAMRIID, Fort Detrick, Maryland, USA, are acknowledged with thanks for providing this figure and Figures 18.4, 5 and 8)

Epidemiology

Domestic cats play a central role in the dissemination of *T. gondii* in nature. Not only domestic cats (*Felis domesticus*) but other members of the Felidae can shed oocysts.[30] Oocysts of *T. gondii* were reported from faeces of naturally infected jaguar (*Panthera onca*),[8] ocelots (*Felis pardalis*),[67] cheetah (*Acinonyx jubatus*), bobcats (*Lynx rufus*),[62] and Canadian lynx (*Lynx conadensis*).[1] Outbreaks of acute toxoplasmosis in humans were attributed to contamination of a water reservoir by oocysts shed by feral domestic cats[7] and oocysts inhaled in a riding stable.[73] A cat may shed millions of oocysts after ingesting a few tissue cysts or bradyzoites.[29, 33] Oocysts are shed in large numbers for two to three weeks following infection. Cats usually develop a solid immunity to reshedding of oocysts after primary infection. Although they may shed oocysts on reinfection, the number of oocysts shed for the second time are fewer than after primary infection.[26] Oocysts are not present on a cat's fur; therefore petting a cat is not the source of *T. gondii* infection for humans but handling faecally contaminated material, such as sand-boxes or garden soil, is.[26] Sporulated oocysts are resistant to environmental factors and can survive freezing and drying.[30]

Herbivorous animals mainly become infected as a result of faecal contamination of pastures, water and feed with oocysts from cats[25] and other felids, and pasture contamination may be of more importance than conserved feed. Sporulated oocysts remain infectious for more than a year

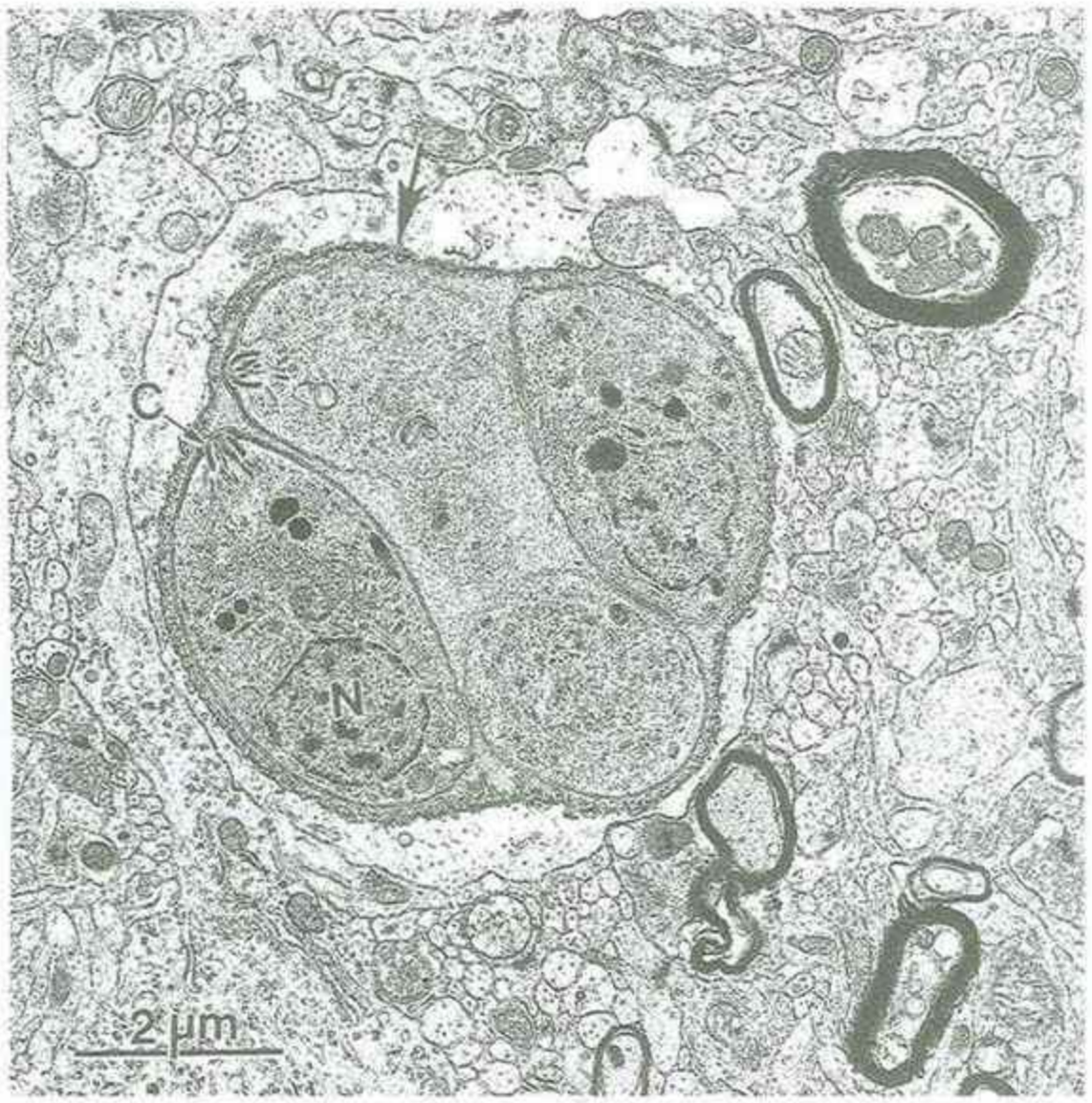


Figure 18.4 Transmission electron micrograph of a young tissue cyst in a mouse brain. Note well-developed cyst wall (arrow) enclosing four bradyzoites. Note conoid (C) and posteriorly located nucleus (N) in one bradyzoite. (This figure was provided by Prof. C.A. Speer and Drs D. Fritz, K. Kuehl and D. Austing; *vide* Figure 18.3)

and, because of their small size, are easily dispersed by wind and rain. Transmission of *T. gondii* to ewes by contact with infected placentas and aborted foetuses is not of practical significance.

Toxoplasma gondii may be transmitted to pigs not only as a result of the ingestion of oocysts, but also of uncooked or undercooked swill, infected rodents or other intermediate hosts, or through cannibalism.

There is possibly also an active sylvatic cycle in which wild felids act as definitive hosts, and their prey, such as rodents and birds, are intermediate hosts. In southern Africa, in which a large population of wild felids and a wide range of potential intermediate hosts exist, such a sylvatic cycle may play an important role in the transmission of *T. gondii* to herbivores.

Very little information is available on the prevalence of toxoplasmosis in animals in countries in southern Africa.[59, 66, 72, 74] However, notwithstanding a high prevalence of serologically positive animals, the occurrence of disease in domestic animals is very rare and only a few cases of toxoplasmosis have been reported in dogs[64, 71, 77] although these might have been due to neosporosis (**see Chapter 23: Neosporosis**). *Toxoplasma gondii* has been reported in a Cape hunting dog (*Lycaon pictus*)[58] and a cheetah[78] in southern Africa. Recently, the finding of antibodies to *T. gondii* in 50 of 68 (74 per cent) of sera from wild felids from South Africa — 37 of 41 lions (*Panthera leo*), three of four leopards (*Panthera pardus*), and 10 of 23 cheetahs — has been reported.

Toxoplasma gondii is an important cause of abortion and related problems in sheep in several countries including the USA, Australia, New Zealand, the UK, and Norway.[3, 9, 30, 39, 44, 46, 56, 57, 58] In these countries toxoplasmosis is a primary cause of loss in 10 to 20 per cent of flocks which have abortion/stillbirth problems. Similarly, toxoplasmosis can cause severe losses in goats.[17, 38, 49]

The consumption of undercooked meat containing tissue cysts appears to be the most common method of infection in humans. Tissue cysts have been isolated from mutton, pork and chicken meat. Cattle are highly resistant to *T. gondii* infection and the consumption of beef is probably not important in the transmission of the infection.[30] Human infection can also occur following the accidental ingestion of oocysts from cat faeces.

In Central America and other countries with a moist tropical climate, the prevalence of serologically positive humans increases from about one year of age to adolescence. This is considered to be due to children in these areas habitually playing in oocyst-contaminated soil.[30] *Toxoplasma gondii* is an opportunistic pathogen in humans with immune system dysfunction. In human immunodeficiency virus infections toxoplasmosis usually presents as a

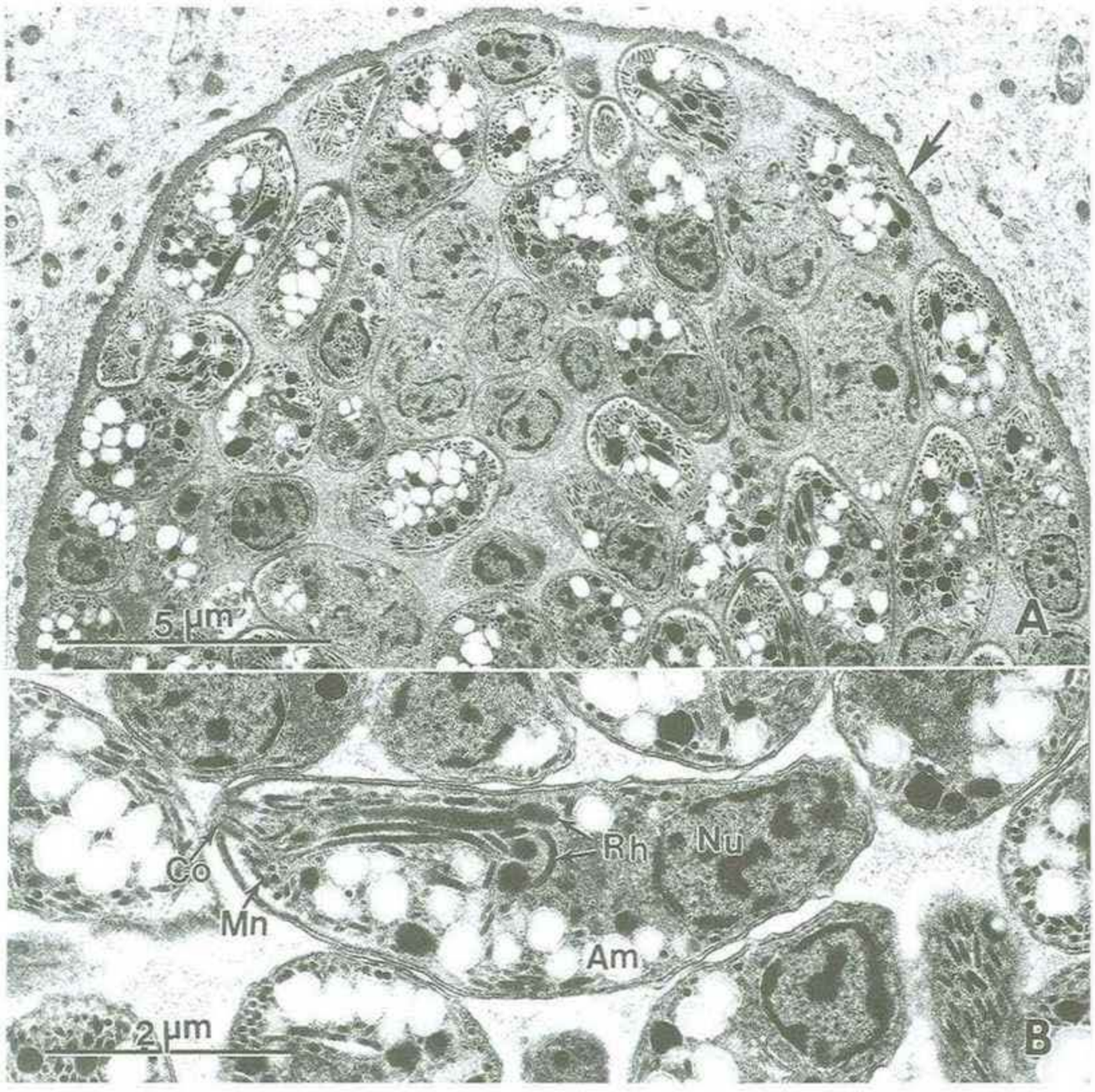


Figure 18.5 Transmission electron micrograph of a large tissue cyst of *Toxoplasma gondii* in a mouse brain

A Note thin cyst wall (arrow) and many bradyzoites

B Higher magnification of a bradyzoite in longitudinal section. Note conoid (Co), micronemes (Mn), electron-dense rhopties (Rh) with convoluted necks, and a terminally located nucleus (Nu). (This figure was provided by Prof. C.A. Speer and Drs D. Fritz, K. Kuehl and D. Austing; *vide* Figure 18.3)

meningoencephalitis. It is also important for pregnant women as tachyzoites can cross the placental barrier and cause defects in the developing foetus.

A wide variation in the occurrence of *T. gondii* antibodies has been recorded in humans in tropical Africa.[30] A limited number of studies concerning human toxoplasmosis has been conducted in southern Africa. In South Africa, in one study, 37 per cent of 806 human sera from Gauteng were positive,[63] while, in another, 29 per cent of the general population in Zimbabwe were serologically positive.[72] However, 47 per cent of the slaughtermen in Zimbabwe (n = 90) were serologically positive, the source of infection possibly being associated with the handling or consumption of raw or undercooked meat.

Pathogenesis

After the ingestion of *T. gondii* by a non-feline host (although the following sequence of events can also occur in cats), it first multiplies in the submucosa of the small intestine and its associated lymph nodes[28, 45] **(Figure 18.9)**. The host may die of enteritis and lymphadenitis before other

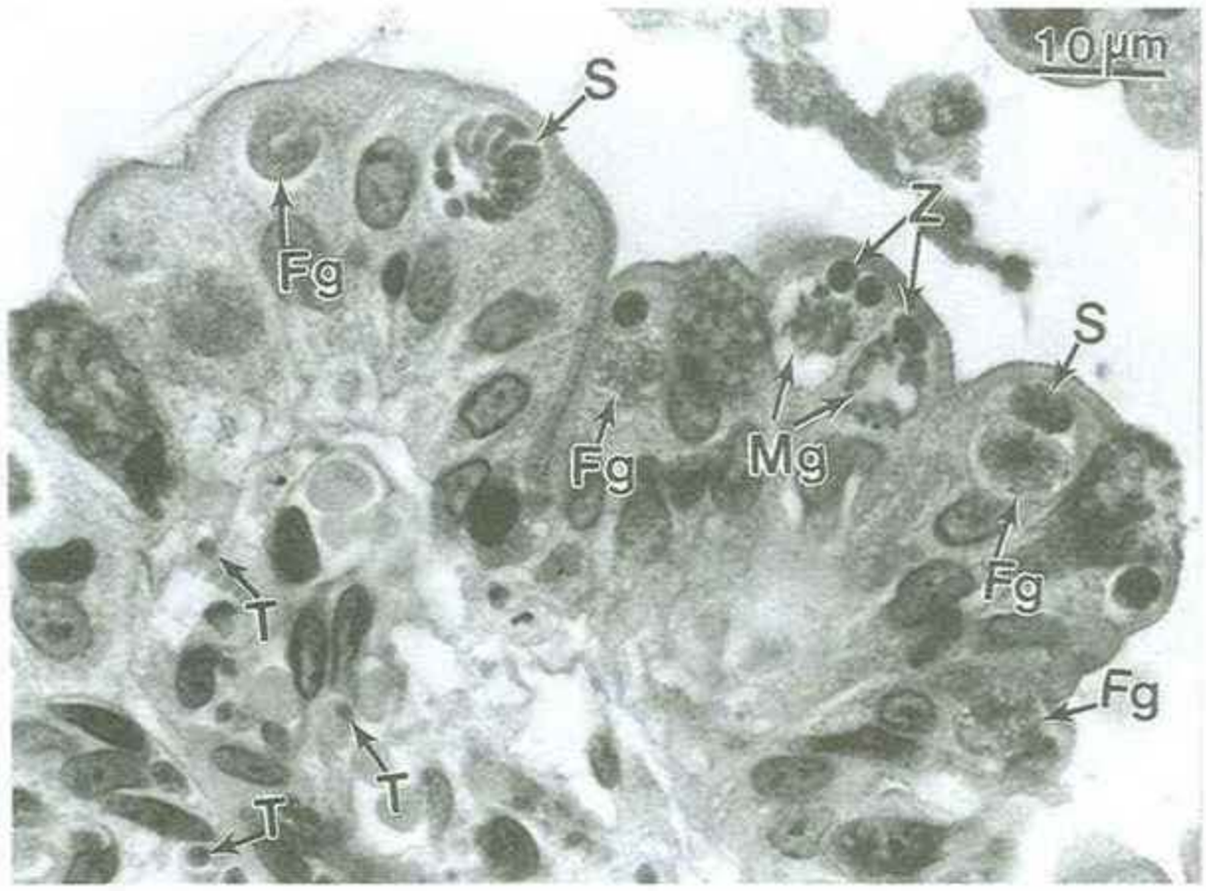


Figure 18.6 Enteroepithelial stages of *Toxoplasma gondii* in ileum of a cat six days after feeding tissue cysts. Note mature schizonts (S), male gamonts (Mg), and female gamonts (Fg) are located in epithelial cells above the host cell nucleus. Zoites (probably developing schizonts) are also present toward the brush border. Tachyzoites (T) are present in the lamina propria. Haematoxylin and eosin stain



Figure 18.7 Unsporulated oocysts of *Toxoplasma gondii* in faeces of a cat

organs are affected.[30] Parasitaemia occurs during the first week, tachyzoites being disseminated in lymphocytes, macrophages and granulocytes, and as free forms via the lymph and blood to the tissues and organs in which they actively invade, or are actively phagocytosed by, host cells.[43] They multiply and, in doing so, destroy the host cell and cell-to-cell transmission may occur within infected organs[43] (**Figure 18.9**). In this way small necrotic foci are formed that elicit an inflammatory reaction in which monuclear cells predominate. In most animals a protective immunity develops before tachyzoites have had time to cause serious deleterious effects. The infection, however, continues to develop subclinically and tissue cysts containing bradyzoites form. Tissue cysts do not usually evoke any host reaction as encystment protects the bradyzoites from both the humoral and cellular immune systems. Inflammation may follow cyst rupture and reactivation of infection may occur in immunosuppressed animals or humans, such as those suffering from the acquired immunodeficient disease syndrome (AIDS).[30]

In susceptible pregnant animals, transplacental infection causes abortion as a result of either placentitis or foetal infection, or both. Parasitaemia and foetal infection occur during the second week after ingestion of oocysts by pregnant

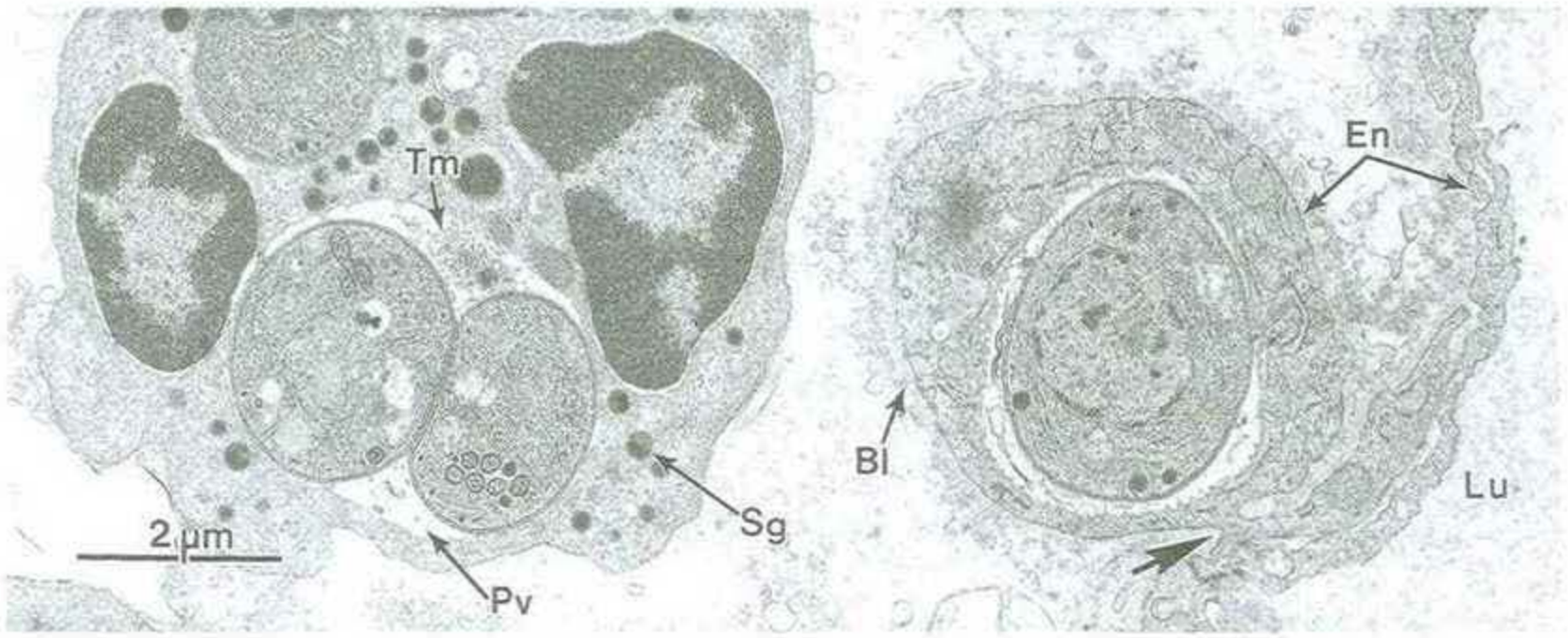


Figure 18.8 Transmission electron micrograph of cells in the ileal lamina propria of the small intestine of a mouse 48 hours after feeding oocysts. Note the cell on the left is a neutrophil showing specific granules (Sg) and a parasitophorous vacuole (Pv) containing two tachyzoites and a well-developed tubulovesicular membranous network (Tm); on the right side a single tachyzoite is located within an endothelial cell (En) that has become displaced but is still connected by a narrow cytoplasmic bridge (arrow) with the capillary endothelium (En); basal lamina of capillary endothelium (Bl); lumen (Lu) of capillary; specific granule (Sg) of neutrophil. (This figure was provided by Prof. C.A. Speer and Drs D. Fritz, K. Kuehl and D. Austing; *vide* Figure 18.3)

sheep and goats.[16] The severity of the lesions in the foetus generally exceeds that in the dam, the effect of the foetal infection largely being determined by the age of the conceptus. Infection during the early stages of gestation may lead to embryonic death and resorption, while infection of ovine foetuses at about 70 to 90 days of gestation is responsible for the death of a large proportion of the foetuses, and they are either aborted or become mummified and are then expelled at a later stage.[46] Some survive the infection for several weeks but are born dead close to term, or else they are born alive but weak and die soon thereafter. Infection of ewes during the last 30 days of gestation generally results in subclinical infection of new-born lambs.

The pathogenicity of *T. gondii* is closely related to the virulence of the strain of the organism and the susceptibility of the host species. New World monkeys, Australian marsupials and Pallas cats are the most susceptible to toxoplasmosis, whereas Old World monkeys, rats, cattle, horses and buffaloes are highly resistant to clinical toxoplasmosis.[30, 40] Other unknown factors vaguely classified as stress may affect the outcome of *T. gondii* infection in a host. More severe infections are found in pregnant or lactating mice than in non-pregnant or non-lactating mice. Concomitant infections may make a host more susceptible or resistant to *T. gondii* infection. Clinical toxoplasmosis in dogs is often associated with canine distemper, ehrlichiosis and lymphosarcoma.[30, 61]

Clinical signs

In livestock, clinical signs are most commonly encountered after transplacental infection has occurred. Infection of sheep and goats during the early stages of pregnancy results in resorption, abortion or mummification of the foetus. Infection between 50 and 120 days of gestation results in abortion or the birth of premature, weak lambs or kids, or in the birth of offspring suffering from subclinical disease. Apart from aborting, ewes and does do not generally manifest any other evidence that they are infected. Clinical toxoplasmosis has, however, been observed in adult goats,[22] pigs[21] and chickens[30, 53] but is rare. Blindness and torticollis have been described in canaries.[80] There is no well-documented case of clinical toxoplasmosis in cattle and horses.[20, 30] Clinical toxoplasmosis occurs in dogs, cats and Australian marsupials.[30, 40] The clinical signs manifested by 100 cases of fatal toxoplasmosis in cats from one hospital in the USA[31] and in wild and zoo animals[40] have been reviewed. Acute systemic toxoplasmosis in animals induces a wide variety of clinical signs because of the many organs and tissues that may be affected. The most common signs observed are those resulting from encephalitis, pneumonia and retinitis.[8] In spite of the reproduction of the parasite in the intestine of cats, clinical evidence of enteric disease has not been recorded.

Most postnatally acquired infections in humans are generally asymptomatic, but fever, lymphodenopathy, headache, myalgia and fatigue may occur.[30, 51] Severe illness may develop in those suffering from immunodeficiency syndromes or who are undergoing immunosuppressive therapy. Congenital infection, on the other hand, may have serious consequences, chorioretinitis, neurological disturbances and hydrocephalus being most commonly manifested. Clinical toxoplasmosis in humans has been reported in South Africa.[70]

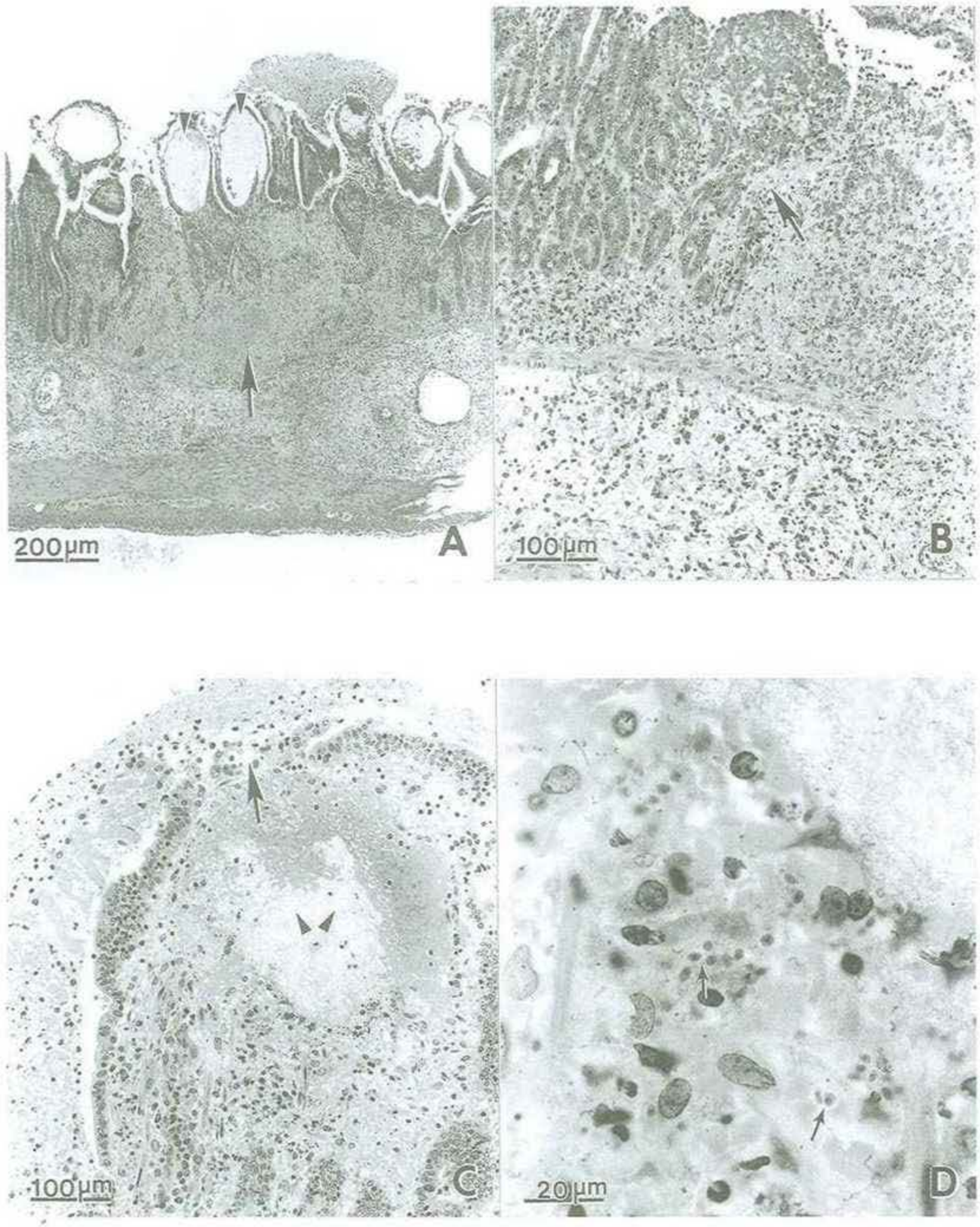


Figure 18.9 Lesions in the small intestine of pigs fed *Toxoplasma gondii* oocysts

A Note transmural necrosis (arrow) and ballooning of villi (arrowheads)
B Higher magnification of an area of inflammation and necrosis (arrow) adjacent to relatively unaffected epithelium
C High magnification of a villus showing oedema of lamina propria (arrowheads) and exudation into the lumen from an ulcer (arrow)
D Higher magnification of lamina propria showing tachyzoites (arrows). Haematoxylin and eosin stain

Pathology

Systemic toxoplasmosis occurs mainly in young animals and immunocompromised hosts. The predominant lesions of systemic toxoplasmosis in animals comprise interstitial pneumonitis, focal hepatic necrosis, lymphadenitis, myocarditis and non-suppurative meningoencephalitis.[19, 22, 23, 30, 31, 40, 54, 61, 68] Macroscopic lesions in the lung vary from irregular grey areas of necrosis to haemorrhagic pneumonia. Necrosis is common and appears to be related to the rapid replication of tachyzoites. The most striking and pathognomonic lesions of toxoplasmosis in animals, however, are in the placenta of sheep and goats.[4, 5, 9, 16, 17, 30, 38, 39, 55, 69] These consist of foci of necrosis varying in size from microscopic to macroscopic in the cotyledons while intercotyledonary areas are unaffected (**Figure 18.10**). The characteristic lesions manifest as white flecks or multiple white, chalky nodules up to 2 mm in diameter and are found in approximately half of the confirmed cases. They may be sparse or dense, may occur in any plane of the cotyledon (**Figure 18.10**) and may be confluent. Not all cotyledons are affected to the same degree. It is often necessary to wash the cotyledons thoroughly in saline solution in order to expose the deeper lesions that may otherwise be overlooked.

The earliest lesions are necrosis of mesenchymal cells, and oedema in the foetal villi with infiltration of mononuclear cells associated with hyperplasia and focal coagulative necrosis of the trophoblastic epithelium. Older lesions consist of foci of caseous necrosis involving foetal and maternal villi which may show mineralization. Most *T. gondii* present in lesions are degenerated and difficult to find in haematoxylin and eosin-stained sections. Immunohistochemical staining is useful in locating *T. gondii*[76] (**Figures 18.10C and D**).

Lesions in foetal tissues are microscopic and occur particularly in the brain, liver and lungs.[5, 30, 55] Lesions in the brain are found in 90 per cent of cases and consist of leukoencephalomalacia and gliosis.

Placental lesions have also been described in toxoplasmosis in cats[36] and pigs.[42] Numerous pale foci of necrosis were seen in the allantochorion of the cats while multiple foci of necrosis of the allantochorion were associated with numerous tachyzoites in the pigs.

Diagnosis

The diagnosis of toxoplasmosis can be made by biological, serological or histological methods, or by a combination of some of these. Clinical signs are not sufficiently specific to enable a definite diagnosis to be made because the disease mimics several other infectious diseases.

Numerous serological procedures are available for the detection of humoral antibodies, including indirect haemagglutination test (IHT), indirect fluorescent antibody test (IFT), direct agglutination test, latex agglutination tests, enzyme-linked immunoabsorbent assay (ELISA), and immunoabsorbent agglutination assay test (IAAT).[30] The IFT, IAAT and ELISA have been modified to enable detection of IgM antibodies which appear sooner after infection than IgG and disappear faster than IgG after recovery. The finding of antibodies to *T. gondii* in one serum sample from an animal merely establishes that the host has been infected at some time in the past; it is therefore preferable to collect two samples from the same animal, the second two to four weeks after the first. A four-fold increase in antibody titre in the second sample indicates an acute infection. In some animals a high antibody titre persists for months after infection. A rise in antibody titre may not be associated with clinical signs. The fact that titres persist after clinical recovery complicates the interpretation of the results of serological tests. The direct agglutination test using formalin-preserved whole tachyzoites and mercaptoethanol has proved very useful in measuring IgG antibodies in livestock, including poultry.[30, 32, 41, 44]

Finding antibodies in foetal fluids or presuckling serum in ruminants is diagnostic of congenital infection because maternal antibodies do not cross the placenta.[32, 47]

Toxoplasma gondii can be isolated from the infected tissues, secretions, excretions and body fluids of animals collected either ante- or post-mortally, by the inoculation of them into laboratory animals and for tissue cultures.[30] Using these methods it is possible not only to isolate *T. gondii*, but also microscopically to visualize the organism or its DNA using the polymerase chain reaction (PCR) test.[50, 65] A rapid diagnosis may be made by the microscopic examination of impression smears of lesions. After drying for 10 to 30 minutes, the smears are fixed in methyl alcohol and stained with a Romanowsky stain, Giemsa being very satisfactory. Well-preserved *T. gondii* are crescent shaped (**Figures 18.2A**). In tissue sections, the tachyzoites usually appear round to oval (**Figure 18.2B**). Tissue cysts are usually spherical and lack septa, and their walls stain with silver stains. The bradyzoites are strongly PAS positive (**Figure 18.2D**). The immunohistochemical staining of parasites with fluorescent or other types of labelled *T. gondii* antiserum can assist in the diagnosis[76] (**Figure 18.10**). Electron microscopy can aid the diagnosis. *Toxoplasma gondii* tachyzoites are always located in vacuoles; they have few (usually four) rhoptries and often have a honeycomb structure (**Figure 18.3**).

In cats, oocysts in faeces are concentrated by salt or sugar floatation techniques.[30] They can be differentiated from the oocysts of *Isospora* spp. by the fact that *T. gondii* oocysts are smaller (10 × 12 µm) and are shed in an unsporulated state (**Figure 18.7**).

Ewes are usually serologically positive at the time of abortion, and a negative test generally excludes toxoplasmosis as a cause of the abortion. A positive result, however, is not necessarily diagnostic, as high titres may persist into the following breeding season.[30]

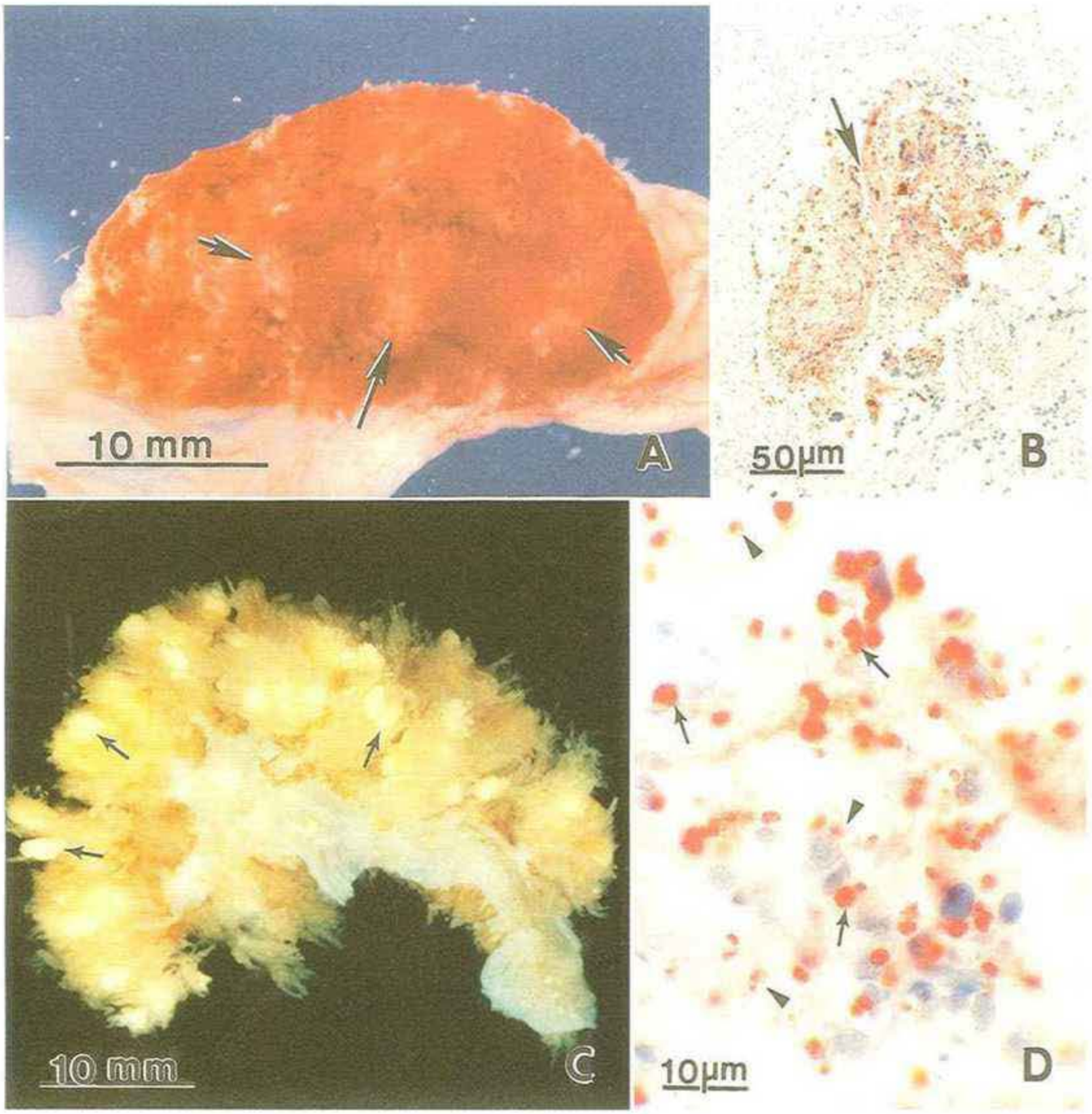


Figure 18.10 Lesions in the placenta of goats and sheep naturally infected with *Toxoplasma gondii*

A Cotyledon of a goat with whitish-yellow areas of discoloration (arrows). The intercotyledonary area is normal. Unstained
B Microscopic lesion in the placenta of a goat. Note necrosis, mineralization, and dispersed *T. gondii* antigen (red coloured areas). Immunohistochemical staining with anti-*T. gondii* serum
C Cut surface of a sheep cotyledon showing yellowish-white areas of necrosis in villi (arrows). Unstained
D Higher magnification of Figure 18.10C showing intact (arrows) tachyzoites and dispersed *T. gondii* antigens (arrowheads)

Differential diagnosis

Foetal and neonatal losses in sheep resulting from *T. gondii* infection should be differentiated from other possible causes of abortion, stillbirths or neonatal deaths, such as those caused by *Chlamydophila abortus* (*Chlamydia psittaci*), *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* serovars, *Brucella* spp., *Coxiella burnetii*, Rift Valley fever and Wesselsbron disease viruses, *Neospora caninum*, and *Sarcocystis* spp. (see the relevant chapters on these diseases).

Control

The control of toxoplasmosis in livestock is aimed at the prevention of infection in pregnant animals. The perpetuation of the life cycle of *T. gondii* in them depends on the availabil-

ity of susceptible definitive hosts, the most important of which is probably the domestic cat, and of infected intermediate hosts, such as rodents. Cats and rodents should therefore be excluded from areas where livestock are fed, or where their feed is stored. If swill is fed to pigs, it should be cooked, and cannibalism should also be prevented.

Treatment of individual cases is usually limited to those suffering from acute infections in high-risk groups, and to immunologically compromised hosts. The treatment of choice is potentiated sulphonamide mixtures, such as sulphadoxine, and trimethoprim.[30] These two drugs act synergistically to inhibit sequential steps in the biosynthesis of folic acid. Monensin at a dose of 5 mg/animal/day incorporated into the feed of pregnant ewes can reduce losses from toxoplasmosis.[12] Experimentally, monensin limits but does not prevent infection; ewes therefore develop protective immunity to subsequent challenge. The level of monensin in the feed should not exceed 30 ppm as otherwise refusal of feed or toxicity may occur. Incorporation of monensin (200 ppm) into feed blocks is a safe way of utilizing this drug.[10] The addition of decoquinate to the feed of pregnant ewes at the daily rate of 2 mg/kg body weight reduces the deleterious effects of *T. gondii*.[11] A live modified *T. gondii* vaccine is marketed in Europe to reduce the prevalence of abortion.[13]

Pregnant women who are serologically negative should avoid gardening without wearing gloves. Cat sand trays should be cleaned daily as oocysts will not have had time to sporulate.

Pregnant women should not eat raw or undercooked meat and should wash their hands after handling raw meat. Immunity follows infection so that a second abortion due to toxoplasmosis is unlikely.

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19

Besnoitiosis

Synonyms: Elephant skin disease, olifantvelsiekte (Afrik.)

R D BIGALKE AND L PROZESKY

Introduction

Besnoitiosis, caused by the protozoan parasite *Besnoitia*, is a relatively common disease of cattle and rarely of equids in southern Africa. Inapparent, mild and severe forms of the disease occur in cattle. The severe form is characterized in the acute stage by fever and anasarca, as well as orchitis in bulls. This is followed by a chronic stage characterized by scleroderma, alopecia and hyperkeratosis. In most cattle, however, the infection is clinically inapparent. The protozoan parasite responsible for the bovine disease *Besnoitia besnoiti* was discovered by Besnoit and Robin.[3] The infection in equids is caused by *Besnoitia bennetti*.

Although bovine besnoitiosis has been known in southern France since the middle of the nineteenth century, it now seems to be of more significance in Africa where its distribution is fairly widespread. It is of economic importance in several countries, including Israel, South Korea, Russia, Nigeria, and South Africa, where it was first discovered and described in 1945 by Hofmeyr in the Rustenburg district in the North-West Province.[21]

Although cattle may die from the disease during the acute anasarca or chronic scleroderma stages, the mortality rate is low. Of more importance are the debility, the possible sterility of bulls (which is usually permanent if the animal has shown frank clinical signs) and the downgrading of carcasses in abattoirs, due to trimming, or even the condemnation of carcasses resulting from the presence of cysts in the fasciae and intermuscular connective tissues.

Besnoitiosis has also been recognized as being economically important in goats in Kenya and Iran, where the disease is characterized by scleroderma, alopecia and the presence of large numbers of cysts in sites similar to those observed in cattle.[11, 12, 34]

Clinical *B. bennetti* infections in horses and donkeys, and subclinical infections in a mule and zebra (*Equus burchelli*) have been recorded in South Africa.[9, 36, 39] Equine besnoitiosis was, however, first recorded in France by Henry and Masson[20] and subsequently in Sudan.[2]

Bovine besnoitiosis is a much more common disease than equine besnoitiosis and has therefore been studied more comprehensively. Caprine besnoitiosis has recently received considerable research attention. However, it is remarkably similar to the bovine disease. Bovine besnoitiosis will thus form the basis of the descriptions in this chapter and be compared to the disease in goats, where appropriate.

Aetiology and life cycle

Although the life cycle of *B. besnoiti* in its presumed final host needs further elucidation, the evidence obtained from other *Besnoitia* spp. and its morphological similarity to *Toxoplasma* and *Sarcocystis* spp. indicate its coccidian nature and justify its classification in the phylum Apicomplexa, family Sarcocystidae and subfamily Toxoplasmatinae.[24]

Based on observations made on other *Besnoitia* spp.,[18, 37] and a single, as yet unconfirmed, observation on *B. besnoiti*,[35] it must be assumed that cattle serve as intermediate hosts and contract besnoitiosis by the ingestion of mature isosporan-type oocysts (*c.* 15 × 13 µm) shed in the faeces of members of the cat family, which presumably serve as final hosts.

The sporozoites apparently enter the bovine circulation and multiply by endodyogeny in endothelial cells of blood vessels, particularly those in the skin, fasciae, upper respiratory tract and testes.[1] Merozoite-like endozoites (tachyzoites), *c.* 5,9 × 2,3 µm in size, reinvade adjacent or more distant endothelial cells to produce further endozoites. This developmental cycle is associated with the anasarca stage of the disease and is superseded by cyst formation.

Cyst formation begins about one week after the initial cycle of proliferation, when activated, hypertrophic cystozoite-containing histiocytic cells become detectable in the same sites where the endozoites were formed. The histiocytic cells are observed either within or in close association with blood vessel walls. The merozoite-like cystozoites (bradyzoites) are *c.* 8,4 × 1,9 µm in size and multiply by endodyogeny[37] in vacuoles of the markedly enlarged host cells with enlarged and multiple nuclei (**Figure 19.1**). The characteristic thick-

walled cysts, with their conspicuous periodic-acid, Schiff-positive hyaline walls (**Figures 19.1 and 19.2**), apparently formed by the host cells, and eccentrically placed host-cell nuclei, reach a diameter of *c.* 400 µm after about six weeks. The ultrastructural morphology of the cystozoites is depicted in **Figures 19.3 and 19.4**. Cyst formation is remarkably synchronous in experimental cases and there is no evidence that cystozoites from disintegrating cysts give rise to further endozoites or cysts in the same animal.[4] Cyst formation is associated with the chronic scleroderma stage of the disease.

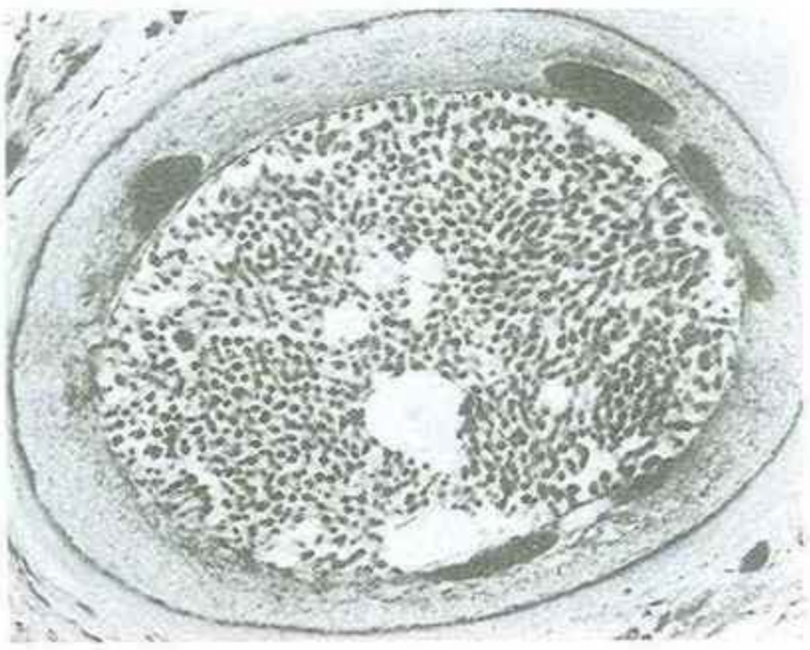


Figure 19.1 *Besnoitia besnoiti* cyst: cystozoites in a cyst are surrounded by a rim of cytoplasm containing multiple nuclei of the host cell (histiocyte) followed by a hyaline wall

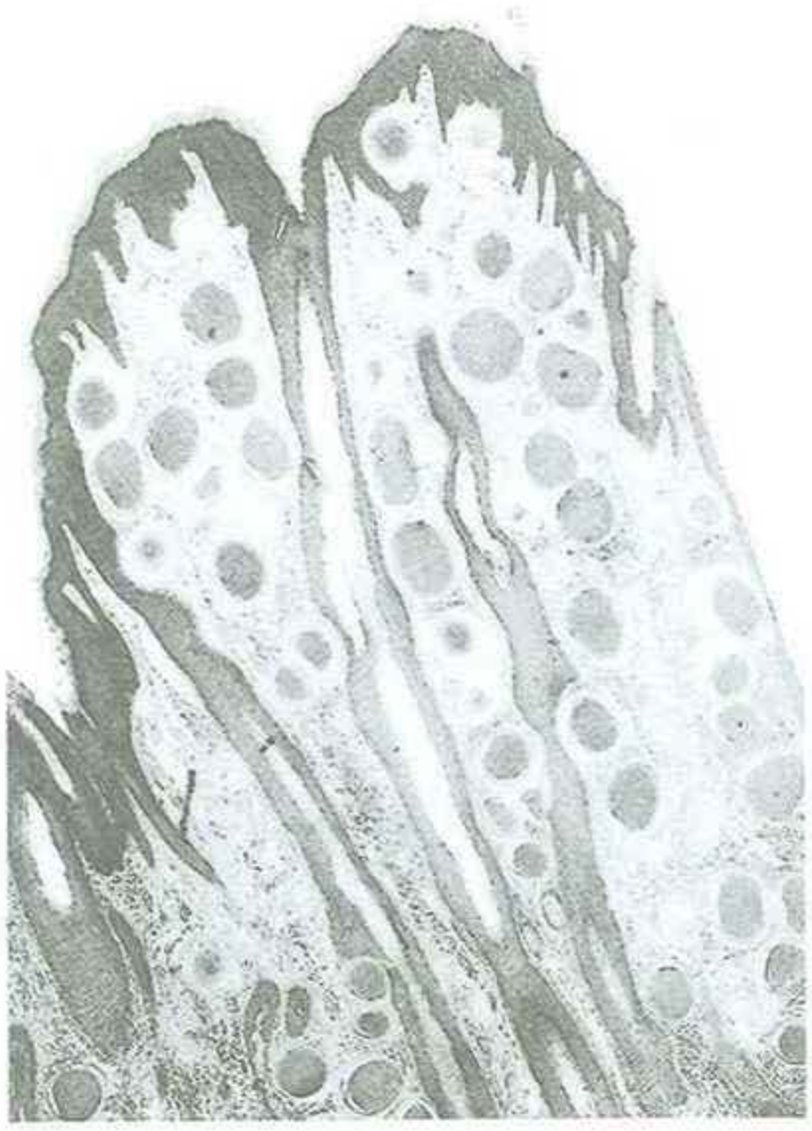


Figure 19.2 Scleroderma: numerous cysts in the dermis





Figures 19.3 (top) and 19.4 (bottom) Ultrastructural morphology of cystozoites (bradyzoites)

Only the cystozoite phase of the life cycle of *B. bennetti* has been described, which is morphologically very similar to *B. besnoiti*. The cysts are slightly larger, reaching a diameter of more than 600 µm, and the hyaline walls of the cysts are thinner than those of the bovine parasite.[9] Cystozoites are ultrastructurally distinguishable from those of *B. besnoiti*.[44]

Besnoitia in blue wildebeest (*Connochaetes taurinus*) and impala (*Aepyceros melampus*) is morphologically and immunogenically similar to *B. besnoiti*, but differs from it in certain biological respects.[25] The taxonomic relationship is obviously close and hence the parasites of antelope origin are regarded as being *B. besnoiti*.[10]

Epidemiology

Besnoitia has a fairly wide host range and several *Besnoitia* spp. have been described. Frank disease caused by *Besnoitia* has only been recognized in cattle, goats, horses and barren-ground (*Rangifer tarandus groenlandicus*) and woodland (*Rangifer tarandus caribou*) caribou,[15, 45] but clinically inapparent infections have been detected in blue wildebeest, impala, zebra, donkeys, a mule and a warthog (*Phacochoerus aethiopicus*).[23]

In South Africa, bovine besnoitiosis is of economic importance mainly in the Bushveld and Lowveld of the Limpopo, North West, Mpumalanga and KwaZulu-Natal provinces. A few cases have been recorded in the Northern Cape Province and western Free State. It has also been recorded in Swaziland, Zimbabwe, Botswana, Namibia, Angola, Zaire, all the East African countries, and Cameroon and Nigeria in West Africa.[6] Outside Africa it has been reported in Israel, south-western Russia, South Korea, Portugal, France and Venezuela.[6]

The disease is more prevalent in less temperate, and even subtropical climates. In South Africa, the majority of new cases occur during the warmer, moister months of the year. This is apparently also the case in Israel.[43]

All breeds of cattle and both sexes seem to be susceptible, although infection in calves under six months of age is rare. A survey on a farm where the disease was prevalent revealed that the highest incidence was in animals 18 months to 6 years old. Of 5 018 cattle surveyed in an endemic area by examination of the scleral conjunctiva for cysts, 427 (8,5 per cent) were infected, of which 74 (17,3 per cent of infected animals and 1,5 per cent of the total number examined) showed clinically detectable signs of chronic infection.[4] Thus, comparatively few animals develop clinical signs of infection. The passive transfer of antibodies from chronically infected cows to their offspring[43] may be an important factor in the above-mentioned epidemiological features of the disease.

There is experimental and circumstantial field evidence that chronically affected cattle harbouring large numbers of cysts serve as sources of infection of bovine besnoitiosis.[4, 43] Although a variety of laboratory animals, such as rabbits, gerbils (*Meriones tristrami shawii*) and hamsters, and sheep, goats and black wildebeest (*Connochaetes gnou*) are susceptible to artificial infection with bovine strains of *B. besnoiti*,[4, 5, 18, 26, 36, 41] no naturally infected hosts, other than cattle, have been found. Strains of *Besnoitia* isolated from blue wildebeest and impala, although antigenically closely related to cattle strains of *B. besnoiti*, are relatively nonpathogenic and reveal viscerotropic rather than dermatotropic affinities in cattle and rabbits.[10] Consequently, it seems unlikely that antelopes infected with these strains could serve as carriers for the typical cattle disease.

Whereas goats were successfully infected by inoculation with cystozoites of the caprine parasite, attempts to similarly infect cattle and a variety of laboratory animals (rabbits, mice, guinea pigs, hamsters, rats or sheep) failed.[13, 29] On the strength of its apparent host-specificity for goats and some ultrastructural differences from *B. besnoiti* of cattle, the parasite of goats was recently named *B. caprae*.[29, 30]

Experimental and circumstantial field evidence indicates that bovine besnoitiosis can be transmitted mechanically from chronically infected cattle to susceptible ones by blood-sucking insects such as tabanids.[4, 43] Although this method of transmission probably plays some part in the epidemiology of the disease, it does not seem to play a major role as only clinically inapparent cases with small numbers of cysts are produced in cattle in this way.[4] The severe form of the disease can consistently be produced experimentally in cattle immunosuppressed with corticosteroids prior to artificial infection with cystozoites or endozoites.[17]

It seems logical that a carnivorous host must exist for *B. besnoiti*. The wild cat (*Felis lybica*), incriminated as a host in Russia,[35] has an almost countrywide distribution in South Africa. Domestic cats, which could be fed raw, infected meat by humans, do not, however, occur on all infected farms. Moreover, it is difficult to understand how a small wild cat, such as *F. lybica*, can become infected on such farms where cattle are the only known potential source of infection for them. It therefore seems probable that a hitherto unknown cycle of transmission involving a carnivorous host occurs in nature, although it has not as yet been proven experimentally by feeding muscles and connective tissues containing numerous cysts to a variety of carnivorous animals.[17] Attempts to transmit *B. caprae* to domestic cats have also produced negative results.[27]

Caprine besnoitiosis is widespread in northern Kenya compared to bovine besnoitiosis, which appears to be limited in its distribution in that country.[28, 33] In Iran, outbreaks of the disease in goats have been limited to the south.[34]

Equine besnoitiosis is a rare, very sporadic disease and its epidemiology is obscure.

Pathogenesis

The endozoites associated with the acute anasarca stage of bovine besnoitiosis proliferate in the cytoplasm of endothelial cells, causing vasculitis, and sometimes thrombosis,

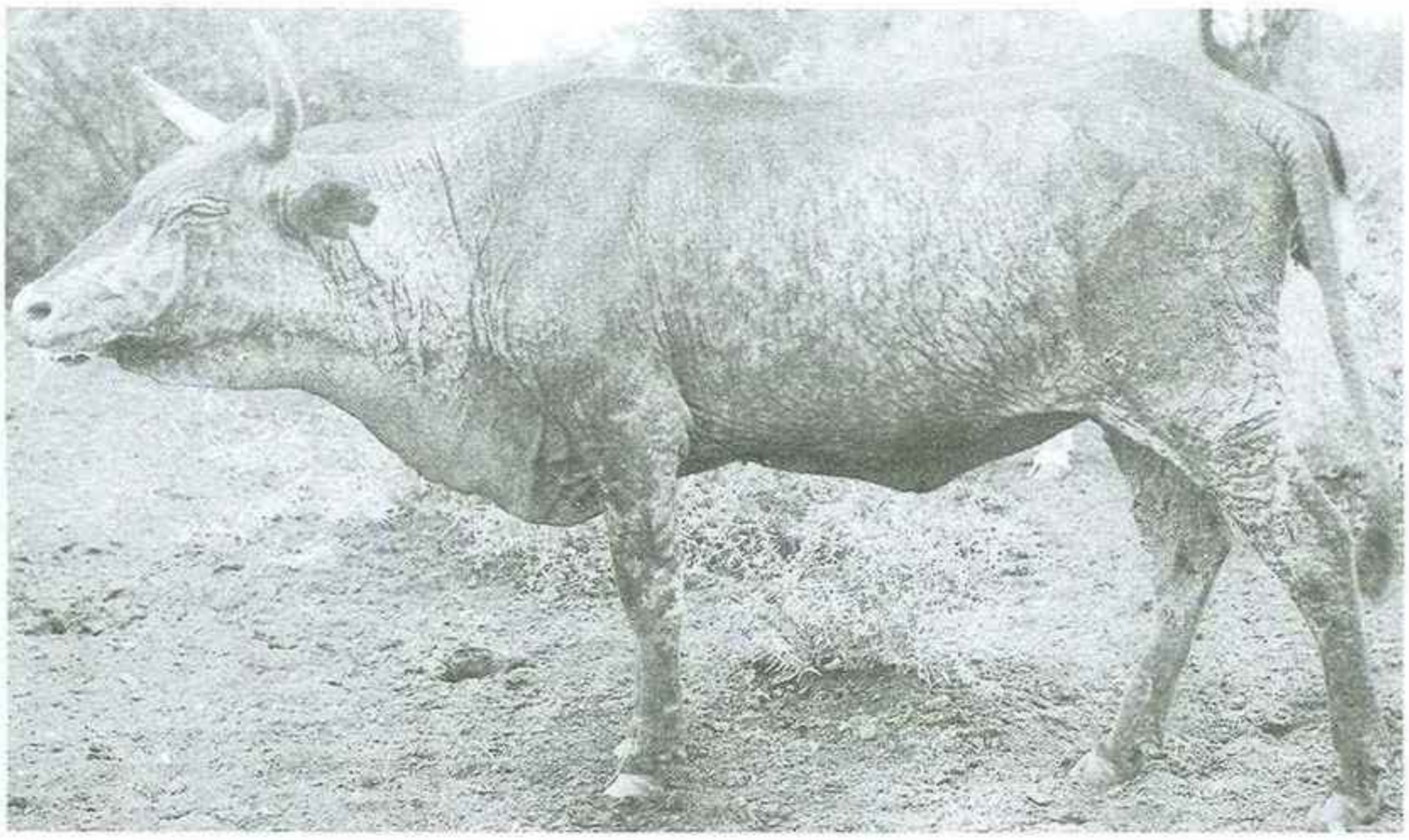


Figure 19.5 Clinical case of the scleroderma stage of besnoitiosis. Note photophobia and salivation



Figure 19.6 Marked scleroderma of hindquarters and legs

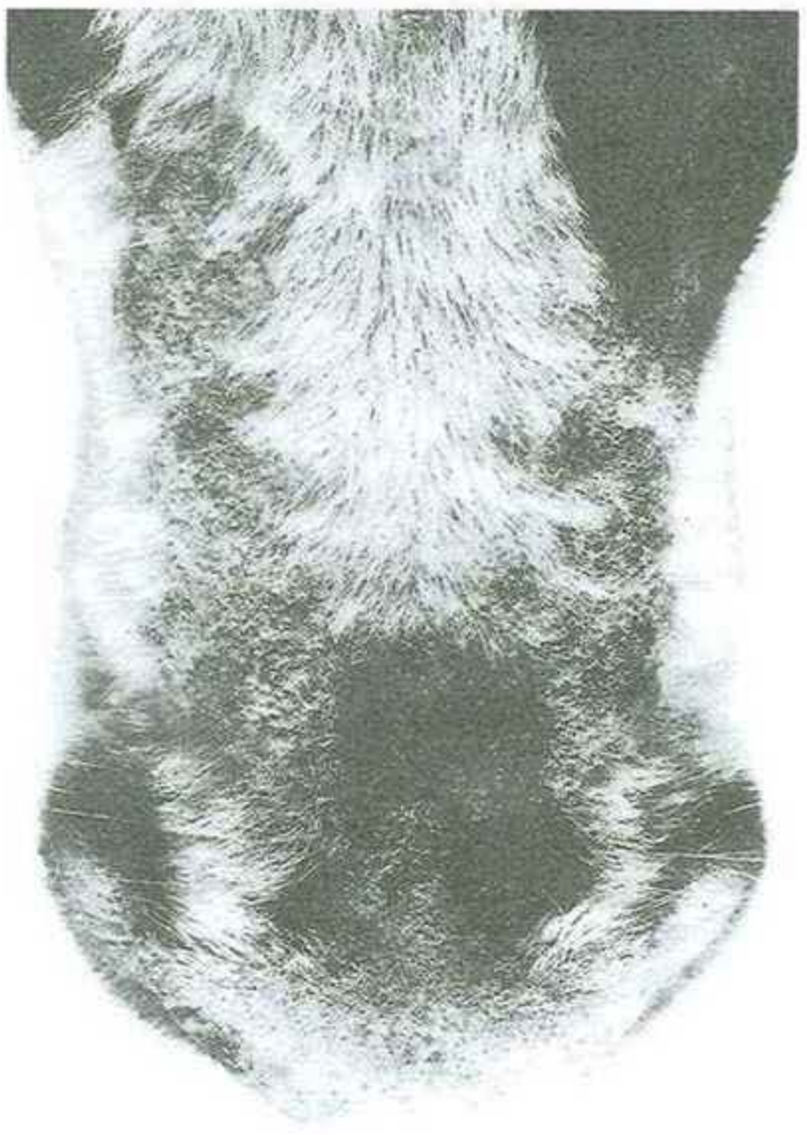


Figure 19.7 Scleroderma of dorsum of nose



Figure 19.8 Scleroderma: close-up of severe dermatitis crustosa

particularly in smaller veins and capillaries in the skin, subcutis, the mucosa of the nasal cavity, larynx, and trachea, and the testes.[1] These lesions, together with a possible toxic effect of the parasite, cause an increased vascular permeability, resulting in oedema and sometimes small haemorrhages and foci of necrosis in the afore-mentioned sites.

The circulatory disturbances are probably also responsible for the necrosis of the epidermis, deeper layers of the skin and of mucous membranes of the upper respiratory tract in the scleroderma stage of the disease. Very large numbers of rapidly growing, thick-walled cysts, especially in the dermal papillae, fibrosis and granulomatous reactions around cysts, oedema of the skin as well as hyperkeratosis and acanthosis probably account for the typical scleroderma.[1]

Clinical signs and pathology

Two sequential stages of the clinical disease are recognized in cattle, namely an acute anasarca stage, which is associated with proliferation of endozoites, and a chronic scleroderma stage (**Figures 19.5 to 19.8**), which is associated with cyst formation.

An incubation period of four days was recorded in cattle after oral infection with sporulated oocysts of feline origin,[35] whereas Bigalke[4] reported a mean incubation period of 13 days after experimentally induced mechanical transmission of the infection by biting flies.

Fever is the first clinical sign and early-morning rectal temperatures may reach 40 to 41 °C or higher and remain at that level for a week or more. Pyrexia is accompanied by progressive inappetence, culminating in complete anorexia in severe cases with consequent loss of weight. Affected animals are generally listless and prefer to lie down; are photophobic (**Figure 19.5**) and hence seek shade; and have an increased respiratory rate. They walk with a slow gait if forced to move.

Hyperaemia of the muzzle, periorbital skin and scrotum is noticeable in light-skinned animals a few days after the rise in temperature. Anasarca also usually appears at this time and may vary from nothing more than a slight swelling of the face, or thickening of skin folds of the neck, back or chest regions, to subcutaneous oedema over the entire body in more severe cases. The testes are swollen and sensitive to palpation. Early anasarca often goes unnoticed and its presence is only revealed when oedema fluid accumulates along the ventral parts of the body such as the brisket, sternum, prepuce and legs. The anasarca stage often goes unnoticed in the field. In cases in which it does occur, there may be a distinct break between this stage and the next, with disappearance of the above-mentioned signs by the third week of the reaction, or the two stages may be sequential. Animals sometimes succumb to the disease in the anasarca stage.

The most characteristic clinical sign of the scleroderma stage in severely affected cattle is progressive thickening, hardening and prominent folding and puckering of the skin, which may be widespread or more localized (**Figures 19.5 to 19.8**). The skin of the legs is often hard and the limbs may be markedly thickened (**Figure 19.6**). In such cases, movement is slow and restricted, apparently due to pain. These features usually appear three to four weeks after the initial rise in temperature.

Progressive loss of hair, accompanied by dermatitis with hyperkeratosis and dermatitis crustosa, also occurs (**Figure 19.8**). Parts of the epidermis may be shed in patches, leaving greyish, hairless, seborrhoeic areas. Sit-fasts are commonly seen over bony prominences as well as on the ventral portion of the scrotum. Often a sero-sanguineous exudate oozes from the skin. This dries to form large scabs which sometimes become secondarily infected. Deep, raw fissures, which usually become maggot-infested, sometimes develop between folds of the skin in sites such as the breech and over bony prominences. The superficial lymph nodes are invariably swollen. A mucopurulent nasal discharge, which forms crusts often clogging the nostrils and causing stridor, may occur in severe cases. These signs are usually accompanied by an undulant low-level fever (which persists for several weeks), poor appetite and emaciation. More pronounced fever and anorexia occur in more severe cases, some of which may be fatal.

Bulls invariably become aspermatogenic, due to the presence of a usually persistent and severe orchitis, followed by uni- or bilateral testicular atrophy and induration in the chronic disease. Acute, subacute and chronic orchitis accompanied by pronounced sperm changes are also observed in goats.[31]

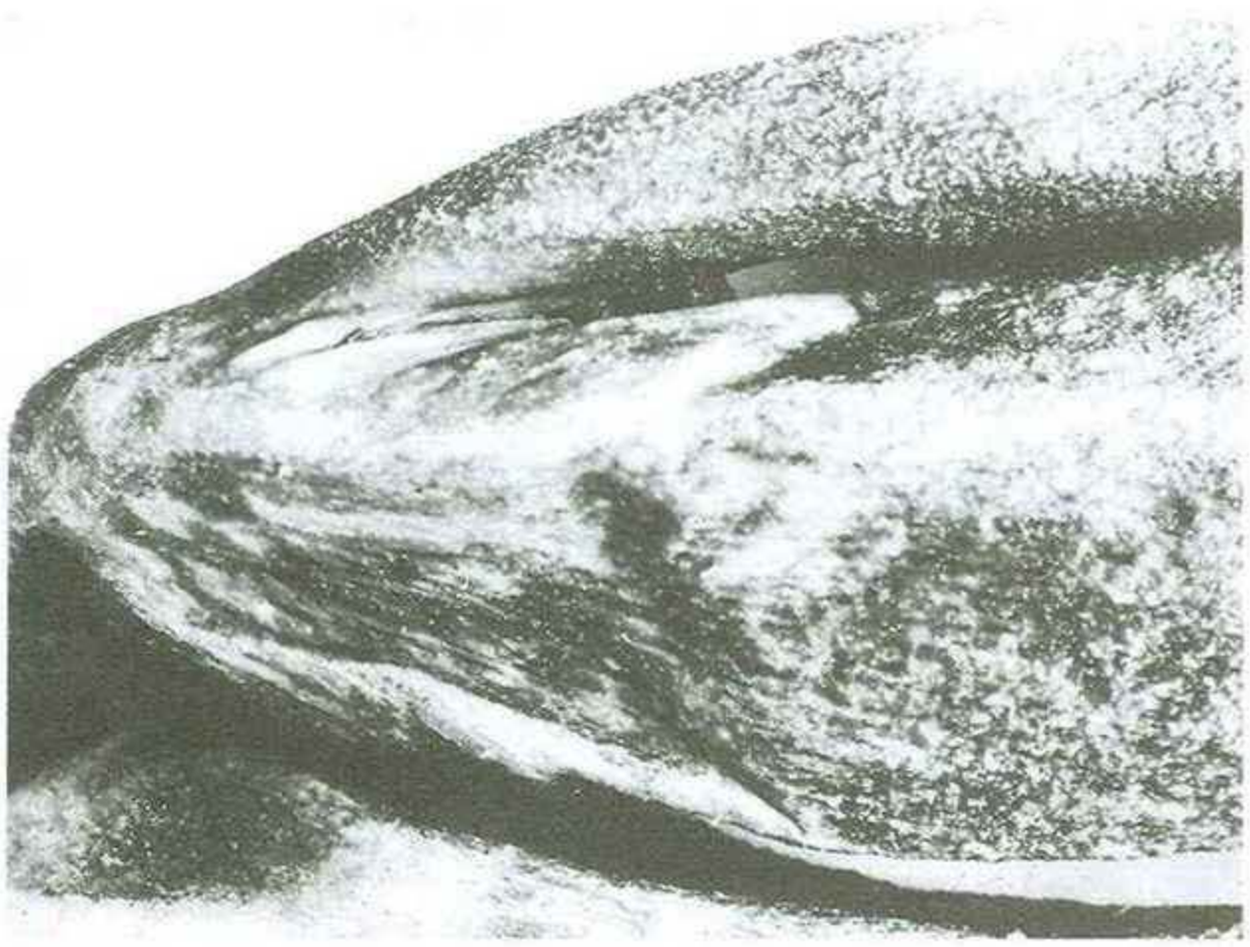


Figure 19.9 Numerous cysts in the mucosa of the turbinates

Cysts become visible to the naked eye in the scleral conjunctiva and nasal mucosa (**Figures 19.9 and 19.10**) about six to seven weeks after the initial rise in temperature.

Most animals survive, but convalescence is slow and scleroderma and alopecia may be permanent. Gradual improvement in the appearance of the animals seems to be associated with the degeneration of cysts and a subsequent decrease in their numbers.

Surveys conducted by scrutinizing the scleral conjunctiva[4] and by serological tests[19, 22, 26] indicate that most animals which become infected in the field contract a clinically inapparent form of the disease.

Only the scleroderma stage has been recognized in horses. The disease is very similar to that observed in cattle, but recovery from the skin lesions is usually more rapid in horses, and outgrowing hooves, apparently due to laminitis, are sometimes observed.[9]

Only the chronic scleroderma stage has been observed in natural cases in goats, but a subcutaneous oedema has been recorded in artificially infected animals.[29]

The pathology of bovine besnoitiosis has been described in a few publications.[1, 21, 25, 36, 38]

At necropsy, apart from the clinically discernible lesions already described for the anasarca stage, widespread petechiae and ecchymoses in the subcutis, oedema of the lymph nodes and testicular parenchyma, and a catarrhal to mucopurulent rhinitis and conjunctivitis are often noticeable. Mild to prominent oedema of the lungs occurs less commonly.

In the chronic stage, cysts are visible macroscopically as small, whitish granules the size of a sugar granule particularly in the muscles, intermuscular fasciae, tendons and



Figure 19.10 Numerous white cysts clearly visible in the mucosa of turbinates

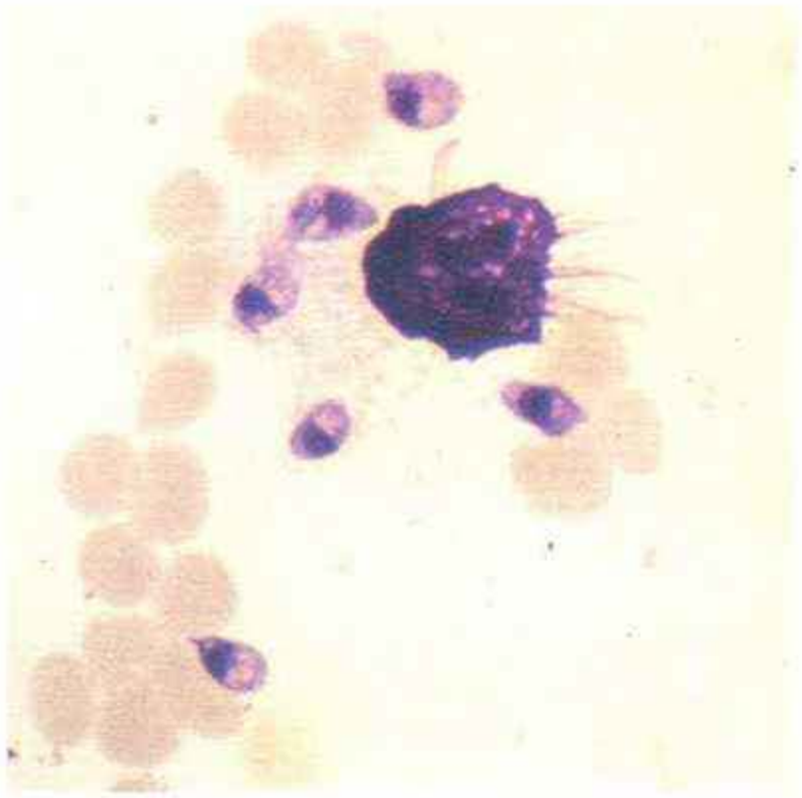


Figure 19.11 Peripheral blood smear containing endozoites

tendon sheaths of the legs, synovial sheaths, periosteum of the metatarsal and metacarpal bones, testicular parenchyma, mucosa of the nasal turbinates, pharynx and upper respiratory tract, bulbar conjunctiva, endocardium, cardiac valves and intima of the more peripherally located larger veins of the legs, head and neck.[1, 25, 38]

Microscopically, in the acute stage, varying numbers of endozoites are found predominantly in endothelial cells of capillaries and small veins, and less frequently in what appears to be mesenchymal cells in the intima of veins and small arteries. The vessels in the dermis and subcutis, nasal cavity, larynx and trachea and testicular parenchyma are most severely affected. Parasitized blood vessels show necrosis of endothelial cells and fibrinoid degeneration and necrosis of their walls with resultant oedema, haemorrhage, thrombosis, and necrosis in surrounding tissues. Macrophages and a few lymphocytes, plasma cells and eosinophils occur perivascularly and in the walls of affected vessels.[1]

On microscopic examination of the skin of chronically affected animals, numerous cysts are discernible particularly in the dermis and subcutis but also in the intermuscular fasciae (**Figure 19.2**). Cysts in blood vessels are mainly located in the intima, but may be present in all parts of the wall. Hyperplasia of the endothelial cells and occasionally sclerosis of the intima may be evident in the immediate vicinity of the cysts.[1]

Most of the cysts are enclosed by a thin collagenous capsule. When the organisms die, they elicit a granulomatous reaction.[25, 38]

The clinical signs and pathology of the disease in goats are remarkably similar to those in cattle.[12, 14, 29, 34]

Reports on the lesions of besnoitiosis in horses are limited and deal only with chronic besnoitiosis.[2, 39] The distribution of cysts and the pathology of the disease in equids are similar to those in cattle.

Diagnosis

Endozoites of *B. besnoiti* that are free in the blood or in monocytes may be demonstrated in peripheral blood smears of cattle for several days at the height of the febrile stage, but they are scarce and difficult to find (**Figure 19.11**). Blood smears made from the ear or tail of chronic cases often contain cystozoites originating from punctured cysts. The coccidian-type endozoites and cystozoites are merozoite-like in appearance, and are therefore not diagnostic for besnoitiosis.

The clinical diagnosis is easily confirmed in scleroderma cases by demonstration in skin sections of large numbers of the typical cysts in the dermis (**Figures 19.1 and 19.2**).

Cysts of *B. besnoiti* are visible to the naked eye in the scleral conjunctivae of cattle from about six to seven weeks after infection. Cysts are plentiful at this site in typical scleroderma cases. They may even be detected in small numbers in the scleral conjunctivae in cattle which show no other evidence of infection.[4, 8] Their identity may be confirmed by examining smears prepared from single cysts isolated from a tissue specimen with the aid of a dissection microscope, or by microscopical examination of tissue sections.

At abattoirs, or at necropsy of cattle with typical scleroderma, large numbers of cysts are visible at the predilection sites.

The indirect immunofluorescence test and the enzyme-linked immunosorbent assay are useful aids in studies on the epidemiology of the cattle disease[19, 22, 26, 41, 43] and on the efficacy of vaccination.[16]

Most of what applies to bovine besnoitiosis is also true for the caprine disease. However, no serological test has been developed for the latter.

Differential diagnosis

Particularly if no subcutaneous oedema is present, the acute febrile stage of the bovine besnoitiosis can be confused with other acute febrile diseases, such as heartwater and redwater, which also occur in the endemic areas. Acute photosensitivity should be differentiated from the anasarca stage. In cases of bovine besnoitiosis where there is oedema of the legs and ventral parts of the body, cardiac failure and acute lumpy skin disease should be considered.

The skin lesions present in the scleroderma stage of bovine besnoitiosis are sometimes very similar clinically to those seen in dermatophilosis (Senkobo disease), sarcoptic mange, lousiness, subacute to chronic sweating sickness, lumpy skin disease, photosensitivity, and poisoning by mercury, chlorinated naphthalenes and hairy vetch (*Vicia* spp.).

Severe dermatophytosis, onchocercosis, and mange can be difficult to distinguish clinically from equine besnoitiosis.

Lousiness and mange should also be considered when dealing with skin conditions in goats.

Control

No suitable drug is available for the treatment of natural cases of besnoitiosis, although oxytetracycline has been found to have a delaying effect on the course of artificial infection in rabbits, if the drug is administered concurrently with the organism.[40] Long-acting oxytetracycline at 200 mg/kg gave complete protection to experimentally infected gerbils.[42]

Tetracyclines, wound dressings, control of secondary infections and infestations, and provision of shade and nutritious food and water *ad libitum*, are advocated as supportive treatment.

A live vaccine against bovine besnoitiosis has been developed by the Onderstepoort Veterinary Institute.[7] Each dose of vaccine issued contains *c.* 1×10^7 organisms of a blue wildebeest strain of *B. besnoiti* grown in cell culture. The vaccine is issued in the frozen form and is recommended for use in weaners and older animals. A single dose of vaccine protects cattle from the clinical form of the disease for up to four years but does not entirely prevent subclinical infection from taking place. The indirect immunofluorescent test has, however, revealed that levels of antibody to a homologous antigen start to decline at 180 days after vaccination.[16] Annual revaccination for two or three years is therefore advocated.

The systematic annual elimination of all chronically infected cattle, detectable by examination of the scleral conjunctiva, in a closed herd on a farm in an endemic area over a period of several years, appeared to result in a decrease of new infections in the herd.[3] Until the epidemiology of the disease is better understood, it will not be possible to determine with certainty whether preventative measures based on these actions will control the disease.

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20

Sarcocystosis

M B MARKUS, J J VAN DER LUGT AND J P DUBEY

Introduction

Sarcocystis is ubiquitous worldwide. The protozoon commonly and characteristically causes a chronic, subclinical infection in the cardiac and/or skeletal muscles of livestock, but, on rare occasions, *Sarcocystis* does give rise to disseminated acute disease, encephalitis (which can occur in the absence of disseminated infection), abortion or premature birth and, possibly, eosinophilic myositis. In southern Africa, cases of acute (pre-muscular) sarcocystosis have so far been seen in goats and cattle[142] **(Figures 20.1 to 20.3)**.

Aetiology and life cycle

The coccidial nature of *Sarcocystis* first became apparent approximately 30 years ago as a result of work by Fayer in the USA and by Heydorn and Rommel in Germany. The classification, history and nomenclature of *Sarcocystis* have been discussed elsewhere.[31, 40, 79, 115, 135, 137] The organism is categorized as follows: phylum Apicomplexa (Sporozoa); class Sporozoasida; subclass Coccidiasina; order Eucoccidiorida; suborder Eimeriorina; family Sarcocystidae; subfamily Sarcocystinae; genus *Sarcocystis*. This generic name is derived from the cyst stage **(Figure 20.4)** in muscle ('sarco' = muscle, from the Greek word for flesh). *Sarcocystis* spp. have the organelles that are characteristic of the phylum Apicomplexa such as apical rings (also called conoidal or preconoidal rings), polar rings, conoid, pellicle, subpellicular microtubules, micropores and micronemes.

Typically, multiple species of *Sarcocystis* are found in domestic animals **(Table 20.1)**. A two-host life cycle is involved **(Figure 20.5)**. The intermediate hosts — water buffalo (*Bubalus bubalis*), cattle, goats, pigs, sheep, or equine hosts — harbour the asexual stages of the parasite, whereas a sexual cycle takes place in the definitive hosts, such as cats, dogs or humans **(Table 20.1)**. The period of time required for completion of the life cycle varies considerably, depending upon the species of *Sarcocystis*.[40] In the following description, the life cycle of *S. cruzi* will serve as an example.[29]

Dogs, coyotes, foxes and possibly wolves, jackals, and raccoons are the definitive hosts of *S. cruzi*, whereas bison (*Bison bison*) and cattle are the intermediate hosts.[29] The definitive host becomes infected by ingesting muscular or neural tissue containing mature sarcocysts. Bradyzoites (synonymous with cystozoites or cystozoic merozoites[88]) liberated from the sarcocyst by digestion in the stomach and gut, penetrate the mucosa of the small intestine and transform into male (micro-) and female (macro-) gamonts (gametocytes). Within six hours of ingestion of infected tissue, gamonts are found within a parasitophorous vacuole (PV) in goblet cells near the tips of villi. Macrogamonts are ovoid to round, 10 to 20 µm in diameter, and contain a single nucleus. Microgamonts are ovoid to elongated and contain one or more nuclei. The microgamont's nucleus divides and, as the microgamont matures, the daughter nuclei migrate towards the periphery of the gamont. Mature microgamonts of *S. cruzi* measure about 7 × 5 µm and contain 3 to 11 slender gametes. The microgametes are about 4 × 0.5 µm in size and have a compact nucleus and two flagella. Microgametes liberated from the microgamont actively move to the periphery of the macrogamont. After fertilization, a wall develops around the zygote and the oocyst is formed. The entire process of gametogony and fertilization can be completed within 24 hours and gamonts and oocysts may be found at the same time. The location of gametogony and the type of cell parasitized, vary with the species of *Sarcocystis* and stage of gametogenesis. *Sarcocystis* differs from other coccidia in that no asexual multiplication takes place in the intestine prior to sexual reproduction.

In contrast to other coccidia, *Sarcocystis* oocysts sporulate in the lamina propria of the gut. The inner mass of the oocyst (sporont) divides into two sporocysts. Four sporozoites are formed in each sporocyst. Sporulated oocysts are generally colourless and thin-walled **(Figure 20.6)**. Occasionally, unsporulated or partially sporulated oocysts are shed in the faeces. The prepatent and patent periods vary (the latter is usually long), but oocysts of most *Sarcocystis* species first appear in faeces between 7 and 14 days after



Figure 20.1a Acute sarcocystosis in a goat; natural infection. Schizont in a vascular endothelial cell in the brain

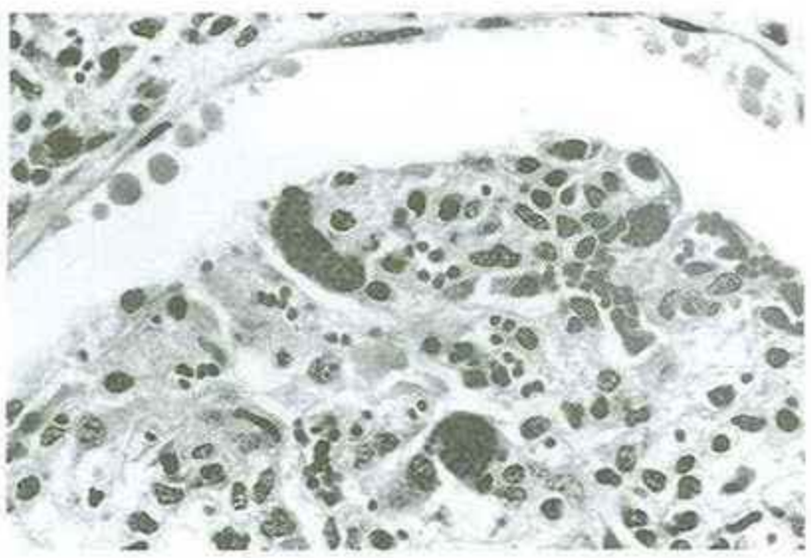


Figure 20.1b Natural infection: glomerulus in the kidney of a goat containing schizonts in vascular endothelial cells. (Tissue by courtesy of Dr J.A. Neser, Onderstepoort Veterinary Institute, South Africa)

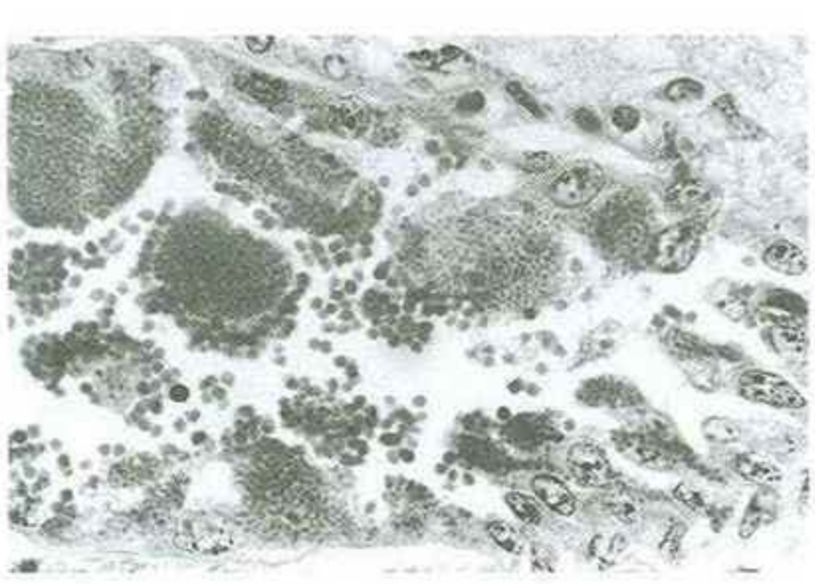


Figure 20.2 Acute bovine sarcocystosis: natural infection. Vascular schizonts in endothelial cells and some apparently lying free in the lumen of the vessel. (Tissue by courtesy of Dr J. Kitching, Regional Veterinary Laboratory, Allerton, Pietermaritzburg, South Africa)

ingestion of sarcocysts. Curiously, both sporulating and fully sporulated oocysts of *Sarcocystis* have been seen in skeletal muscles of baboons in South Africa, which are frequently both intermediate and definitive hosts in terms of the life cycle.[84]

The intermediate host becomes infected by ingesting sporocysts. Sporozoites excyst from sporocysts in the small intestine. The fate of the sporozoite from the time of ingestion of the sporocyst until initial development in arteries in the mesenteric lymph nodes, is not known. First-generation schizogony (merogony) begins in endothelial cells of arteries as early as seven days post-inoculation[29] (DPI) and may be completed as early as 15 DPI. Second-generation schizonts (meronts) have been seen in vascular endothelium from 19 to 46 DPI, predominantly in capillaries but also in small arteries virtually throughout the body. These schizonts seem to be most numerous in the glomeruli of the kidney (**Figure 20.7**). Cell division in schizonts is by endopolygeny,[40] which is a specialized form of schizogony. The nucleus becomes lobulated and divides into a number of daughter nuclei (up to 37). Merozoites (tachyzoites) form at the periphery. The shape and size of schizonts vary considerably. Both first- and second-generation schizonts develop within the host cell's cytoplasm and are not surrounded by a PV. Merozoites of *Sarcocystis* have the same organelles as do *Toxoplasma gondii* merozoites (tachyzoites), except that there are no rhoptries[40] (**Figure 20.8**). Merozoites are found in peripheral blood from 24 to 46 DPI, coincident with the maturation of second-generation schizonts.[27] Merozoites in blood are extracellular or located within unidentified mononuclear cells. Intracellular merozoites contain one or two nuclei and some divide into two, apparently by endodyogeny. The number of generations of schizogony and the type of host cell in which it occurs, varies, depending on the species of *Sarcocystis* concerned. Nevertheless, trends are apparent. All species of *Sarcocystis* of large domestic animals form first- and second-generation schizonts in vascular endothelium, whereas only a single precystic generation of schizogony has been found in *Sarcocystis* species of small mammals (mice and deer mice), and this is usually in hepatocytes.[40] Merozoites that are liberated following the final cycle of schizogony initiate development of sarcocysts in muscle. Cysts of some species of *Sarcocystis* also occur in neural tissue, whereas those of other species are possibly not formed there.[3, 40, 67] The intracellular merozite surrounded by a PV becomes circular to ovoid, forming a metrocyte which undergoes repeated divisions (see below). Eventually, the sarcocyst is filled with bradyzoites, which are the infective stage for the predator. Sarcocysts generally become infectious at about 75 DPI but in this there is considerable variation among species of *Sarcocystis*. Sarcocysts may disappear from a host with passing of time.[30] Neither schizonts nor immature sarcocysts containing only metrocytes give rise to intestinal infection in the definitive host. There is no lactogenous transmission.

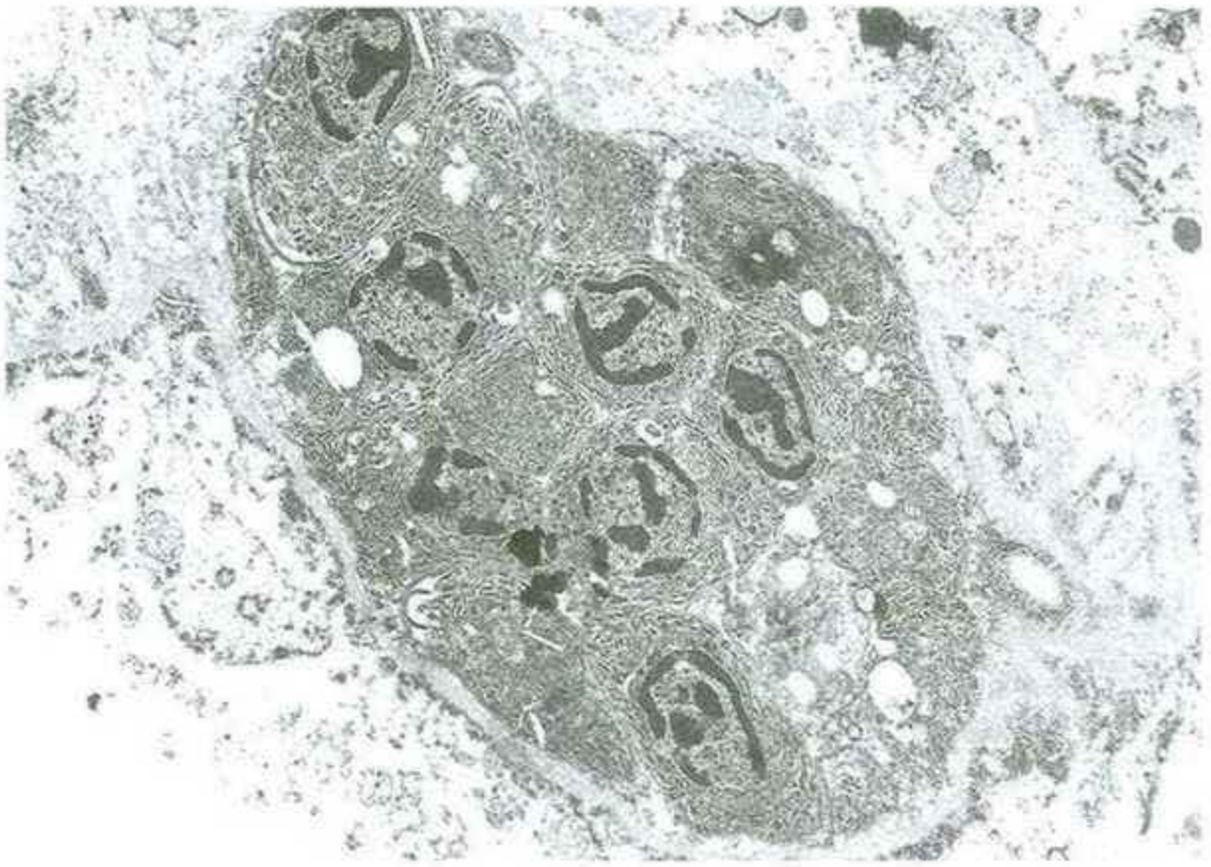


Figure 20.3 Natural infection: electron micrograph of a *Sarcocystis* schizont in a vascular endothelial cell in the kidney of a goat, x 12 000. Note the absence of rhoptries and of a parasitophorous vacuole. (Tissue by courtesy of Dr J.A. Neser, Onderstepoort Veterinary Institute, South Africa)

Sarcocysts vary in size and shape, depending on the species of the parasite. Some always remain microscopic, whereas others become macroscopic. Sarcocysts usually take a long time to develop to a macroscopic size in the musculature of horses[100] and other livestock, and so, for instance, it tends to take more than a year for *S. gigantea* (**Figure 20.4**) and *S. medusiformis* to become macroscopically detectable in sheep.[53, 114] Microscopic sarcocysts vary from very long and narrow to short and wide. Macroscopic sarcocysts appear filamentous, like rice grains, or globular (**Figure 20.4**). Sarcocysts are always located within a PV in the cytoplasm of the host cell. More than one sarcocyst may be found in the same host cell. The sarcocyst consists of a cyst wall that surrounds the parasite's metrocyte or bradyzoite stages (**Figure 20.9**). The structure and thickness of the cyst wall differs among species of *Sarcocystis* and within each species as the sarcocyst matures. A connective tissue wall (secondary sarcocyst wall) surrounds sarcocysts of *S. gigantea, S. hardangeri,* and *S. rangiferi*. Histologically, the sarcocyst wall is smooth, striated or hirsute; or it may possess complex, branched protrusions. Internally, groups of bradyzoites are frequently seen to be divided into compartments by septa that originate from the sarcocyst wall. Septa are present in all but a few species of *Sarcocystis*. The structure of the parasites within the sarcocysts changes with the maturation of the sarcocyst. Immature sarcocysts contain metrocytes (mother cells). Each metrocyte produces two daughter metrocytes by endodyogeny. After what appears to be several such divisions, some of the metrocytes, through the process of endodyogeny, produce banana-shaped parasites that are now termed bradyzoites. They contain prominent amylopectin granules that stain bright red in the periodic acid-Schiff reaction. Even mature sarcocysts may contain some peripherally arranged metrocytes.

The ultrastructure of the mature sarcocyst wall is of taxonomic value, but the fine structure thereof may vary with the age of the cyst. Sarcocyst development begins when a merozoite enters a muscle or nerve cell, as explained above. The merozoite resides in a PV and is surrounded by a parasitophorous vacuolar membrane (PM) which appears to develop into a primary sarcocyst wall (PW). The PW is the PM plus an underlying electron-dense granular layer situated immediately beneath the PM. Sarcocyst walls have been categorized into 37 types (types 1 to 37) to aid species determination.[33, 40]

Species of *Sarcocystis* of domestic animals are generally more specific for their intermediate hosts than for their definitive hosts.[40, 49, 144, 145] Certain wild carnivores can serve as efficient definitive hosts for domestic dog-transmitted species of *Sarcocystis* such as *S. cruzi, S. tenella* and *S. capracanis*. However, none of the species infective for dogs are infective for cats, and *vice versa*.

Carnivores can harbour *Sarcocystis* spp. in their muscles, but the host specificity of such species has yet to be determined.[15, 40, 87] In southern Africa *Sarcocystis* occurs commonly in lions[93] and several other carnivores.[16, 87, 91, 143] It has also been seen in the domestic dog in South Africa.[113]

Epidemiology

Sarcocystis occurs commonly in the muscles of cattle, sheep, pigs, horses and goats in southern Africa, as well as in wild vertebrates in the region.[2, 16–18, 76, 77, 87, 91, 92, 95, 97, 98, 116, 131, 143] For instance, the prevalence of *S. cruzi* approaches 100 per cent in the hearts of adult cattle.[86] It is interesting to note that

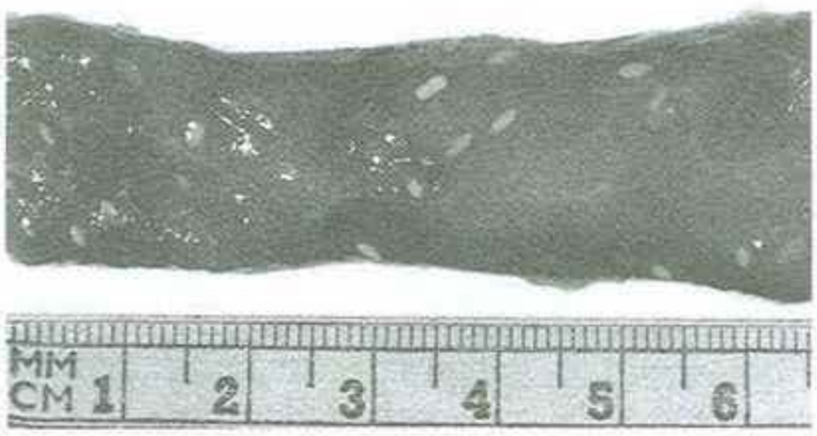


Figure 20.4 Two examples of macroscopic *Sarcocystis gigantea* cysts in ovine oesophagi. The ages of the cysts in the respective oesophagi are different: the larger cysts are older

because of its high prevalence in the skeletal muscles of cattle in South Africa, *Sarcocystis* was for a short while mistakenly considered to be concerned in the aetiology of botulism.[64]

Although no active attempt has been made to ascertain which species of *Sarcocystis* occur in livestock in southern Africa, the following have been identified and life-cycle studies have been carried out locally on some of them: *S. arieticanis* of sheep;[90] *S. bertrami* of horses;[18] *S. capracanis* of goats;[94] *S. cruzi* of cattle;[86] *S. hirsuta* of cattle;[91] *S. gigantea* and *S. tenella* of sheep;[86] and *S. miescheriana* of pigs.[91] *Sarcocystis hominis* of cattle and/or *S. suihominis* of pigs, both of which have humans as the definitive host (**Table 20.1**), must occur in southern Africa because sporocysts are often observed in human faeces.[4] A kind of cyst which closely resembled *S. suihominis* has been seen in pork from an abattoir in the former Transvaal Province of South Africa.[91] Other species of *Sarcocystis* will unquestionably be found in domestic livestock in southern Africa if looked for. Human muscular sarcocystosis is known in South Africa[96] but, ultrastructurally, the wall of a sarcocyst in the person concerned was found to be unlike those of domestic animals, being more of the general type with elaborate cyst wall protrusions that sometimes occurs in sarcocysts in non-human primates.[97]

The long patent period of several months in definitive hosts contributes to the high prevalence of *Sarcocystis* infection in livestock as it provides ample opportunity for contamination of pastures with sporocysts, which are resistant to environmental conditions.[101, 102, 127] Long-term production of sporocysts by definitive hosts is enhanced by the fact that the latter are susceptible to repeated reinfection. It is not only roaming cats and dogs but also wild carnivores which help to maintain the life cycle if they have access to dead animals. Sporocysts of *Sarcocystis* have been seen in the faeces of the black-backed jackal (*Canis mesomelas*),[82] which may act as an alternative definitive host in southern Africa for the pathogenic, dog-transmitted species of *Sarcocystis* of livestock (**Table 20.1**). Nevertheless, the main definitive hosts in southern Africa are cats, dogs and humans. Dispersal of sporocysts by coprophilic arthropods, such as flies,[85] is probably of considerable epidemiological importance.

Pathogenesis

The pathogenicity of the different species of *Sarcocystis* in livestock varies (**Table 20.1**), acute clinical sarcocystosis being associated with the schizogonic phase of the life cycle. The pathogenesis of the acute disease has yet to be elucidated, but ascites and oedema in tissues are probably caused by hypoproteinaemia and destruction of vascular endothelium during schizogony, whereas the fever which occurs is most likely related to the release of pyrogens from bursting schizonts, which either act directly on the hypothalamus or stimulate the release of prostaglandins.[7, 10, 40] There is evidence that prostanoids are important in the pathogenesis of acute sarcocystosis,[22] and it is speculated that destructive immune responses such as local delayed hypersensitivity reactions could be involved. The mechanisms responsible for the anaemia which is a prominent feature of acute sarcocystosis are not known,[10] nor has the pathogenesis of *Sarcocystis*-induced abortion or the manner in which the parasite affects foetal health been clarified.

A complex and incompletely defined interaction between nutrition and the endocrine and immune systems is responsible for the growth perturbation that is often a consequence in animals which have recovered from acute sarcocystosis. Connective tissue breakdown and muscle wasting might be correlated with the abnormal nitrogen metabolism that appears to result from the malabsorption and internal recycling of nitrogen.[48] The host's immune response to the infection leads to changes in the concentration of the cytokine signal. There is thought to be a tumour necrosis factor alpha response by macrophages, which is localized within tissues and not reflected by a raised level in the plasma. Hormonal changes occur, particularly in regard to secretion of growth-associated hormones, such as

somatostatin, thyrotropin-releasing hormone, insulin-like growth factor-1 (somatomedin C) and growth hormone (somatotropin),[48] which eventually lead to inhibition of growth. Interleukin-1 and other cytokines may also be involved in growth retardation.

Sarcocystis has traditionally been regarded as the cause of eosinophilic myositis. This is partly because sarcocysts are visible in the centre of some lesions[51, 56] and there appears to be immunohistochemical support for their occurrence there.[62] *Sarcocystis*-specific type-1 hypersensitivity may be involved in the pathogenesis.[62] However, when examined by light microscopy, many cases of eosinophilic myositis are found not to be obviously related to *Sarcocystis* infection.[84] Consequently, the sarcocystic aetiology of

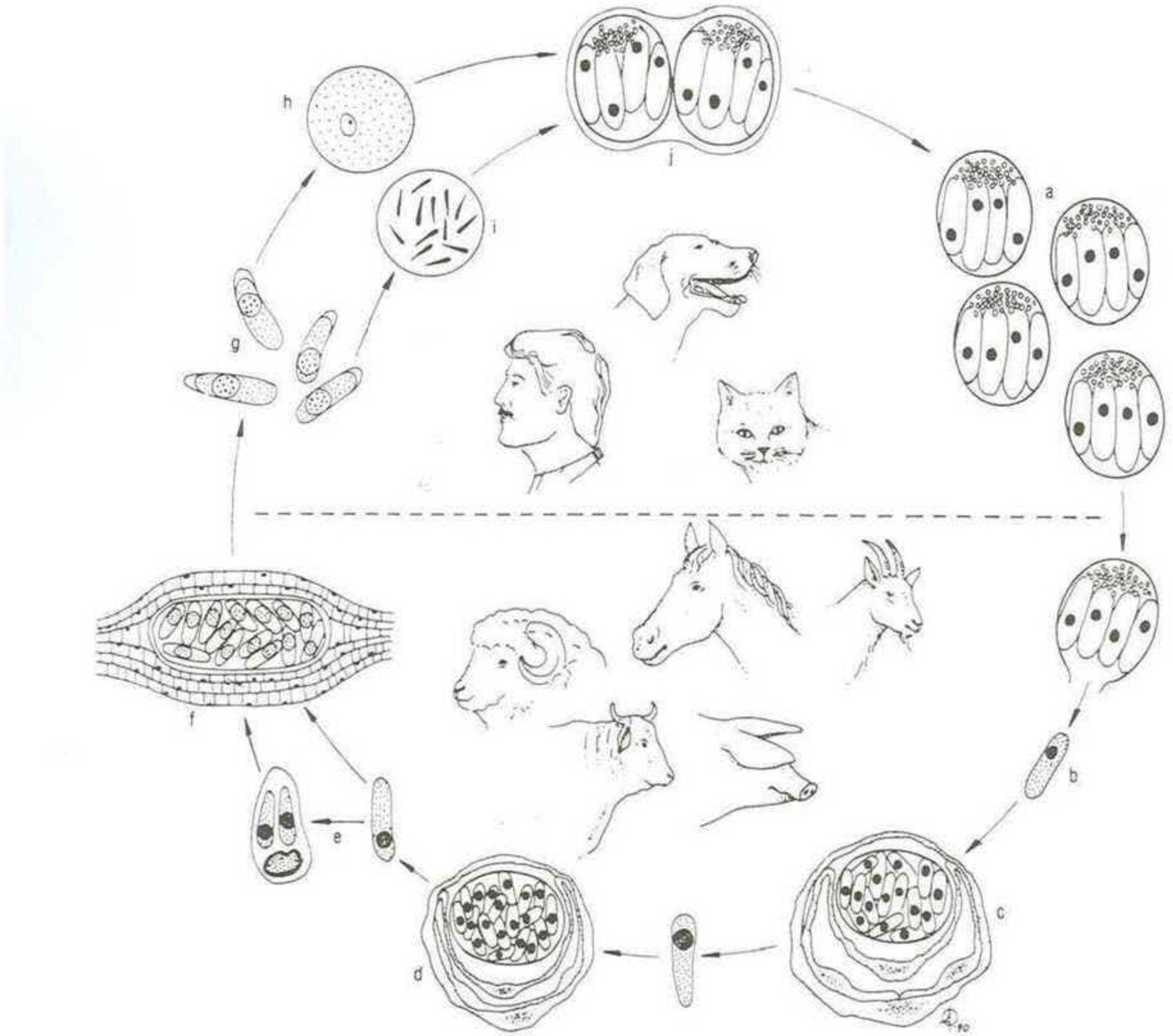


Figure 20.5 Life cycle of *Sarcocystis*

a = sporocysts
b = sporozoite
c and d = parasites undergoing schizogony in endothelial cells of blood vessels
e = tachyzoic merozoites (free or inside mononuclear cells, in the bloodstream)
f = sarcocyst in muscle
g = bradyzoic merozoites
h = macrogametocyte
i = microgametocyte
j = oocyst

eosinophilic myositis remains unproven, particularly since the condition has not been observed in any animals which have suffered from experimentally induced disease. These have, however, usually been young individuals that were infected on only one occasion. It remains a possibility that the development of eosinophilic myositis is dependent upon repeated ingestion of sporocysts of *Sarcocystis*.

Some species of *Sarcocystis* can cause transplacental infection: and congenital sarcocystosis has been induced in cattle, sheep, goats and pigs.[40] Animals infected with large doses of sporocysts during pregnancy may abort, have stillbirth, or give birth to weak animals. *Sarcocystis* schizonts have been demonstrated in foetal membranes and foetal tissues. The exact mechanism of abortion is not known because the presence of schizonts in the foetus is rare, certainly in experimentally induced infections.[40] Under natural conditions, neonatal death has been recorded once in a goat in Australia[81] and in one lamb.[32] There are, however, several reports of congenital infections in cattle[14] and these reports up to 1988 have been summarized.[40] In a planned survey, *Sarcocystis* was identified in only one of 498 aborted foetuses in California,[1] indicating that *Sarcocystis* is not a common cause of abortion in cattle. Earlier reports may have been of misdiagnosed *Neospora* infections (**see Chapter 23: Neosporosis**).

Clinical signs

Virtually all *Sarcocystis* infections in livestock are subclinical, because animals do not usually ingest large numbers of sporocysts at any one time.[19, 23, 29, 38–40, 52, 53, 109, 126, 129] It is when numerous sporocysts of a pathogenic species of *Sarcocystis* (**Table 20.1**) are ingested simultaneously in feed or water contaminated by definitive hosts that clinical disease develops. Under natural conditions, clinical sarcocystosis has been documented only in cattle,[40] sheep[32, 50, 108, 132] and goats.[81] Concurrent infections might predispose animals to clinical sarcocystosis.[30]

Naturally occurring acute sarcocystosis has so far been recognized only in goats and cattle[142] in southern Africa (**Figures 20.1 to 20.3**).

In the acute illness, the body temperature may rise to over 40 °C between two and three weeks after infection, although an increase in temperature has been noted in experimentally induced disease in horses 10 to 12 days after they were given large numbers of sporocysts.[99, 128] Apart from fever, clinical signs are not noticed until about 24 days after infection, when animals may exhibit anaemia, diarrhoea, anorexia, weakness, mild dehydration and loss of weight.[11, 14] Other signs may include excessive salivation (a prominent feature in affected cattle), muscle tremors, and loss of hair, which in some cattle is particularly noticeable at the tip of the tail.[14, 40, 39] Pregnant animals may abort or give birth prematurely. If the disease progresses, many affected animals become recumbent, showing signs of central nervous system involvement, and die. The duration of clinical signs varies from a few days to several weeks. Growth may be retarded in individuals that survive an acute infection[20, 21, 40, 45, 48, 71] and wool growth may be adversely affected.

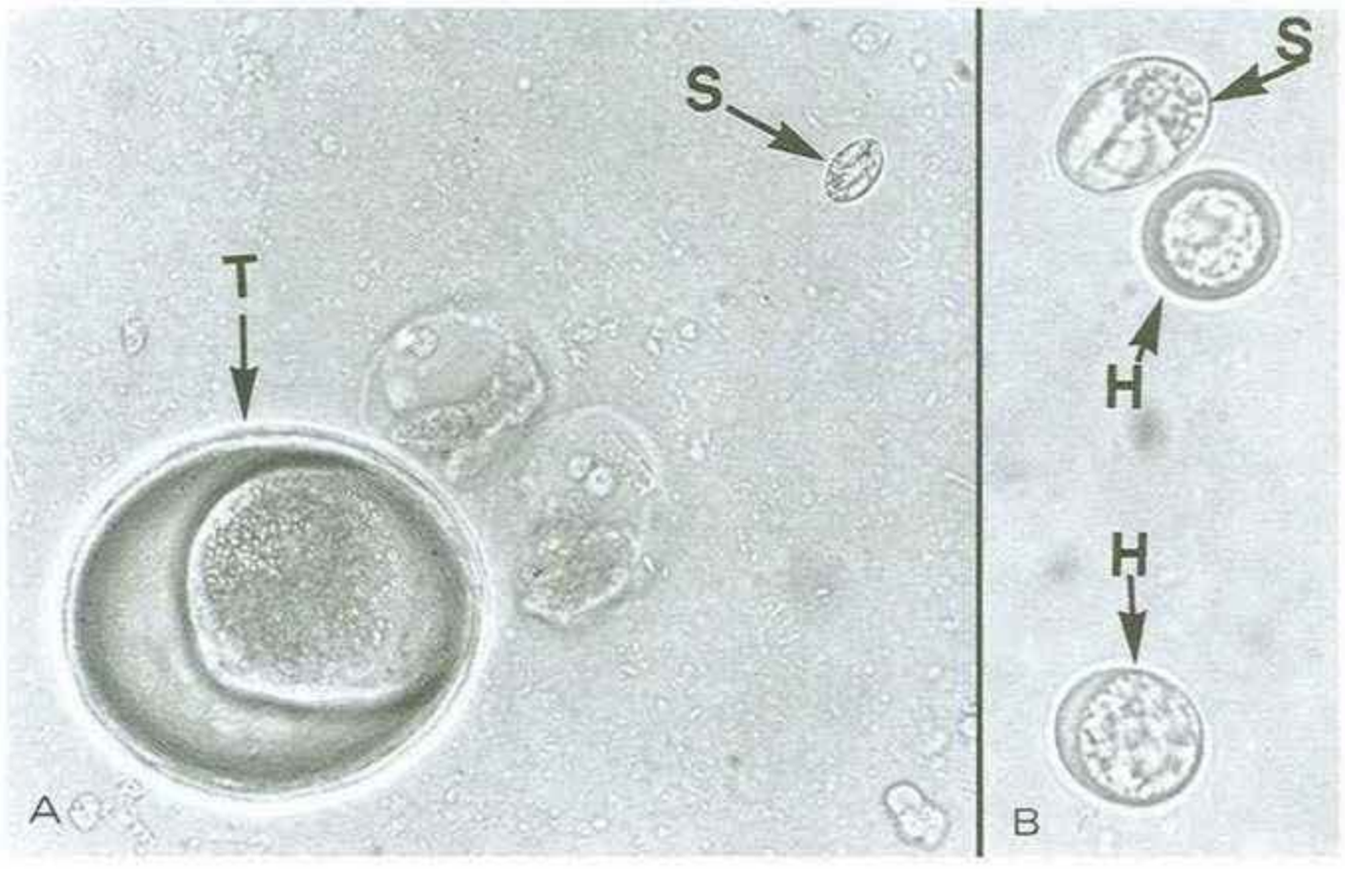


Figure 20.6 Faecal flotation from a dog naturally infected with *Sarcocystis*. A: Low magnification, to show the size of a *Sarcocystis* sporocyst (S) compared with a *Toxascaris* egg (T), x 430. B: High magnification, showing a *Sarcocystis* sporocyst (S) and two *Neospora* or *Hammondia*-like oocysts (H), x 1 250. (From reference number 26, with permission)

Table 20.1 *Sarcocystis* species in livestock

INTER-MEDIATE HOST	*SARCOCYSTIS* SPECIES	DEFINITIVE HOSTS	PATHOGENICITY IN LIVESTOCK	CYST TYPE IN LONG-STANDING INFECTION	LIGHT MICROSCOPIC APPEARANCE OF CYST WALL	ULTRA-STRUCTURAL APPEARANCE OF PRIMARY CYST WALL PROTRUSIONS	PERTINENT LITERATURE, INCLUDING REFERENCE(S) IN WHICH FINE STRUCTURE OF CYST WALL IS ILLUSTRATED
Cattle	*S. cruzi*	Dog, coyote, fox, jackal, raccoon, wolf	Pathogenic	Microscopic	Thin	Filamentous, flattened over cyst wall	16, 27, 118
	S. hirsuta	Cat	Mildly pathogenic	Macroscopic	Thick	Villus-like, constricted at base, laterally expanded in the middle and tapered distally	28, 37
	S. hominis	Human, other primates	Mildly pathogenic	Not known	Thick	Palisade-like, not constricted at base	37, 103
Sheep	*S. arieticanis*	Dog	Pathogenic, but less so than *S. tenella*	Microscopic	Thin	Filamentous, long and flattened over cyst wall	69, 118
	S. tenella	Dog, coyote, fox	Pathogenic	Microscopic	Thick	Palisade-like	16, 36, 118
	S. gigantea	Cat	Mildly pathogenic	Macroscopic	Thick secondary cyst wall formed	Cauliflower-like	16, 110, 111, 118
	S. medusiformis	Cat	Unknown	Macroscopic	Thin	Snake-like	12, 114, 118
	S. mihoensis	Dog	Unknown	Macroscopic	Thick	Palisade-like	123
Goat	*S. capracanis*	Dog, coyote, fox	Pathogenic	Microscopic	Thick	Palisade-like	39, 44, 65, 118
	S. hircicanis	Dog	Possibly pathogenic	Microscopic (but cysts can be up to 2,5 mm long)	Thin	Filamentous, long and flattened over cyst wall. (The cyst wall is similar in appearance to that of *S. arieticanis*)	70
	*S. moulei**	Cat	Non-pathogenic	Macroscopic	Thick secondary cyst wall formed	Cauliflower-like	58, 68
Pig	*S. miescheriana*	Dog, fox, jackal, raccoon	Pathogenic	Microscopic	Thick	Palisade-like	60
	*S. suihominis***	Human, other primates	Pathogenic	Microscopic	Thick	Palisade-like	71, 80, 104
	S. porcifelis+	Cat	Pathogenic	Macroscopic	Not known	Not known	26
Horse	*S. bertrami*++ *S. equicanis*++ *S. fayeri*++	Dog	Mildly pathogenic	Macroscopic	Both thick- and thin-walled cysts have been seen	Villus-like, conical with corrugated outline	18, 42, 46, 95, 117
Water buffalo	*S. buffalonis*	Cat	Unknown	Macroscopic	Thick	Palisade-like	72
	S. dubeyi	Unknown	Unknown	Macroscopic	Thick	Palisade-like	74
	S. fusiformis	Cat	Non-pathogenic	Macroscopic	Thin	Cauliflower-like	103, 105
	S. levinei	Dog	Unknown	Macroscopic	Thin	Filamentous	25, 73

* In the absence of evidence that two cat-transmitted species of *Sarcocystis* occur in goats, *S. caprifelis*[79] is provisionally considered to be a synonym of *S. moulei*

** Li and Lian[80] noted possible biological and structural differences between the organism they studied and *S. suihominis* in Europe

\+ The validity of this species is uncertain and confirmation of the pig–cat life cycle[26, 61] is needed. A macrocyst in a warthog (*Phacochoerus aethiopicus*) from the former Transvaal Province in South Africa had cauliflower-like cyst wall protrusions. It differed ultrastructurally from a microcyst in warthog from Namibia, KwaZulu-Natal Province in South Africa, and Zimbabwe. This microcyst was not unlike *S. miescheriana*.[92] However, there is not as yet any evidence that either species of the warthog is transmissible to the domestic pig. Two species of *Sarcocystis* have since been described from warthog in South Africa[131]

++ It is not yet clear whether the thick-walled cysts in horses belong to different species or not

Clinical myopathy, which may be accompanied by chronic illness, has been observed in horses with muscular *Sarcocystis* infection, but there is evidence that there may not be an association between the presence of sarcocysts and clinical myositis.[55, 141]

Intestinal *Sarcocystis* infections in definitive hosts are usually asymptomatic.[40, 84] However, a dog in South Africa that was clinically ill (cause unknown) was shedding vast numbers of oocysts and sporocysts, detectable upon direct examination of faecal specimens.[107] This massive intestinal infection was probably the result of repeated meals of *Sarcocystis*-infected meat.

Pathology

A haemolytic, normocytic, normochromic anaemia, with few or no reticulocytes in evidence, is characteristic of acute sarcocystosis.[8–10, 40] The packed cell volume begins to fall between three and four weeks after infection and reaches a level of below 20 per cent in animals with moderate to severe disease. Infection with as few as 1 000 sporocysts can cause reduced haematocrits between four and five weeks after infection in lambs.[109] Clinical pathological findings have not been uniform in the experimental situation as far as parameters of blood coagulation, such as partial thromboplastin time, prothrombin time and thrombin time, are concerned.[19]

The serum bilirubin level is elevated during the acute infection, and in terminal disease the blood urea nitrogen level also rises. Serum levels of enzymes such as aspartate aminotransferase, creatine kinase and lactic dehydrogenase tend to be elevated during acute and early chronic sarcocystosis. In pigs, an increase in creatine kinase activity is detected from 28 days after infection.[23] Serum creatine kinase levels in horses increase 4- to 40-fold some 63 days after oral administration of sporocysts and may remain high until approximately 147 days after infection.[128]

Nutrition is affected in acute sarcocystosis. There is a marked reduction in retained nitrogen, percentage nitrogen retained per unit consumed, hydroxyproline excretion and creatine excretion, and an increase in the ratio of 3-methylhistidine to creatine excreted by parasitized cattle.[48]

The pathology of acute sarcocystosis has been well described, with much of the early work having been done on cattle infected with *S. cruzi*. In all livestock species, gross lesions at necropsy include serous atrophy and necrosis of fat, pallor of the mucous membranes, an icteric appearance of tissues and organs, hydropericardium, hydrothorax, ascites, and widely distributed petechiae and ecchymoses (**Figures 20.10 and 20.11**), especially on serosal surfaces of the viscera, and throughout the skeletal and cardiac musculature, which may also show multiple pale foci of variable size or haemorrhagic streaks. Skeletal muscles are often pale pink with dark red mottling. Lymph nodes are enlarged and oedematous and often contain petechiae. The lungs are oedematous and congested. There may be small erosions or shallow ulcers in the mucosa of the buccal cavity, oesophagus and abomasum.

The predominant microscopic lesion in acute sarcocystosis is inflammatory rather than degenerative.[40, 47] Medium to large numbers of lymphocytes and macrophages, together with a few plasma cells and eosinophils, are found perivascularly and in the interstitial tissues of the heart, skeletal muscles, lungs, liver, kidneys and, to a lesser extent, other organs. The predominantly mononuclear cell infiltrate is frequently accompanied by haemorrhage, oedema, multifocal degeneration and necrosis (especially in the cardiac and skeletal musculature and kidneys), vasculitis and, less commonly, thrombosis. The haemorrhages may be generalized, and are often not associated with inflammation. Muscular lesions in acute sarcocystosis vary in severity from muscle to muscle and within individual muscles. In the brain and spinal cord, focal haemorrhages sometimes surround areas of necrosis, and scattered foci of gliosis and of mononuclear cell infiltration in the meninges are evident. Enlargement of lymph nodes results from lymphoid hyperplasia with attendant distension of medullary cords, oedema, and histiocytosis of the sinuses in the medulla. Schizonts may or may not be present in organs and, regardless of location, are not always associated with tissue changes, particularly not in infections with non-pathogenic species of *Sarcocystis* of livestock (**Table 20.1**).

Some species of *Sarcocystis* form macroscopically visible sarcocysts in the muscles of chronically infected livestock (**Table 20.1**). Macroscopic cysts of *S. gigantea* are sometimes seen in sheep, being particularly conspicuous in oesophageal, laryngeal and tongue muscles. They are oval or round (**Figure 20.4**). Meat with macroscopic sarcocysts is generally condemned in abattoirs as unfit for human consumption, despite the fact that a zoonotic risk has not been identified.[6, 52, 43]

In chronically infected livestock animals, sarcocysts occur within myocytes of the myocardium and skeletal muscles, and sometimes in the brain.[3, 40, 84] A tissue response is not normally apparent in muscular *Sarcocystis* infections.[40, 84] Rarely, a degenerating sarcocyst, or sarcocysts, may be encountered in muscle. The resulting reaction may be macroscopically visible as one or more dull white foci about 1 mm in diameter. The dead parasites are surrounded by mononuclear cells, neutrophils, eosinophils, giant cells or a combination of these cells. However, the cellular response is usually mainly mononuclear and neutrophilic, a different reaction to that of eosinophilic myositis. How sarcocysts are removed from host tissue is not yet clear, but there is phagocytic involvement.[40, 120]

Eosinophilic myositis is a specific and apparently subclinical inflammatory condition which is sometimes encountered in livestock (particularly cattle and sheep)

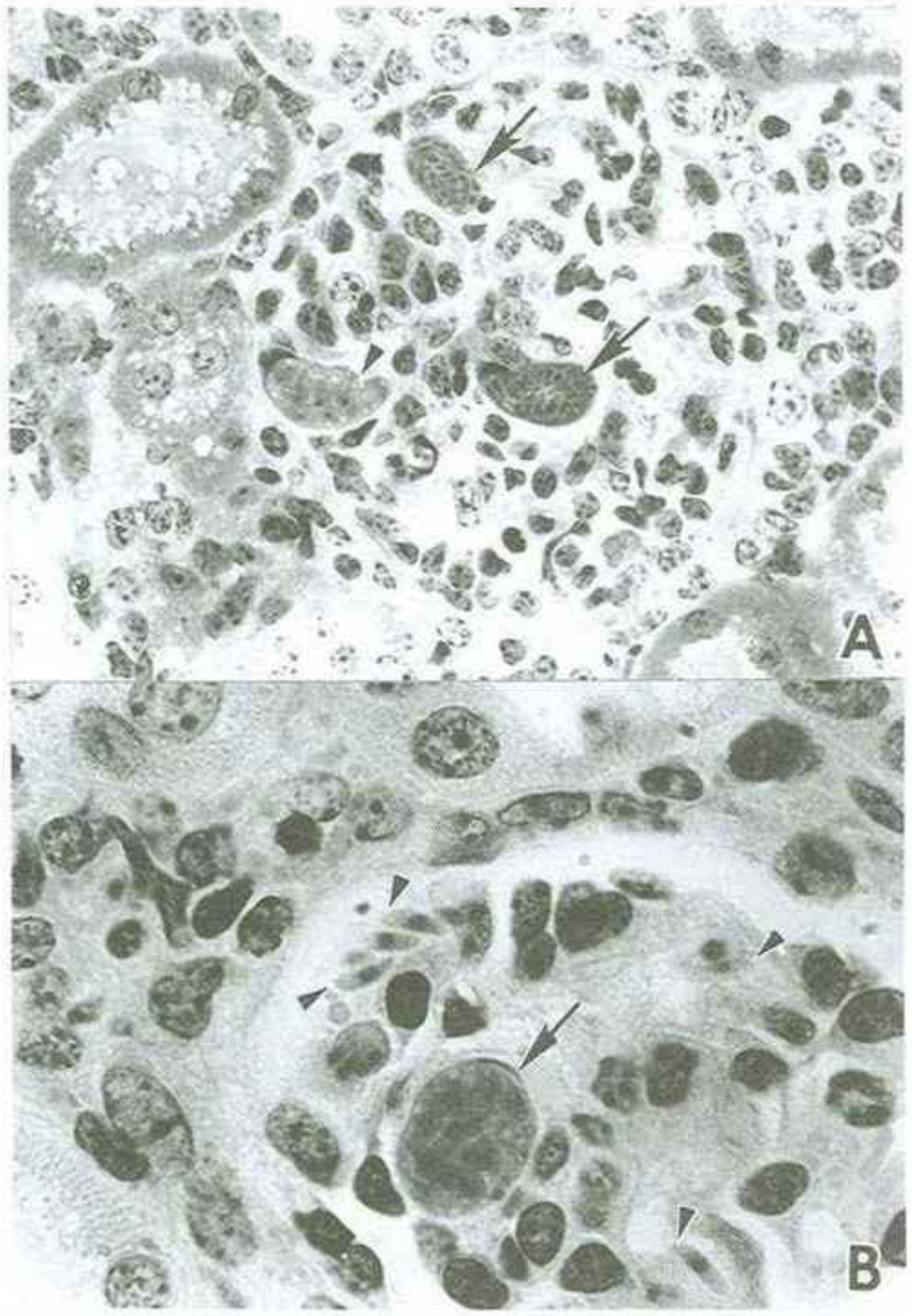


Figure 20.7 Second-generation schizonts in section of bovine kidney, 24 days after ingestion of *S. cruzi* sporocysts
A: An immature (arrowhead) schizont with nuclear lobes and two schizonts that are almost mature (arrows). × 630
B: Individual merozoites (arrowheads) and an immature schizont (arrow). × 1 000

slaughtered in abattoirs. The macroscopically visible lesions consist of greenish or yellowish areas, patches or granulomas (measuring up to 5–15 × 1–3 mm) in cardiac and skeletal muscles. There is no predilection for particular muscles. The presence of the granulomas often results in condemnation of the affected carcasses. Early lesions consist of numerous eosinophils (which are responsible for the green colour) lying between muscle fasciculi and fibres. Eventual degeneration, necrosis and disappearance of muscle fibres occur, their place being taken by eosinophils and connective tissue. The granulomas have a central area containing necrotic cellular debris, which is surrounded by eosinophils, epithelioid cells, fibrocytes, giant cells and lymphocytes.

The granulomas of eosinophilic myositis should not be confused with the sometimes superficially similar cysts of *Sarcocystis*, such as *S. gigantea* of sheep (**Table 20.1**), which may also be macroscopically visible (**Figure 20.4**).

Diagnosis

Identification of the species of *Sarcocystis* that are present in muscles of chronically infected livestock animals (there is frequently more than one species in the same individual) can often be based mainly on the ultrastructural appearance of the cyst wall (**Table 20.1**). Identification of *Sarcocystis* to the specific level is, however, usually an academic exercise rather than a matter of practical importance; the reason being that chronic *Sarcocystis* infections in livestock are invariably subclinical. The prevalence of sarcocysts in the musculature of domestic herbivores is high, the occurrence of mature sarcocysts in an animal with acute sarcocystosis normally being an incidental finding, indicative of a (previous) chronic infection. It is difficult to diagnose acute sarcocystosis because the clinical signs of disease are non-specific and there are, at present, no definitive tests to confirm the diagnosis in the living animal. Parasitological

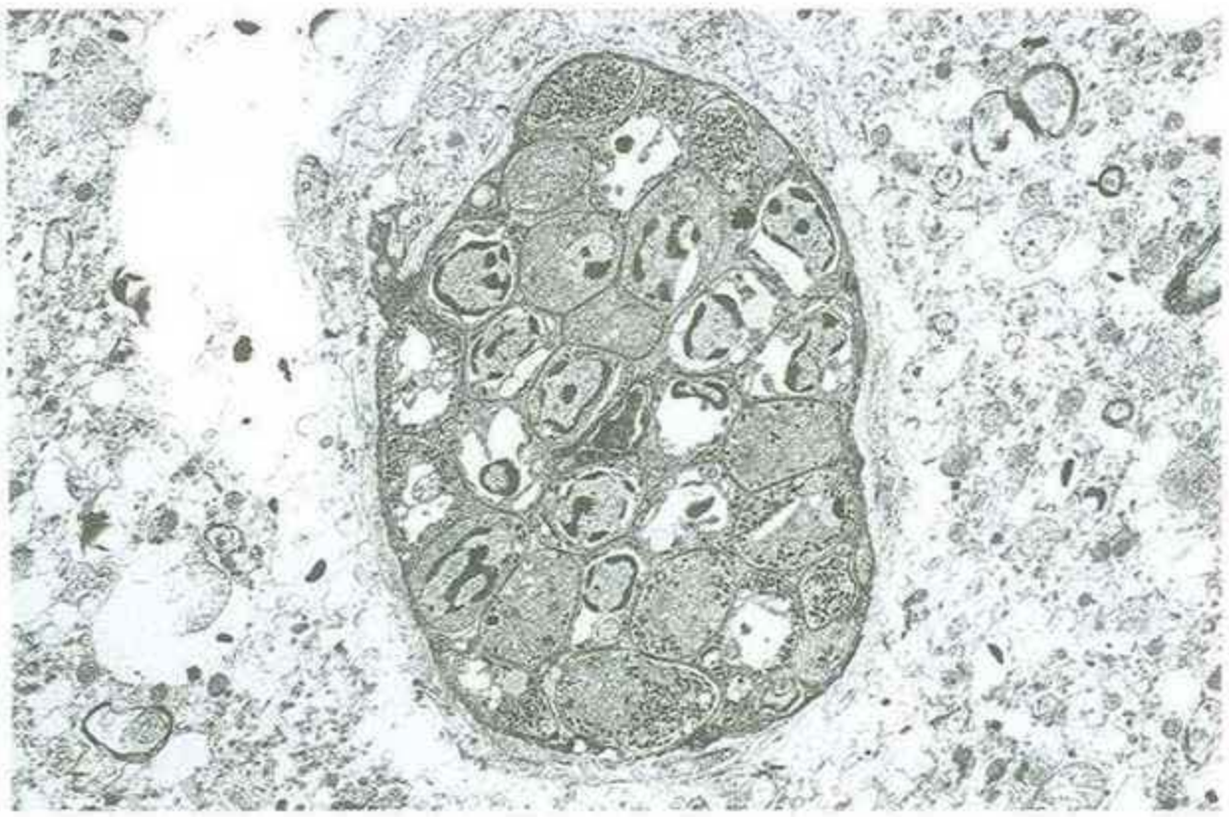


Figure 20.8 Experimental bovine sarcocystosis: electron micrograph of a schizont in a vascular endothelial cell in the brain, x 6 000. Note that rhoptries are absent and that no parasitophorous vacuole is visible



Figure 20.9 Typical histopathological appearance of a mature sarcocyst in striated muscle. Cysts occur commonly in the musculature of livestock. (By courtesy of Dr J.A. Neser, Onderstepoort Veterinary Institute, South Africa)

confirmation of clinically suspected disease is based on the finding of schizonts (**Figures 20.1 to 20.3 and 20.7**) in endothelial cells of blood vessels, on microscopy of biopsy material (such as muscle or lymph node), or, more commonly, on histopathology after a necropsy. However, schizonts or free merozoites are not always detected in infected animals, either because they are too few in number to be found or because schizonts have ruptured by the time that the infection becomes clinically apparent. It is particularly in foetuses that the parasites are not consistently present, and the chances of diagnosing *Sarcocystis*-induced abortion are improved if a variety of foetal tissues are examined microscopically. These tissues should include samples from the brain and placenta, where sarcocystosis gives rise to gliosis and necrosis, respectively. Schizonts may occur in vascular endothelial cells in the subepithelial connective tissue of placental cotyledons. Ultrastructurally, the merozoites of *Sarcocystis* are similar to other apicomplexan zoites (**see Differential diagnosis**), except that they lack rhoptries. Immunohistochemistry is helpful in identifying schizonts in tissue sections.

The parasitaemia during acute sarcocystosis is not useful for diagnosis because the detection of parasites in blood or buffy-coat smears is too time-consuming.

Because of the size of the sarcocysts, a diagnosis of chronic infection with species of *Sarcocystis* such as *S. gigantea* (**Figure 20.4**) or *S. medusiformis* can readily be made macroscopically in long-standing infections in older



Figure 20.10 Experimental bovine sarcocystosis: petechial and ecchymotic haemorrhages in the kidney

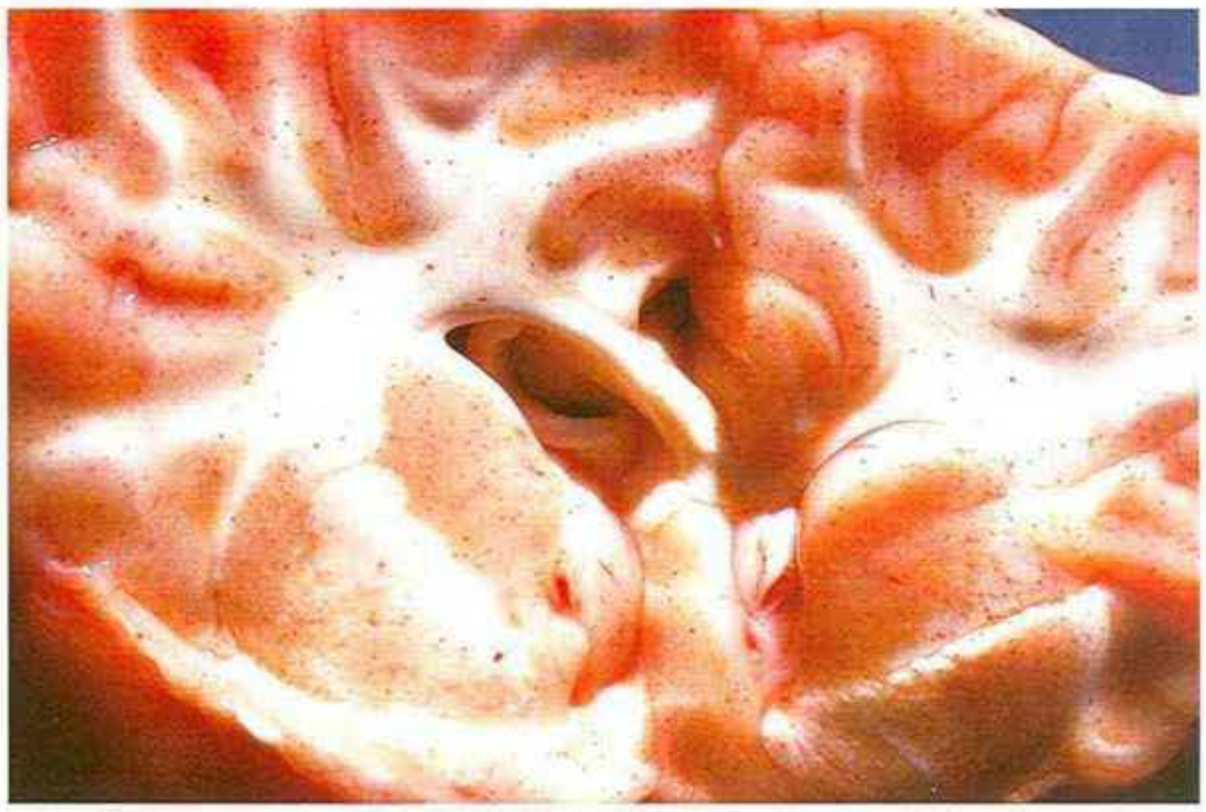


Figure 20.11 Experimental bovine sarcocystosis: petechial haemorrhages in the brain

animals. Cysts of some species of *Sarcocystis*, such as *S. cruzi*, never become large enough to be clearly visible to the naked eye (**Table 20.1**).

Although various serological tests (indirect fluorescent antibody test and enzyme-linked immunosorbent assay) have been described, they are of limited value. Few laboratories keep antigen as it is not commercially available. Results are difficult to interpret because subclinical *Sarcocystis* infections are very common in livestock. Furthermore, there is cross-reaction in serological tests between pathogenic and non-pathogenic *Sarcocystis* spp., although *Sarcocystis* is largely antigenically distinct from other coccidia.[31, 40, 83, 84] Antibody titres can nevertheless assist the making of a diagnosis, especially if those in the serum of clinically healthy animals from the same farm are compared with the titres of individuals which are ill. Research is being carried out in an attempt to refine techniques for the diagnosis of acute sarcocystosis.[40, 57, 63, 75, 106, 112, 119, 125, 130, 134, 136, 138, 139]

A search for the small sporocysts of *Sarcocystis* in the faeces of carnivores (particularly dogs) by centrifugal flotation, using a super-saturated sodium chloride solution, can help in the aetiological evaluation of suspected acute sarcocystosis. What should be taken into consideration is that the prepatent period in carnivores is one to two weeks, and that the duration of the patent period can be several months. Although it is possible to stain sporocysts in faeces (they are acid fast, as are oocysts of *Cryptosporidium* and other coccidia[89]), this is not necessary because they are clearly visible

on light microscopy at ×400 (or even lower) magnification in fresh preparations, provided that the illumination used is not too bright. The internal structure of a sporocyst (containing four sporozoites and a granular sporocystic residuum) can be seen when an oil immersion objective is used.

Differential diagnosis

Known parasites of livestock which closely resemble *Sarcocystis* morphologically are *Toxoplasma gondii* **(see Chapter 18: Toxoplasmosis)** and the newly described *Neospora caninum*, which has been reported as being a pathogen in cattle, sheep, goats, dogs, and horses **(see Chapter 23: Neosporosis)**. In histological sections, *Sarcocystis* can be differentiated from these protozoa by the presence of schizonts in vascular endothelial cells (they often spuriously appear to be free in the blood vessel lumen), whereas *Neospora* and *Toxoplasma* can develop in a wide range of cell types. The presence of schizonts in numerous endothelial cells of blood vessels is suggestive of a *Sarcocystis* infection.

On electron microscopy of *Sarcocystis* schizonts, merozoites are seen to be free in the host cell cytoplasm **(Figures 20.3 and 20.8)** (i.e. no parasitophorous vacuole is evident). Groups of *Toxoplasma* organisms, on the other hand, are surrounded by a parasitophorous vacuolar membrane. In *Neospora* infections, a parasitophorous vacuole may or may not be present. Merozoites of *Sarcocystis* do not have the organelles known as rhoptries which are observed in *Neospora* and *Toxoplasma* tachyzoic merozoites, being more numerous in the former (dividing tachyzoic merozoites contain fewer rhoptries than non-dividing merozoites).

Immature schizonts with multiple nuclei not surrounded by discrete cytoplasmic areas are seen in sarcocystosis. Multinucleated stages do not occur in *Neospora* and *Toxoplasma* infections because division is by endodyogeny, each parasite giving rise to two daughter organisms.

Tissue cysts of both *Neospora* and *Toxoplasma* lack the cyst wall protrusions which are characteristic of sarcocysts. Cysts of some species of *Sarcocystis* (e.g. *S. cruzi*) are divided into compartments by septa, unlike tissue cysts of *Neospora* and *Toxoplasma*. There are metrocytes (ovoid, rapidly multiplying organisms) in young sarcocysts (and sometimes a few in older ones) but these do not occur in cysts of *Neospora* or *Toxoplasma*.

Sarcocystis parasites can be distinguished immunohistochemically from *Toxoplasma*, *Neospora* and other apicomplexan parasites.

Control

The life cycle of *Sarcocystis* is dependent upon the predator–prey relationship that exists between its definitive and intermediate hosts. Consequently, if meat eaten by the definitive hosts has been cooked, the transmission cycle is broken. However, freezing of meat for long periods does not necessarily kill *Sarcocystis*[13] and is, therefore, less effective as a means of interrupting transmission.

To effectively control *Sarcocystis* infection on farms, carcasses of dead livestock should be buried or burned. Carnivores that eat uncooked meat of domestic animals should not be allowed on to premises where their faeces can contaminate the drinking water, feed or bedding of livestock.

At present, no vaccine is available to protect livestock against acute sarcocystosis.

Although prophylactic administration of some drugs (amprolium, salinomycin and monensin) can reduce the clinical effects of acute sarcocystosis, none of these regimes is practical because exposure to *Sarcocystis* is unpredictable.[54, 78]

For chemotherapy, only halofuginone has been used with success in livestock but the drug is toxic and careful dosing is necessary. Halofuginone (0.66 mg/kg body weight) given once or more often has prevented death in goats and sheep with acute sarcocystosis.[66] Some of the potentiated sulphonamides are effective in eliminating both immature and mature cysts of *S. muris* in mice, but their effects on sarcocysts in livestock have not yet been studied.[121]

Sarcocystis species with unusual life cycle or biology

Sarcocystis species are rarely fatal to wildlife.[33] However, *S. falcatula*, which has an avian–opossum cycle, is known to be biologically different from other species of *Sarcocystis*. It appears to have a wide range of intermediate avian hosts but apparently only the opossum (*Didelphis* spp.) has so far been identified as the definitive host.[122] There are several reports of fatal acute sarcocystosis in passerine birds, at least some of which are likely to have been caused by *S. falcatula*. An unusual feature of pathogenesis is that schizogony may last for up to six months. Pneumonia is the predominant lesion, with intravascular schizonts being present.

Another group of *Sarcocystis*-like organisms with unknown life cycles have been reported to kill naturally infected capercailliae, minks, chinchillas, sea lions, harbour seals, raccoons, monkeys, black bears, skunks, sheep, cattle, horses, cats and dogs.[140] The disease is often confined to the central nervous system or liver and schizonts are not intravascular. Sarcocysts of these parasites are not known. The life cycle of one of these organisms in the horse (*S. neurona*) has been partly resolved **(see Chapter 24: Equine protozoal myeloencephalitis)**. *Sarcocystis canis* is another protozoon belonging to this group.[35] The parasites affect mainly hepatocytes, and dogs, cats, bears, chinchillas, a horse and a sea lion are known to have died of acute severe hepatitis.[24] Reports of organisms causing myeloencephalitis in sheep[5, 41, 124] and cattle[34, 133] probably belong to this group.

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21

Balantidiosis

B T SPENCER

Introduction

Balantidium coli is a ubiquitous protozoan of the class Ciliata which inhabits the lumen of the large intestine of many pigs, but may also be found in other species of animals, including guinea pigs, dogs, rats, non-human primates[7] and birds, as well as in humans. It is a commensal, but under certain circumstances, may apparently act as an opportunistic pathogen.

Aetiology and epidemiology

The vegetative form, or trophozoite of *B. coli* is a relatively large, motile, oval to ellipsoidal organism varying in size from 30 to 150 µm in length and 25 to 120 µm in width. Its surface is entirely covered with cilia arranged in slightly oblique rows, and internally within the endoplasm are two nuclei, a large kidney-shaped macronucleus and a much smaller, spherical micronucleus which lies in the notch of the macronucleus.[9] The nuclei are readily identifiable and their presence assists in confirming the identity of the parasite.[2] The parasite also contains one contractile vacuole and several food vacuoles. Reproduction is generally by transverse binary fission, but conjugation may also take place.[9]

Cysts are produced. These are ovoid to spherical and measure 45 to 65 µm in diameter. The peroxisomes of the trophozoites of *B. coli* found in subclinical cases are smaller than those isolated from pigs with acute balantidiosis.[8] The organism can be recognized within the cyst by the macronucleus.[9]

Both cysts and trophozoites are passed to the external environment in the faeces. Generally, transmission is by ingestion of cysts which may remain viable for days or weeks in moist pig faeces, but trophozoites may also play a role in this respect.[9] Trophozoites, however, are much less resistant than the cysts.[9] The faeces of sows are the major source of infection for piglets.[5]

No reports of clinical disease caused by *B. coli* have been recorded in South Africa,[10] but the organism has been the subject of incidental identification in histological sections of the colon of pigs.[4, 10]

Pathogenesis, clinical signs and pathology

Under normal circumstances, *B. coli* lives in the intestinal contents and on the mucosa of the large intestine, and does not appear to have any deleterious effect upon the host. On occasion, it may invade the intestinal mucosa when this becomes damaged by, for example, a primary pathogen (**Figure 21.1**). In some animals *B. coli* will penetrate to the submucosa or even as deep as the muscular layer.[1] The organism produces hyalonuridase which facilitates its penetration into tissues.[11] The response to the tissue invasion is inflammation of the part concerned, with the development of superficial and deep ulceration. Clinical manifestations in such affected animals are those associated with mild to severe, even fatal, enteritis.[1] The faeces may be bloodstained. In piglets, anaemia, enteritis and decreased growth rate have been reported.[5]

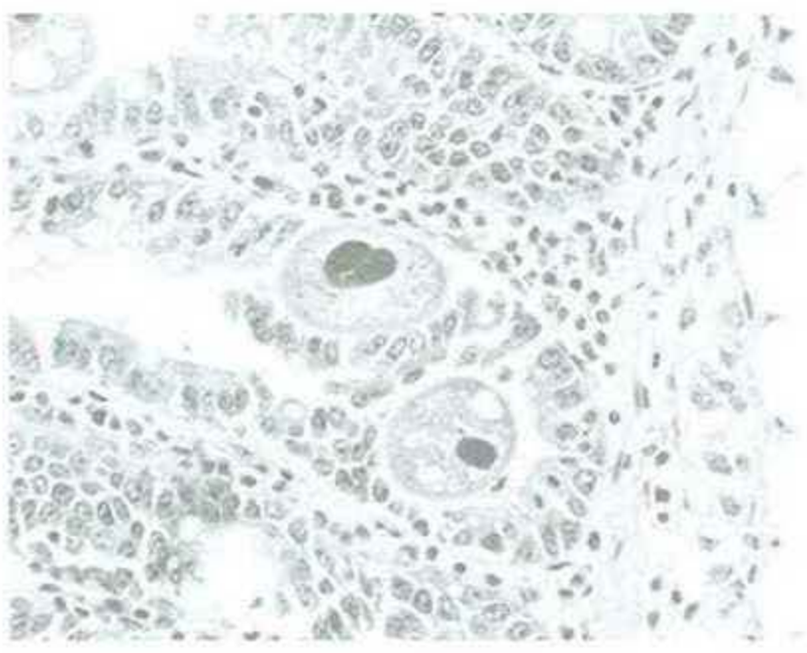


Figure 21.1 Balantidiosis in a pig: *Balantidium coli* parasites in the mucosa of the colon

Infection of humans is usually acquired by the contamination of the hands or food with pig faeces.[9] In some humans harbouring the parasite, there is severe diarrhoea or dysentery. Ulceration of the large intestine may develop, which can be complicated in some cases by haemorrhage,[6] perforation of the gut wall and peritonitis.[2] The ulcers are similar to those of amoebic dysentery.[2]

Diagnosis

The diagnosis of frank disease caused by *B. coli* infection is based on the clinical signs, lesions and presence of large numbers of organisms in affected areas. Before making a definitive diagnosis of balantidiosis, however, the pathogenesis of the disease process must be borne in mind; the fact that the parasite is present does not necessarily indicate that it is the primary cause of the condition unless additional evidence can be provided.[9]

Control

Tetracyclines have been used both for the treatment of clinically affected animals and for the prophylactic treatment of pigs so as to prevent transmission to humans. The addition of 100 g oxytetracycline per tonne of pig feed appears to be efficient in clearing the faeces of *B. coli* cysts.[5] Metronidazole at 30 mg/kg twice a day for five days administered *per os* can be used for treatment of dogs, cats[8] and humans.

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22

Leishmaniosis

J J VAN DER LUGT AND C G STEWART

Introduction

Leishmaniosis is caused by obligatory intracellular parasitic protozoa of the genus *Leishmania* that affect various mammalian hosts, but disease occurs most commonly in humans and dogs. Cutaneous, mucocutaneous and visceral forms of the disease are recognized; the visceral form being chronic and often fatal in humans and dogs.

Leishmania spp. are widely distributed in Central and South America, East and West Africa, Asia, the Mediterranean basin and some neighbouring European countries, but they are rare in southern Africa. The cutaneous form of the disease has recently been diagnosed in two sheep from different localities in South Africa.[15, 22]

Aetiology, life cycle and epidemiology

Members of the genus *Leishmania*, which comprises a considerable number of species, belong to the order Kinetoplastida and family Trypanosomatidae.[11] Identification of species within the genus is complex and is based on clinical and pathological features of the disease, geographical distribution, morphology and behaviour of the parasite, and the results of biochemical and immunological tests.[10]

Diseases caused by *Leishmania* spp. are transmitted by blood-sucking sandflies of the genera *Lutzomyia* in the New World and *Phlebotomus* in the Old World. The disease in dogs is caused by *L. infantum*.

There is usually a primary reservoir host for a given *Leishmania* sp. in a particular area where the parasite is maintained by a cycle between sandflies and the mammalian hosts (**Figure 22.1**). Secondary and incidental mammalian hosts also become infected.[2] Both domestic and wild dogs (*Lycaon pictus*), as well as rodents and other wild animals, are reservoir hosts, but occasionally humans may act as the main reservoir.

The parasite occurs in two forms: a promastigote stage which is found in the sandfly; and an amastigote stage which occurs in the mammalian host. During feeding, the infected sandfly injects promastigotes into the dermis of the mammalian host and these are engulfed by phagocytic cells before transforming into amastigotes which multiply by binary fission. Heavily parasitized host cells eventually rupture, and the liberated amastigotes invade other macrophages in which they may be spread locally or systemically.[17] In tissues, amastigotes of all *Leishmania* spp. are morphologically similar (**Figures 22.2 and 22.3**): they are round to oval bodies, 1,5–3 × 2,5–6,5 µm in diameter, containing a single nucleus and a rod-shaped kinetoplast.[17]

Leishmaniosis has rarely been reported in livestock. There have been reports of infection in calves from Africa in the early literature, but no details are given.[13] The cutaneous form of the disease occurs frequently in horses, donkeys and mules in endemic areas of human and canine leishmaniosis in Venezuela and Brazil.[3, 4] In 1989 and 1990 cutaneous leishmaniosis was diagnosed in sheep in South Africa — in one instance in two adult ewes in a flock near Barberton in Mpumalanga, and in the other in one sheep near Greytown in KwaZulu-Natal.[15, 22]

Canine leishmaniosis is endemic in the Mediterranean countries, and is occasionally seen in North Europe and in the USA in dogs that have previously been in endemic areas.[20]

In South Africa, visceral leishmaniosis has been diagnosed twice in dogs: in 1964 in a dog from Durban whose life history is uncertain;[18] and in 1987 in an animal from the Free State which had never left the country.[23] In Namibia, 34 cases of cutaneous leishmaniosis in humans have been diagnosed since 1970,[9] while only a single case of the cutaneous form was reported from the Northern Cape Province in 1979.[1]

The epidemiology of leishmaniosis in southern Africa is not known. In Namibia, apart from humans, *Leishmania* parasites have also been isolated from the skin of hyraxes (*Procavia capensis*) and from naturally infected *Phlebotomus rossi* sandflies (which are possible vectors of the human disease). The isolates made from humans and sandflies are identical, but are distinctly different from those recovered from the hyraxes. The two Namibian strains of parasite are also dissimilar to *L. tropica* and *L. major* which cause cutaneous leishmaniosis in the Old World and are afforded separate status.[9]

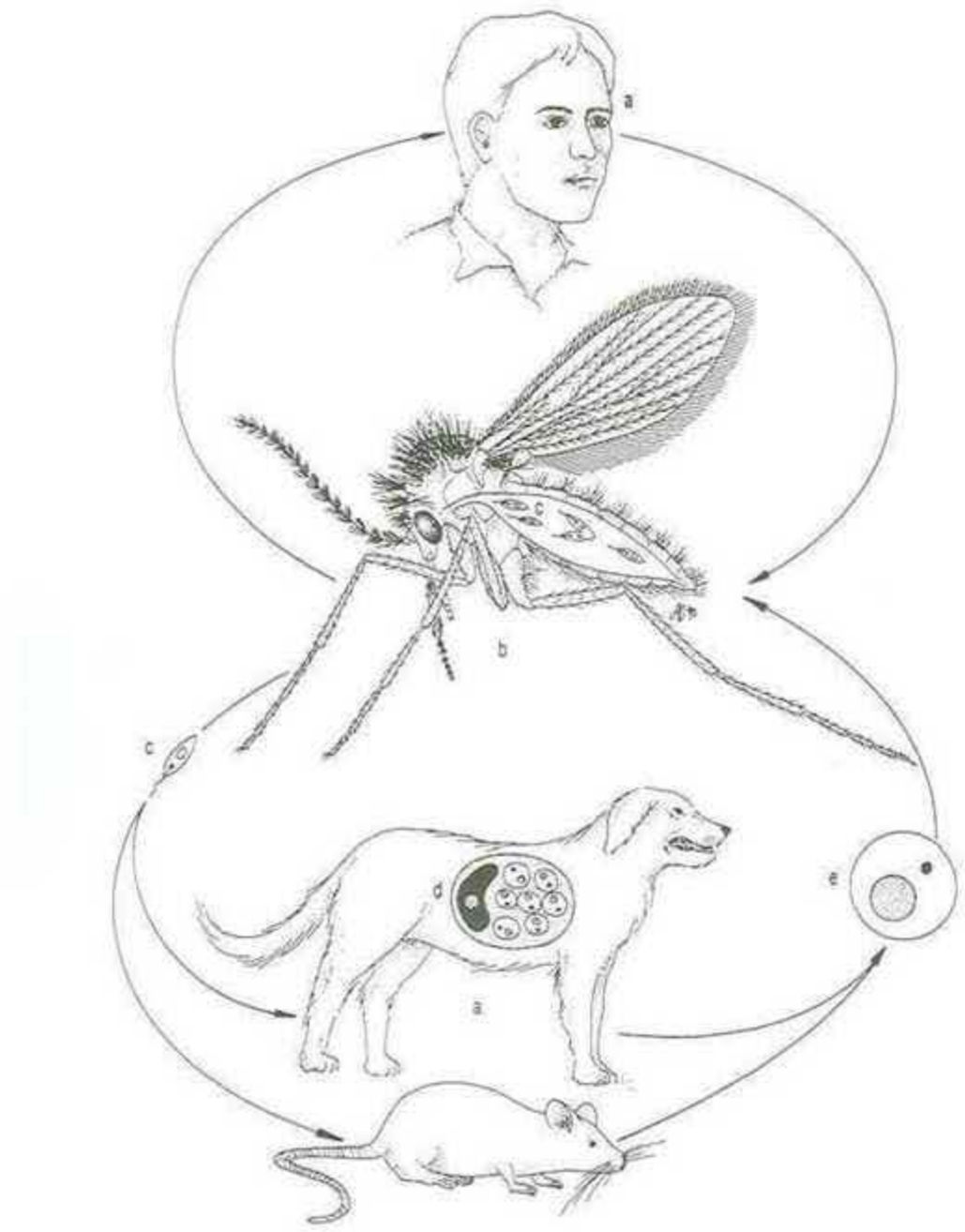


Figure 22.1 Life cycle of a *Leishmania* sp.

a = mammalian hosts
b = *Phlebotomus* sp.
c = promastigote
d = macrophage containing amastigotes
e = amastigote

Pathogenesis, clinical signs and pathology

Leishmaniosis in humans comprises several syndromes depending primarily on the species of *Leishmania* concerned. The clinical spectrum ranges from asymptomatic infections to those with a high mortality. Lesions may be confined to the skin (cutaneous leishmaniosis) and result, *inter alia*, in nodule and ulcer formation, known in some parts of the world as the 'Oriental sore'; to the mucocutaneous tissues (mucocutaneous leishmaniosis, sometimes referred to as espundia), in which the organisms are found in endothelial and mononuclear cells of the skin and mucous membranes of the nose, mouth, pharynx and elsewhere and may result in severe disfigurement of the invaded tissues; or they may involve several tissues especially the liver, spleen, bone marrow and lymph nodes (the highly fatal visceral leishmaniosis or 'Kala azar').[21]

In dogs, the parasites multiply within macrophages and other cells of the mononuclear-phagocyte-system and cause chronic inflammatory processes.[5, 6] Canine leishmaniosis is a systemic disease, and, in contrast to the situation in human cutaneous leishmaniosis, the skin of dogs is affected in the course of a generalized dissemination of the parasite. Chronic renal failure due to an immune-mediated glomerulonephritis is the main cause of death in affected dogs.[20]

Clinically, canine leishmaniosis is a chronic wasting disease accompanied by anaemia, intermittent pyrexia and generalized lymphadenopathy. Skin lesions, of which four forms have been described, are common: symmetrical alopecia with dry desquamation; a generalized nodular disease; ulcerative dermatosis; and a form characterized by the presence of multiple pustules. Keratoconjunctivitis, epistaxis and diarrhoea may also be present.[8, 12]

At necropsy, in addition to the lesions visible clinically in dogs, there is generalized lymphadenopathy, and hepato- and splenomegaly. Microscopically, large numbers of macrophages filled with the amastigote form of *Leishmania* are present in the spleen, bone marrow and lymphoid tissues. In non-lymphoid tissues, a granulomatous inflammatory infiltrate composed of parasitized macrophages, plasma cells and lymphocytes is evident.[12]

Leishmaniosis was diagnosed in two adult sheep born and reared on a farm in Mpumalanga. The clinical signs in

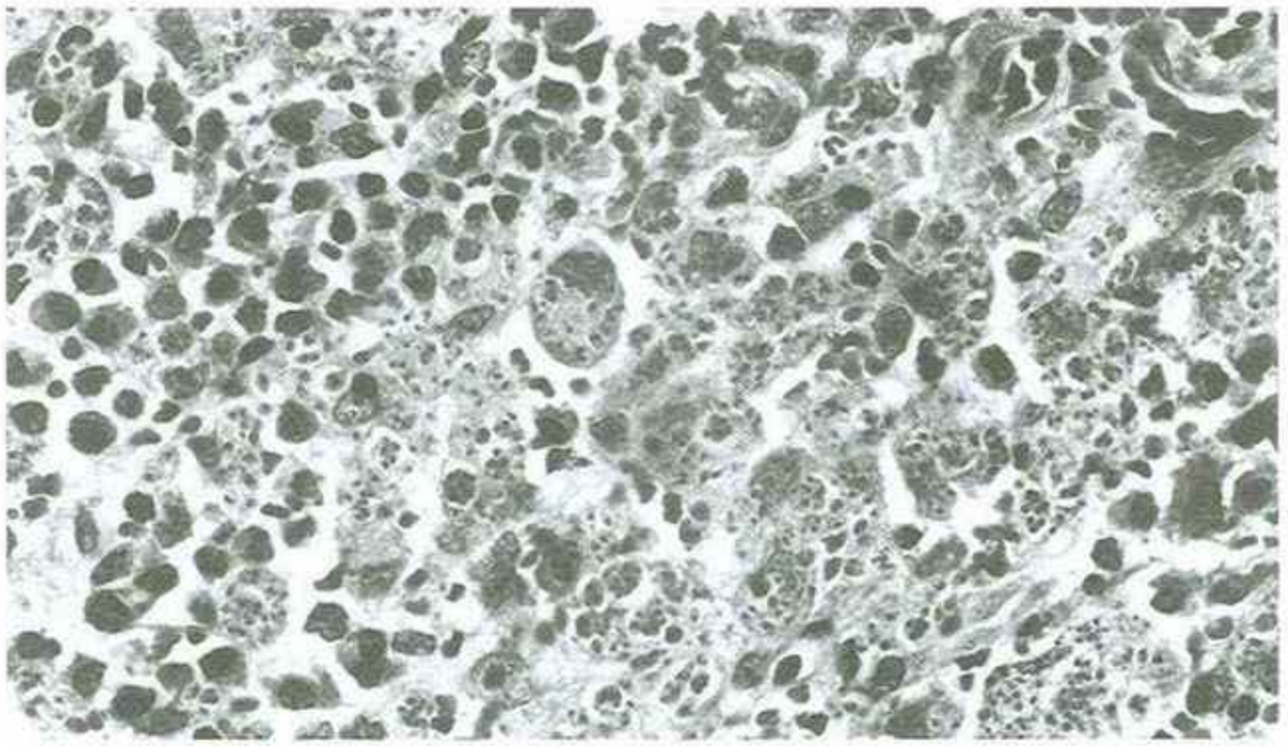


Figure 22.2 Leishmaniosis in a sheep: numerous macrophages in the skin containing amastigotes of a *Leishmania* sp.

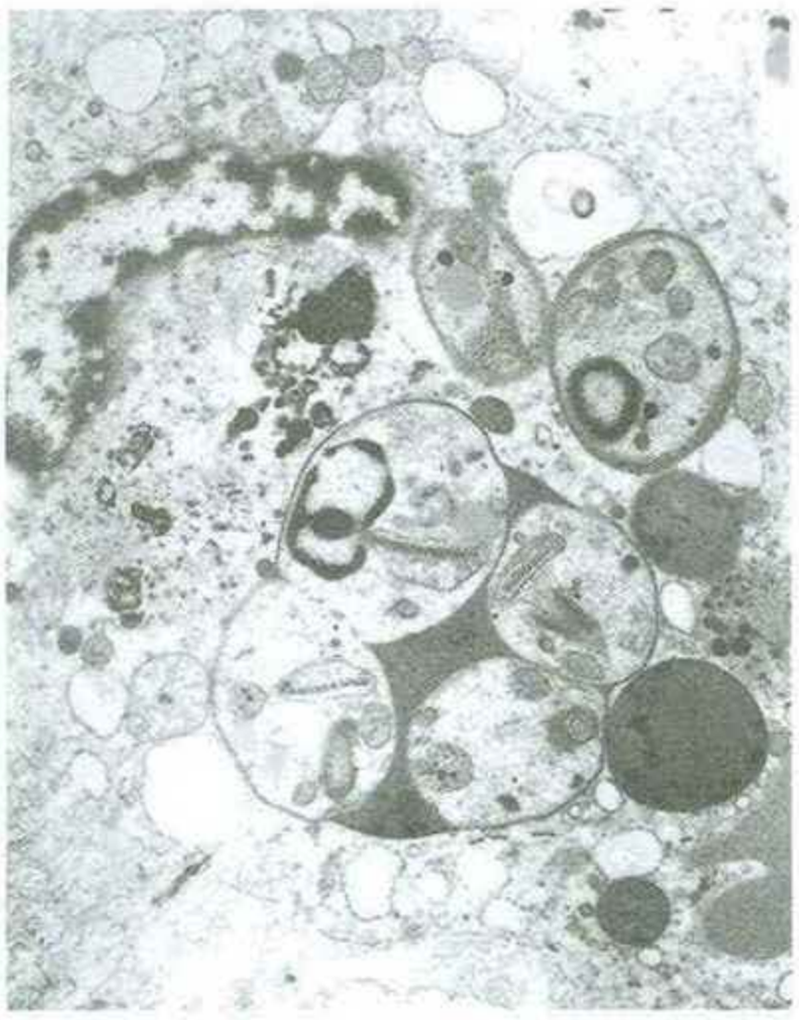


Figure 22.3a Group of *Leishmania* organisms in a macrophage (x 12 750). (By courtesy of Drs J. Soley and S. Yates, Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, Onderstepoort)

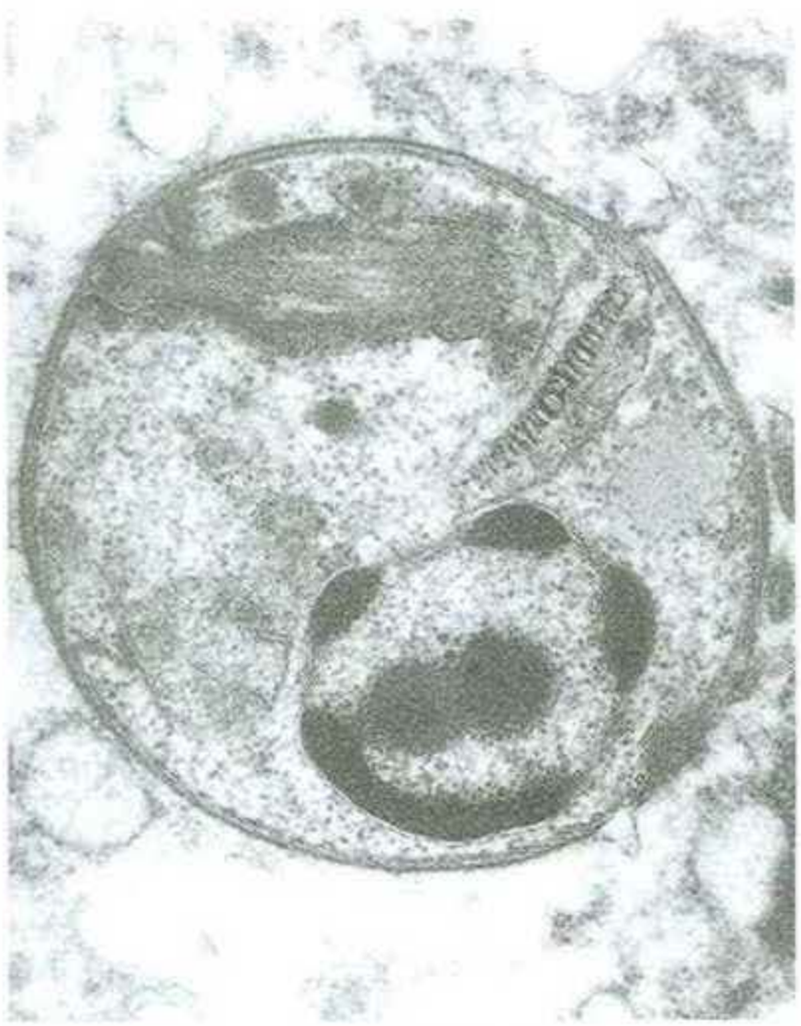


Figure 22.3b Higher magnification (x 44 000) of a single *Leishmania* organism. (By courtesy of Drs J. Soley and S. Yates, Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, Onderstepoort)

both cases were similar. The pinna of one ear of each animal was severely swollen and the overlying skin diffusely thickened and covered with a crusty exudate. Small crusts also occurred in the skin around the external nares and eyes, and in that of the upper lip, muzzle and lateral aspects of the face. Both animals recovered spontaneously.

Another case was diagnosed in an adult sheep from KwaZulu-Natal. It showed alopecia, hyperpigmentation, hyperkeratosis and crust formation of the skin of the dorsum of the nose and both ears, and around the eyes. It also displayed a focally disseminated alopecia and the presence of nodules in the skin of the ventral aspects of

the limbs, abdomen and base of the tail. Its recovery was spontaneous.

Diagnosis

The diagnosis of leishmaniosis is based on identification of the agent and serological tests. Demonstration of the amastigotes in Giemsa-stained smears of splenic, bone marrow and lymph node aspirates, and of skin scrapings, gives a positive diagnosis, while an indirect immunoperoxidase staining technique of paraffin-embedded section is also available. If the infection is of low grade, detection is possible only by attempting *in vitro* or *in vivo* isolation. Isolated *Leishmania* organisms are characterized by isoenzyme, DNA and antigen methods at present. Serology is the preferred method for the diagnosis of visceral and canine leishmaniosis with the indirect fluorescent antibody test and the enzyme-linked immunosorbent assays the most suitable. Serology is of less value for the cutaneous and mucocutaneous forms of the disease.[7, 14, 16, 19, 20]

Control

Pentavalent antimonials, especially meglumine antimonate, are the most effective drugs for the treatment of canine leishmaniosis. With therapy, the majority of cases in dogs show remarkable improvement, provided renal glomerular filtration is not seriously decreased. Treatment is, however, expensive and relapses are the rule, with the development of complications such as iridocyclitis or end-stage renal failure being common. Effective vaccines are not available.[20]

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23

Neosporosis

J P DUBEY

Introduction

Neospora caninum is a recently recognized protozoan parasite of livestock and companion animals. It is not a new parasite[42] but until 1988 was misdiagnosed as *Toxoplasma gondii* because of structural and biological similarities.[34] The disease was first recognized in dogs by Bjerkås, Mohn and Predsthus[15] in Norway in 1984 but the parasite was not named. Dubey and his co-workers[34] subsequently described the parasite and proposed a new genus, *Neospora*, with *N. caninum* as the type species. *In vitro* isolation of the parasite in cell culture inoculated with tissues of paralysed dogs led to newer knowledge of its biology.[38]

Aetiology and life cycle

Neospora caninum is a coccidian parasite, closely related to *Toxoplasma gondii* (**Figure 23.1**). Dogs are both an intermediate host and a definitive host.[34, 76] In addition to dogs, cattle,[31] sheep,[37] goats,[10, 33] horses,[48] deer[49, 102] and water buffaloes (*Bubalus bubalis*),[31, 54, 59] are also intermediate hosts. Antibodies to *N. caninum* have been found in foxes (*Vulpes vulpes*),[23] coyotes (*Canis latrans*),[69] camels (*Camelus dromedarius*)[55] and white-tailed deer (*Odocoileus virginianus*),[39] suggesting that these hosts may be natural intermediate hosts for *N. caninum*. Cats, mice, pigs, rats, foxes, gerbils, and monkeys may be induced to be experimental intermediate hosts.[31, 66] Only an asexual cycle occurs in the intermediate hosts which consists of tachyzoites and tissue cysts. Both tachyzoites and tissue cysts are microscopic and intracellular. Tachyzoites are ovoid, lunate or globular and measure 3–7 µm × 1–5 µm, depending on the stage of division (**Figure 23.2**). They divide into two zoites by endodyogeny (**Figure 23.3**). In infected animals, tachyzoites are found in many cells including neurons, macrophages, fibroblasts, vascular endothelial cells, myocytes, hepatocytes and dermal cells. Tachyzoites are usually located within the host cell cytoplasm within a parasitophorous vacuole. Ultrastructurally, *N. caninum* tachyzoites are similar to *T. gondii* tachyzoites except that the rhoptries in *N. caninum* are electron-dense whereas in *T. gondii* they are electron-lucent.[42, 71]

Tissue cysts are often round to oval in shape (**Figure 23.4**), up to 107 µm long, and have been observed only in neural tissues (brain, spinal cord, nerves and retina)[34] with the single exception of a solitary tissue cyst in the ocular muscles of a foal.[72] The tissue cyst wall is smooth and up to 4 µm thick, presumably depending upon how long the infection has existed. In most tissue cysts, the cyst wall is 1 to 2 µm thick. Septa are absent and there is no secondary cyst wall (**Figure 23.5**). Bradyzoites are slender (6–8 µm × 1–1,8 µm) and contain the same organelles as are found in tachyzoites except that there are fewer rhoptries and more periodic-acid Schiff (PAS)-positive (amylopectin) granules in the bradyzoites. Tissue cysts may degenerate and cause a host reaction (**Figure 23.6**).

Dogs fed tissue cysts may shed unsporulated oocysts.[76] Oocysts can sporulate outside the host within 24 hours. Sporulated oocysts contain two sporocysts each with four sporozoites. *Neospora caninum* oocysts are 10 to 11 µm in diameter (**Figure 23.7**) and are morphologically indistinguishable from *Hammondia heydorni* found in canine faeces, and *Toxoplasma gondii* and *Hammondia hammondi* in cat faeces. At present, nothing is known regarding the frequency of shedding of oocysts, the survival of the oocysts in the environment, and whether canids other than domestic dogs are also definitive hosts for *N. caninum*.

Susceptible hosts can become infected by ingesting food and water contaminated with *N. caninum* oocysts from dog faeces. Experimentally, animals may become infected lactogenically.[26, 98]

Neosporosis in cattle

Neosporosis affects both dairy and beef cattle.[47, 58, 96] Bovine *N. caninum* infection has been reported from Europe, Australia, Asia, Africa, New Zealand, and the Americas.[22, 31, 79, 81, 82, 96] It is a major cause of abortion in

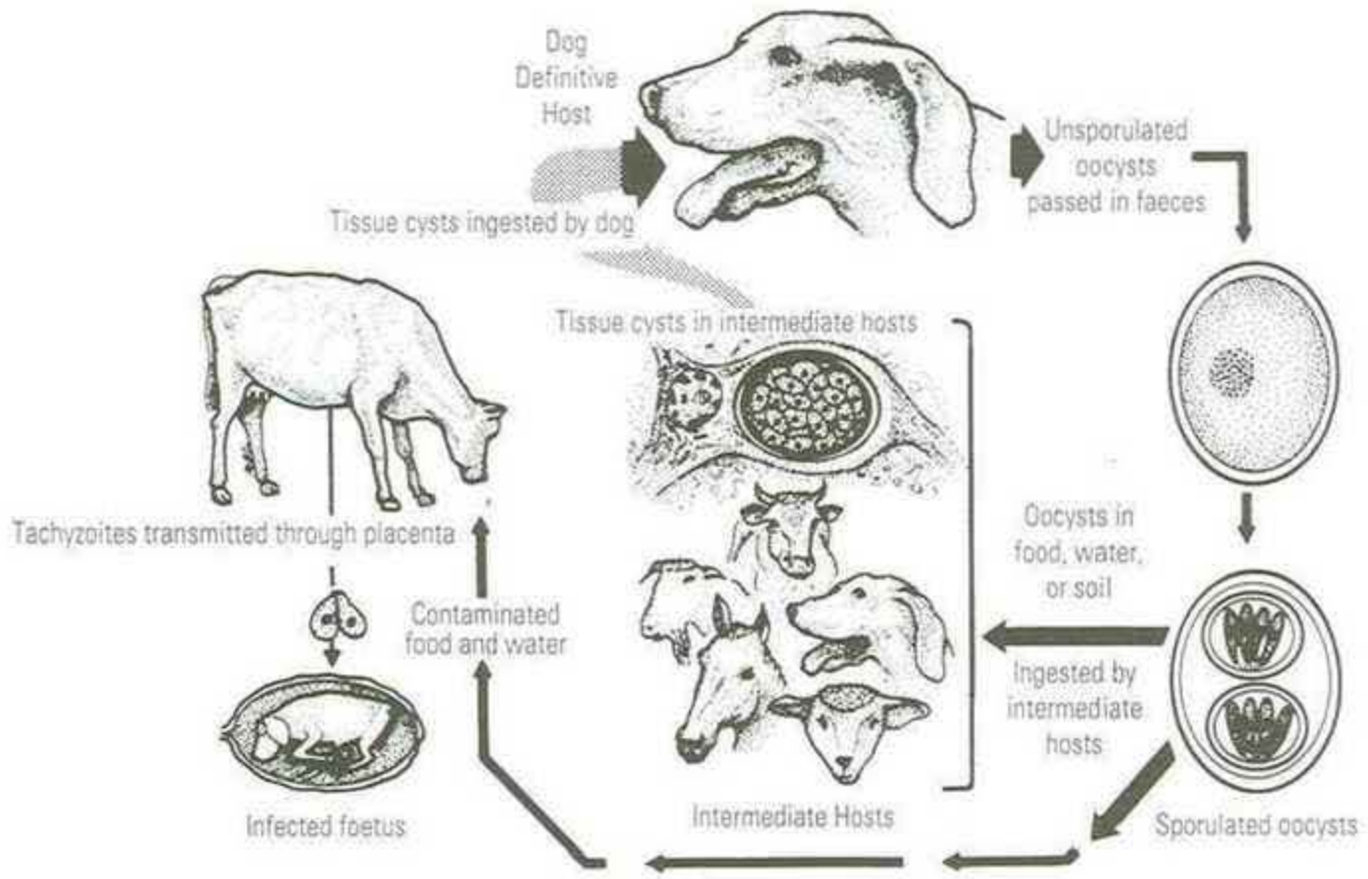


Figure 23.1 Life cycle of *Neospora caninum*

dairy cattle in the USA,[1, 2, 8, 96] New Zealand,[97] and the Netherlands.[103] Based on serological surveys, up to 100 per cent of cattle in some herds have been exposed to *N. caninum*.[40, 85–88, 101] Neoporosis has been reported in cattle from South Africa,[62, 63] and Zimbabwe.[64]

Neospora caninum is efficiently transmitted vertically in cattle, even for several generations, but horizontal transmission is necessary to introduce new infections in the herd.[3, 17]

Neospora caninum can be transmitted transplacentally in naturally infected cattle, sheep, goats, deer and horses, and experimentally in several other species of animals.[31] Transplacental infection can occur repeatedly in the same animal and through its progeny for several generations. The mechanisms of primary and repeat congenital transmission of infection are unknown.

Whether the repeat congenital infections which occur in dogs and cattle are due to relapse of the primary infection or reinfection is unclear.

Abortion is the only clinical sign observed in adult cows.[31, 42, 103] Cows of any age may abort from three months of gestation to term. Most *Neospora*-induced abortions occur at five to six months of gestation.[1, 103] Foetuses may die *in utero* and be resorbed, mummified or aborted, or may be stillborn, born alive but diseased, or born clinically normal but chronically infected. Within herds, abortions may be clustered, sporadic or epidemic.[77, 80, 96, 103, 105] *Neospora caninum*-induced abortions occur year round. Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows.[78, 88, 103]

Neospora caninum-infected calves may be born underweight, unable to rise and with neurological signs. Hind limbs and/or forelimbs may be flexed or hyperextended and neurological examination may reveal ataxia, decreased patellar reflexes, and loss of conscious proprioception.[11, 12, 36] Exophthalmia or an asymmetrical appearance of the eyes may also be observed.

The presence of specific antibodies in serum from a cow that has aborted is only indicative of exposure to *N. caninum*. Several serological tests can be used to detect *N. caninum* antibodies, including enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody test (IFAT), and the direct agglutination test.[14, 16, 18, 28, 40, 43, 66, 84, 86, 92, 101, 103] Reagents for some of these tests are available commercially. An IgG avidity ELISA with the potential to discriminate between recent and chronic *N. caninum* infections in cattle has been described.[20, 21] It has recently been found by the use of a newly developed ELISA that there are serological differences between *N. caninum*-associated epidemic (abortion storm) and endemic (scattered) abortions.[95] Within the group of animals that tested seropositive by IFAT and immunoblot, dams from herds with *N. caninum*-associated endemic abortions had significantly higher ELISA indices than did those from herds with *N. caninum*-associated epidemic abortions.

A definitive cut-off titre for serodiagnostic purposes has not been established for cattle because of the uncertainty of

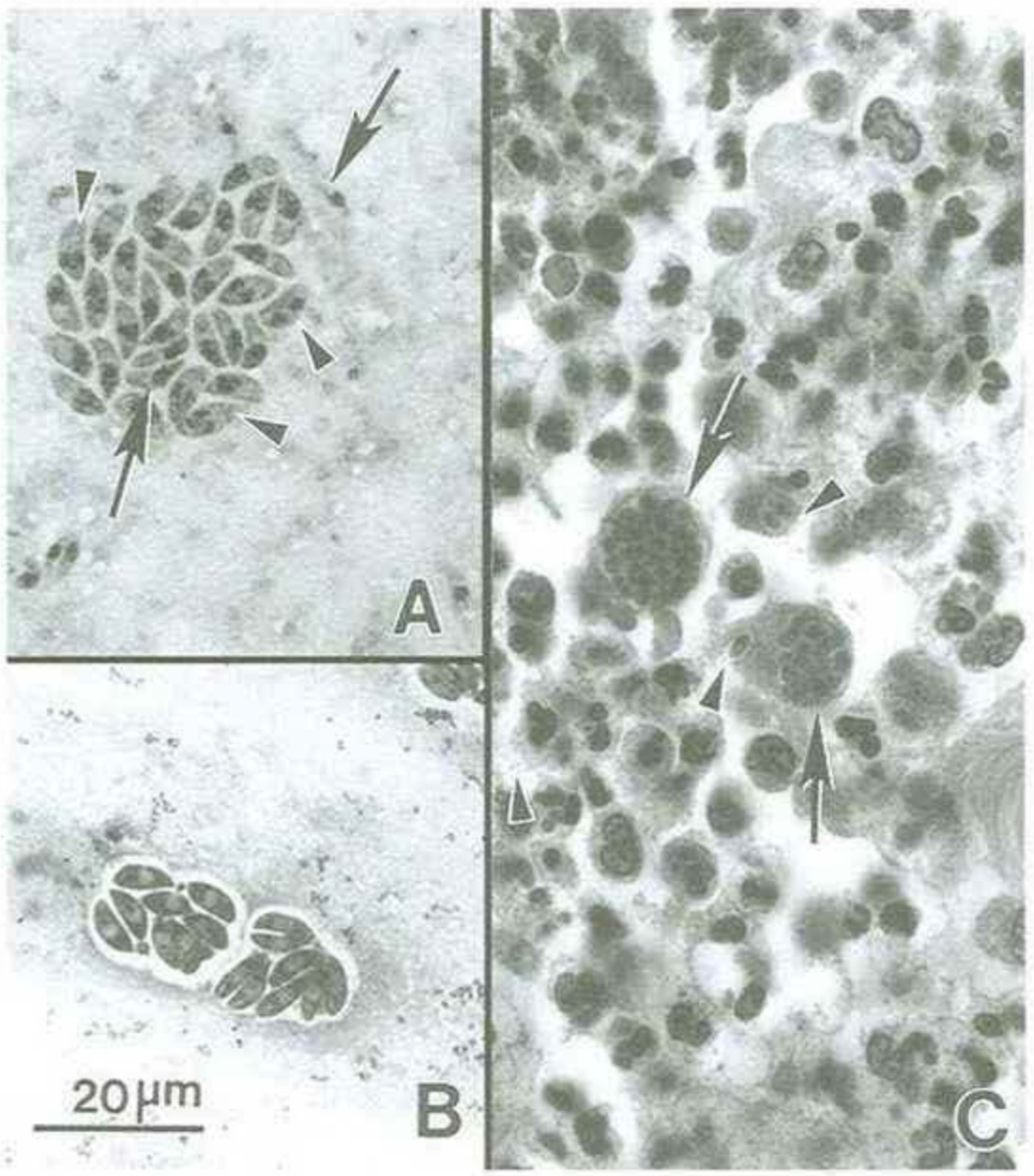


Figure 23.2 Tachyzoites of *Neospora caninum*

A Impression smear. Note organisms dividing (arrowheads) into two are bigger than single tachyzoites (arrows). Giemsa stain
B Tachyzoites in parasitophorous vacuoles in cell culture. Giemsa stain
C Sections of the skin of a dog. Note suppurative inflammation associated with two groups of tachyzoites (arrows) and individual tachyzoites (arrowheads). Tachyzoites in sections are much smaller than those in smears. Haematoxylin and eosin stain

serological diagnosis in chronically infected animals and the limited availability of sera from non-infected cattle. In serological assays, titre and absorbance values are dependant on antigen composition, secondary antibodies and other reagents. Further, cut-off values can be arbitrarily selected to provide sensitivity and specificity requested for the particular application. The age of the animal may also affect selection of a given cut-off value. For example, an IFAT titre of 1:640[28] or 1:200[40] has been considered indicative of *N. caninum* infection in adult cattle, whereas much lower values (1:80) have been selected as cut-off values for samples from bovine foetuses.[9] The situation may be different for adult cattle sera probably because they have been exposed to a very great diversity of antigens. Although *N. caninum* is closely related to *T. gondii*, *Sarcocystis* spp. and other apicomplexans, cross-reactivity has not been a major issue in animals experimentally infected with *N. caninum* and related apicomplexans.[43] Recent evidence indicates that even low IFAT titres (1:25) are specific for *Neospora* infection.[100]

Examination of the foetus is necessary for a definitive diagnosis of neosporosis. Ideally, the entire foetus should be submitted but if this is not possible then samples from the brain, heart and liver should be examined for histopathological changes, and body fluids or blood serum for serological evaluation. Although *N. caninum* infection can cause lesions in several organs, foetal brain is the most consistently affected tissue.[1, 8, 44] The most characteristic lesion is focal encephalitis characterized by necrosis (**Figure 23.8**) and non-suppurative inflammation (**Figure 23.9**).[13] Hepatitis is found more commonly in foetuses aborted during epidemic events than in those in which abortions are sporadic.[103] Because most aborted foetuses are likely to be rapidly autolysed, even semi-liquid brain tissue should be fixed in 10 per cent buffered neutral formalin for histological and immunohistochemical (IHC) examination[1, 25] (**Figure 23.10**). There are no significant gross lesions of neosporosis. Although a presumptive diagnosis may be made by examination of haematoxylin and eosin (HE) stained sections, IHC is necessary because there are often only a few *N. caninum* present in autolysed tissues and these are often not visible in HE stained sections.[42, 68] Only rarely are *N. caninum* organisms sufficiently numerous to be found in each histological section.[32, 47] The sensitivity of even the most efficient method (IHC) to detect *N. caninum* in tissues is low.

Finding *N. caninum* antibody in foetal serum or precolostral calf serum indicates infection, but a negative result in a foetus is less useful as antibody production in the foetus is

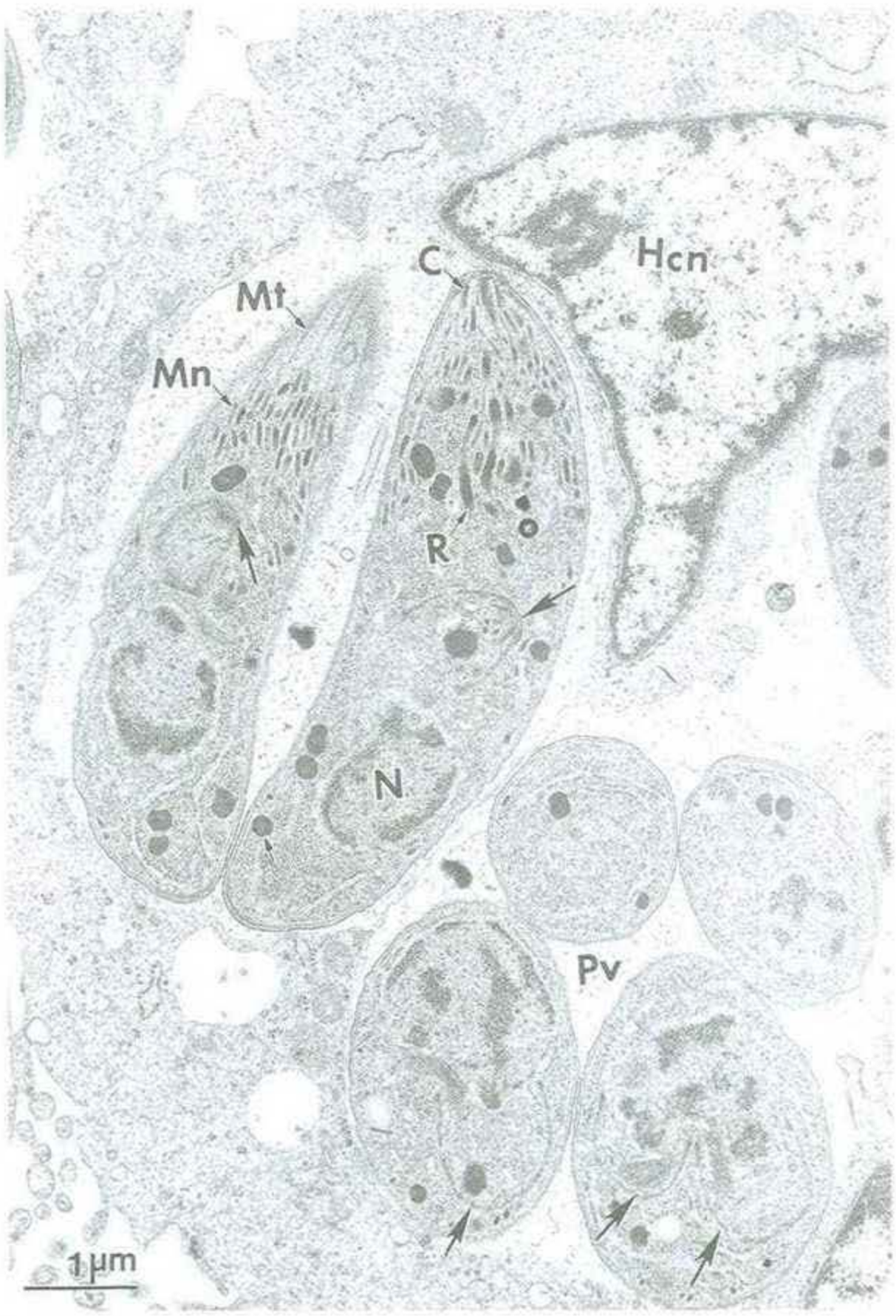


Figure 23.3 Transmission electron micrograph of dividing tachyzoites in two parasitophorous vacuoles (Pv). The Pv have many tubular networks. Arrows point to conoidal end of daughter tachyzoites forming inside the mother tachyzoites. Note electron-dense rhoptries (R), micronemes (Mn), microtubules (Mt) or subpellicular tubules, conoid (C), host cell nucleus (Hcn). Cardiopulmonary artery endothelial cell culture. (Courtesy of Dr C.A. Speer, Montana State University, Bozeman, Montana, USA)

dependent on the stage of gestation, level of exposure, and the time between infection and abortion.[9, 102] In one study, IFAT titres (1:80) were found in 50 per cent of neosporosis-confirmed foetuses and in only one of 64 foetuses that were aborted due to other causes,[9] while in another low IFAT titres (1:25) were found in 65 per cent (31 of 48) of foetuses immunohistochemically positive for *N. caninum* and in none of the 39 foetuses aborted due to other causes.[104] Thus, even a low IFAT titre of 1:25 should be considered specific in foetuses. In congenitally infected calves, precolostral serum from live calves, and brain and spinal cord from dead calves are the specimens of choice for establishing a diagnosis.

Isolation of *Neospora* in cell culture is rarely possible because most organisms in bovine foetuses die when the host cells become autolysed.[27] Several polymerase chain

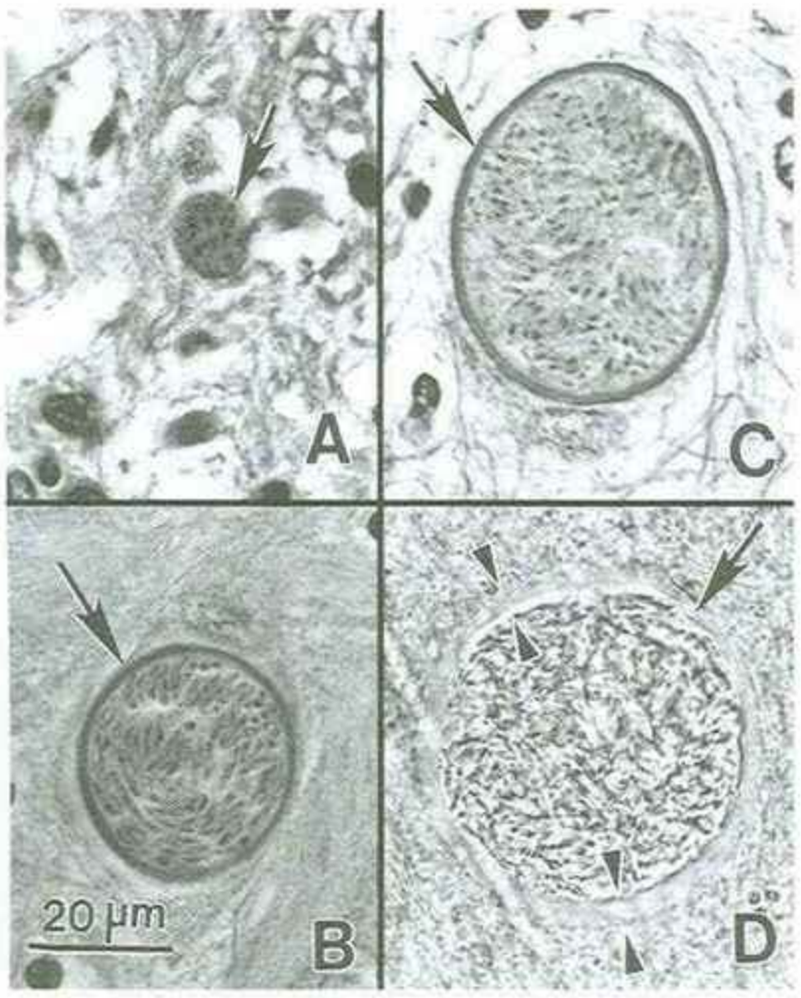


Figure 23.4 Tissue cysts (arrows) of *Neospora caninum*

A Section of a calf brain. Note small tissue cyst with thin cyst wall. Haematoxylin and eosin stain

B Intraneuronal tissue cyst in a section of the spinal cord of a calf. Haematoxylin and eosin stain

C Intraneuronal tissue cyst in a section of spinal cord of a calf. Note silver-positive cyst wall. Silver stain

D Tissue cyst in a brain squash of an experimentally infected mouse. Note thick cyst wall (opposing arrowheads). Unstained

reaction (PCR) methods have been reported with which to detect *N. caninum* DNA,[51, 52, 57] but have not yet been evaluated critically for the diagnosis of *N. caninum*-induced abortion in cattle.

Neosporosis in horses

Clinical neosporosis has been reported in seven horses, all from the USA,[24, 30, 48, 53, 55, 73, 74] the first of which was in 1985 in a two-months-premature aborted foetus. Only lung tissue was available from this case and numerous *N. caninum* tachyzoites were observed in the lesions that were present.[48] Recently *N. caninum* was identified by PCR in an aborted foetus in France.[90]

Neonatal neosporosis was diagnosed in a one-month-old Quarter horse filly in Wisconsin.[72] She had circled her dam since birth and frequently tripped over obstacles. If separated from her dam she could not find her way back. Encephalitis associated with tissue cysts was revealed on histopathological examination.

An unusual case of visceral neosporosis has been reported in a 10-year-old Appaloosa mare which had a history of weight loss and anaemia. Lesions associated with *N. caninum* tachyzoites were confined to the intestines and mesenteric lymph nodes. This is the first reported case of *Neospora*-associated enteritis in any animal species.[53]

Neosporosis has been diagnosed in an aged Pinto mare suffering from a pituitary adenoma which had a history of hind limb paralysis, bizarre behaviour and masticatory difficulty.[30] Lesions were present in the central nervous system, peripheral nerves and myocardium, and consisted of multifocal non-suppurative encephalomyelitis, polyradiculoneuritis and multifocal myocarditis. *Neospora* tachyzoites and tissue cysts were demonstrated by IHC examination in the brain, spinal cord and peripheral nerves. Neosporosis has been observed in a 20-year-old horse suffering from a pituitary tumour.[55]

Neospora organisms were identified recently in the brain and spinal cord of a Californian horse which had manifested neurological signs.[73, 74] The parasite was isolated in cell cultures inoculated with neural tissue of the horse, and was named *N. hughesi*.[74] *Neospora hughesi* has also been isolated from the spinal cord of a 13-year-old horse from Alabama.[24]

Antibodies to *N. caninum* were found in 23.3 per cent of 296 horses in the USA slaughtered for food for export [50] and, in another study, in 11.5 per cent of 536 horses in the USA.[24]

Neosporosis in goats

Abortion associated with *N. caninum* has been reported in herds of pygmy goats in California[10] and Pennsylvania,[33] and in dairy goats in Heredia, Costa Rica.[46] In the Californian episode, a foetus from each of two different flocks was examined histologically. Tissue cysts were present in encephalitic lesions in both cases.

One neosporosis-infected foetus from a small flock of pygmy goats in Pennsylvania was examined. The flock had experienced abortions, stillbirth, and birth of weak kids. The foetus that was examined was full-term, and the lesions present were predominantly in the brain, especially the midbrain in which an encephalitis characterized by numerous microglial nodules with minimal necrosis and severe perivascular infiltration of mononuclear cells was noted. Numerous tissue cysts were observed, some of which were undergoing degeneration. Tachyzoites were not noticed.

In Heredia, Costa Rica, the farmer had also experienced an undiagnosed abortion problem in his flock of goats. The foetus that was examined was of 3.5 months' gestational age and a Saanen-Toggenberg cross. Lesions were found mainly in the brain and were characterized grossly by hydrocephalus and a hypoplastic cerebellum, and microscopically by meningoencephalitis which manifested as a ventriculitis, gliosis, necrosis, mineralization and vasculitis. An unusual feature of this case was the presence of large numbers of tissue cysts and the rarity of tachyzoites. In addition, myositis

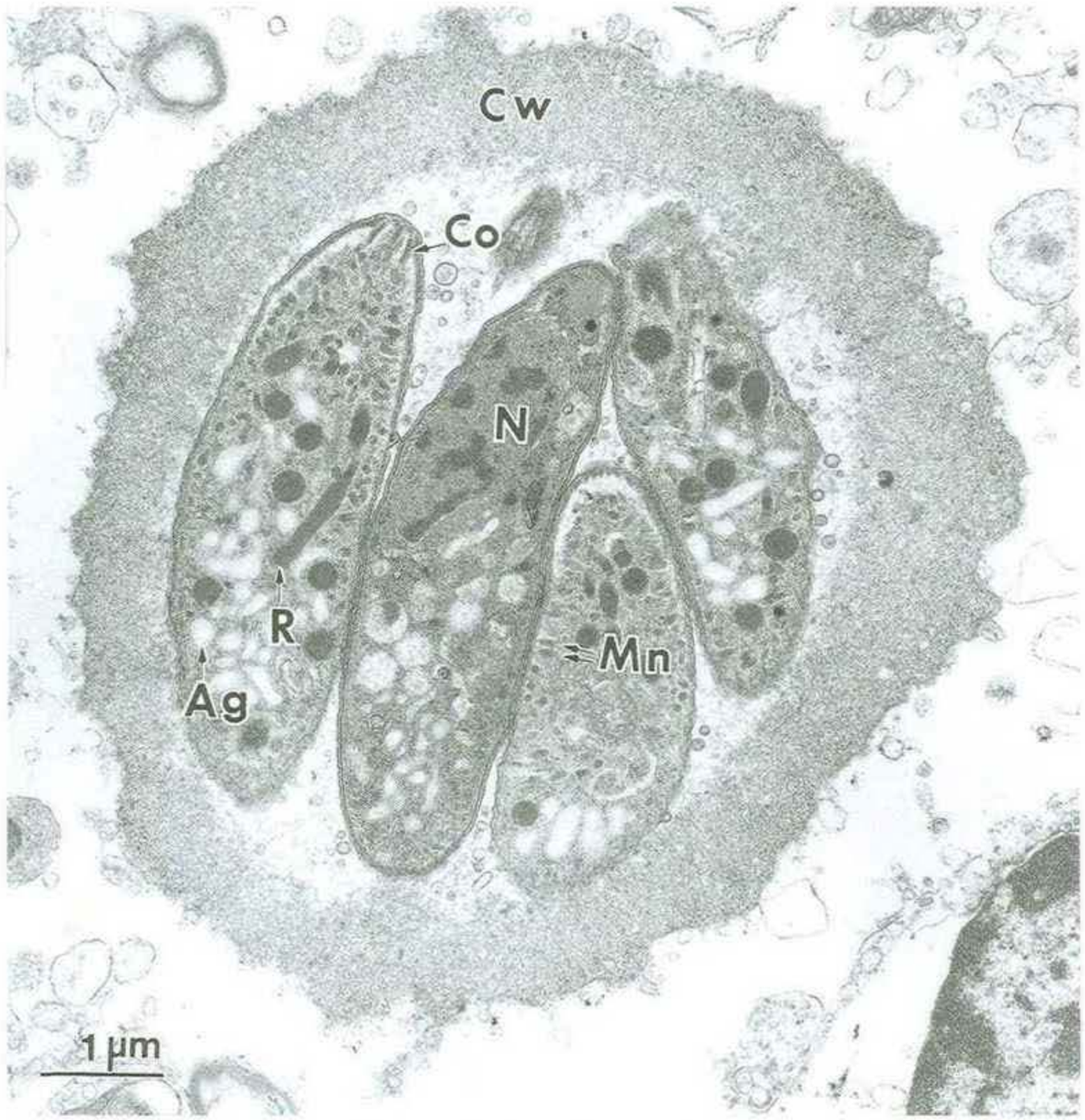


Figure 23.5 Transmission electron micrograph of a *Neospora caninum* tissue cyst in mouse brain. Note thick, smooth cyst wall (Cw), absence of septa, micronemes (Mn) perpendicular to the bradyzoite plasmalemma, amylopectin granules (Ag) that stain red with PAS, electron-dense rhoptries (R), and a subterminal nucleus (N). (Courtesy of Dr C.A. Speer, Montana State University, Bozeman, Montana, USA)

associated with the presence of tachyzoites was noted. The foetus's dam had an *N. caninum* IFAT titre of 1:800. Five of 77 other does in this herd also had IFAT antibody titres to *N. caninum*; cross-reactivity with *T. gondii* was obtained. Experimentally, goats have been proved to be susceptible to *N. caninum* infection.[70]

Neosporosis in sheep

There is only one report of congenital neosporosis in a sheep lamb.[37] The affected lamb was born weak, partially ataxic, and died at one week of age. The most prominent findings were in the spinal cord which showed a myelitis, unilateral reduction of grey matter in the ventral horn, and focal cavitation and were associated with intact and degenerating *N. caninum* tissue cysts; tachyzoites were not seen.

Based on the results obtained in one study, *Neospora* infections are not considered as a cause of abortion in sheep in England.[83]

Neosporosis in deer

Neosporosis has been diagnosed in a two-month-old female black-tailed deer (*Odocoileus hemionus columbianus*) that

was found dead in California, USA.[102] Lesions and *N. caninum* were present histopathologically in sections of the lungs, liver, and kidneys. Tachyzoites were most numerous in the lungs and were associated with interstitial pneumonia, while in the kidneys an interstitial nephritis associated with their presence in tubular epithelium and lumens was noted. Tachyzoites within hepatocytes and Kupffer cells were found in the liver.

Neosporosis was also been encountered in a full-term stillborn brown-antlered deer (*Cervus eldii siamensis*) from the Paris Zoo, France.[49] *Neospora caninum* tissue cysts associated with a non-suppurative encephalitis were present. Neosporosis was thought to be the main disease involved in the decline of the population of this endangered species in the zoo. Antibodies to *N. caninum* were found in 162 of 400 (40.5 per cent) white-tailed deer (*Odocoileus virginianus*) in Illinois, USA.[39]

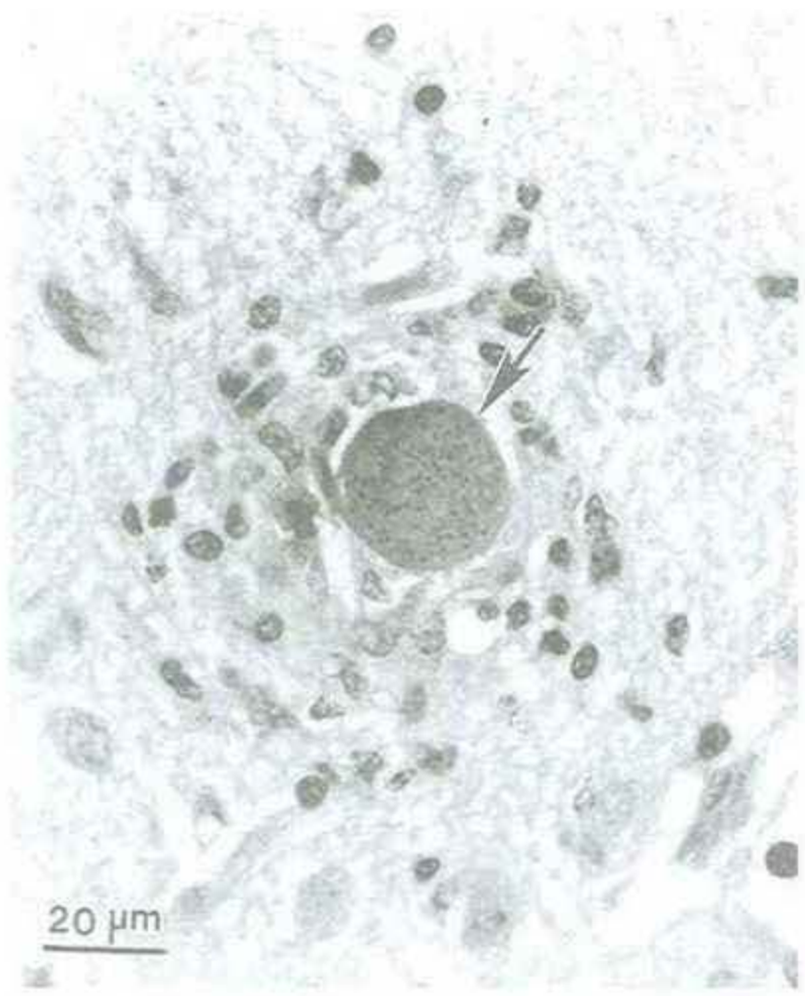


Figure 23.6 Degenerating tissue cyst (arrow) in the brain of a goat. Note glial cells and mononuclear cells surrounding the tissue cyst. Haematoxylin and eosin stain

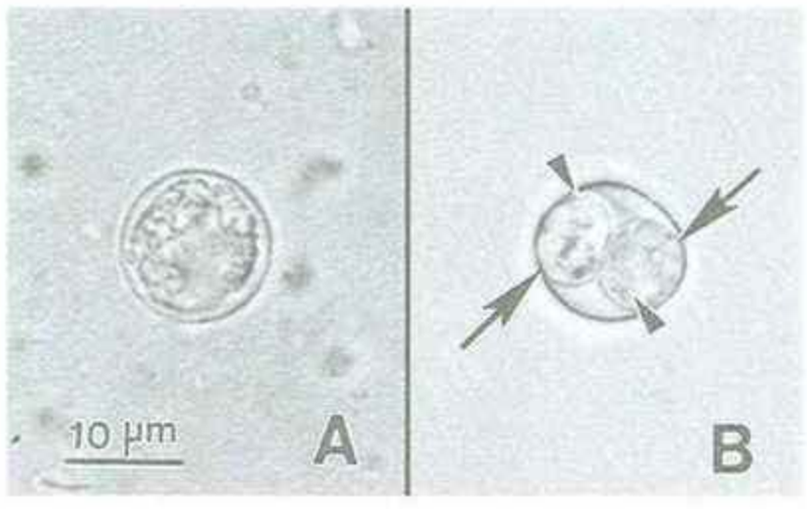


Figure 23.7 Oocysts of *Neospora caninum*

A = Unsporulated
B = Sporulated
Note two sporocysts (arrows) and sporozoites (arrowheads). Unstained

Neosporosis in dogs

Dogs of any age may be affected. Fatal neosporosis has been reported in 8- to 15-year-old dogs.[34, 42] The disease has been reported in dogs in South Africa.[60]

Subclinically infected bitches can transmit the parasite to their foetuses, and successive litters from the same bitch may be born infected.[7, 42] Whether there is breed predisposition and differential sex susceptibility to neosporosis in dogs is not known. Most described cases have been in Labrador Retrievers, Boxers, Greyhounds, Golden Retrievers and Basset Hounds.[42]

Only limited information is available concerning the prevalence of *N. caninum* infection in dogs. Using an IFAT titre of 1:50, 0.25 to 29 per cent of dogs in several countries have been found to have *N. caninum* antibodies.[4, 19, 42, 67, 89, 93]

The most severe cases of neosporosis occur in young, congenitally infected pups. The disease in young dogs most frequently manifests as hind limb paresis (**Figure 23.11**) that develops progressively into paralysis[6, 29, 34, 41, 60, 61, 89, 99] but

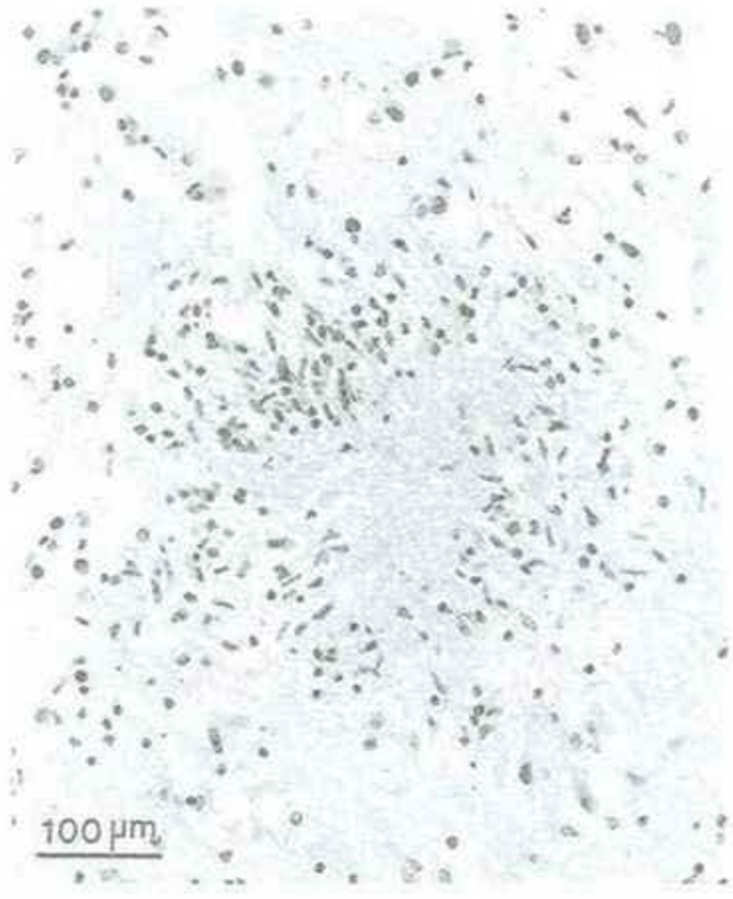


Figure 23.8 Foetal bovine brain infected with *Neospora caninum*. Note a central necrotic focus with glial cells and mononuclear cells at the periphery. Haematoxylin and eosin stain

the neurological signs are dependent on the site parasitized in the central nervous system.[5, 34] The hind limbs are generally more severely affected than the fore limbs and often exhibit rigid hyperextension. The cause of the hind limb hyperextension is not known, but is most likely caused by a combination of upper motor neuron paralysis and myositis, which results in rapidly progressive fibrous contracture of the muscles that may cause fixation of joints. Other dysfunctions which occur include difficulty in swallowing, paralysis of the jaw, muscle flaccidity, muscle atrophy and even heart failure. Dogs with hind limb paralysis may be alert and survive for months.

The disease may be localized or generalized and virtually all organs and tissues may be involved, including the skin.[34, 45] Dermatitis may be severe and large numbers of *N. caninum* tachyzoites may be associated with it.

For the ante-mortem diagnosis in young dogs, the clinical sign of an ascending paralysis, particularly if several litter mates are affected, should arouse suspicion of neosporosis. Haematological values are usually not altered but there may be increased levels of the serum enzymes associated with necrosis of myocytes and, occasionally, hepatocytes.

The finding of *N. caninum* antibodies in serum can aid the diagnosis. The IFAT has been used most often to diagnose canine neosporosis and a high titre (1:800) is often indicative of an acute infection. It should be noted, however, that high titres (1:12 800) have been shown to persist for at least four years in two dogs,[7] and clinical neosporosis (verified histologically and by isolation of the parasite) was diagnosed in two dogs with IFAT titres of 1:50.[35] There is little information on IgM production in postnatally infected dogs. In two congenitally infected animals, IgM was not detectable at a 1:10 serum dilution.[35]

The detection of *N. caninum* in biopsy tissues or cytological preparations can confirm a diagnosis. *Neospora caninum* has been detected in biopsies of muscles, lung aspirates and dermal pustular exudate. Immunohistochemical staining, however, is required to differentiate the parasite from *T. gondii* because tachyzoites of the two parasites are indistinguishable from each other by light micros-

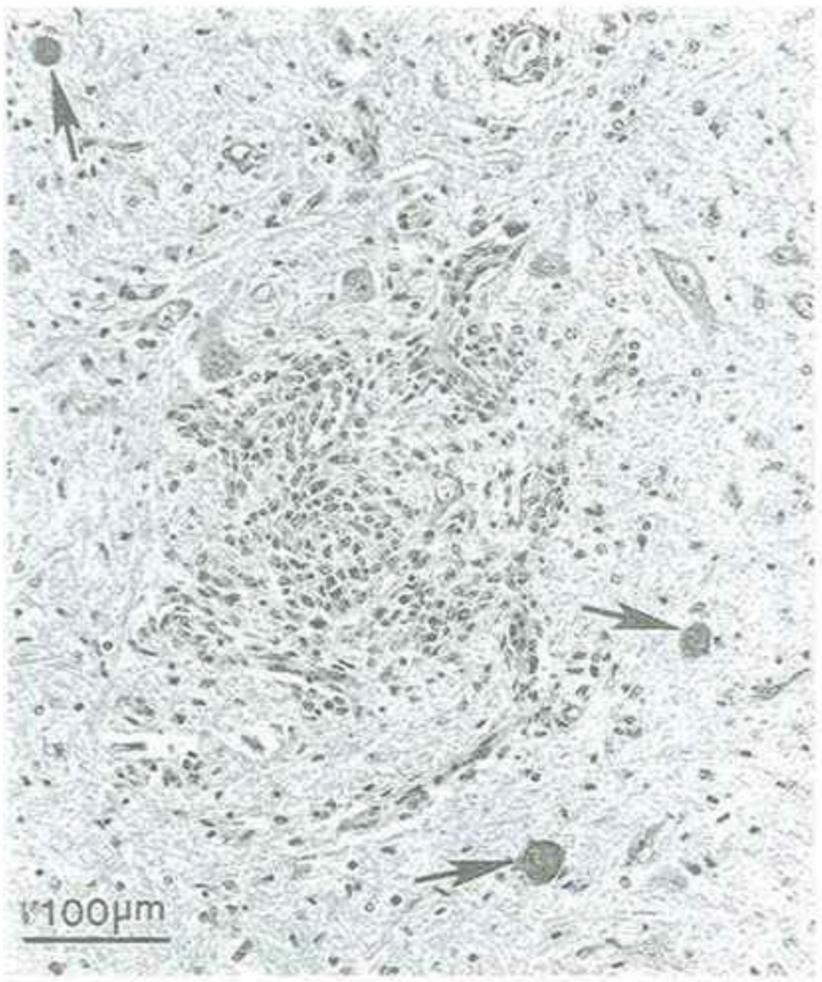


Figure 23.9 Section of the spinal cord of a calf congenitally infected with *N. caninum*. Note glial proliferation, perivascular inflammation and three tissue cysts (arrows). Immunohistochemical stain with *N. caninum* antibody

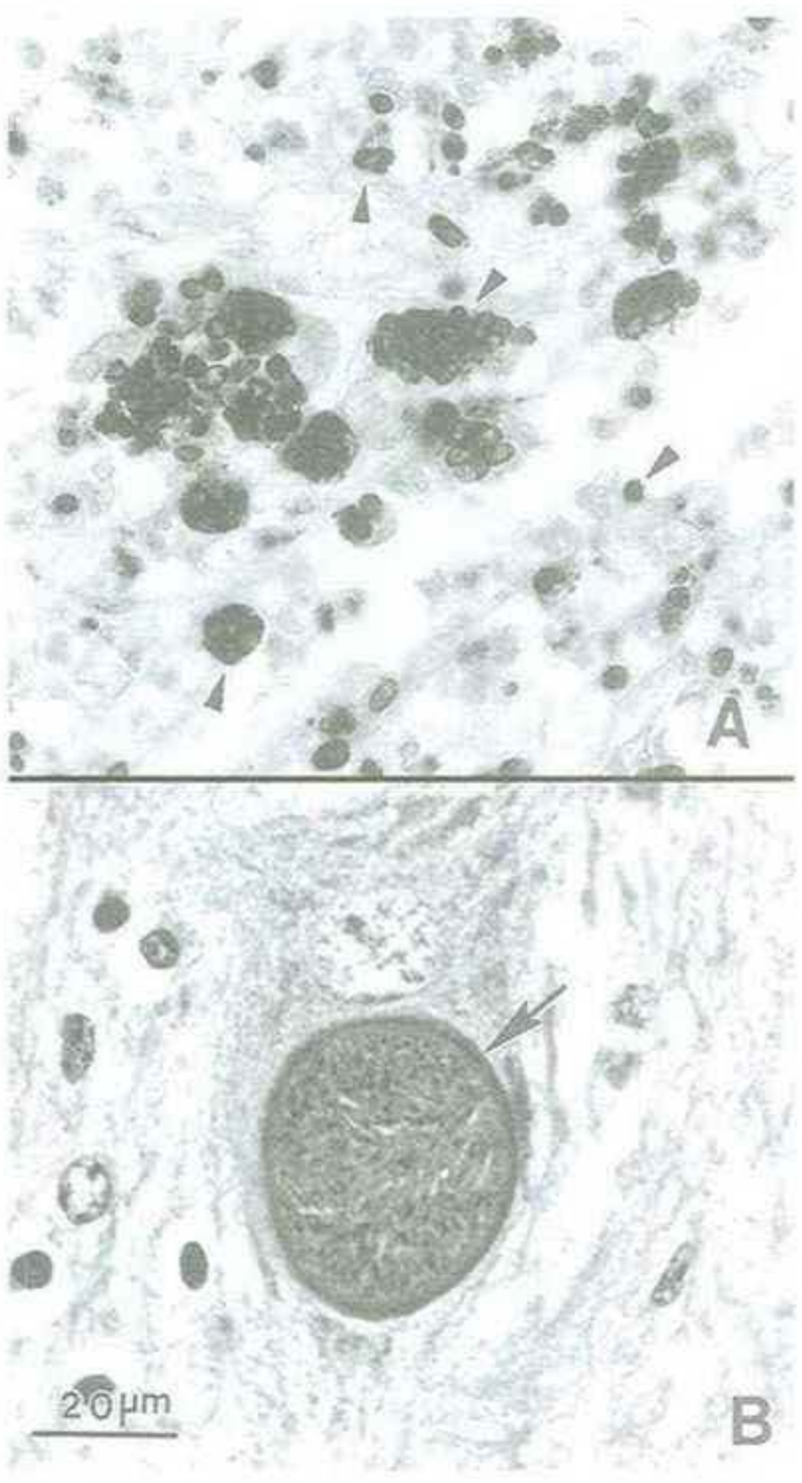


Figure 23.10 *Neospora caninum* tachyzoites and a tissue cyst stained immunohistochemically with *N. caninum* antibody

A Numerous tachyzoites (arrowheads) in the skin of a dog
B A tissue cyst (arrow) in a neuron in a congenitally infected calf



Figure 23.11 *Neospora caninum* infected dog with hind limb paralysis. The first isolate of *N. caninum* was obtained from the neural tissues of this dog in 1988. Immunohistochemical staining, however, is required to differentiate the parasite from *T. gondii* because tachyzoites of the two parasites are indistinguishable from each other by light microscopy. Cerebrospinal fluid should be examined for tachyzoites in cases manifesting hind limb paralysis

copy. Cerebrospinal fluid should be examined for tachyzoites in cases manifesting hind limb paralysis.

Post-mortem diagnosis is based on demonstrating the parasites in lesions of affected dogs. Gross lesions of neosporosis that have been reported are necrosis in the central nervous system and liver, granulomas (up to 10 mm in diameter) in visceral tissues, yellowish white streaks in muscles, particularly those of the diaphragm, cerebellar atrophy and ulcerative dermatitis.[42] Immunohistochemical staining using specific antibodies will confirm the diagnosis.

The parasites most likely to be confused with *N. caninum* are *T. gondii* and *Sarcocystis canis*. The latter divides by endopolygeny into many organisms, whereas *T. gondii* and *N. caninum* divide into two organisms by endodyogeny.

The response to treatment is dependent on the stage of disease when treatment is initiated. Treatment has been successful in some dogs suffering from early neosporosis-induced limb weakness.[6, 42, 75] Sulfonamides and/or pyrimethamine, and clindamycin have been used with partial success for the treatment of canine neosporosis. A combination of trimethoprim and sulfadiazine (Tribrissen®, Coopers Pitman-Moore) at the standard combined dosage rate of 15 mg/kg twice daily, and pyrimethamine (Daraprim®, Wellcome) at 1 mg/kg daily, all for four weeks has reversed *N. caninum*-associated paralysis in some dogs. Various preparations of clindamycin alone or in combination with trimethoprim plus sulfonamide have been used to treat neosporosis in dogs.[6, 35, 45, 74] There is no treatment currently available that will prevent a bitch from transmitting *N. caninum* to her offspring.

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24

Equine protozoal myeloencephalitis

J P DUBEY

Introduction

Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses in the Americas caused by *Sarcocystis neurona*. The clinical syndrome was recognized in the USA by Rooney[86] in the 1960s and protozoa were first demonstrated in lesions in 1974.[2, 12, 26] Initially, the protozoa were considered to be *Toxoplasma gondii*.[12, 37, 93] A protozoan was first cultured *in vitro* from the spinal cord of an affected horse in 1991 and was named *Sarcocystis neurona* by Dubey *et al*.[27] The development of a serological test in 1993[56] based on antigen prepared from *in vitro* cultured *S. neurona* merozoites led to the discovery that *S. neurona* infection is common in horses in the Americas.[3, 4, 34, 45, 100] The history of EPM and its biology have recently been reviewed.[37]

Aetiology and life cycle

Sarcocystis neurona is a coccidian belonging to the family Sarcocystidae. Opossums of the genus *Didelphis* (*D. virginiana* and *D. albiventris*) are the definitive hosts and the horse is an aberrant intermediate host (**Figure 24.1**).[21, 25, 35, 36, 49, 52] Sea otters and several other mammals are also aberrant intermediate hosts.[37]

The natural intermediate hosts of *S. neurona* are unknown. The sarcocyst stage of *S. neurona* and an experimental intermediate host were recently identified.[41] Laboratory-raised cats fed sporocysts from a naturally infected opossum developed sarcocysts in their skeletal muscles. The sarcocysts were microscopic (about 700 µm long) with a 1 to 2 µm thick cyst wall. The bradyzoites were slender and tiny (about 5 µm long). Laboratory-raised opossums fed infected cat muscles shed sporocysts. The sporocysts were 10 × 8 µm in size.

A sexual cycle occurs in the lamina propria of the intestines of opossums. After gametogony, oocysts sporulate in the lamina propria of the small intestine and are excreted in the faeces. Sporocysts contain four sporozoites and a residual body.

Horses become infected by ingesting food and water contaminated with sporocysts. The only stages of *S. neurona* found in the horse are schizonts and merozoites which are confined to the brain and spinal cord[27] (**Figure 24.2**). The time period and the route of dissemination of the parasite in the horse are unknown. In an experimental aberrant host, the immunodeficient gamma interferon knockout (KO) mouse, sporozoites excyst in the small intestine and can be found in peripheral blood within one day of feeding sporocysts.[22, 23] *Sarcocystis neurona* first multiplies in visceral tissues and eventually resides in the central nervous system (CNS).[22]

Sarcocystis neurona schizonts are found in neurons, various glial cells and resident macrophages in the brain,[26, 53, 94] and divide by endopolygeny.[94] In endopolygeny, the parasite nucleus becomes multilobed and eventually each nuclear lobe is incorporated into two budding merozoites (**Figures 24.3 and 24.4**). Schizonts in tissue sections are approximately 5–35 × 5–20 µm in size and contain up to 40 merozoites, often arranged in a rosette sometimes around a residual body[37] (**Figure 24 4**). Merozoites are approximately 5 × 1 µm in size. Ultrastructurally, *S. neurona* schizonts are located in the host cell cytoplasm without a parasitophorous vacuole (**Figure 24.4**). Merozoites contain conoid, micronemes, a nucleus, a prominent lipid body posterior to the nucleus, but no rhoptries[37, 94] (**Figure 24.4**).

Epidemiology

Equine protozoal myeloencephalitis has been reported only in horses born and raised in the Americas, including Canada, the USA, Panama and Brazil.[1, 5–8, 18, 26, 38, 39, 46, 48, 51, 55, 57, 61, 63, 72–74, 81, 89] Although fatal EPM has been diagnosed in other parts of the world, including South Africa,[85] it occurs only in horses imported from the Americas. The distribution of EPM follows the geographic range of the

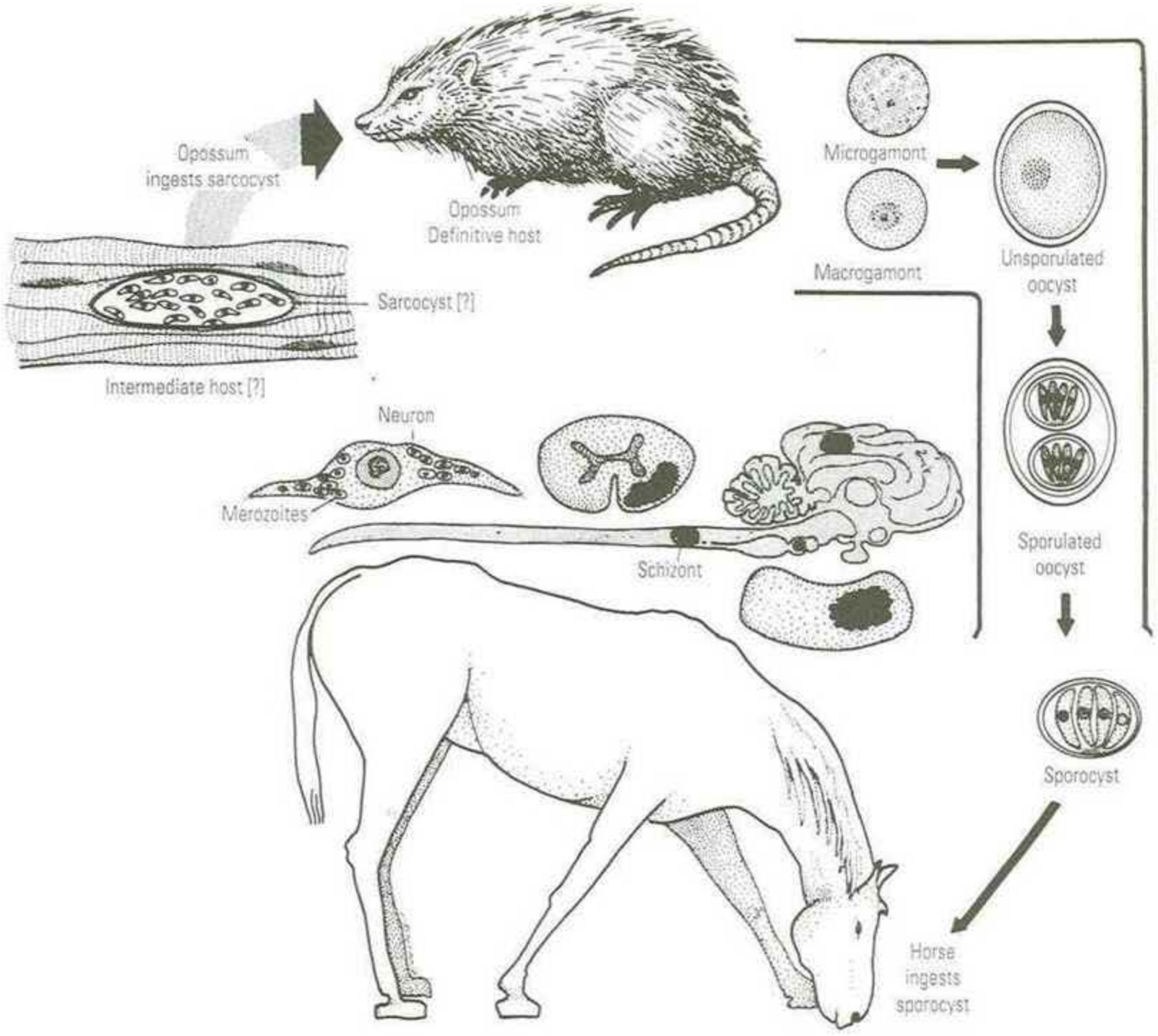


Figure 24.1 Life cycle of *Sarcocystis neurona*

opossum (*D. virginiana*) in North America and its relative *D. albiventris* in Central and South America.[36] Serological surveys indicate that about 30 to 50 per cent of horses in the USA, Brazil, and Argentina have been exposed to *S. neurona*.[3, 4, 34, 45, 99] Although antibodies to *S. neurona* have been found in horses, donkeys, and mules,[88] bona fide clinical disease has not been reported in animals other than horses, with the exception of a Grant's zebra (*Equus burchelli bohmi*) in a zoo.[40, 80] The prevalence of *S. neurona* antibody increases with age and is not related to breed, sex, and category (race/draught) of the horse.[88] Although horses of any breed or age may suffer from EPM, clinical disease occurs most frequently in three- to five-year-old Thoroughbreds and Standardbreds. There are no documented cases of congenital *S. neurona* infection.

Sarcocystis neurona-like organisms have been found in the CNS of raccoons (*Procyon lotor*)[29, 31, 44, 96, 98] (**Figures 24.5 and 24.6**), mink (*Mustela vison*),[32] skunks (*Mephitis mephitis*),[30] a domestic cat,[33] Pacific harbour seals (*Phoca vitulina richardsi*),[65] Southern sea otters (*Enhydra lutris nereis*),[71, 87] and a rhesus monkey (*Macaca mulatta*).[64] The significance of these hosts in the life cycle of *S. neurona* is unknown. *Sarcocystis neurona* infection in a sea otter was confirmed by isolation of the live parasite.[71] Experimentally, an EPM-like disease can be induced in immunodeficient mice.[22, 35, 37, 75] Up to the present, *S. neurona* has been recovered from the CNS of only horses and sea otters; the other reports are based on immunohistochemical examination with the exception of the parasite in the monkey which was not confirmed by this means.[29]

Opossums are also definitive hosts for at least two other species of *Sarcocystis*, *S. falcatula* and *S. speeri*.[21, 35, 37, 79] Sporocysts of all three species have not been morphologically distinguished but they are genetically related.[13, 16, 37, 97]

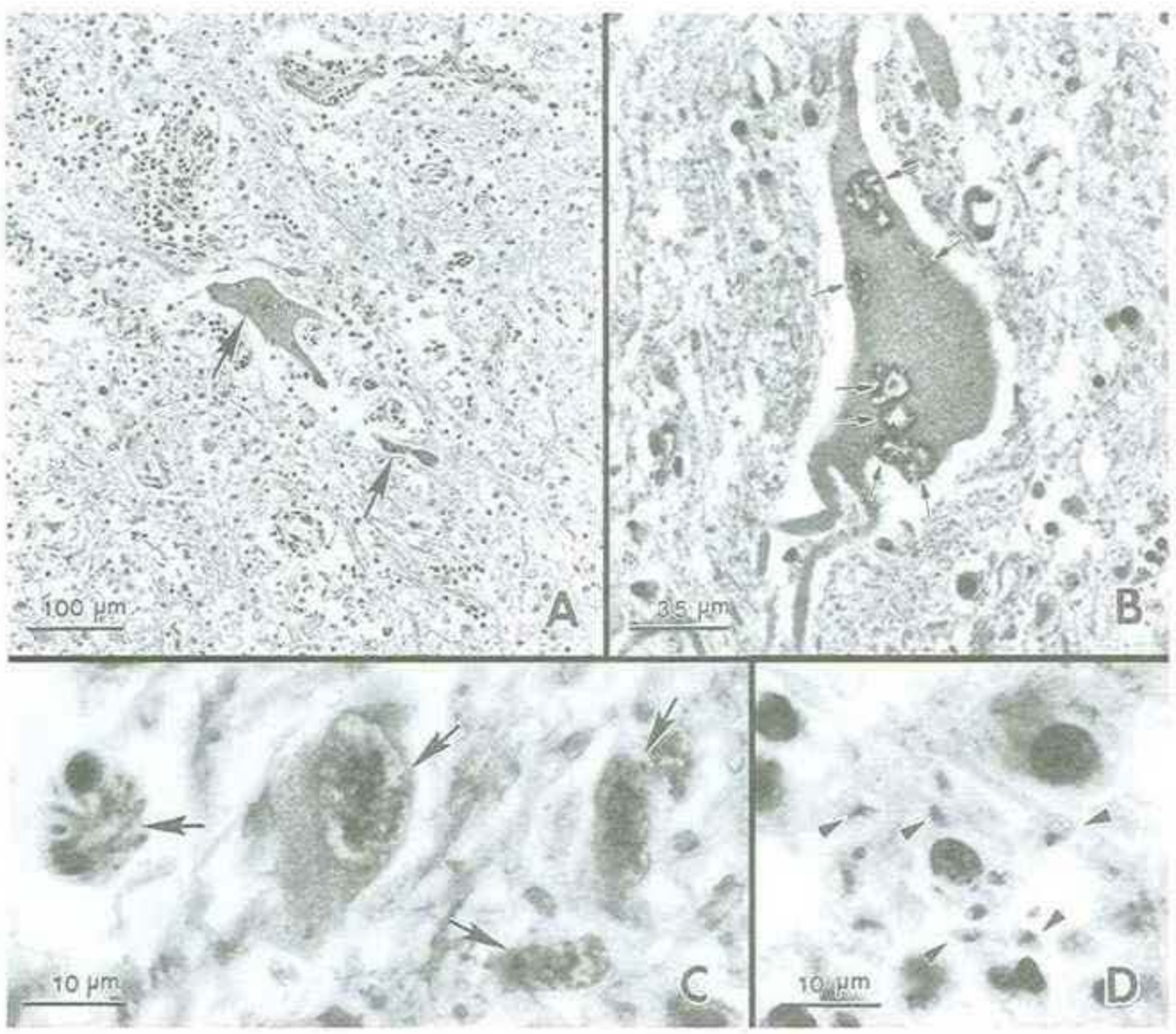


Figure 24.2 *Sarcocystis neurona* in sections of the spinal cord of a horse. Haematoxylin and eosin stain

A Perivascular infiltration and necrosis. Two infected neurons (arrows) but parasites are barely visible at this magnification
B Neuron containing several schizonts (arrows)
C Four schizonts (arrows) in various stages of development
D Intracellular and extracellular individual merozoites (arrow heads)

The prevalence of *S. neurona* in the faeces of opossums is unknown. In one survey, *Sarcocystis* species sporocysts were found in 51 per cent of 47 road-killed opossums. Based on bioassays of 23 infected opossums, using immunodeficient mice and budgerigars (*Melopsittacus undulatus*), 21 had *S. falcatula*, eight had *S. neurona* and eight had *S. speeri*.[21] In another survey, *S. neurona* was identified in the intestines of 19 of 72 opossums from Mississippi, USA.[25]

Clinical signs

Because *S. neurona* can infect any region of the brain and spinal cord, clinical signs are related to the region affected.[37, 72, 73] For example, EPM lesions in the spinal cord may result in demarcated areas of spontaneous sweating or in loss of reflexes and cutaneous sensation.[72] There may be foot dragging, difficulty in backing, or ataxia of the hind limbs or of all the limbs. Horses with lesions in the brain stem may be depressed, and have vestibular nerve dysfunction.[72] There may be muscular atrophy, particularly of the gluteal muscles. Clinical signs may be sudden or progress over a period of weeks; horses are known to have collapsed while racing.

Pathology

Lesions in horses are confined to the CNS but occur more commonly in the spinal cord than in the brain. In the brain, the brain stem is affected more frequently than the cerebrum. Gross lesions consist of focal haemorrhages and areas of discoloration (from red to dark brown) 1 to 30 mm in extent (**Figure 24.7**). Any region of the spinal cord may be affected, and lesions may be confined to one segment of the spinal cord or be dispersed.[2, 6, 10, 12, 20, 26, 37]

Microscopically, an encephalomyelitis characterized by haemorrhages in, and necrosis of, the neuropil as well as an infiltration of mononuclear cells in the parenchyma and perivascularly, is observed. Chronic lesions consist of glial nodules and scattered areas of mononuclear cell infiltrations. *Sarcocystis neurona* parasites are usually associated with the lesions and may be scant or numerous, but some may be located away from the lesions. In most cases of EPM

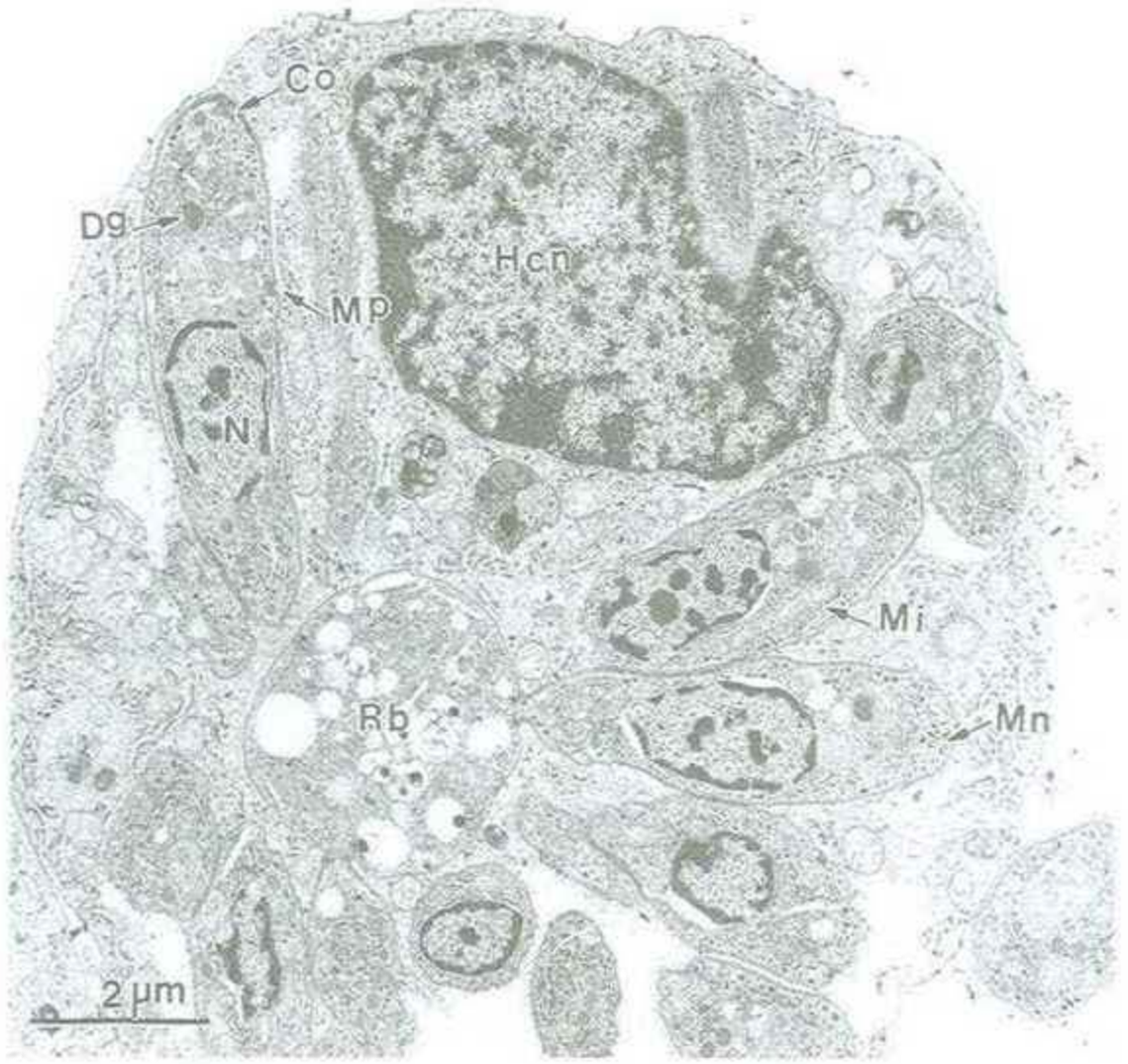


Figure 24.3 Transmission electron micrograph of a schizont of *Sarcocystis neurona* in a bovine monocyte showing merozoites budding from residual body (Rb). Some of the merozoites are still attached (arrowheads) to the residual body; conoid (Co); dense granule (Dg); host cell nucleus (Hcn); mitochondrion (Mi); microneme (Mn); micropore (Mp); nucleus of merozoite (N)

only a few organisms are found in the lesions. Schizonts and merozoites are more generally detected in the cytoplasm of cells (neurons, glial cells and macrophages) and rarely in vascular endothelial cells. In sections stained with haematoxylin and eosin (HE) organisms are not distinctive. Immunohistochemistry (IHC) reveals many more organisms than in sections stained only with HE (**Figure 24.8**).

Diagnosis and differential diagnosis

The ante-mortem diagnosis of EPM is based on the clinical signs and the detection of *S. neurona*-specific antibodies in the cerebrospinal fluid (CSF).[11, 37, 56, 72, 73, 83] The detection of antibodies in serum only indicates exposure and not necessarily clinical disease, but their detection in CSF indicates a local production of specific antibodies due to invasion of the CNS by the organism, or their non-specific presence resulting from the contamination of CSF by blood or leakage of antibodies from blood associated with increased permeability of the blood–brain barrier (caused by e.g. trauma, or encephalitis or myelitis due to other causes).[11, 72] A knowledge of the prevalence rate of subclinical *S. neurona* infection in the local equine population assists in the evaluation of serological results.

The diagnostic test used commercially to detect *S. neurona*-specific antibodies is an immunoblot using culture-derived *S. neurona* merozoite antigen.[37, 56] Antibodies to bands of molecular weights 14.5, 13 and 7 KD are considered diagnostic for *S. neurona* infection.[37] Because other species of *Sarcocystis* in the horse (e.g. *S. fayeri*) may cross-react serologically in the indirect fluorescent antibody test (IFAT) or other tests, these are not reliable for the diagnosis of EPM.

The polymerase chain reaction (PCR) may also be used for the detection of *S. neurona* DNA in CSF.[72] Although the test is specific, it has low sensitivity. However, PCR may be positive in acute cases before antibodies are detectable in the CSF. The combination of immunoblot and PCR increases sensitivity. Several tissue culture cell lines support the growth of *S. neurona* for *in vitro* isolation of the parasite from equine tissues.[37]

At present, there is no commercially available *S. neurona*-specific serum for the identification of *S. neurona*. Because polyclonal *Sarcocystis* sera produced against other species of *Sarcocystis* (e.g. *S. cruzi* of cattle) cross-react with *S. neurona*, these polyclonal sera have been used to identify *S. neurona*-like organisms.[57] Recently, high titre *S. neurona*-specific sera have been prepared in rabbits that specifically stain *S. neurona* in equine tissues.[29]

Other protozoa that may be confused microscopically with *S. neurona* are other *Sarcocystis* spp.,[60] *Neospora* spp.[15, 62, 77, 78] and *Toxoplasma gondii*.[24] *Sarcocystis* spp.[60] schizonts are generally found in vascular endothelium.[42] *Neospora* spp. divide by endodyogeny and there is no schizont stage, whereas *Sarcocystis* spp. schizonts divide by

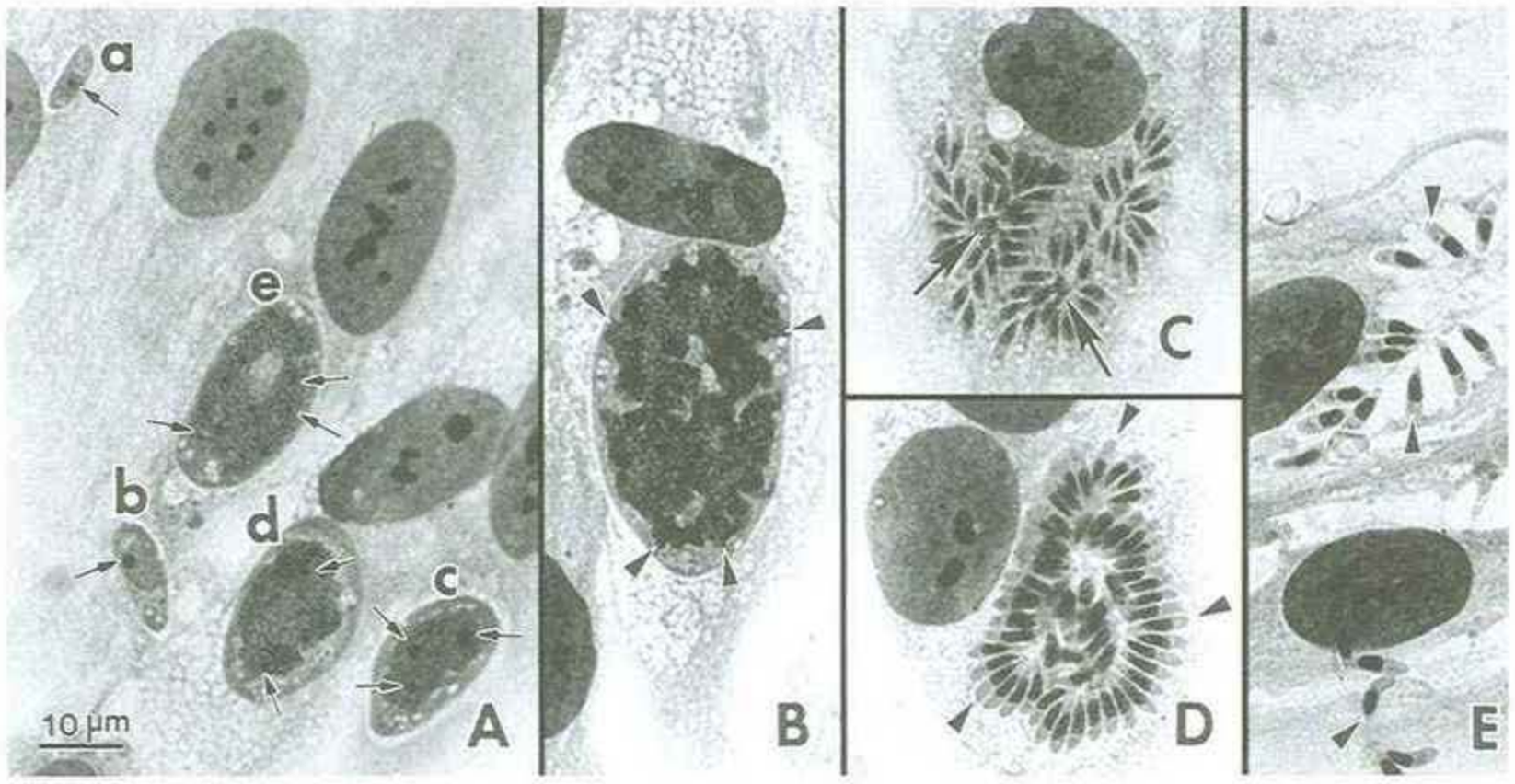


Figure 24.4 *In vitro* developmental stages of *Sarcocystis neurona* in bovine turbinate cells. Giemsa stain

A Immature schizonts with progressively differentiating nuclei from *a* to *b*. The nucleus in *a* and in *b* contains a single nucleolus whereas in *c* to *e* the nucleus has become lobed and has several nucleoli (arrows)
B Immature schizont with multilobed (arrrowheads) nucleus
C Three schizonts with merozoites arround a residual body (arrows)
D Mature schizont with peripherally situated merozoites (arrowheads). Some merozoites are at the centre of the schizont
E Free merozoites (arrowheads)

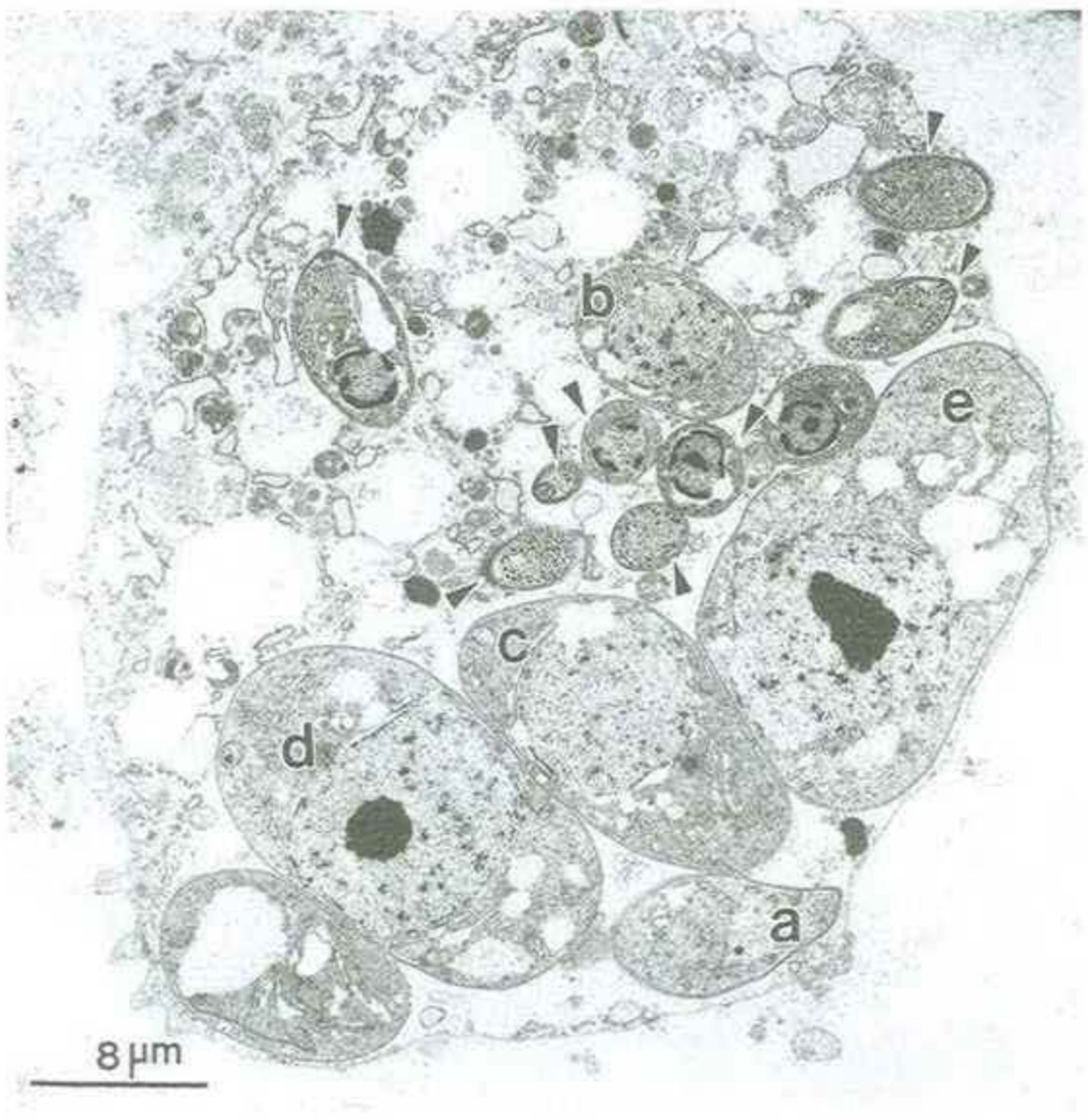


Figure 24.5 Degenerating host cell with eight individual merozoites and five developing schizonts (*a* to *e*) with single large nucleus. *Sarcocystis neurona* merozoites can form new schizonts without leaving the host cell. (From Dubey *et al.*[44])

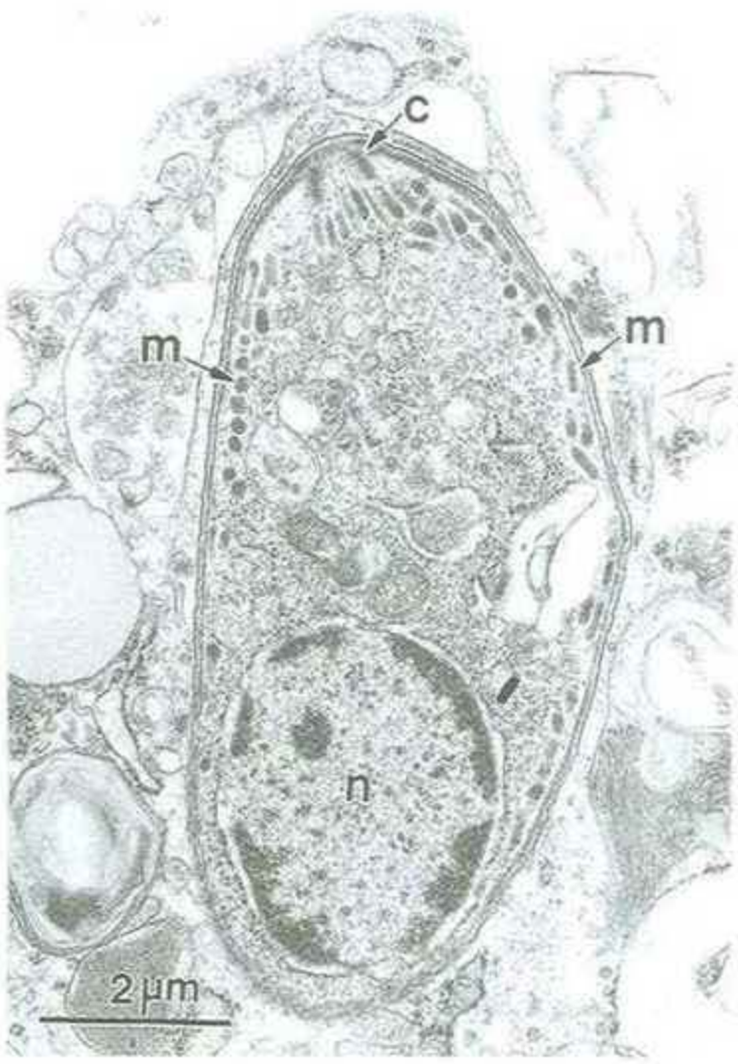


Figure 24.6 Transmission electron micrograph of a *Sarcocystis neurona* merozoite in the brain of the raccoon in Figure 24.5. Longitudinal section of a merozoite. Note the conoid (c), micronemes (m) and nucleus (n) but no rhoptries. (From Dubey *et al.*[44])

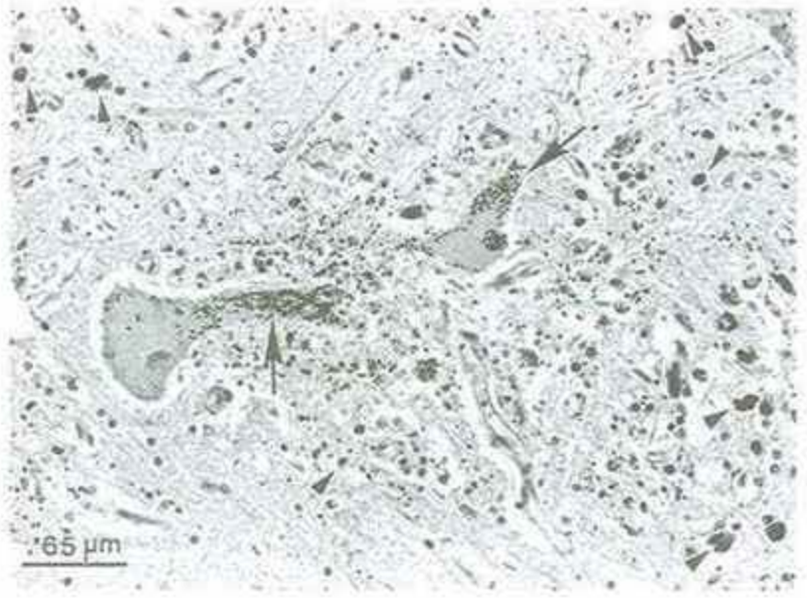


Figure 24.8 Section of a spinal cord of a horse heavily infected with *Sarcocystis neurona*. Numerous merozoites (arrows) and schizonts (arrowheads) are present in neurons (arrows). All black areas are *S. neurona*. Immunohistochemical stain with *S. neurona* antibody



Figure 24.7 Cross-section of the spinal cord of a horse that had suffered from equine protozoal myeloencephalitis. Note area of discoloration (arrow)

endopolygeny. As yet, no clinically confirmed case of toxoplasmosis in the horse has been diagnosed.[42] *Sarcocystis neurona* schizonts in the horse are confined to the CNS. A case of hepatic sarcocystosis has recently been recognized in a horse;[17] this protozoan did not react with anti-*S. neurona* antibodies.

Equine protozoal myeloencephalitis should be considered in the differential diagnosis of any disease of the horse associated with impairment of function of the CNS, such as neosporosis **(see Chapter 23: Neosporosis)**,[15, 62, 77, 78] neurological involvement as a result of equid herpesvirus-1 infection, rabies, equine encephalitides caused by alphavirus infections, and cervical stenosis.[72, 73]

Control

Horses suffering from EPM have been treated empirically with sulfadiazine and pyrimethamine, although these compounds have not been specifically tested against *S. neurona*-induced infections in horses.[37, 72, 73, 101] Current recommendations are 20 mg/kg of sulfadiazine and 1 mg/kg of pyrimethamine orally in liquid formulations daily for 90 days. Prolonged use of these drugs may induce toxicity of haematopoietic tissues, and supplementation with 40 mg daily of folinic acid may be needed in horses suffering from such toxicity. Diclazuril, an anticoccidial agent used in poultry, has also been used empirically to treat EPM.[59] The dosage rate recommended is 2.5 to 5.5 mg/kg of diclazuril for 28 days. Prophylactic treatment with diclazuril prevents clinical EPM infection in KO mice.[28] Sulfadiazine, pyrimethamine, and diclazuril can prevent multiplication, or even kill, *S. neurona* merozoites in a cell culture system[67, 68] but they have not been scientifically evaluated *in vivo* in animals known to be infected with *S. neurona*.

There is no proven vaccine to control EPM but there is work in progress to identify protective antigens.[37, 66]

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BABESIOSES

Babesiosis is caused by infection with species of tick-borne, intra-erythrocytic and generally host-specific protozoan parasites of the genus *Babesia* which belong to the phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, suborder Piroplasmorina and family Babesiidae. It occurs in a wide variety of vertebrate hosts, including humans, and has a very wide distribution around the world. In southern Africa the resulting disease is often given one or more colloquial names in the regions where it occurs, such as redwater or rooiwater (Afrik.) in cattle, and biliary fever or galkoors (Afrik.) in horses and dogs. The general disease manifestations are similar in all vertebrate host species and, as the popular names imply, tend to be characterized in varying degrees by intravascular haemolysis, haemoglobinaemia and the development of haemoglobinuria (which is responsible for the urine being coloured red), and icterus. *Babesia equi* is thought also to have a stage of development in lymphocytes of the equine host.

The genus *Babesia* is named after Dr Victor Babes who, in 1887, established the aetiology of a cattle disease in Romania associated with haemoglobinaemia. The disease in cattle is particularly prevalent in tropical and subtropical countries where the vector ticks are widespread.

In southern Africa, bovine babesiosis and other tick-borne diseases such as heartwater, gallsickness (anaplasmosis) and some of the theilerioses (e.g. East Coast fever, Corridor disease, and Zimbabwe theileriosis) are of considerable economic importance. The true importance of equine babesiosis in the subcontinent in Africa has not been adequately assessed, but it remains an important disease. In addition, it is a hindrance to the free movement of horses, as many countries prohibit their importation from southern Africa. Outbreaks of porcine babesiosis are rare and are not of economic importance. *Babesia motasi* and *Babesia ovis* infections in sheep and goats have not been recorded in southern Africa. Biliary fever in dogs caused by *Babesia canis* remains a very important, if not the most important, infectious disease of dogs and is widespread in southern Africa. On the other hand, the prevalence of *Babesia felis* infection in cats is low and encountered mainly in the coastal parts of KwaZulu-Natal and the Eastern and Western Cape provinces of South Africa. Babesiosis is a zoonosis, but cases of disease in humans caused by *Babesia* spp. are rare and usually occur in immuno-compromised (e.g. splenectomized) patients. However, on Nantucket Island and the island off the coast of Massachusetts and New York in the USA, babesiosis caused by the rodent parasite *Babesia microti* has been reported in immunocompetent humans.

25

Bovine babesiosis

Synonyms: Redwater, Asiatic (European) redwater (*Babesia bovis* infection), African redwater (*Babesia bigemina* infection), rooiwater (Afrik.)

A J DE VOS, D T DE WAAL AND L A JACKSON

Introduction

Bovine babesiosis, or redwater as it is commonly known, is a tick-borne disease caused by the intra-erythrocytic protozoan parasite *Babesia*. Four species are known to infect cattle in southern Africa, namely *Babesia bovis*,[39, 48] *Babesia bigemina*,[39, 48] *Babesia occultans*[65] and an as yet unnamed species.[49] Of these species, only *B. bovis* and *B. bigemina* are economically important. The acute disease is characterized by haemolysis, circulatory disorders in the case of *B. bovis*, and death may follow in some instances. European breeds of cattle are particularly susceptible although the disease can also be economically important in Sanga and Zebu breeds. A clinically inapparent form of the disease is common in young animals, and recovered animals become latent carriers for variable periods. Recovery is followed by a lasting immunity to the infecting parasite. Cross-immunity between the two organisms is limited.

Babesia bovis was first reported in southern Africa in 1941[110] although clinical evidence of its presence in South Africa was recorded as early as 1905.[119] It was probably introduced with the Asian blue tick *Boophilus microplus* during the latter part of the nineteenth century (**see Chapter 1: Vectors: Ticks**).

Babesia bigemina is principally transmitted by *B. microplus* and by the common, indigenous African blue tick *(Boophilus decoloratus)*. This parasite would therefore have been endemic in the subcontinent for centuries. Indeed, redwater is said to have been the cause of high cattle mortality in the present-day KwaZulu-Natal Province of South Africa prior to European settlement in the 1830s.[48]

Several accounts have been published of the history of redwater and other tick-borne diseases in southern Africa, as well as of the people involved in the early battles against these diseases. These reports are well worth reading.[67, 87, 137, 139, 146]

Redwater had a marked effect on the livestock industries in the subcontinent early in the twentieth century, especially in imported cattle.[87, 139] However, its impact was masked by a devastating East Coast fever epidemic (**see Chapter 29: East Coast fever**). The campaign to control this epidemic by intensive regulatory control of the vector *Rhipicephalus appendiculatus* lasted half a century and had a big effect on the single-host *Boophilus* spp., ensuring that redwater remained of secondary importance for many decades.

In the latter part of the twentieth century, relaxation or breakdown of tick control measures and the advent of acaricide resistance in *Boophilus* spp. caused redwater to increase in prevalence and significance to the point where it became recognized as one of the most important livestock diseases in southern Africa.[8, 48, 86, 112] During 1972 the disease caused the loss of 10 000 cattle in the Transkei region of the Eastern Cape alone[39] and contributed to the death of a million cattle during the war in Zimbabwe.[112] A survey of farmers in the heartwater-endemic regions of South Africa in 1993 indicated that losses of 0.3 per cent of cattle were ascribed to babesiosis[50] (**see Chapter 1: Vectors: Ticks**).

Although the diseases caused by *B. bovis* and *B. bigemina* are clinically very similar, it is important to separate them for a number of reasons, hence the names Asiatic (European) and African redwater, respectively, have come into common use. While *B. bovis* is the more virulent of the two parasites, *B. bigemina* is probably more important in southern Africa because of its wide distribution. In this review, we pay more attention to *B. bovis*, mainly because it has been studied more intensively throughout the world and is much better understood than *B. bigemina*. Information obtained from southern and eastern Africa is emphasized but reference to relevant literature from elsewhere is also made.

Aetiology and life cycle

The genus *Babesia* belongs to the phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, suborder Piroplasmorina and family Babesiidae.[3, 88, 89] The *Babesia* spp. known to infect cattle in southern Africa, and their proven vectors, are listed in **Table 25.1**.

Table 25.1 Bovine *Babesia* spp. and their vectors in southern Africa

BABESIA SPP.	VECTORS	REFERENCES
Babesia bovis	*Boophilus microplus*	119
Babesia bigemina	*Boophilus decoloratus*	111, 119, 121
	Boophilus microplus	119
	Rhipicephalus evertsi evertsi	22
Babesia occultans	*Hyalomma marginatum rufipes*	55
Unnamed *Babesia* sp.	*Hyalomma truncatum*	49

Babesia bovis is classically known as a 'small' *Babesia* measuring up to 2 µm in diameter, while *B. bigemina* is larger and can extend to the full diameter of an erythrocyte.[119] However, the two species both show considerable morphological variation, making it difficult to identify one from the other.[24] Large forms of *B. bovis* are quite common.[24, 119]

Single *B. bovis* organisms are round, oval or irregular in shape while paired forms are piriform or club-shaped. The angle between the paired organisms is often, but not invariably, obtuse (**Figure 25.1**).[71, 119]

Single forms of *B. bigemina* are elongated or amoeboid in shape and contain fine cytoplasmic filaments. Paired forms are typically piriform with an acute angle between the merozoites (**Figure 25.2**).[71, 119]

Boophilus microplus is the only known tick vector of *B. bovis* in southern Africa.[119] Transmission is transovarial, with engorging adult ticks ingesting the parasites and the larval ticks of the next generation transmitting the infection to the host[57, 119] (**Figure 25.3**). Ensuing stages are not infected.

Confirmed vectors of *B. bigemina* include *B. microplus*,[22] *B. decoloratus*[119, 121] and *Rhipicephalus evertsi evertsi*[22] (**Table 25.1**). Earlier reports included *Rhipicephalus appendiculatus* as a vector, but this has not been confirmed.[22, 125] Transmission by *Boophilus* spp. is transovarial, with engorging adult ticks becoming infected in the same way as *B. bovis*, but in this instance the infection is transmitted to cattle by the nymphal and adult stages of the next generation (**Figure 25.3**). There is some evidence of vertical transmission in the tick, with infection passing from one tick generation to the next in the absence of reinfection.[57, 66] In addition, wandering male *Boophilus* are known to migrate to uninfested cattle[100] and may play a part in the transmission of *B. bigemina*.[36] Transmission by *R. e. evertsi* is transovarial, and only the nymphal stage infects the bovine host.[22]

Hyalomma marginatum rufipes is the only known vector of *B. occultans;* transmission is transovarial with infection of cattle by nymphal and adult stages of the next generation.[65]

Extracts of infected ticks have been used to infect cattle experimentally.[124] Both *B. bovis* and *B. bigemina* are easily transmitted by subinoculation of infected blood, a fact which forms the basis of vaccination using live parasites.

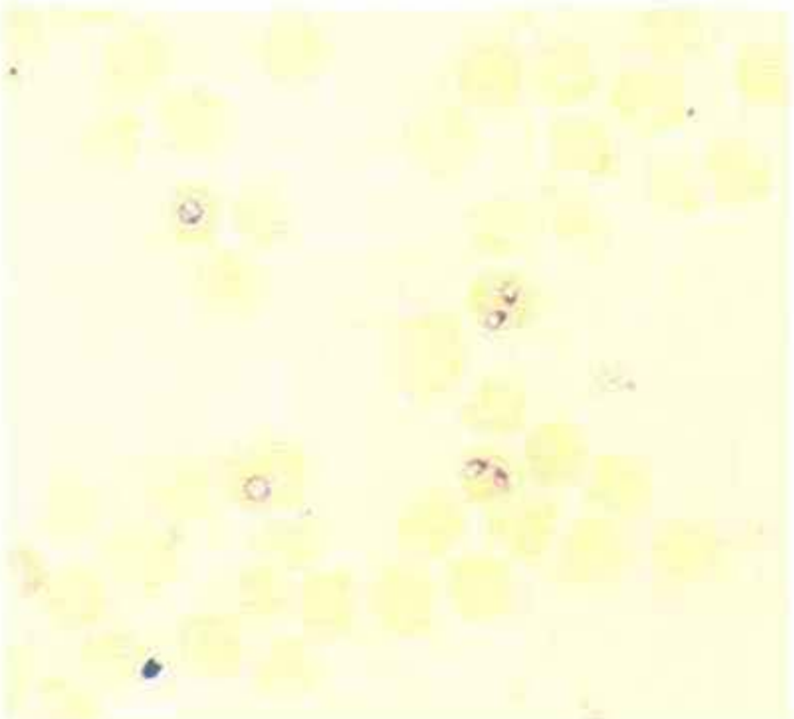


Figure 25.1 *Babesia bovis* in a blood smear

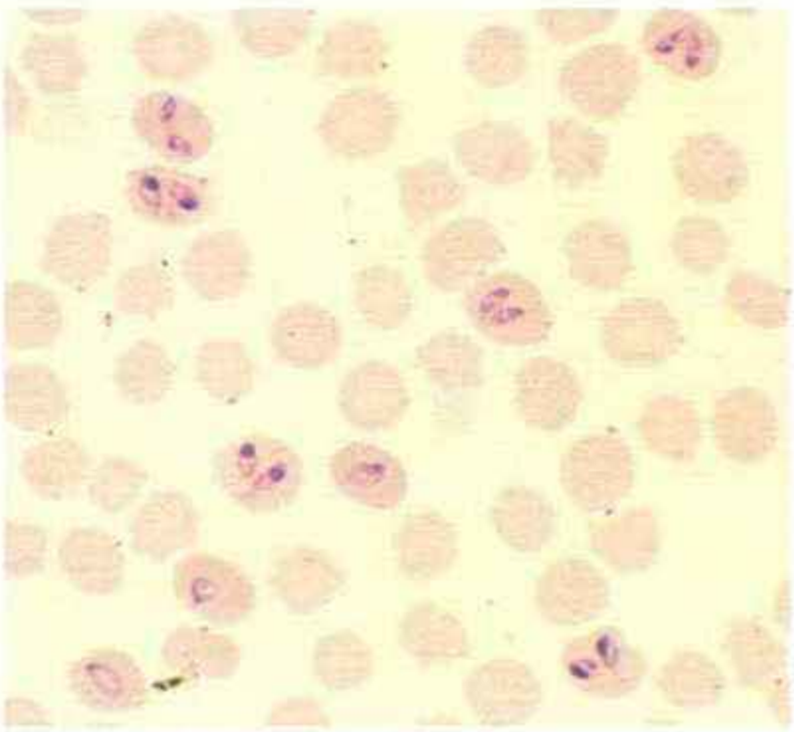


Figure 25.2 *Babesia bigemina* in a blood smear

Despite the fact that one parasite inoculated intravenously is capable of inducing infection,[60] there is no evidence for iatrogenic or mechanical transmission by biting flies. Intrauterine infection has been reported, but is rare.

It appears that isolates of *Babesia* spp. are mixtures of subpopulations differing genotypically and in biological characteristics.[32] Selection of different subpopulations or strains may occur during artificial passage in cattle or during growth *in vitro* causing some of these characteristics to change, including virulence, transmissibility by ticks and immunogenicity. These changes induced by passaging or maintenance have particular significance in the production and effect of live blood vaccines.[10, 37] Cloning experiments have shown these subpopulations to be separable and to differ in virulence, protein profiles and antigenic composition.[60, 141]

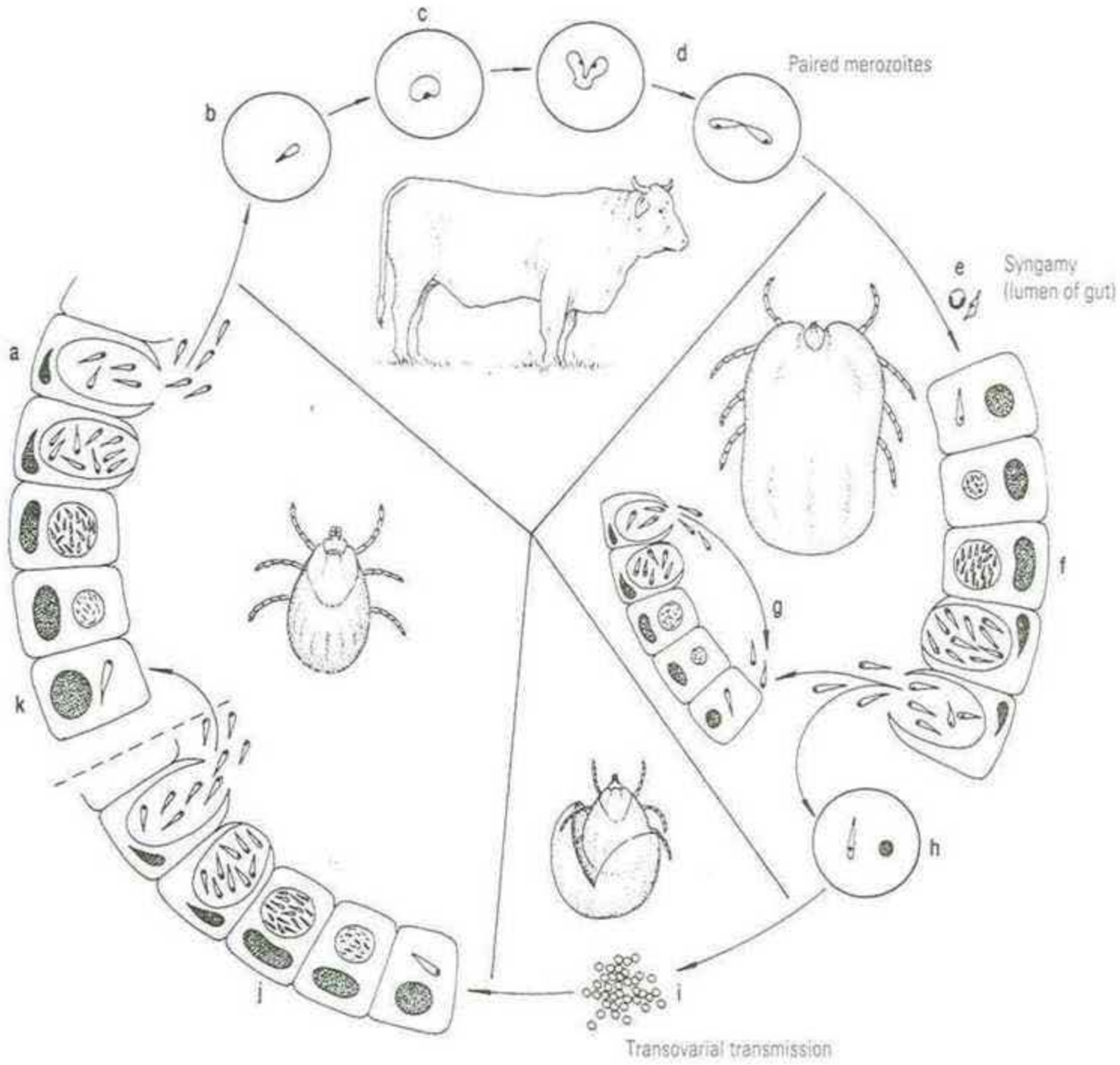


Figure 25.3 Life cycle of bovine *Babesia* spp.

a = sporozoites released from tick salivary glands
b = sporozoites invade erythrocytes
c = sporozoites in erythrocytes become trophozoites
d = limited merogony (schizogony) whereby a pair of merozoites is produced
e = syngamy follows in the tick gut after ingestion of infected erythrocytes by the engorging female
f = ookinetes penetrate gut epithelial cells and undergo schizogony (merogony) to produce merozoites
g = merozoites (sporokinetes) undergo secondary schizogony (merogony) and also find their way into developing ova (h)
i = oviposition constitutes infected eggs (transovarial transmission)
j = replication in infected larvae by schizogony in the gut of the infected larva
k = sporokinetes eventually invade the salivary glands where they undergo sporogony to produce sporozoites

Repeated serial passages of infected blood exert selection pressures on the parasite populations, which may result in reduced infectivity of *B. bovis* for ticks[101, 132] and reduced virulence of both species for cattle.[33, 40]

The development of *Babesia* spp. in ticks has been reviewed by Friedhoff.[57] Despite many detailed studies, our understanding of the life cycles of *Babesia* spp. is still incomplete.[24] Some of the more recent investigations indicate that sexual reproduction does take place.[104] To prevent confusion about the terminology used in the literature to describe different developmental stages in the life cycle, alternatives are given below, in parentheses.

Babesia bovis and *B. bigemina* follow similar developmental patterns in adult *Boophilus* spp.[57, 119, 120] Initial development takes place in basophilic epithelial cells of the gut wall[1] where, after reported fusion (syngamy) of gametes in the intestinal lumen, schizogony (multiple fission) occurs with the formation of distinctive, large merozoites (vermicules, sporokinetes)[119] (**Figures 25.4 and 25.5**). Successive cycles of schizogony then occur within a variety of cell types

and tissues, including the oocytes. Thus, transovarial transmission occurs with further development taking place in the larval stage. In the case of *B. bovis*, a final cycle of schizogony (sporogony) takes place within cells in the salivary glands of feeding larvae leading to the formation of small merozoites (sporozoites)[120] **(Figure 25.6 and 25.7)**. These small merozoites are infective for cattle **(Figure 25.3)**. In *B. bigemina*, some development takes place in the feeding larvae, but schizogony in the salivary glands only occurs in the nymphal and adult stages[121] **(Figure 25.3)**.

In cattle, *Babesia* spp. develop only in the erythrocytes. Each merozoite penetrates the cell membrane with the aid of a specialized apical complex[122, 123] **(Figure 25.8)**. Once inside, it transforms into a trophozoite from which two new merozoites develop by a process of merogony[57, 122, 123] **(Figure 25.9)**. In stained blood films of acutely affected cattle, single trophozoites and typical paired merozoites are readily detected, but dividing forms are less common. Cells containing more than two parasites are rare. Parasitaemias of up to 1 and 5 per cent respectively are common for *B. bovis* and *B. bigemina*, but can exceed 20 per cent at the peak of the reaction. Recovered cattle retain a latent infection varying from six months to several years with detectable recrudescences of parasitaemia occurring at irregular intervals.[92] The mechanisms used by these parasites to evade immune mechanisms of the host have been reviewed.[2]

Epidemiology

Numerous factors influence the prevalence of redwater. In this discussion, the factors affecting the vector, host and parasite, as well as the concepts of endemic (enzootic) stability and epidemic (epizootic) spread, will be considered.



Figure 25.4 Large merozoites in a smear made from the haemolymph of a tick

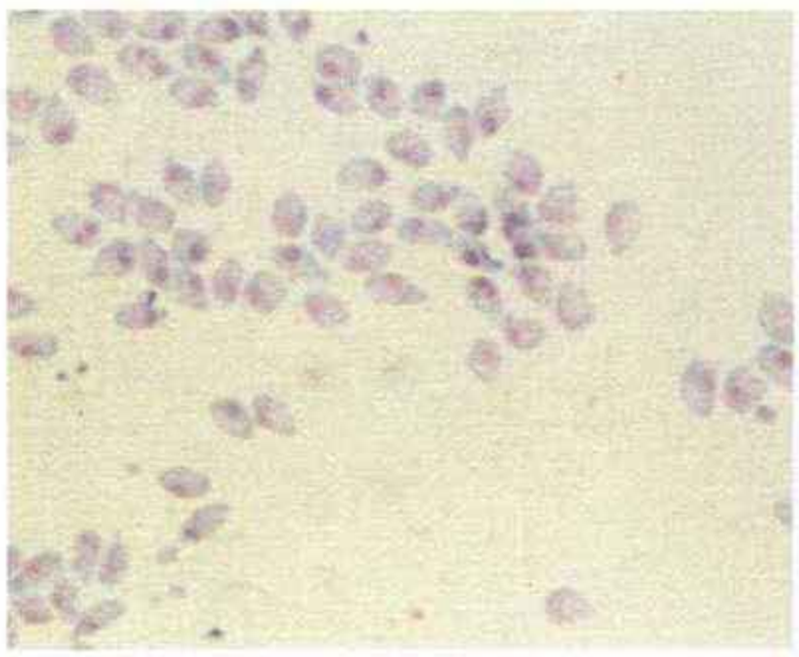


Figure 25.6 Small merozoites in a smear made from the salivary gland of a tick

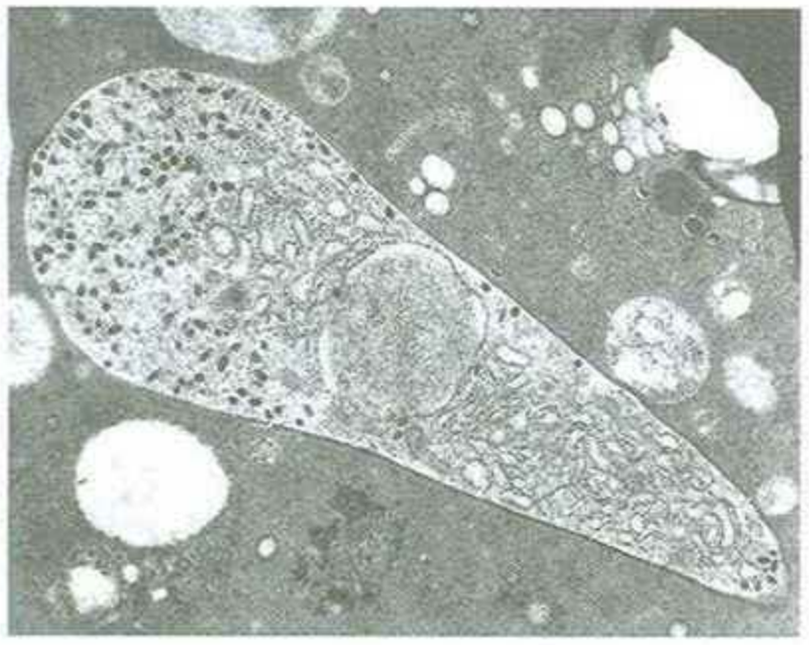


Figure 25.5 Longitudinal section through a mature, large merozoite of *Babesia bovis* in the tick gut, × 43 000

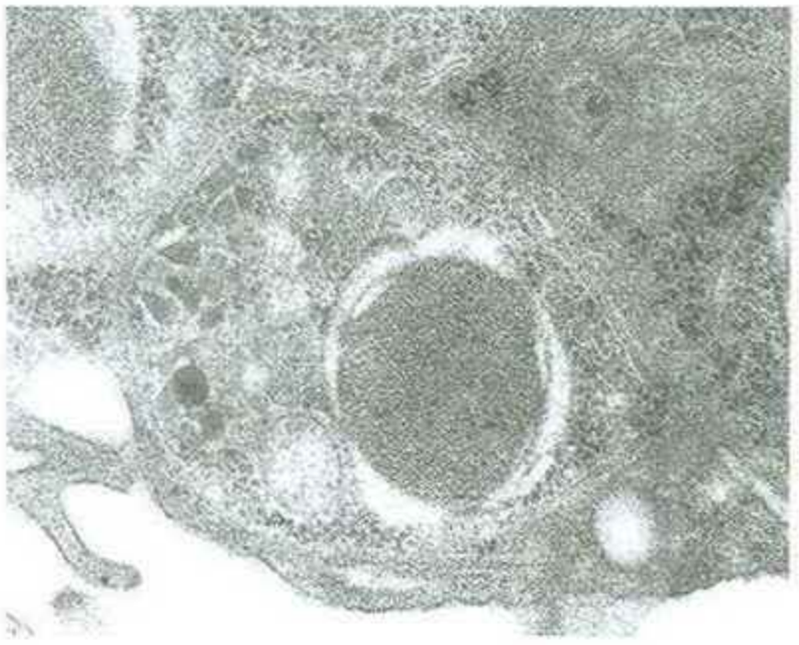


Figure 25.7 Longitudinal section through a single merozoite of *Babesia bovis* in a schizont in the tick salivary gland, × 48 400

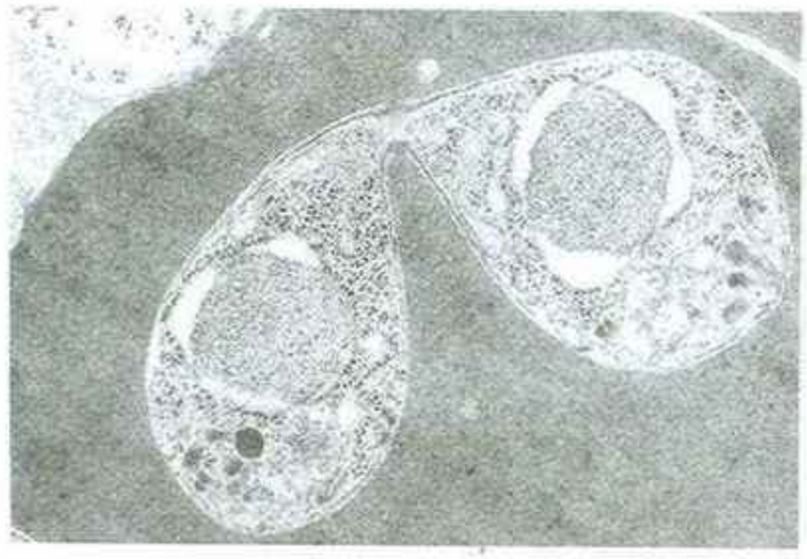


Figure 25.8 Section through typical paired *Babesia bovis* merozoites in an erythrocyte, x 50 000. Note ultrastructural features of apical complex

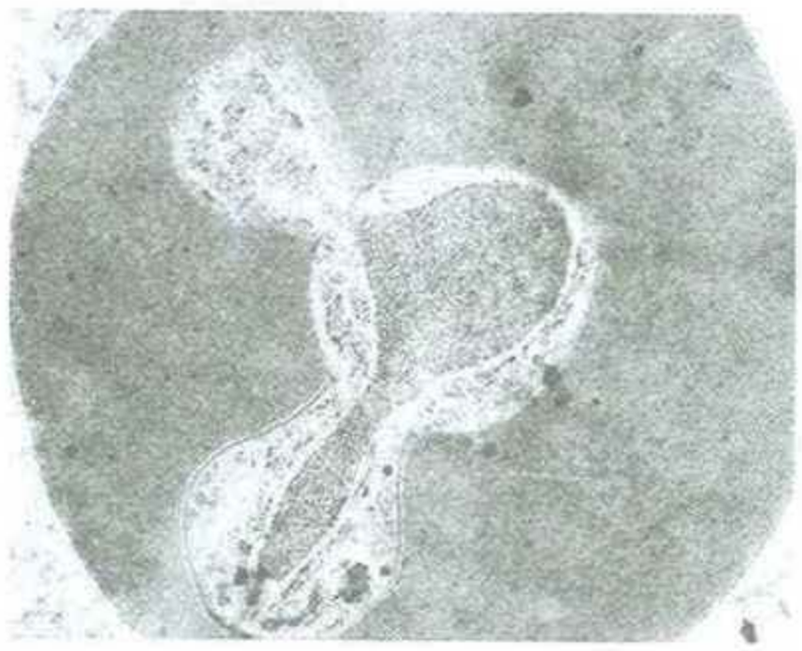


Figure 25.9 Section through an intra-erythrocytic dividing form of *Babesia bigemina* showing the development of two daughter cells (merozoites) from the mother cell (trophozoite), x 37 500. Note dividing nucleus and cytoplasmic differentiation of the developing merozoite

The distribution of *B. bovis* and *B. bigemina* is determined by the distribution of the vectors. *Babesia bovis* is restricted to areas where *B. microplus* is prevalent, usually the higher rainfall areas in the eastern parts of the subcontinent, including the Eastern Cape, KwaZulu-Natal and Mpumalanga provinces of South Africa,[39] Swaziland, Mozambique,[25] parts of eastern Zimbabwe,[113] the north-eastern parts of Zambia,[75] and Malawi. *Babesia bigemina* is much more widespread in the subcontinent than *B. bovis* because of its wider vector range **(see Chapter 1: Vectors: Ticks)**. It is present throughout most of the subcontinent, except for the arid parts of the Western and Northern Cape and the western Free State of South Africa, Namibia and Botswana, as well as some high-lying parts of the Drakensberg and Lesotho.[39, 75, 103, 113] In South Africa, its distribution appears to coincide with that of *B. decoloratus* and *B. microplus*.[39] The role of the more drought-resistant vector, *R. e. evertsi*,[70] in the epidemiology is unclear.

Inter-specific matings may occur between *B. microplus* and *B. decoloratus*, resulting in the production of sterile eggs[130] and the creation of a 'hybrid zone' where populations of the two species overlap.[135] This zone may have prevented *B. microplus* from fully exploiting all the climatologically favourable areas in the subcontinent. Its width and stability are unknown, but it is likely to have a significant effect on the epidemiology of *B. bovis*. *Babesia bigemina*'s lack of vector specificity makes it unlikely that this species will be affected by competition between tick species.

One infected larval tick is sufficient to transmit *B. bovis*, but tick infection rates are usually low and the rate of transmission to cattle is therefore low. Studies in Australia found only 0.04 per cent of field ticks to be infected with *B. bovis* where European cattle breeds were involved, and even less in the case of Zebu cattle.[94, 97] Tick infection rates with *B. bigemina* are higher (0.23 per cent in the Australian study) and therefore transmission rates of this species are also higher than those of *B. bovis*. As a result, *B. bigemina* infections are usually more prevalent in those herds where both species are present[39] and less readily affected by factors such as climate or management, which reduce tick numbers.

Babesia bovis and *B. bigemina* have high degrees of host specificity. European, Sanga and Zebu breeds are all susceptible, and all develop latent infections after recovery which persist for various lengths of time. European breeds of cattle can retain *B. bovis* infections for life[111] and remain infective for ticks for up to two years,[92] while most cattle with a significant Zebu content lose the infection within two years.[73] *Babesia bigemina* infections rarely persist for more than a year, regardless of the host, and infected cattle remain infective for ticks for only four to seven weeks.[73, 92] African buffalo (*Syncerus caffer*) and Asiatic buffalo (*Bubalus bubalis*) can develop latent infections.[42] Antibodies and transitory infections have also been demonstrated in other animals, but there is little evidence to suggest that non-familial hosts are important reservoir hosts[42, 79] even though *R. e. evertsi* infected with *B. bigemina* have been collected off a sable antelope (*Hippotragus niger*) with clinical babesiosis.[69]

Both *Babesia* spp. probably evolved in cattle in the tropics, and well-developed relationships therefore exist between Zebu and Sanga cattle and these parasites. As a result, *Babesia* spp. are less important in these breeds while European breeds are known to be highly susceptible.[12, 32] However, literature on the resistance of these breeds and crossbred cattle is rather ambiguous.[11, 12, 91] In a large controlled field trial in Australia, 32 per cent of 56 pure *Bos indicus* showed detectable clinical signs after natural exposure to *B. bovis* and *B. bigemina* while 2 per cent (one animal) was severely affected.[12] In comparison, 87 per cent of 52 crossbreds (halfbred *Bos indicus*) were clinically affected, 19 per cent of them severely. Typing of the parasites in severely affected cattle suggested that *B. bovis* was the main cause of the clinical reactions. In a parallel pen trial, *Bos indicus* and

crossbred cattle were more resistant to *B. bigemina* than *Bos taurus* cattle (none of six *Bos indicus* and six crossbred cattle was severely affected compared with six of the seven *Bos taurus* cattle).[11] However, susceptibility of individual *Bos indicus* and crossbred animals varied considerably. The reason for this is unknown but it may help to explain the ambiguity in the literature with regard to breed susceptibility to *B. bigemina*.

The virulence of *Babesia* is not a stable characteristic. Mild and virulent isolates of both species have been identified from the field and are known to be capable of rapid change. A major factor in parasite virulence is a history of recent tick transmission; long residence in a bovine reduces virulence while frequent cyclical transmission in ticks may increase the severity of the ensuing reactions.[24]

Isolates and selected strains of *B. bovis* and *B. bigemina* also differ antigenically[32] but this appears to have little epidemiological significance. Cross-immunity experiments have shown that recovered cattle are more resistant to challenge with the same (homologous) isolates than with different (heterologous) ones.[32, 42] Antigenic variation is also known to occur during *B. bovis* and *B. bigemina* infections with variants emerging at regular intervals during the latent phase of the infection associated with slight recrudescences of parasitaemia.[42]

It is possible to have both redwater and its vectors present in a cattle population without measurable economic losses or clinical disease. This situation is known as endemic stability and defined as the state where the relationship between host, agent, vector and environment is such that clinical disease occurs rarely or not at all.[115] An important factor in the establishment of endemic stability is the age of first exposure. Calves have a natural resistance during the first six to nine months of life and rarely show clinical signs, yet develop solid, long-lasting immunity.[32, 42] The occurrence of redwater in a herd depends entirely on the frequency with which the causative species is transmitted. If there is frequent transmission, infection of all animals will take place at a young age and immunity will develop without evidence of disease.[24, 39, 95]

Under conditions of endemic instability, some animals will fail to become infected for a considerable period after birth and will therefore be susceptible to clinical disease. *Babesia bigemina* has higher infection rates in ticks than *B. bovis* and endemic instability is therefore less likely to develop to it than to *B. bovis* in regions where both are present.[46]

A simple mathematical model has been used to predict the level of stability in a herd.[95] It utilizes the rate at which infection occurs in calves (determined serologically at a specific age), thereby providing a means of estimating the status of *B. bovis* infections.[46, 95] It has proved useful in predicting stability levels in European breeds but less so in those of other breeds. More recently, a disease prediction spreadsheet model was developed to more accurately calculate incidence risk from age-specific seroprevalence.[126] The model predicts the proportion of animals in each age and sex class that would be affected by different severities of the disease, and the information is then converted into a herd model to estimate the number of animals in each severity class. This model has been used to predict the potential impact of *Babesia* spp. on some large properties in Australia and the cost/benefit of control measures.[14]

Babesia bovis is presumed to be absent or endemically unstable in most of southern Africa.[46] This is mainly due to a large part of the subcontinent being ecologically unsuitable for *B. microplus*, the large zone of hybrid interference between the two *Boophilus* spp., and prevailing tick control measures in the high rainfall areas.

Babesia bigemina is endemically stable in large parts of southern Africa but, where cattle are not exposed at a young age, e.g. in situations associated with increasing aridity[39] or with tick control,[113] it may become important.

Severe losses may occur when susceptible cattle, especially *Bos taurus* breeds, are brought into an area where *Boophilus* spp. are prevalent. Mortality rates of 5 to 10 per cent are quite common under these conditions.[39]

The distribution of the vectors of *B. bovis* and *B. bigemina* is limited by ecological factors such as rainfall and also by the extent of tick control measures. Removal of these barriers may result in the ticks becoming established temporarily or permanently in previously uninfested areas. This happened during a period of unusually high rainfall between 1974 and 1976 in the normally arid Northern Cape Province of South Africa, when *B. decoloratus* moved several hundred kilometres in a westerly direction.[39] Similarly, a breakdown in tick control measures during the pre-independence war in Zimbabwe between 1973 and 1978 enabled *B. microplus* to spread from a few foci near the Mozambique border to the centre of Zimbabwe.[112, 113] Both tick migrations caused large outbreaks of babesiosis in susceptible cattle populations (**see Chapter 1: Vectors: Ticks**).

It is interesting that introduction of *B. microplus* in the latter half of the nineteenth century into Australia resulted in a major epidemic of redwater[24] paralleling the southern African experience with East Coast fever. This raises the question why the same did not happen when *B. microplus* was introduced into southern Africa. Some possible explanations are: *B. bigemina* was already present and therefore only *B. bovis* was associated with the spread of *B. microplus*; competitive interference by *B. decoloratus* suppressed *B. microplus* in some areas; the East Coast fever campaign in the early part of the twentieth century prevented the unchecked spread of *B. microplus*; and *B. bigemina* gave partial protection against the introduced *B. bovis*.

Immunology

Calves less than two months old born to previously unexposed cows are susceptible to infection and the effects of the disease,[24] while offspring of immune mothers are resistant, presumably because of a passive transfer of immunity via

the colostrum.[42, 154] After the age of two months a natural, non-specific, innate resistance, which persists for at least a further four to six months and is not dependent on the immune status of the cow, protects calves.[24, 144]

Severe attacks of redwater in cows at calving have been observed, and may be related to the perinatal immunosuppression which occurs in other host-parasite systems.[24] Aged animals may also suffer severely, presumably as a result of a general decline in immune competence.

Most cattle develop a durable immunity after recovery. This immunity is not absolute but may last for life, even in the absence of reinfection. Following recovery, the infection persists for varying periods in a latent form without detriment to the host. The presence of this latent infection, known as premunity, was long considered to be a prerequisite for protective immunity.[111] It is now known that persistence of infection is not necessary to ensure immunity. For instance, cattle which naturally eliminate the infection retain strong and lasting immunity,[10, 73, 90] while cattle cured of the infection by drugs show levels of resistance more closely related to the degree and duration of antigenic stimulation than to the presence of parasites.[26, 90] The concept of premunity in *Babesia* infections has therefore been abandoned.

The quality and duration of the resistance to reinfection after recovery is not affected by the age of the host at the time of initial infection, nor by the virulence of the strain involved.[24, 96, 98] Cattle recovered from *B. bigemina* infections show some resistance to *B. bovis* challenge,[153] but the reverse is not true. Furthermore, cattle harbouring latent infections of *Theileria buffeli*, a benign parasite in Australia, have increased resistance to *B. bovis* but not to *B. bigemina* infections.[93] A similar effect of African *Theileria* spp. has not been demonstrated but is worth exploring. Non-specific stimulation of immunity in cattle with agents such as levamisole and BCG has been shown to moderate laboratory *Babesia* infections,[42] but does not appear to have a significant effect on field infections.

The mechanisms of immunity in babesiosis are complex and involve both antibody and cell-mediated components. Antibodies develop after infection, but not all are protective and attempts to correlate antibody levels with immunity have failed.[26, 93] However, some indirect evidence for a protective role for antibodies is provided by the passive transfer of maternal immunity to *B. bovis* and *B. bigemina* in calves by colostrum intake.[42, 154]

Live *B. bovis* inoculated into cattle induces antibodies that remain at high levels for at least six months.[142] These antibodies are strain specific[96] and are of the IgM and IgG1 isotype.[61] IgG2 antibodies to *B. bovis* have not been detected.[61] Specific IgG1 antibodies reach high titres and are reactive in a complement fixation test, an indirect fluorescent antibody test and an antibody-dependent cell-mediated cytotoxicity assay.[62] In addition to *B. bovis* antigens being immunogenic for bovine B lymphocytes, *B. bovis* DNA is mitogenic for bovine B lymphocytes and enhances immunoglobulin G1 and G2 production by B lymphocytes.[17]

Specific and non-specific cell-mediated mechanisms involving T lymphocytes and natural killer (NK) cells appear to be involved in laboratory *Babesia* infections: intracellular death of parasites occurs which is not caused by antibodies and which is non-specific with other parasites, micro-organisms and even microbial extracts exerting the same effect on rapidly dividing *Babesia* organisms. A possible explanation of this phenomenon is the non-specific action of NK cells activated by either a specific immune response involving lymphocytes, or by non-specific agents.[42]

Macrophages are important for immunity to *B. bovis* because they act as antigen-presenting cells (APC) for T helper (Th) lymphocytes and remove parasitized erythrocytes by phagocytosis. Macrophages activated by interferon-gamma are thought to play a significant role in resistance to *B. bovis* by parasite suppression via secretory products released from macrophages.[152] This has been demonstrated *in vitro* where supernatant from macrophages stimulated with *B. bovis* antigens inhibited the growth of *B. bovis*.[107] These inhibitory products were nondialysable, resistant to heat and inactivated by freezing and thawing; but no attempts to identify the inhibitory factors were made.

The generation of reactive nitrogen intermediates (RNI), such as nitric oxide (NO) or reactive oxygen intermediates (e.g. H_2O_2), is a mechanism whereby activated macrophages release microbicidal products. NO has been shown to be produced by bovine macrophages following stimulation either with *B. bovis* merozoites or with antigen-stimulated *B. bovis*-immune T lymphocytes.[133] NO has been shown to inhibit the growth of *B. bovis in vitro* whilst H_2O_2 does not.[72] The effect of NO appears to be directly on the parasite and not on the erythrocytes.

There is no direct evidence that *B. bovis*-specific T lymphocytes are protective because adoptive transfer studies using T lymphocytes from immune animals are difficult to perform in cattle. Indirect evidence, however, suggests that T lymphocytes are important for immunity. In many protozoal infections, T lymphocytes are the primary effector cells in protective immunity.[54, 82] Peripheral blood mononuclear cells from immunized cattle exhibit weak and short-lived proliferative responses to a crude soluble parasite extract;[140] however, they may proliferate in response to merozoite membrane antigen for up to two years after infection.[20] Brown and co-workers have been studying T lymphocytes from cattle immune to *B. bovis* in an effort to identify antigens of *B. bovis* which may induce protective immunity.[20] They have shown that $CD4^+$ T lymphocytes (Th lymphocytes), but not $CD8^+$ T lymphocytes (T suppressor lymphocytes) or γδ T lymphocytes, are preferentially stimulated by crude parasite antigens *in vitro*.[18] Further work analysing cytokine profiles of Th lymphocyte clones derived from immune animals has

shown that interferon-γ, interleukin-2, interleukin-4 or tumour necrosis factor may be produced in response to *in vitro B. bovis* stimulation.[21]

The role of the spleen in immunity to babesiosis is well known.[42] Splenectomy of latent carriers usually results in fatal *B. bigemina* relapses and in *B. bovis* relapses that are sometimes fatal. Infection of susceptible splenectomized cattle results in fulminating infections with both parasite species. Splenic lesions have been shown to reduce the ability of intact calves to contain attenuated *B. bigemina* infections.[51] The spleen is not essential for the development of protection, but immunity is established more rapidly in intact than in splenectomized animals, suggesting that localization of immune cells in the spleen and the splenic microcirculation facilitates the process.

Pathogenesis

Babesia bovis infection

The acute disease is characterized by a hypotensive shock syndrome with vascular stasis and accumulation of parasitized red blood cells in the peripheral circulation. It is accompanied by activation of a coagulation/complement cascade and the release of vasoactive compounds resulting in vasodilation and circulatory stasis as well as generalized organ damage due to anoxia and toxic products of both parasites and damaged host tissue.[151]

Parasite proteases cause hydrolysis of fibrinogen which results in the accumulation of large quantities of soluble fibrin complexes which are not cross-linked, as well as in altered fibrinogen in the circulation. Thus, the coagulability and viscosity of blood increases but insoluble fibrin is evidently not produced, suggesting that classic disseminated intravascular coagulopathy is not a feature of *B. bovis* infections.[150]

The cause of cytoadherence[129] in *B. bovis* infections may be related to the formation of erythrophylic fibrinogen and soluble fibrin complexes adhering to infected erythrocyte membranes,[63] or due to the adhesive interaction between infected red blood cells and receptors on endothelial cells caused by vascular cell adhesion molecules induced by inflammatory cytokines.[31] It has further been suggested that *B. bovis* could express variable surface antigens functionally similar to those expressed by *Plasmodium falciparum*.[34]

Parasite proteases also activate macrophages to release pharmacologically active agents, such as histamine and 5-hydroxytryptamine, causing vasodilation, hypotension, increased capillary permeability, oedema and vascular collapse.[149] This hypotensive shock state has much in common with endotoxic shock, suggesting that *B. bovis* and endotoxin cause macrophages to activate the same harmful mediators.[29, 30, 149] In fact, the susceptibility of laboratory animals to endotoxin increases dramatically after they have been infected with *Babesia* spp.[30] Laboratory evidence also suggests that *Babesia* spp. cause leukocytes to release free oxygen radicals.[30] These radicals are toxic and contribute to endothelial damage thus augmenting the effects of circulatory stasis and hypotension.[30, 152]

Progressive haemolytic anaemia develops during the course of *B. bovis* infections. While this is not a major factor during the acute phase of the disease, it will contribute to the disease process in more protracted cases.

Babesia bigemina infection

Pathogenesis is almost entirely related to rapid, sometimes massive, intravascular haemolysis.[24] Coagulation disorders and the hypotensive state seen in acute *B. bovis* infections are not features of *B. bigemina* infections.[34, 150]

Clinical signs

The clinical signs which develop as a result of *B. bovis* and *B. bigemina* infections are similar, but the course and outcome are often very different.[24] The incubation periods of natural infections usually vary from 8 to 15 days.[67]

Babesia bovis infection

In acute infections, fever (>40°C) is usually present for several days before other signs become obvious. This is followed by inappetence, depression, weakness and a reluctance to move. Haemoglobinuria is often present, hence the name redwater. Anaemia and icterus develop and are especially obvious in more protracted cases. Diarrhoea is common and cows may abort. Muscle wasting, tremors and recumbency develop in advanced cases and are followed by coma in terminal cases. Affected animals may die from one to several days after the onset of clinical signs. Non-fatal cases may take several weeks to regain condition but recovery is usually complete.[24, 27] Surviving bulls may show reduced fertility lasting six to eight weeks as a result of the fever.[55]

Cerebral babesiosis develops in some *B. bovis* infections and is manifested by a variety of signs of central nervous system involvement. These include hyperaesthesia, nystagmus, circling, head pressing, aggression, convulsions and paralysis, and may or may not accompany other signs of acute redwater. The course of the disease is usually short and the outcome is almost invariably fatal.

In subacute *B. bovis* infections, clinical signs are less pronounced and sometimes difficult to detect. Calves infected before they reach eight months of age often develop an inapparent form of the disease.[24]

Babesia bigemina infection

Acutely affected cattle are usually not as severely affected as those with *B. bovis* infections, but the disease can develop very rapidly with sudden and severe anaemia, icterus and death, which may occur with little warning.[27] Haemoglobinuria is present earlier and more consistently than in *B. bovis* infections. Signs of cerebral involvement are not seen and recovery in non-fatal cases is usually rapid and complete.

Pathology

Clinical pathology

***Babesia bovis* infection** Haemolytic anaemia, which is characteristically macrocytic and hypochromic, is a feature of *B. bovis* infections. Packed cell volumes (PCV) may fall to less than 0,10, total erythrocyte counts to less than $3,0 \times 10^6$/ml, and total haemoglobin to less than 50 g/ℓ.[24, 147] Severe anaemia is particularly evident in protracted cases, while very acute cases may die with little evidence of anaemia.

In cattle which survive the infection, parasitaemia levels start to fall three to five days after the onset of patency (the first microscopic detection of parasites), and evidence of a response indicative of erythrocytic regeneration can be detected two to four days later. These signs include anisocytosis, polychromasia, punctate basophilia, macrocytosis and reticulocytosis.[24] Recovery is slow and it may take several weeks before normal red blood cell counts are restored. Leukocytic changes are variable, ranging from leukopenia to leukocytosis.[157]

Changes in blood chemistry reflect the consequences of circulatory stasis and hypotension, including renal and liver damage, and muscle degeneration.[150] Characteristically, the following occur:[147, 150, 151, 156, 157]

- significant increases in blood urea nitrogen and plasma creatinine levels;
- marked increases in unconjugated bilirubin levels, plasma creatine kinase and lactate dehydrogenase levels;
- the presence of haemoglobin in the serum to a level that may be as high as 5 g/ℓ;
- increased plasma concentrations of fibrinogen and soluble fibrin;
- proteinuria during the acute phase of the infection of which 15 to 20 per cent is haemoglobin and 70 to 75 per cent albumin;
- metabolic and respiratory alkalosis as shown by elevated bicarbonate, excess base levels and a lowered pCO_2; and
- a terminal increase in lactate and pyruvate levels.

***Babesia bigemina* infection** Haemolytic anaemia is the outstanding feature and is very similar to that seen in *B. bovis* infections. However, red blood cell destruction occurs more rapidly in severe cases and is accompanied by precipitous falls in PCVs, red blood cell counts and haemoglobin values. Osmotic fragility of the red blood cells increases during the acute phase of the infection,[150] and serum haemoglobin levels are high in acute cases. Evidence of kidney and liver damage is similar to that seen in *B. bovis* infections.[147]

Macroscopic and microscopic pathology

***Babesia bovis* infection** In acute infections, congestion of most organs and tissues is intense with subserosal petechiae and ecchymoses occurring in many internal organs. Conversely, protracted cases show evidence of anaemia and icterus. Haemoglobinuria is often seen at necropsy as a light to dark red discoloration of the urine. The spleen is commonly enlarged, sometimes to several times its normal size. The liver is swollen and may be yellowish-brown, with the gall bladder containing copious amounts of thick granular bile. The kidneys and lymph nodes are also enlarged. Pulmonary oedema may be present but is uncommon. In the cerebral form of the disease, the grey matter of the cerebrum and cerebellum has a characteristic cherry-pink colour (**Figure 25.10**).[24, 27, 127, 155]

Microscopically, sequestration of parasitized red blood cells in the peripheral circulation (**Figure 25.11**) and evidence of vascular stasis are striking in acute *B. bovis* infections. Large numbers of parasitized red blood cells fill the capillaries, sinuses and sinusoids of various organs, and can even pave the intima of larger blood vessels. Accumulations of haemosiderin and phagocytozed red blood cells, sometimes containing parasites, are common in cells of the reticuloendothelial system, especially in the spleen, liver and lymph nodes.[27, 127, 147] Other lesions include:[24, 127, 147]

- degeneration and necrosis of the epithelium of the convoluted tubules in the kidneys and an accumulation of hyaline or granular casts in the tubular lumens;
- centrilobular hydropic or fatty degeneration to extensive centrilobular and midzonal hepatic necrosis and bile stasis;
- marked congestion of the sinusoids of the spleen and a reduced ratio of white to red pulp with the germinal centres containing few cells;
- oedematous and congested sinuses in the lymph nodes and depletion of lymphocytes in the germinal centres;
- oedema of the lungs in some cases;
- marked distension of the capillaries of the brain by parasitized red blood cells (perivascular haemorrhages are uncommon);
- haemorrhages in the myocardium and hyaline degeneration of some myocytes; and
- degeneration of skeletal muscle fibres in the hind limbs.

Ultrastructurally, capillaries in the brain are dilated and filled with closely packed red blood cells, most of which are parasitized. These red blood cells have scalloped edges with fine strands apparently connecting adjacent red blood cells, as well as connecting erythrocytes and endothelial cells (**Figure 25.12**). Masses of lysed red blood cells which have undergone lysis but still contain intact parasites are frequently seen in capillaries. Changes in the capillary endothelium of the affected parts in the brain range from swelling of the cytoplasm and nucleus to necrosis. Perivascular and perineuronal spaces are enlarged.[148]

In the kidneys, capillaries are not packed as tightly with red blood cells as in the brain and the parasitaemia does not exceed 50 per cent of red blood cells. Other changes are similar to those seen in the brain. Capillaries in the lungs are packed with red blood cells, but only a small proportion of the cells are parasitized.[155]

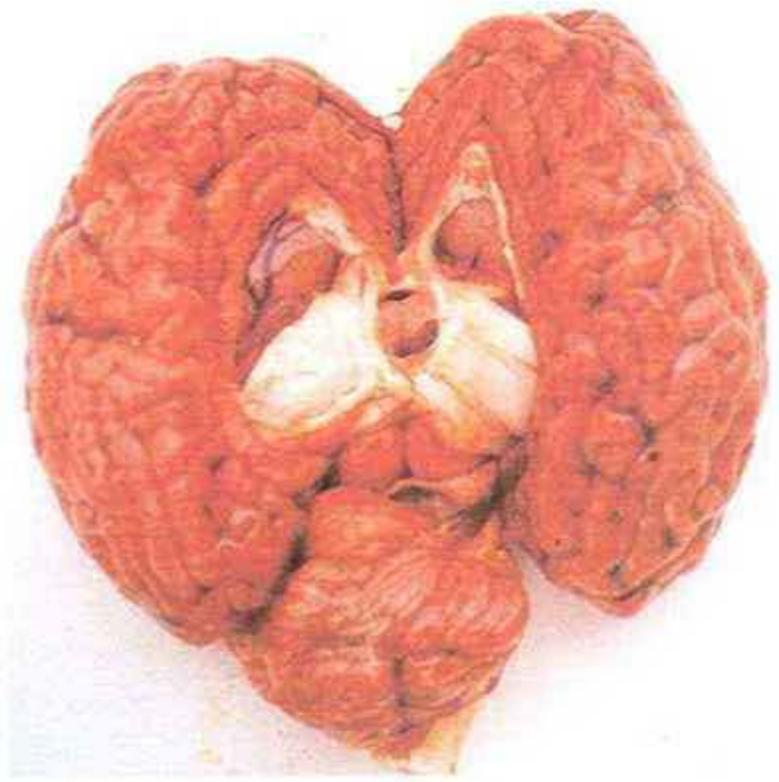


Figure 25.10 Cerebral babesiosis caused by *Babesia bovis*. Note marked congestion of the grey matter of the brain

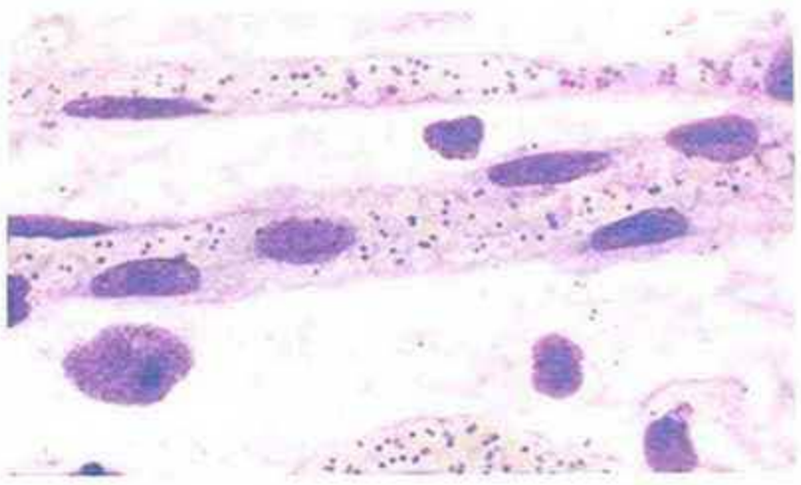


Figure 25.11 Erythrocytes parasitized by *Babesia bovis* in a brain squash smear

***Babesia bigemina* infection** Lesions seen at necropsy resemble those in animals which have died after an anaemic crisis. There is evidence of severe haemolysis, such as a pale carcass, watery blood and haemoglobinuria. Haemorrhages in internal organs and splenomegaly are not as marked as in *B. bovis* infections, but pulmonary oedema is a common finding. Icterus is evident in protracted cases.[24, 27]

Histological changes are less pronounced than in *B. bovis* infections, and sequestration of infected red blood cells and vascular stasis are not features of the infection. Changes in the kidneys and liver are similar to those caused by *B. bovis*. Extensive necrosis of the red pulp of the spleen is common and large thrombi may be present.[27, 147]

Immunopathology

Evidence of auto-antibodies as mediators of anaemia in babesiosis comes mainly from experimental studies with laboratory infections of *Babesia* spp. In bovine babesiosis,

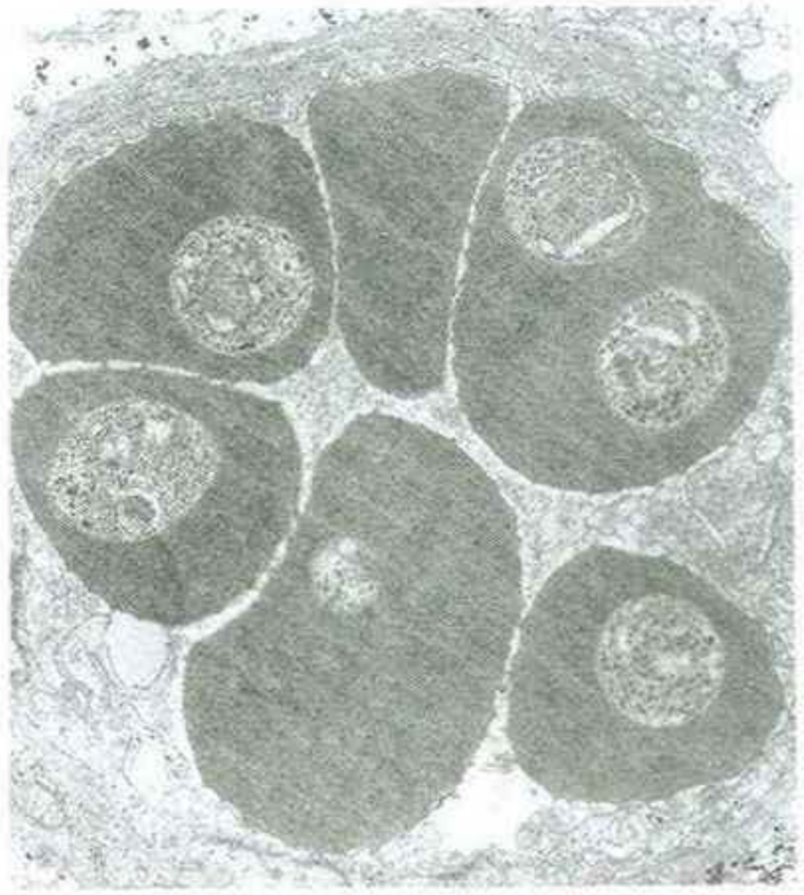


Figure 25.12 Cross-section through a brain capillary packed with parasitized erythrocytes. Note the projections from infected cell surfaces and the large interstitial spaces which are prominent in the neuropil

however, anaemia closely parallels the parasitaemia and there is no evidence of auto-immune anaemia having a significant pathogenic role, even though phagocytosis of non-parasitized erythrocytes is known to occur.[32]

Immune complexes have been implicated as a cause of the anaemia, splenomegaly and glomerulonephritis in *Babesia* models in the laboratory, but their contribution to the disease in cattle has not been conclusively established.[32, 42] *Babesia bovis* antigen has been detected in glomeruli and the endothelium of blood vessels, and is known to be intimately associated with host fibrinogen.[64]

Immune responses to heterologous antigens are suppressed during *Babesia* infections. The most convincing evidence of this immunosuppression comes from laboratory *Babesia* models. It is manifested by an impaired ability of the host to counter superimposed parasitic and other infections, as well as by decreased antibody responses to inoculated antigens.[32, 42] *Babesia bovis* causes a prolonged increase in the susceptibility of cattle to *B. microplus* infestation if the host is exposed to both parasites at the same time.[28] Cortisol values also become markedly suppressed during *B. bovis* infections and may be followed by transient adrenocortical dysfunction.[53]

Diagnosis

History, clinical signs and macroscopical lesions may often be suggestive of redwater, yet are not absolutely characteristic of the disease. Differentiation between *B. bovis* and *B. bigemina* infections is also impossible on these grounds

but it is highly advisable that it is done because of differences in pathogenesis, prognosis and control. The traditional method of identifying the agent in infected animals is by microscopical examination of thick and thin blood films stained with, for example, Giemsa's stain.[27] Details of these techniques are recorded in the literature[4, 27] and the sensitivity is such that parasites can be detected in most clinically affected animals. Species differentiation is good in thin films but poor in the more sensitive, thick films. This technique is usually adequate for detection of acute infections but is not suitable for detection of carriers where the parasitaemias are usually well below levels detectable by this method. Parasite identification and differentiation can be improved by using a fluorescent dye, such as acridine orange, instead of Giemsa's stain.[58] A Quantitative Buffy Coat method using acridine orange to stain parasites in capillary tubes was developed to demonstrate *Plasmodium* in human blood and has the potential to detect low *Babesia* parasitaemias as well, but differentiation is likely to be poor.[15]

For the best results, blood films should be prepared from capillary blood collected, for instance, after pricking the tip of the tail or margin of an ear. The temptation to use blood of the general circulation should be resisted as these specimens may contain up to 20 times fewer *B. bovis* than capillary blood.[27] In *B. bigemina* infections, parasitized cells are evenly distributed throughout the blood circulation. Mixed infections of the two *Babesia* spp. are uncommon. *Babesia bovis* parasitaemias are often low (<0,1 per cent), even at the peak of the reaction, while *B. bigemina* parasites are usually more numerous and therefore easy to detect. The most common method of staining these films is to apply Giemsa's stain at 10 per cent in phosphate buffered saline (pH 7,4) for 20 minutes after fixation in absolute methanol.[27]

Thick blood films are especially useful for the detection of low level *B. bovis* infections.[4] These films differ from thin ones in that the blood is not spread over a large area and is not fixed before staining (fixing in acetone is optional), thus allowing lysis of the red blood cells and concentration of the parasites. These films are usually stained with a 5 to 10 per cent Giemsa's solution for 20 minutes. A high degree of cleanliness is necessary when preparing thick films.[27]

Several factors may make it either difficult or impossible to identify *Babesia* spp. in blood films: prior treatment of the animal with a babesiacide will cause the parasites to degenerate and disappear, often within 24 hours; exposure of the films to moisture or formalin will make identification of the parasites difficult; and dirty smears may mask the presence of parasites.[27]

Diagnosis at necropsy is possible if decomposition is not too advanced. The specimens should include thin films of blood from the peripheral and general circulation, a squash preparation of a small piece of cerebral cortex and impression smears of, in order of preference, kidney, liver, lung and spleen. These are stained in the same manner as blood films. *Babesia* parasites assume a round shape within a few hours of death, thus making identification impossible on the basis of morphology alone. However, vast accumulations of parasites in organ smears, or big differences in the parasitaemias of the general and peripheral circulations, are indicative of *B. bovis* infections. In *B. bigemina* infections, parasites are readily detected in the general circulation. Accurate diagnoses are usually possible as long as intact erythrocytes are recognizable in the preparations. In cool weather this may be as long as three days after death.[27, 45, 58]

For histopathological examination, specimens of brain, spleen, liver, lung and spleen should be submitted. *Babesia bovis* will be identifiable by virtue of its accumulation in the capillaries of organs, but a diagnosis of *B. bigemina* may be difficult to confirm unless the parasitaemia is very severe.

Labelling of parasites with fluorescein or horse-radish peroxidase conjugated anti-*B. bovis* and anti-*B. bigemina* IgG is a sensitive, specific laboratory tool to identify parasites in blood and organ preparations, provided adequate numbers of parasites are present.[74, 83] Probes have also been used to detect DNA of bovine and other *Babesia* spp. but are generally not more sensitive than direct microscopy and application in routine diagnostics is limited.[15, 58] PCR assays have proven to be very sensitive particularly in detecting *B. bovis* and *B. bigemina* in carrier cattle.[23, 32, 56] However, PCR assays generally do not lend themselves well to large-scale testing and are unlikely to supplant serological tests as the method of choice for epidemiological studies. PCR assays may be useful as confirmatory tests, in some cases for regulatory testing, and also as markers for vaccine strains.[13]

In vitro culture methods have been used to demonstrate the presence of carrier infections of *Babesia* spp.[68, 158] The minimum parasitaemia detectable by this method will, to a large extent, depend on the facilities available and the skills of the operator[15] but could be as low as 10^{-10}, making it a very sensitive method for the demonstration of infection.[58] An added benefit is that it is 100 per cent specific.

A variety of serological tests has been used to demonstrate the presence of antibodies to *Babesia* spp. Of these, the indirect fluorescent antibody (IFA) test is widely used to detect antibodies to *Babesia*.[25, 40, 113] The IFA test is specific for *B. bovis*, but cross-reactions with antibodies to *B. bovis* in the *B. bigemina* IFA are a particular problem in areas where the two parasites coexist.[7] Unfortunately, in the standard IFA test the degree of serological cross-reaction that occurs between the four *Babesia* spp. present in southern Africa is such that accurate differentiation is sometimes difficult.[47] The detailed methodology of the IFA test for babesiosis is available in the literature.[4, 27]

To overcome the disadvantages of the IFA test, such as low sample throughput and subjectivity, the development of alternative tests has been intensively researched. Internationally validated enzyme-linked immunosorbent assays (ELISAs) for the diagnosis of *B. bovis* infection have been developed[38, 105, 145] but, despite concerted efforts, there is still

no similarly validated ELISA for *B. bigemina*. Typically, ELISAs for antibodies to *B. bigemina* suffer from poor specificity[52] but an assay recently developed in Australia shows considerable promise.[106]

Differential diagnosis

The diagnosis of suspected clinical cases of babesiosis showing fever, haemoglobinuria, anaemia and icterus, should be confirmed by demonstrating the parasites microscopically in erythrocytes. Efforts should be made to differentiate between *B. bovis* and *B. bigemina* infections, as specific diagnoses lead to a better understanding of the epidemiology with regard to species distribution, prevalence in specific areas, and their respective importance in disease outbreaks. Disease control strategies certainly benefit from such recorded information.

Species differentiation is essential in handling redwater outbreaks as it influences the choice of prophylactic treatment to be used, and the recommendations for appropriate follow-up vaccination programmes.

Babesiosis should be differentiated from anaplasmosis in animals showing anaemia and icterus, especially in the absence of haemoglobinuria. Positive identification of parasites in blood smears, especially *Anaplasma* spp., may be difficult in advanced cases because of massive haemopoiesis and low numbers of parasites at this time. It is common practice in South Africa to treat such clinical cases with drugs directed against both infections.

Haemolysis, haemoglobinuria and icterus may also occur in cattle suffering from leptospirosis as well as from non-infectious conditions such as chronic copper poisoning and intoxications with *Brassica* spp. and *Allium* spp.[81]

In southern Africa, cerebral babesiosis caused by *B. bovis* can be confused with other nervous conditions in cattle, such as heartwater, cerebral theileriosis, sporadic bovine encephalomyelitis (caused by *Chlamydophila pecorum*), plant poisonings (such as those caused by *Albizia* spp., *Cynanchum* spp., *Sarcostemma viminale*),[81] chlorinated hydrocarbon pesticide poisoning, vitamin B_1 deficiency responsible for cerebrocortical necrosis, and bacterial meningitis and meningoencephalitis (such as infections with *Haemophilus somnus*, *Pasteurella* spp. and *Streptococcus* spp.).

It is often speculated that new infections or stress might 'revive' a latent infection of different haemotropic parasites, because mixed infections of *Theileria*, *Babesia* and *Anaplasma* spp. are frequently encountered in blood smears of clinically affected animals. However, such conditions are difficult to simulate in the laboratory and have not yet been studied adequately under local conditions.

Control

There are several options for control of tick-borne diseases in southern Africa. Eradication of the tick vectors (the so-called minimum disease situation) is the most desirable, permanent solution to the problem but is rarely considered practical or economical on either a national or local basis.[8] The alternative approach of allowing natural endemic stability to develop by practising limited or no tick control, is similarly unrealistic in areas where *R. appendiculatus* and *Amblyomma* spp. are well established.[116] These regions are also endemic for *Boophilus* spp. and essential control of other tick species will inevitably affect the epidemiology of redwater. It is recognized, however, that the livestock industries in the region can be guided along cost-effective pathways by 'managing' the ticks and diseases associated with them.[8, 134] In the long term, this approach can be achieved by integrating the strategic use of acaricides, the application of vaccines in endemically unstable conditions and the use of tick-resistant breeds of cattle.[8]

Tick control

Control of ticks as a method of preventing babesiosis is feasible in situations where ticks have made temporary incursions into environments which are normally unsuitable for long-term survival.[39] Many farmers in regions endemic for *Boophilus* spp. also believe that very few or no ticks should be seen on cattle.[50] However, on the basis of cost and benefit, tick control in these regions for the purpose of preventing redwater can rarely be justified.[102]

Vaccination

Cattle develop a durable immunity after a single infection with *B. bigemina* and *B. bovis*. This feature has been exploited in some countries with the use of live attenuated vaccines to immunize cattle.[16, 44, 48, 117] Traditionally, these vaccines have been produced in government-supported laboratories as services to the livestock industries, amongst others in South Africa and Zimbabwe.[48, 80, 138] Malawi also has the capability to produce vaccines.[143] These vaccines are similar in composition to those produced in Israel[117] and Australia.[44, 45, 76] A survey of farmers in heartwaterendemic regions of South Africa showed that about 12 per cent used the vaccine.[50]

Early redwater vaccines involved carrier–donor systems whereby blood of recovered carriers was used as vaccine. These vaccines were simple to produce but suffered from variable infectivity and poor quality control in general. From 1973 until 1998, a standardized chilled vaccine was produced at the Onderstepoort Veterinary Institute (OVI) in South Africa, using attenuated strains, which overcame the problem of poor infectivity but not that of post-production quality control, including exclusion of contaminants.[128] Since 1989, frozen *B. bigemina* and *B. bovis* vaccines, which are subjected to full quality control procedures before release, have also been produced at OVI. These frozen vaccines steadily gained popularity and since 1998 are the only vaccines produced.[5] A similar frozen *B. bigemina* vaccine is also produced in Zimbabwe.[80]

More details on the use of these vaccines are provided below. Considerable progress has been made in the setting of international standards for the production and quality control of *Babesia* vaccines.[45, 118] The Onderstepoort *B. bovis* and *B. bigemina* vaccines consist of blood collected from animals in the acute stage of infection with these parasites.[5, 48] The intensity of the infections is measured and blood is collected in anticoagulant at the peak of the reactions. The blood is then diluted to contain a standardized number of parasites per dose. The vaccine is packed in five-dose containers and issued on demand. It is dispatched on dry ice and each dose is 1 mℓ injected intramuscularly irrespective of the size of the animal. It should be used as soon as possible after receipt and specific procedures are used when thawing the vaccine. Thawed vaccine cannot be refrozen. Frozen vaccines are currently produced in monovalent form but indications are that the production of multivalent vaccines is feasible.[99]

The strains of *B. bigemina* and *B. bovis* used in the vaccine are of reduced virulence, but are not entirely safe. Reactions to *B. bigemina* may be seen within seven days and to those *B. bovis* within 10 to 14 days. A practical recommendation is therefore to limit the use of vaccine to calves when non-specific immunity will minimize the risk of reactions. When older animals have to be vaccinated, there is a risk of severe vaccine reactions. These reactions occur infrequently, but valuable animals obviously warrant close attention, and should be observed daily for three weeks after vaccination. Because of the risk of abortions, vaccination of pregnant cows is rarely advised. Ideally, rectal temperatures of vaccinated cattle should be taken and animals treated when significant fever develops. Prophylactic treatment, including use of low doses of babesiacides, tetracyclines and aminoguanidine have also been used to reduce the prevalence of vaccine reactions.[48, 59, 78]

The main features of the live redwater vaccines are as follows:

- Protective immunity develops in four to six weeks.
- The immunity lasts for several years in the case of *B. bovis*,[39] but in the absence of natural challenge, it may break down in the case of *B. bigemina*.[111] However, the latter does not seem to pose a problem under local conditions and revaccination is therefore rarely advocated.
- Redwater vaccines can be given at the same time as anaplasmosis and other vaccines, except heartwater vaccine.
- Attenuated strains of *B. bovis* and *B. bigemina* impart a high degree of immunity to other strains, even in other countries and continents.[40, 41]
- Factors influencing the development of protective immunity includes potency of the vaccine (viability), development of 'breakthrough' field isolates, poor immunogenicity of the vaccine strain and poor responsiveness of the vaccinated animals.[10]

Details on the use of redwater vaccines are provided with the vaccine.

The technical and biological shortcomings of these live vaccines have provided impetus to improve them and also to develop inactivated vaccines. *In vitro* methods have been used experimentally to grow organisms for preparation of live vaccine[77, 140] and alternative cryopreservatives have been studied to improve the viability of frozen products.[131] Research on inactivated vaccines in the past decade has focused mainly on three approaches in identifying target antigens. The first approach was to sequentially fractionate merozoite antigens.[154] The second was to identify babesial molecules as potential targets of the protective immune response (such as the surface of sporozoites and extracellular merozoites and the surface of parasitized erythrocytes). The third approach has been based on the proposed function of the babesial molecules (antigens) in erythrocyte invasion (such as apical complex organelles), followed by epitope characterization and ability to induce protective immunity.[114] Experimental vaccines containing crude or purified *Babesia* antigens, extracted from infected cattle or cultures have been developed but the level and duration of protection against heterologous challenge have varied.[6, 32, 108] Progress in the development of subunit vaccines has been hampered by polymorphism among isolates and species, and a lack of knowledge of the immune effector mechanisms responsible for protection against haemoparasitic diseases,[109, 114] but antigens that stimulate strong anamnestic immune responses have been identified.[19] For any subunit vaccine to be successful it should produce an immunity that is at least comparable to that of the live vaccines in degree of protection as well as the longevity of the immunity. No killed or subunit vaccines were available in the year 2000 for routine use against *B. bigemina* and *B. bovis*.

Treatment

Attempts at treating redwater feature amongst the earliest studies on the control of livestock diseases in southern Africa. A number of compounds have been used through the years, some of which are shown in **Table 25.2.** Unfortunately, none is ideal for the purpose and while some of the diamidine compounds have proven to be both safe and effective, problems with residues have affected availability in some countries, and potentially also export markets for beef and dairy products in others. To complicate matters further, the supply by some manufacturers has been erratic in recent years, making it difficult at times to obtain the drug of choice. Dose rates and instructions of the manufacturers must be adhered to at all times. The chemotherapy of babesiosis is comprehensively reviewed in the literature.[4, 84, 85]

Drug resistance to bovine *Babesia* spp. has not been confirmed. Some tolerance by *B. bovis* to imidocarb has been reported,[35] but did not affect the therapeutic value of the drug.

Recovery is the rule if specific treatment is given early in the course of the infection. However, if treatment is delayed, supportive therapy may be essential if the animal is to

Table 25.2 Chemotherapy of bovine babesiosis

DRUG	TRADE NAME	DOSAGE AND ROUTE OF ADMINISTRATION*	USES AND ADVANTAGES	DISADVANTAGES
DIAMIDINE DERIVATIVES				
Diminazene	Berenil Ganaseg Trypazen Veriban Babezene Dimisol	3,5 mg/kg i/m	Rapid activity against both *Babesia* spp.; well tolerated	
Amicarbalide	Diampron Pirodia	5–10 mg/kg s/c, i/m	Rapid activity against both *Babesia* spp.; well tolerated	
Imidocarb	Imizol Forray 65	1,2–3,0 mg/kg s/c or i/m	Rapid activity against both *Babesia* spp.; well tolerated	Nephro- and hepatotoxic at high doses
Phenamidine	Phenamidine	12 mg/kg s/c or i/m	Greatest activity against *B. bigemina*	Cholinesterase inhibition
QUINOLINE DERIVATIVES				
Quinuronium sulphate	Babesan Ludobal Acaprin Pirevan	1 mg/kg s/c	Greatest activity against *B. bigemina*	Slow effect on *B. bovis*. Cholinesterase inhibition: dose rate should not be exceeded (atropine counteracts toxic effects)
ACRIDINE DERIVATIVES				
Euflavine	Gonacrine Euflavine	2–4 mg/kg i/v	Rapid activity against both *Babesia* spp.	Highly irritant if not given strictly i/v
Trypan blue	Trypan blue	0,1 mg/kg i/v	Active against *B. bigemina*	Little effect against *B. bovis*, irritant if not given i/v; discoloration of milk and carcass
Antibiotics				
Tetracycline	Terramycin LA	20 mg/kg i/m	Mitigates *Babesia* vaccine reactions	Doubtful efficacy in clinical disease

* i/m = intramuscular; s/c = subcutaneous; i/v = intravenous

survive. Non-specific support includes the use of haematinics, vitamins, intravenous administration of fluids, good nutrition and provision of shade. Blood transfusions may be indicated in cattle with heavy parasitaemias and low PCVs (<0,10); histo-incompatibility is seldom seen at the first transfusion. In acute *B. bovis* infections, use of antioxidants such as vitamin E, and high doses of corticosteroids may help to offset the hypotensive and hypercoagulable state of the animal.[14] In cases of cerebral babesiosis, intravenous use of hypertonic solutions of mannitol or glucose may provide temporary relief.

Chemoprophylaxis

Imidocarb and diminazene are the only babesiacides with useful prophylactic properties for the short-term control or prevention of babesiosis. Treatment with imidocarb (3 mg/kg) will prevent overt *B. bovis* infections for at least four weeks and *B. bigemina* infections for at least eight weeks.[5, 136] Diminazene (3,5 mg/kg) will protect cattle against the two diseases for one and two weeks respectively.[5]

Short-term control of outbreaks by chemoprophylaxis may be valuable, particularly if the morbidity rates are high. Treatment of all affected and exposed cattle will be costly but will bring about rapid, although temporary, relief.[39] It is also useful when susceptible cattle are transported through or temporarily reside in an endemic area, and when pregnant cows need temporary protection.[39]

The use of prophylactic compounds may be combined with an intensive dipping programme aimed at eradicating the vector ticks, or with subsequent vaccination to stabilize the situation.[39] Unfortunately, the prophylactic use of imidocarb may interfere with the development of immunity following vaccination because the residual effect of the drug may eliminate or suppress the infection.[39, 43] The interval between the use of imidocarb and vaccination should be at least eight weeks if immunity to *B. bovis* is required and 16 weeks in the case of *B. bigemina*. If diminazene is used, the intervals for the two parasites should be about four and eight weeks respectively.[5]

Genetic resistance

It is well known that Sanga and Zebu cattle carry less ticks than European breeds and these breeds also have a degree of natural resistance to babesiosis. This trait has, no doubt, contributed to the popularity of indigenous breeds such as the Afrikaner, Nguni and Mashona in southern Africa and Zebu breeds generally here and in other endemic parts of the world. Unfortunately, the resistance of

these cattle is not absolute and significant losses can result from exposure to babesiosis. While the literature on the topic is conflicting, it is clear that pure *Bos indicus* cattle have a high degree of natural resistance to *B. bovis* and *B. bigemina* infections while crossbred cattle are sufficiently susceptible to warrant preventive measures.[9, 11, 12] Using Ramsay's disease prediction model[126] on data from Queensland,[14] the benefit to cost ratio of vaccination is obviously very favourable in the case of *Bos taurus* cattle. It is also favourable for crossbred cattle except at the 90 per cent seroprevalence level, while, in the case of *Bos indicus* cattle, it is only positive when the seroprevalence is 30 to 50 per cent and only calves were vaccinated.

Control of outbreaks

Procedures to be followed during an outbreak will depend largely on the number and manageability of the animals concerned, and the availability and cost of labour, drugs, vaccine and acaricides. One or more of the following actions can be taken to limit losses:[5]

- Treat sick animals and separate them, if possible, from the rest of the herd.
- Have the diagnosis confirmed at a reputable laboratory.
- Treat unaffected cattle for ticks to prevent exposure.
- Consider immediate vaccination of all unaffected cattle.
- Consider use of a prophylactic treatment programme as mentioned above.

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26

Equine piroplasmosis

Synonyms: Biliary fever, equine babesiosis, *Babesia equi* infection, *Theileria equi* infection, *Babesia caballi* infection, galkoors van perde (Afrik.)

D T DE WAAL AND J VAN HEERDEN

Introduction

Equine piroplasmosis, more commonly known as biliary fever, is an acute, subacute or chronic tick-borne disease of equids caused by the intra-erythrocytic protozoa *Babesia equi* and *Babesia caballi*. It is characterized by fever (which is sometimes intermittent), progressive anaemia, icterus, hepatomegaly and splenomegaly. Bilirubinuria and haemoglobinuria may be present in the later stages of the disease. Pregnant animals may abort as a result of these infections.

In 1883, Wiltshire, as cited by Henning,[35] described a fever of horses, which he referred to as 'anthrax fever'. This is apparently the first record of equine piroplasmosis in South Africa. At about the same time, Hutcheon[45, 46] observed a similar condition in the then Cape of Good Hope Colony, which he named 'biliary fever'. Nunn[81] first regarded it as the 'bilious form' of African horse sickness, but Hutcheon[47, 48] insisted that biliary fever should be regarded as a separate disease entity. In West Africa, the disease was described under the name of equine malaria.[35] Theiler[103, 104] demonstrated that biliary fever is not identical to African horse sickness, but pointed out that a horse may suffer from the two diseases concurrently.

At the turn of the century, Laveran,[60] having examined blood smears from South African horses received from Theiler, classified the organisms as *Piroplasma equi*. Some controversy has accompanied the classification of this organism over the years,[25, 61, 80, 115] and more recently it was placed in the genus *Theileria*,[73] based on elucidation of the life cycle. However, general acceptance of these changes by the scientific community still needs to be assessed and therefore the name *B. equi*[61] will be retained in this chapter.

In 1904 Koch[56] identified two morphologically distinct equine *Babesia* spp. in Zimbabwe. Later Nuttall and Strickland[82, 83] clearly demonstrated that either one of two distinct species of parasites occurs in horses suffering from biliary fever. They suggested the name *Piroplasma caballi* for the larger parasite.

Furthermore, Nuttall and Strickland[83] showed, by means of cross-immunity tests, that horses which had recovered from infections with *B. caballi* were still susceptible to *B. equi*.

No published record of the first cases of *B. caballi* infection diagnosed in South Africa can be found, apart from the casual reference by De Kock[11] in 1920 to the presence of *B. caballi* in the blood of some experimental horses.

It is difficult to assess the economic importance of equine piroplasmosis in southern Africa, as a suitable reporting system is not in use, but in one survey it was found that the number of cases of piroplasmosis treated exceeded that of every other infectious disease of horses, including infectious respiratory diseases and African horse sickness.[66] It is also an important cause of wastage (days lost by racehorses due to not training or being withdrawn from a race) in Thoroughbreds in South Africa.[85]

In earlier reports on equine piroplasmosis little effort had been made to determine whether the infection was caused by *B. equi* or *B. caballi*; the infections were consequently described as a single disease. In time, however, distinct differences between them became evident, particularly with regard to their morphology, life cycle, vectors and susceptibility to chemotherapy. It is therefore important to differentiate between *B. equi* and *B. caballi* infections.

This chapter deals mainly with *B. equi* because almost all clinical cases of piroplasmosis in equids in South Africa are caused by this parasite, *B. caballi* infections being only rarely associated with clinical signs. *Babesia caballi* is seldom diagnosed because it causes an extremely low parasitaemia which is difficult to detect even in blood smears of clinically affected animals.[12] Equine piroplasmosis is one of the major reasons why many countries outside the region prohibit the importation of horses from southern Africa.

Aetiology and life cycle

The genus *Babesia* belongs to the phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order

Eucoccidiorida, suborder Piroplasmorina, and family Babesiidae.[61] However, the recent discovery of apparent exo-erythrocytic schizogony by *B. equi* has cast some doubt on its taxonomic position[98] and it has therefore recently been reclassified as *Theileria equi*[73] (see above).

The occurrence of possible sexual stages in the life cycle of *Babesia* spp. in their tick vectors was first considered by Koch in 1906.[57] Recent work indicates that a sexual stage probably does occur in ticks; it has been reported to occur in a number of *Babesia* spp., such as *B. bigemina*,[32, 113] *B. microti*,[94, 95] *B. canis*[74] and *B. equi*.[72]

Babesia equi

Holbrook and his colleagues described the intra-erythrocytic developmental stages of *B. equi*.[42] Recent studies further indicate that, unlike most other *Babesia* spp., *B. equi* apparently also has a stage of schizogony in lymphocytes in the vertebrate host.[77, 98] No schizonts have, as yet, been observed in blood smears or tissues of *B. equi*-infected horses in South Africa.[12] Merozoite formation in the lymphocytes takes at least nine days after inoculation of sporozoites into lymphocyte cultures before it is complete, or 12 to 14 days after infected ticks first attach to susceptible animals before merozoites are released from lymphocytes to invade erythrocytes.[77] The intra-erythrocytic stages probably include trophozoites, dividing forms, and merozoites. These different developmental stages are difficult to differentiate by light microscopy. The trophozoites of *B. equi* assume a variety of forms (oval, round, elliptical, or spindle) and are up to 3 µm in diameter.[99] The merozoites occur either as two or four pyriform parasites within a red blood cell (**Figure 26.1**). In the latter case, they are arranged in the form of a Maltese cross. The parasites are smaller than *B. caballi*, being only *c.* 1,5 µm long. The fine structure of the erythrocytic stages of *B. equi* resembles that of other *Babesia* spp. and has been described by Holan.[36]

The development of *B. equi* in its vector ticks (*Rhipicephalus evertsi evertsi*, *Hyalomma detritum*, *Hyalomma anatolicum excavatum*, *Rhipicephalus turanicus* and *Boophilus microplus*) has only been studied in detail in their salivary glands.[34, 52, 78, 116] On the eighth day after nymphal ticks detach, sporonts of *B. equi* are found in the salivary glands. They persist unchanged in these glands during the nymphal to adult moult. The maturation of sporozoites does not begin until the infected adult tick attaches to a vertebrate host, after which the process is completed within five days. The sporozoites of *B. equi* are 3 to 3,4 µm in length.[72] Sporozoite development of *B. equi* in the salivary gland is very similar to that of several *Theileria* spp.[90, 97]

Babesia caballi

Babesia caballi develops in the vertebrate host exclusively in erythrocytes.[29, 42] The merozoites are pear-shaped (pyriform), pointed at their posterior ends, vary in size by between 2 and 5 µm in length and by 1 and 1,5 µm in width, and often form pairs. The angle between these pairs is usually acute.[36] The trophozoites are polymorphic, but round, oval or eliptical forms varying between 1,5 and 3 µm in diameter predominate (**Figure 26.2**).[36]

The fine structure of the trophozoites and merozoites is essentially similar to those for other large *Babesia* spp.[36, 99]

Red blood cells rarely contain more than two parasites in *B. caballi* infections, unlike the situation in *B. equi* infections. The parasitaemia in the case of *B. caballi* infections is invariably low — usually less than 0,1 per cent of erythrocytes are infected even in splenectomized horses[12] and, even though the parasitaemia caused by *B. equi* may exceed 20 per cent, levels between 1 and 5 per cent are more common.

The initial development of *B. caballi* in *Dermacentor nitens* takes place in epithelial cells of the gut in the replete female tick. Successive cycles of schizogony occur and a

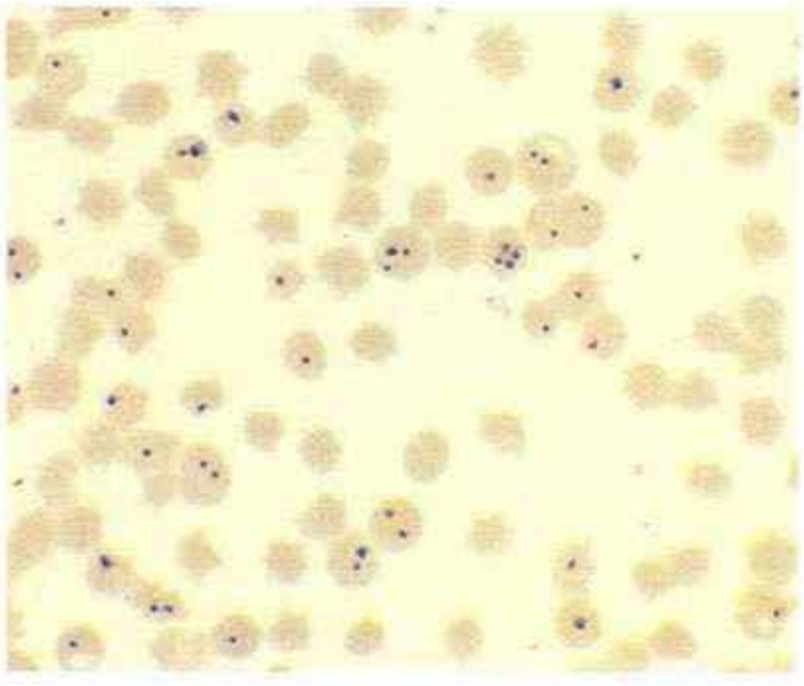


Figure 26.1 Blood smear: *Babesia equi*

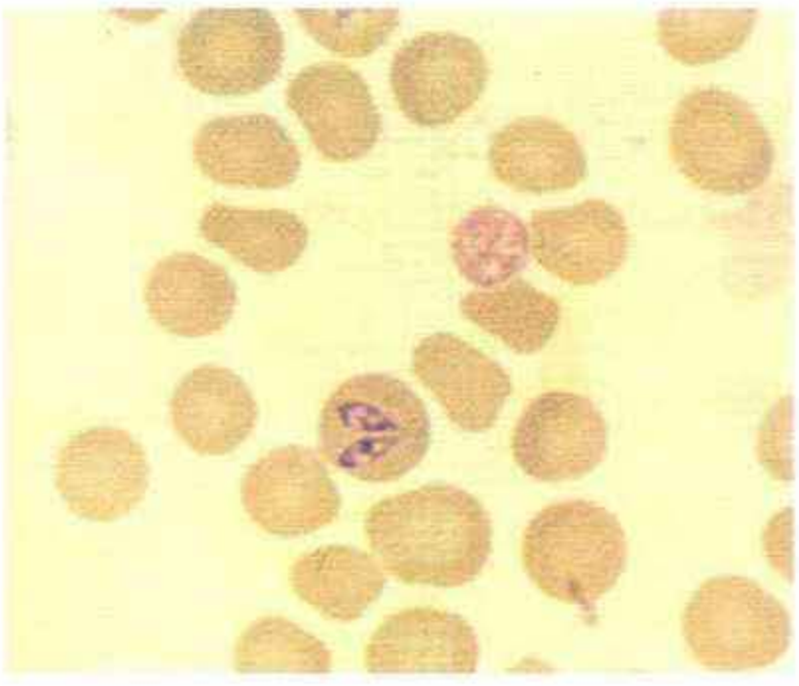


Figure 26.2 Blood smear: *Babesia caballi*

variety of cell types, including ova, become infected. *Babesia caballi* ookinetes (vermicules) enter the salivary glands at about the time of larval moult (6 to 10 days after attachment) and develop to become sporozoites (the infective forms) shortly after nymphal ticks begin feeding. These sporozoites are liberated into the lumen of the salivary gland and are oval or pyriform bodies, 2,5 to 3 µm long. They are thought to be injected while the nymphs are feeding. There are also indications that the infection may be carried over from the nymphal to the adult stage and, transovarially, to the following generation.[38]

No evidence has been published that either confirms or contradicts the existence of a sexual cycle in the development of *B. caballi*, which has been studied in the tropical horse tick (*Dermacentor nitens*) and in other ticks such as *Dermacentor marginatus*, *Dermacentor pictus*, *Dermacentor silvarum* and *Hyalomma plumbeum*.[38]

Epidemiology

Both *Babesia* species occur in Portugal, Spain, France, Italy, and in large parts of the former USSR. Equine piroplasmosis probably does not occur in the UK although *Dermacentor reticulatus* does occur in parts of that country.[50] Autochthonous infections have not been observed in Germany, Switzerland and Austria, although the potential vectors also occur in those countries.[64] Equine piroplasmosis is probably indigenous to Asia.[30] Australia was free of this disease until 1976 when *B. equi* was brought into the country through the importation of Spanish horses, but because of the lack of a suitable tick vector, it has remained localized.[71] The disease occurs throughout the African continent and Madagascar, while South and Central America are also endemic regions. Both *Babesia* spp. were introduced into Florida in the USA in about 1958 when Cuban horses were imported, but, with minor exceptions, the disease has remained restricted to this area.

The distribution of the disease in southern Africa has not been studied; it probably coincides with the distribution of the respective tick vectors. For this reason *B. caballi* is probably more widespread in the subcontinent than *B. equi*[107, 108] **(see Chapter 1: Vectors: Ticks)**.

Horses, donkeys, mules and zebras are susceptible to both *B. equi* and *B. caballi* infections.[80, 96, 116] Foals born in endemic areas usually receive maternal antibodies in the colostrum against *B. equi* and *B. caballi*. Colostral antibodies can be detected for up to five months of age in foals.[14, 19] As in the case of calves, foals are protected from *Babesia* infections by passively acquired and non-specific factors during the first six to nine months of life.[14, 19, 30] Infection occurring during this time will cause immunity without any overt signs of disease developing, and an endemically stable situation will develop.[14, 30] On the other hand, an endemically unstable disease situation develops when, due to infrequent exposure, some animals fail to become infected for a considerable period (probably more than 12 months) after birth and are then fully susceptible.[14] For more information on endemic stability and instability, **see Chapter 25: Bovine babesiosis**.

In pregnant mares, infection of the foetus at any stage of gestation, particularly with *B. equi*, is quite common[24, 76, 80, 89] and 11 per cent of reproductive failures in Thoroughbred mares in South Africa have been found to result from intrauterine infections.[15] Infection usually results in abortion with the foetus showing the lesions characteristic of equine piroplasmosis. Infected foals, however, may be born alive, showing signs of the disease, or they may develop signs within a few days. Due to the persistent nature of the infection, a carrier mare can produce more than one infected foetus which occurs at random in her life-time. In a recent study it was shown that foetuses can be infected with *B. equi* without their developing disease and that they can be born as carriers of the parasite.[63]

After being infected with *B. caballi*, animals may remain carriers of the parasite for up to four years, while the carrier state and immunity is probably life-long after infection by *B. equi*.[12, 30] Carrier horses, donkeys, mules and zebras are infective to ticks. *Babesia caballi* infections may persist for up to five generations in *Rhipicephalus sanguineus* (probably *R. turanicus* as this species could have been incorrectly identified as *R. sanguineus* by earlier workers).[22] There is no cross-immunity between *B. equi* and *B. caballi*.

The occurrence of equine piroplasmosis coincides with the seasonal activity of the adult stages of its vector ticks and therefore clinical cases are encountered more frequently during the summer months.[12] Both parasites frequently share the same vectors in a given region and are therefore closely associated, although *B. equi* is generally distributed more widely throughout the world than *B. caballi*.[61]

Rhipicephalus evertsi evertsi, a two-host tick, is the only confirmed tick vector of *B. equi* in South Africa.[14, 106] Transmission only occurs transstadially. Two tick species have been identified as vectors of *B. caballi* in South Africa, namely *R. e. evertsi* and *Hyalomma truncatum*. *Babesia caballi* is transmitted transovarially by *H. truncatum*, also a two-host tick,[13] and transstadially by *R. e. evertsi*.[16] Both these tick species are widely distributed in South Africa. *Rhipicephalus evertsi evertsi* is present throughout the Limpopo, North West, Gauteng, Mpumalanga and KwaZulu-Natal provinces, the northern part of the Free State, and along the coast of the Eastern Cape and Western Cape. It is present in Swaziland, Zimbabwe and eastern Botswana, bordering South Africa.[107] *Hyalomma truncatum* occurs throughout the western and the northern parts of South Africa. It is absent from the higher and wetter parts of the midlands and the Eastern Cape, Free State and Mpumalanga provinces and most parts of KwaZulu-Natal and Swaziland. It is not present in Lesotho. In Zululand (the north-eastern part of KwaZulu-Natal) and the adjoining lower lying areas of Swaziland, it occurs in pockets where drier climatic conditions prevail in valleys. *Hyalomma*

truncatum is present throughout Namibia (including the Caprivi Strip), Botswana, Zimbabwe and Zambia. It has also been recorded in Mozambique.[108]

Transmission of *B. equi* and *B. caballi* by *R. sanguineus* (probably *R. turanicus* — see above) and *B. equi* by *R. turanicus* and *Boophilus microplus* has been reported.[21, 22, 34, 55, 77, 80] Infected blood inoculated into a susceptible animal via the intravenous (i/v), intramuscular (i/m), or subcutaneous (s/c) routes is capable of inducing infection. Iatrogenic spread of *B. equi* by the use of contaminated hypodermic needles has been reported,[4, 8] but there is no evidence of mechanical transmission by haematophagous insects.

Pathogenesis

Very little is known about the pathogenesis of *B. equi* and *B. caballi* infections in horses. These parasites, however, are probably able to evoke the same pathophysiological events as described for the other *Babesia* spp. (**see Chapter 25: Bovine babesiosis**). This may well explain the diverse clinical manifestations of disease.

The progressive development of anaemia is typical of disease caused by *B. equi* infections.[41] Peaks in parasitaemia are accompanied by varying degrees of thrombocytopenia, hypophosphataemia, hypoferronaemia as well as bilirubinaemia.[17]

Although the pathogenesis of the anaemia has not been elucidated, hypophosphataemia may play a role. *Babesia* parasites depend on red blood cells for their energy supply, and the increased uptake of phosphorus by red blood cells may well be responsible for the development of a hypophosphataemic state. This may contribute to increased fragility of parasitized red blood cells.[27] Hypophosphataemia in humans has resulted in ATP depletion as a result of the metabolism of glyceraldehyde-3-phosphate being inhibited. A deficiency of ATP predisposes to haemolysis. The same mechanism seems to be responsible for postparturient haemoglobinuria in dairy cows.[84] Hypophosphataemia has also been reported in malaria in humans.[62] The haemolysis may result in haemoglobinaemic nephrosis and uraemia in severely affected horses.

Disseminated intravascular coagulopathy may develop in cases of equine piroplasmosis complicated by other infections, such as African horse sickness or worm infestations. *In vitro* studies have indicated disturbances in the clotting mechanism but uncomplicated piroplasmosis in horses has not been associated with disseminated intravascular coagulopathy.[109] Thromboses have been observed in the liver and lungs of a splenectomized horse.[71]

The pathogenesis of the disease in horses infected with *B. caballi* has been related to an accumulation of parasitized red blood cells in small blood vessels and capillaries.[37] It is possible that the development of an acute hypotensive state may be the cause of peracute deaths. Hyperacute infections and death in horses have been associated with pulmonary oedema.[67]

The development in some cases of laminitis with gastrointestinal stasis may well be due to the absorption of endotoxins.[110]

Experimental infection of horses with *B. equi* or *B. caballi* often results in a subclinical form of the disease.[17, 58] Parasitaemias in these animals are usually accompanied by a drop in packed cell volume and platelet count.

Clinical signs

Most clinical cases of equine piroplasmosis in southern Africa are caused by *B. equi*. *Babesia caballi* infection is usually not clinically apparent and is only rarely responsible for severe anaemia and other signs typical of piroplasmosis. It occasionally causes chronic inappetance, poor performance, loss of body weight, pale-pink mucous membranes and mild tachycardia. Splenomegaly is usually present in these cases.

The incubation period of equine piroplasmosis varies from 5 to 21 days.[65, 71, 91, 93, 105] A rare, peracute form of the disease resulting in horses being found either dead or moribund, has been reported in South Africa.[65] Acute cases are characterized by a fever, usually exceeding 40 °C, varying degrees of anorexia and malaise, and elevated respiratory and pulse rates. Fever is often accompanied by sweating, congestion of mucous membranes, tachycardia and palpitating cardiac sounds. The faecal balls are smaller and drier than normal and are yellowish-green.

Subacute cases show varying degrees of anorexia and malaise, weight loss, an elevated or normal rectal temperature, and increased pulse and respiratory rates. Heart rates of 60 beats per minute may be accompanied by medium-grade systolic murmurs. The fever is sometimes more intermittent in *B. equi* than in *B. caballi* infections.[65] The mucous membranes may vary from pale-pink or pale-yellow to bright yellow. Petechiae or ecchymoses may also be visible on the mucous membranes. Normal bowel movements are sometimes slightly depressed, and the animal may show signs of colic, such as pawing, looking at the flanks and lying down. The faecal balls are smaller and firmer in consistency than normal and are coated with a thin layer of mucus. Constipation may be followed by diarrhoea. The urine is often dark-yellow to orange or even brown, but in some cases it is reddish-brown as a result of haemoglobin and bile pigments.[12, 35] Rectal examination usually reveals an enlarged spleen. Untreated or neglected cases become severely anaemic and show signs of general weakness, such as drooping of the head and neck, stumbling, muscle trembling, swaying and lying down. There may be a mild oedematous swelling of the distal parts of limbs and marked praeputial oedema,[110] but oedema of the head, limbs and ventral abdomen has not been observed in horses suffering only from piroplasmosis in South Africa although this has been reported in other countries.[55, 112] African horse sickness or

infestations of helminths occurring concurrently with equine piroplasmosis may explain atypical clinical signs, such as oedema of the supraorbital fossae, and oedema of the ventral abdomen and thorax, sometimes associated with the disease. Clinical signs are apparently less severe at ambient temperatures below 15 °C.[101]

Chronic cases usually present with a history of non-specific clinical signs including mild inappetance, poor performance and a drop in body weight. Some cases reveal pale-pink mucous membranes and mild tachycardia. The spleen is usually enlarged.

Neonatal piroplasmosis in foals is characterized by weakness at birth or the rapid onset of listlessness and the development of anaemia, severe icterus and malaise soon thereafter, often prior to the ingestion of colostrum.[24] Affected foals become progressively lethargic and are ultimately unable to stand on their own or to suck. There is usually a fever and petechiae may be evident in the mucous membranes. Some foals, however, are apparently normal at birth, but develop signs of the disease two to three days later.

Nervous signs are rarely seen in equine piroplasmosis.[40] Marked ataxia, generalized trembling and mild to moderate tonic-clonic spasms have been observed in young foals associated with high rectal temperatures and positive *B. equi* smears.[110]

A variety of complications have been described in equine piroplasmosis. Acute renal failure causing marked elevations in serum urea and creatinine concentrations have been observed.[110] Some horses may show signs of colic and enteritis.[35, 110] The prognosis is poor when the latter occurs together with laminitis. Pneumonia may develop.[101] In stallions, partial or complete loss of fertility, temporary or permanent, has been reported.[65] Mares may abort at any stage of pregnancy as a result of systemic disease and pyrexia, but often there is no history of previous illness in mares that abort; abortion occurs most commonly in the last three months of gestation after intra-uterine infection[20, 65] and is generally accompanied by simultaneous expulsion of the placenta. Uterine involution is normal and subsequent conception is not affected.

Pathology

Clinical pathology

Reduction in the haemoglobin concentration and in red blood cell and platelet counts are typical of *B. equi* and *B. caballi* infections. Acute infections are characterized by lymphopenia and neutropenia.[92] Neutrophilia and lymphopenia have been reported in splenectomized donkeys.[86]

Decreased plasma fibrinogen concentrations and platelet counts,[1] as well as elevated bilirubin concentrations, have been reported in horses suffering from piroplasmosis. Lowered serum phosphorus and iron concentrations[17] and varying degrees of haemoglobinuria are also present. The serum of horses suffering from haemoglobinuric nephrosis is characterized by low sodium and chloride concentrations, and elevated urea and creatinine concentrations. There is a drop in the specific gravity of the urine.[110]

Macro- and microscopical pathology

Gross pathological findings include anaemia and icterus; oedema of subcutaneous and subserosal tissues; varying degrees of emaciation; hepatomegaly and splenomegaly; enlarged pale or, in haemoglobinuric cases, red-brown kidneys with subcapsular and cortical haemorrhages; ascites, hydrothorax and hydropericardium with epi- and endocardial haemorrhages; congestion and oedema of the lungs; and enlargement of the lymph nodes. Catarrhal enteritis, myocardial degeneration, peri- or epicarditis and pneumonia have also been recorded.[65]

Histopathological findings include congestion and oedema of the lungs, and fatty changes and bile stasis in the liver. The nephrosis is characterized by hydropic and fatty degeneration of the tubular epithelium, and by protein and haemoglobin casts in many tubules in the cortex and medulla. Congestion, haemosiderosis, and a depletion of lymphocytes are observed in the spleen.[71, 93] In addition to these changes, marked proliferation of reticulo-endothelial cells in the liver, kidneys, lungs and lymph nodes is evident. Thrombi in the blood vessels of the liver and lungs have been reported in splenectomized horses.[71]

Foetuses aborted relatively early in gestation are usually moderately to severely autolysed. The lesions in aborted foetuses and neonatal foals include anaemia, moderate to severe icterus (**Figure 26.3**), petechiae on visceral and serosal surfaces, hydrothorax, congestion and oedema of the lungs, splenomegaly and hepatomegaly (**Figure 26.4**). The microscopical changes are very similar to those in adult animals. While the parasitaemia in adult animals that die of the disease is usually low, it may often be more than 50 per cent in aborted foetuses and neonatal foals (**Figure 26.5**). The placenta is often oedematous and contains scattered small haemorrhages or large haemorrhagic patches. Many of the red blood cells in the placenta are parasitized.

Diagnosis

Clinical signs, examination of blood smears, serology, subinoculation of blood into a susceptible animal, and xenodiagnosis are all useful in making a diagnosis of equine piroplasmosis. It is not possible to differentiate between disease caused by *B. equi* and *B. caballi* infections on clinical signs alone.

The parasites can best be demonstrated by staining blood smears with Romanovsky-type dyes, such as Giemsa.[3] In peracute, acute and subacute piroplasmosis it is often difficult to demonstrate the parasites in thin blood smears. Even in acute clinical cases of *B. caballi* infection, the parasitaemia is very low (<0,1 per cent) and parasites may be difficult to find in a thin blood smear. The thick blood smear

technique[70] is useful in detecting parasites, particularly in those cases where the parasitaemia is very low.

Identification of carrier animals by means of blood smear examination is not only difficult but also inaccurate. Serological techniques such as the complement fixation (CF)[6, 28, 39] and the indirect fluorescent antibody (IFA)[9, 69, 79] tests are preferred, especially for the purpose of screening horses before exportation to countries which are free of the disease.[3, 101]

In recent years, the enzyme-linked immunosorbent assay (ELISA) has also been used to detect antibodies to both species of parasites in experimentally infected horses.[33, 75] Serological cross-reactions between *B. equi* and *B. caballi* in the ELISA[33, 75, 114] and false-negative or false-positive serological reactions in CF and IFA tests, may be encountered.[18, 28, 102] To overcome the problems associated with cross-reactions and antigen purity, ELISAs based on recombinant antigens and specific monoclonal antibodies have been developed for *B. equi*,[53, 54] and *B. caballi*.[7] Both appear to be more sensitive than the CF test, but further field validation is necessary before these tests will become generally accepted.

Although a cumbersome and expensive procedure, the subinoculation of large quantities of blood (*c.* 500 ml) into a susceptible (preferably splenectomized) animal can be used to identify carriers. The subsequent close observation of the recipient animal for clinical signs of disease and the presence of parasites in the red blood cells is essential.

DNA probes for both *B. equi* and *B. caballi* may lead to the development of a rapid, sensitive and specific diagnostic test.[2, 87, 88] Such tests will be invaluable in the screening of animals for export, but, to date, no such tests are routinely available for detection of either *B. equi* or *B. caballi* infection.

Carrier animals can also be identified by xenodiagnosis. In this technique, a specific vector is fed on the suspected carrier, and the organism is thereafter identified either in the vector or in a susceptible animal after the vector has been allowed to feed on it.

Recent advances in the *in vitro* culture method of equine piroplasmosis has enabled researchers/investigators to identify carrier animals of either *B. equi* or *B. caballi* infection, some of which were microscopically and serologically negative.[44, 119] Depending on the skills of the operator and the facilities available,[5] parasitaemias as low as 10^{-10} may be detected,[31] making this a very sensitive technique for the demonstration of infection with the added benefit that it is 100 per cent specific.

An accurate diagnosis of the specific parasite involved in equine piroplasmosis is essential because of the difference in drug sensitivity of the two parasites. Thorough screening of animals for the presence of parasites or antibodies is required before they can be exported from countries where the disease is endemic to countries free of it. Epidemic spread of the disease may occur if carrier animals are introduced into countries free of the disease and in which the tick vector is prevalent.

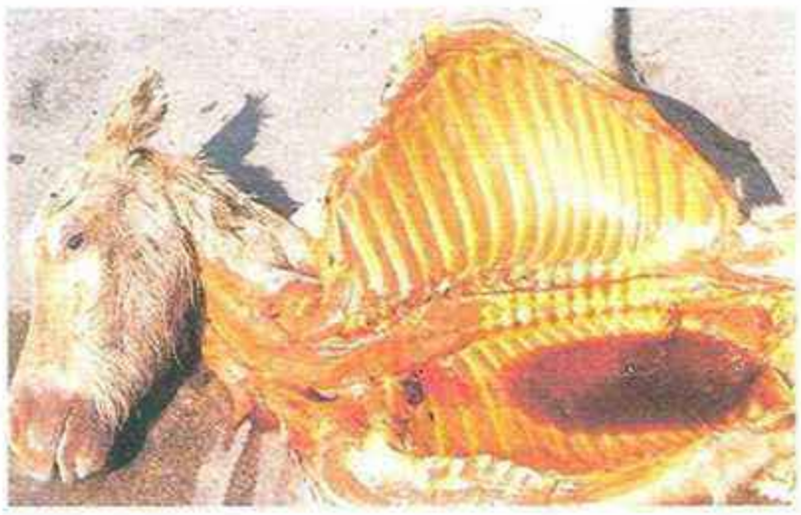


Figure 26.3 Aborted foetus showing severe icterus



Figure 26.4 Aborted foetus: note severe hepatomegaly, splenomegaly and icterus



Figure 26.5 Spleen smear from an aborted foetus as a result of *Babesia equi* infection: 80 to 90 per cent parasitaemia

Differential diagnosis

The clinical signs of equine piroplasmosis are often non-specific, and the disease may be confused with a variety of conditions.

In the febrile stage of equine influenza before typical clinical signs have developed, it may be difficult to differentiate from piroplasmosis.

A low percentage of horses suffering from equine encephalosis may exhibit fever and mild icterus, which should be differentiated from those occurring as a result of equine piroplasmosis.[23] The development of anaemia and icterus in horses showing classical signs of African horse sickness should prompt the examination of blood smears for *Babesia* parasites, since concurrent infections do occur.[35] Equine infectious anaemia, which has not been reported in southern Africa, may be very difficult to differentiate clinically from equine piroplasmosis.

In the new-born foal, piroplasmosis should be differentiated from both equid herpesvirus-1 infection and iso-erythrolytic neonatal icterus (iso-immune erythrolysis). The latter occurs within a few days of the ingestion of colostrum and is often afebrile.

Abortions as a result of piroplasmosis most often occur when pregnant mares are affected after the fifth or sixth month of gestation, and should be differentiated from other potential causes of abortion such as equid herpesvirus-1.

Control

A wide variety of drugs has been used to treat equine piroplasmosis with varying success.

The small (*B. equi*) and large (*B. caballi*) babesias of equids follow a pattern of drug susceptibility similar to that of the small (*B. bovis*) and large (*B. bigemina*) babesias of cattle; the small babesias being generally more resistant to treatment than the large. Acute infections respond to many of the same drugs used in cattle, but higher dosages are usually required.

Aromatic diamidines and related compounds including diminazene diaceturate (Berenil, Hoechst) at 11 mg/kg body weight administered deeply i/m; amicarbalide diisethionate (Diampron, May Baker) at 9 mg/kg body weight i/m and imidocarb diproprionate (Imidocarb, Wellcome) at 2 mg/kg body weight s/c or i/m, are effective against both organisms. Repeated treatments, however, may be required to control *B. equi* infections.[100]

The acridine dyes (euflavine and others) at a recommended dose of 4 to 8 ml/100 kg body weight of a 5 per cent solution with a maximum dose of 20 ml, administered i/v very carefully,[8] are effective against both *B. equi* and *B. caballi*. Subcutaneous administrations of these dyes cause severe irritation of the tissues at the site of injection. Resistance of *B. equi* to euflavine has been reported.[49] The simultaneous i/m administration of atropine is recommended to counteract the potential adverse parasympathomimetic effects (primarily abdominal pain) of imidocarb diproprionate and euflavine.[111]

The bisazo dye, trypan blue, administered in a single dose of 150 to 200 ml i/v of a 2 per cent solution, only has an effect on *B. caballi*.[11] This drug also has the disadvantage of producing a discoloration of many of the animal's tissues. For this reason, and because more effective drugs have been developed, it is now rarely used.

Tetracyclines such as chlortetracycline hydrochloride (Aureomycin, Lederle Laboratories) and oxytetracycline hydrochloride (Terramycin, Pfizer) are effective only against *B. equi* when given i/v daily for two or more days at a dosage rate of at least 5.5 mg/kg body weight.[49]

Preliminary results indicate that parvaquone, an anti-theilerial drug, can be effective in the treatment of acute *B. equi* infections in horses,[39] but it does not eliminate infection.[117]

Depending on the severity of the disease, supportive therapy is often necessary and may include blood and/or fluid infusions and i/v administration of essential phospholipids and antibiotics. Polyionic electrolyte infusions may be considered for severely dehydrated horses showing anorexia or suffering from diarrhoea. Oral laxatives may also be indicated.

Chemosterilization of *Babesia* spp. infections is rarely recommended, but may be indicated when horses are to be moved from an endemic country to one free of the disease. A few drugs will effectively sterilize *B. caballi* infections, but elimination of *B. equi* is very difficult.[100] Drugs used for the elimination of *B. caballi* include diminazene diaceturate administered i/m at a daily dose of 11 mg/kg body weight on two consecutive days;[51, 60] amicarbalide administered i/m at a daily dose of 8.8 mg/kg body weight for two consecutive days; and imidocarb given i/m at a daily dose of 2.2 mg/kg body weight on two consecutive days. Diminazene diaceturate causes swelling and necrosis at the injection sites. Toxic doses result in respiratory distress, depression and other signs of intoxication.[100]

Amicarbalide fails to sterilize *B. equi* infections at dose rates of 8.8 mg/kg and 11.0 mg/kg given i/m daily on four consecutive days.[10, 100] In one trial, four i/m treatments of imidocarb at a dosage rate of 10 mg/kg at 72-hour intervals eliminated *B. equi* from 5 of 10 ponies, but severe side-effects (colic, diarrhoea, and anorexia) and even death, were also reported.[10] In another experiment, four individual treatments of imidocarb 72 hours apart, given at a rate of 4 mg/kg, eliminated *B. equi* infection in 13 of 14 horses.[26] However, eight similarly treated donkeys died from drug toxicosis.[26] Yet, in another study, imidocarb given at these dosages was ineffective in eliminating *B. equi*-carrier state infections in nine mature geldings.[59] Adams and Corrier, as cited by Kutler *et al.*,[58] showed that for horses, the LD_{50} for imidocarb administered twice at an interval of 48 hours is 16 mg/kg.

Babesia equi-carrier geldings have been treated with the anti-theilerial drug, parvaquone, at a dosage rate of either 10 or 20 mg/kg for two or four treatments seven days apart, without sterilizing the infection.[59]

In summary, it would appear that none of the existing babesiacidal drugs is satisfactory in the sterilization of *B. equi* infections.

In highly intensive management systems where animals receive daily individual care, it is probably feasible to control the disease by eliminating the contact between the tick vector and equine host by means of the regular application of an acaricide. However, under free-ranging systems it is difficult, if not impossible, to control these multi-host tick vectors effectively. Strategic tick control will expose foals to ticks and allow them to develop immunity early in life, which will result in endemic stability.

No vaccine is as yet available, and therefore fully susceptible animals introduced into endemic areas should be carefully watched and treated when necessary.

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27

Porcine babesiosis

Synonyms: Porcine piroplasmosis, *Babesia trautmanni* infection, *Babesia perroncitoi* infection

D T DE WAAL

Introduction

Porcine babesiosis is a tick-transmitted, protozoal disease caused by either *Babesia trautmanni* or *Babesia perroncitoi* and characterized by fever, anaemia, icterus and haemoglobinuria. *Babesia perroncitoi* does not occur in southern Africa.

The disease was first reported in Russia in 1911 by Dementjew.[7] According to Knuth and Du Toit the first reliable description of the disease was given by Trautmann who studied the condition in Tanzania in 1914.[7] The protozoan concerned was later named *Babesia trautmanni*.[7] In 1939, Cerruti identified another protozoan parasite in the red blood cells of pigs in Italy which were exhibiting clinical signs of babesiosis, and he named this parasite *Babesia perroncitoi*.[1] *Babesia trautmanni* also occurs in pigs in Zaire[9] and Nigeria.[3, 14] More recently, *B. perroncitoi* has been identified in pigs in Egypt[5] and Senegal.[19]

In southern Africa, porcine babesiosis was described in 1948 in pigs near the Pongola River in the south-eastern region of the Limpopo Province, South Africa,[6] and in Zimbabwe.[8] It was also reported in 1958 in the Soutpansberg district in the Limpopo Province.[12] In all three outbreaks the causative organism was *B. trautmanni*.

Although the disease is probably endemic in large parts of southern Africa, it is seldom reported and is not considered economically important.

Aetiology and life cycle

Babesia trautmanni is a large *Babesia* found in the red blood cells of infected pigs. The merozoites (which are usually paired) measure 2,5–4 × 1,5–2 µm and are oval or pyriform in shape. Single forms, probably trophozoites, are amoeboid or round. The host cell usually contains between one and four parasites.

Babesia perroncitoi is smaller than *B. trautmanni*, the majority of parasites being pleomorphic, annular and measuring 0,7 to 2 µm in diameter. A variety of other forms, such as oval, quadrangular, and pyriform, also occur. These vary in size from 1,2 to 2,6 µm in length and 0,7 to 1,9 µm in width.[1]

The life cycles of the organisms in the tick vector are as yet unknown.

Epidemiology

Clinical cases of babesiosis caused by *B. trautmanni* have only been observed in domestic pigs in southern Africa. The bushpig (*Potamochoerus porcus*) has been shown to be capable of harbouring the parasite for at least 24 days after artificial infection, but without apparent disease occurring.[17]

The disease could potentially occur in those parts of southern Africa where the tick vector(s) and bushpigs are found. Bushpigs are particularly associated with forests, thickets, riparian undercover, reed beds or heavy cover of tall grass where there is water.[18] Agricultural developments have favoured them. In South Africa, bushpigs occur in the Limpopo Province, eastern parts of Mpumalanga, central and eastern KwaZulu-Natal, and along the coast in the Eastern Cape Province. They also occur in Zimbabwe, Botswana and Mozambique south of the Zambezi River. They are widespread but do not occur in the more arid west of Zimbabwe, while in Botswana they are mainly confined to the Okavango Swamps and adjacent river systems.[18] They have not been recorded in Namibia.[18]

Infection is probably endemic in domestic pigs kept under free-ranging systems of management in endemic areas, but clinical disease is rare. Although intensely farmed pigs are usually not exposed to the parasites, clinical cases of babesiosis may occur when they are turned out to pasture, or when infected ticks are introduced into pigsties via grass used for bedding.

Circumstantial evidence suggests that the ticks *Boophilus decoloratus*[7] and *Rhipicephalus turanicus*[13] are the vectors of *B. trautmanni* in Africa, but, it was only relatively recently

that *B. trautmanni* was succesfully transmitted with *Rhipicephalus simus*[2] and *Rhipicephalus turanicus*.[10] The nymph and adult progeny of experimentally infected female *R. simus* and *R. turanicus* ticks transmitted the infection to pigs. The prepatent period varied from 6 to 15 days after tick infestation. Attempts at transmitting the disease with *Hyalomma marginatum*, *Hyalomma truncatum*, *Hyalomma marginatum rufipes*, *Rhipicephalus sanguineus*, *Rhipicephalus maculatus*, *Rhipicephalus rossicus*, *Amblyomma hebraeum* and *Dermacentor silvarum* have failed.[10, 13] The possibility of mechanical transmission by haematophagous flies, such as tabanids and *Stomoxys* spp., has been suggested.[3]

Although not proven, *R. sanguineus*, *Dermacentor reticulatus* and *Hyalomma aegyptium* have been suggested as vectors of *B. perroncitoi* in Europe.[1]

Clinical signs and pathology

Babesiosis caused by *B. trautmanni* is a mild disease[4, 11, 12] which, in endemic situations, usually only results in severe clinical signs when the animals are under stress, such as that caused by malnutrition or helminth infestations. Healthy adult pigs exposed to infection for the first time, however, may contract a severe or even fatal disease.

The disease is less severe in younger pigs than in adults. Clinical signs include listlessness, fever, anorexia and anaemia. Icterus and haemoglobinuria may appear during the later stages of the disease and abortions may result.

Necropsy findings include anaemia, icterus, a slight increase in volume of the fluids in the body cavities, pulmonary congestion and oedema, petechiae on the serous membranes, epi- and endocardial haemorrhages, splenomegaly, and hepatomegaly with pigmentation.

Diagnosis

The history and clinical signs may raise suspicion of porcine babesiosis, but a specific diagnosis can only be made when *Babesia* parasites are demonstrated in red blood cells. Blood smears, preferably stained with Romanovsky-type stains, are therefore essential. The percentage of parasitized erythrocytes may be as high as 60 per cent.

Differential diagnosis

Porcine babesiosis should be differentiated from eperythrozoonosis (yellow belly) and leptospirosis.

Of the two species of eperythrozoon which occur in pigs, only *Eperythrozoon suis* is pathogenic. These rickettsia-like parasites can easily be distinguished from *Babesia* spp. by blood smear examination. Eperythrozoonosis is an acute disease of fattening pigs characterized by anorexia and pyrexia in the early stage of the disease, and by anaemia and icterus in the later stages. It has a low morbidity and mortality. The distribution of porcine babesiosis in southern Africa is restricted to areas where bushpigs occur.

Acute leptospirosis in pigs is manifested by septicaemia, fever, anorexia, haemolytic anaemia and icterus. Appropriate laboratory procedures must be taken in order to exclude it.

Control

Parenteral administration of diminazene leads to a rapid regression of clinical signs.[15] Treatment with phenamidine isothionate is effective against both *Babesia* spp., while trypan blue is only effective against *B. trautmanni*.

It seems that animals which have recovered from the disease will have a life-long immunity, but there is a chance that relapses may occur.[9] It has been claimed that *B. trautmanni* can persist in pigs for up to eight months after infection.[16]

Control of the disease should be aimed at tick control as well as the prevention of contact between domestic pigs and bushpigs.

No vaccine is available.

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28

Ovine babesiosis

Synonyms: *Babesia ovis* infection, *Babesia motasi* infection

I YERUHAM AND A HADANI

Introduction

Ovine babesiosis is a tick-borne disease caused by the intra-erythrocytic protozoan parasites *Babesia ovis*[9] and *Babesia motasi*.[62] The acute form of the disease is characterized by fever, progressive anaemia, icterus and bilirubinaemia. Haemoglobinuria is rare but may occur in the late stage of the disease. Pregnant animals may abort. Susceptible flocks may suffer a high rate of mortality. Recovered animals that are latently infected are usually immune for a certain period. There is no cross-immunity between the two parasites.

Ovine babesiosis caused by *B. ovis* is of considerable economic importance in the southern part of the Palaearctic zone (Mediterranean basin, Balkans, Kazakhstan, Kirghizstan, Turkmenistan, Iraq and Iran) overlapping the distribution area of the vector tick, *Rhipicephalus bursa*.[14] The presence of *B. ovis* in sheep in Madagascar[86] is questionable.

Rhipicephalus bursa is widely distributed between the 31 and 45° parallels north, including the Mediterranean basin,[38, 40, 48, 50, 79, 92] the Balkans,[22, 61, 69] southern former USSR,[66] Iraq[74] and Iran.[41] It has not been found in the southern hemisphere.

The geographical distribution of *B. motasi* is very wide and includes western and northern Europe, the Mediterranean basin, Asia (Turkey, India, China) and Africa (Somalia, Nigeria, Madagascar and southern Africa) and is transmitted probably by *Haemaphysalis* spp. ticks. *Babesia motasi* was first reported in southern Africa in 1902,[42] and subsequently ovine babesiosis was diagnosed in other countries in the African continent (Somalia, Nigeria, Madagascar).[18, 24, 47, 55, 76, 86] Since the report of Hutcheon and Robertson in 1902,[42] there are no other records on the occurrence of ovine babesiosis in southern Africa. *Babesia motasi* causes a severe disease and mortality in sheep in Asia[11, 51] and Africa.[18] It is regarded as a benign parasite in western Europe,[19, 49, 67, 87] but occasionally causes anaemia in lambs.[15]

Babesiosis caused by *B. ovis* and *B. motasi* affects all breeds of sheep and, more rarely, goats, with *B. ovis* being the more pathogenic. It appears that some isolates of *B. motasi* are infective for sheep and goats while others only infect sheep.[6] Three other *Babesia* species have been reported to occur in sheep — *B. sergenti*,[90] *B. foliata*[73] and *B. crassa*[35] (in Algeria, India and Iran respectively). These have a restricted local importance and their validity is still debated.

Aetiology and life cycle

Babesia ovis is a small parasite of 1,0 to 2,5 µm in length. The majority of parasites are oval or pyriform, tending to occur at the periphery of the erythrocytes.[32, 84, 97] The angle between the paired pyriform parasites is obtuse. Cross-forms are uncommon[93] (**Figure 28.1**). *Babesia crassa*, described in Iran,[35] has a similar morphology.

Babesia motasi is a relatively large parasite measuring 2,2 to 3,8 µm in length by about 2,0 µm in width. It commonly appears as a pair of pear-shaped organisms, separated by an acute angle, occupying two-thirds or more of the affected erythrocyte.[7, 87] Ring, oval, elongated and budding forms are less common. *Babesia motasi* often possesses a double nucleus, which is invariably single in *B. ovis*. The intra-erythrocytic stages include single tropozoites, dividing forms and paired merozoites. The fine structure of the tropozoites and merozoites are apparently similar to those described for other species of *Babesia*.[26]

Rhipicephalus bursa, a two-host tick,[100] is the only known vector of *B. ovis*.[97] *Haemaphysalis punctata*, a three-host tick, is the only proven vector of *B. motasi* in Europe[7] while *H. bispinosa* and *H. intermedia* are the only proven ones in India.[3, 51]

Markov (cited by Erashov[21] and Warnecke[89]) reported on the transmission of *B. motasi* by *Haemaphysalis otophila*, a three-host tick. The possibility that *R. bursa* may be a vector of *B. motasi* is extremely doubtful.[87] Opasina[68] suggested that *Amblyomma variegatum*, a three-host tick, is a vector of *B. motasi* in goats in Nigeria.

Babesia ovis and B. *motasi* develop only in erythrocytes and multiply by binary fission into merozoites (merogony).

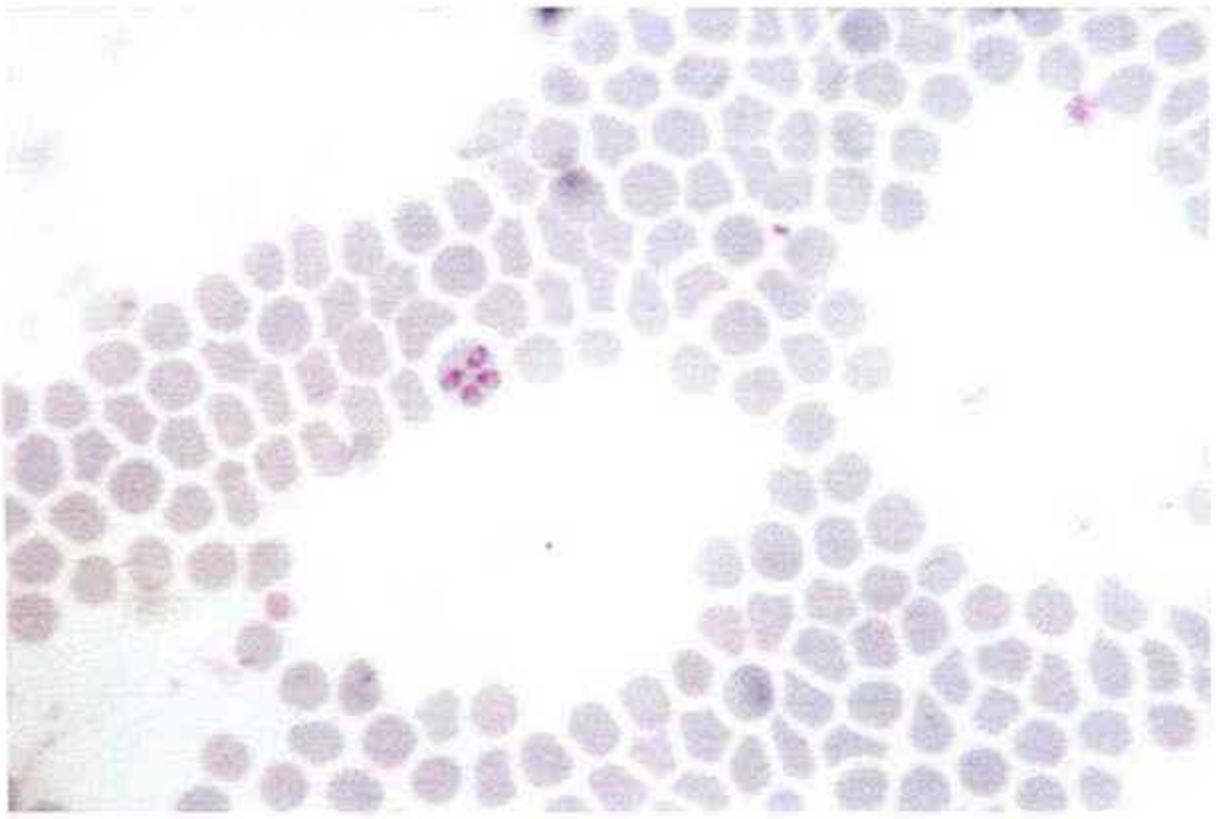


Figure 28.1 Cross-form of *Babesia ovis* in a blood smear

The life cycle of *B. ovis* in the vector *R. bursa* has been thoroughly studied[1, 12, 25, 27, 59, 60] and consists of gametogony (sexual cycle) in the gut of engorged female ticks, which results in the formation of ookinetes (zygotes). A primary schizogony (= sporogony) produces sporokinetes. A number of schizogonic cycles occur in the gut and various tissues. Schizonts have been observed in the haemolymph, eggs and salivary glands. During the final schizogony in the salivary glands, the infective forms (merozoites) are produced. Young kinetes of *B. ovis* in haemolymph and eggs of *R. bursa* are round in shape, but subsequently become elongated and cigar-shaped (**Figures 28.2 and 28.3**).

Transmission of *B. ovis* by *R. bursa*[99] and of *B. motasi* by *H. punctata* is transovarial as well as transstadial.[7, 12, 97] All instars of *H. punctata* are capable of transmitting *B. motasi* parasites.[7]

Adult *R. bursa* ticks become infected with *B. ovis* during a blood meal. Transmission to a susceptible host occurs by all instars of the succeeding generation.[97] *Rhipicephalus bursa* can be infected with *B. ovis* both alimentarily and vertically. Alimentary transmission gives rise to a higher rate and degree of tick infection than that following vertical transmission. Eggs infected with *B. ovis* appear earlier in horizontally (alimentary) infected ticks than in vertically infected ones.[101]

Infection rates in ticks can be estimated by examining their haemolymph 7 to 12 days after their detachment, and those of eggs 5 to 10 days after the commencement of oviposition.[95] In the salivary glands, infective particles can be observed and are transmitted to the host during a blood meal.

Babesia ovis infection in sheep has been observed 11 to 12 days post-infestation by the tick vector.[57] It is readily transmitted by inoculation of infected blood.[98] Successive serial passages of infected blood in splenectomized lambs result in a decrease in the degree of parasitaemia and apparent attenuation of the parasite; this feature has been employed in the production of a blood-based vaccine.[77, 88, 98] Intra-uterine infection of the foetus in pregnant sheep by *B. ovis* also occurs.[83]

Epidemiology

The larvae, nymphs and adults of *R. bursa*, a two-host tick, parasitize the same animal species, and their natural hosts are wild sheep and goats.[41] This tick is one of the most important species in endemically infected countries (Cyprus, Syria, Israel, the Balkans, Turkey)[2, 8, 44, 48, 69, 71, 92, 98] and favours a habitat characterized by rendzinas and terra rossa soils, low average precipitation and a long, dry summer.[39] It has a strictly seasonal life cycle with one generation annually. Larvae are found on the pasture, and larvae and nymphs on the animals during the winter period (October to February) in the northern hemisphere, with a peak prevalence in November to December. The adult ticks first appear in April, their numbers reaching a peak in May, and persist in the field and on the animals until July.[96]

There is a striking seasonal incidence of ovine babesiosis caused by *B. ovis*. The disease occurs annually in *R. bursa*-infested areas during the activity period of the adult ticks.[97] The prevalence of babesiosis reaches a peak in May, corresponding to a peak in the infestation rate of *R. bursa* on the animals. It thereafter decreases and, by the end of July, disappears.[2, 10, 44, 50, 61, 69, 80, 97] Ovine babesiosis occurs two weeks after the first appearance of the *R. bursa* adult ticks on pasture.[94] The disease may reappear annually in the same flocks in endemic areas. This epidemiological pattern differs from the state of enzootic stability known to develop in bovine babesiosis.[54] Sporadic clinical cases of ovine babesiosis

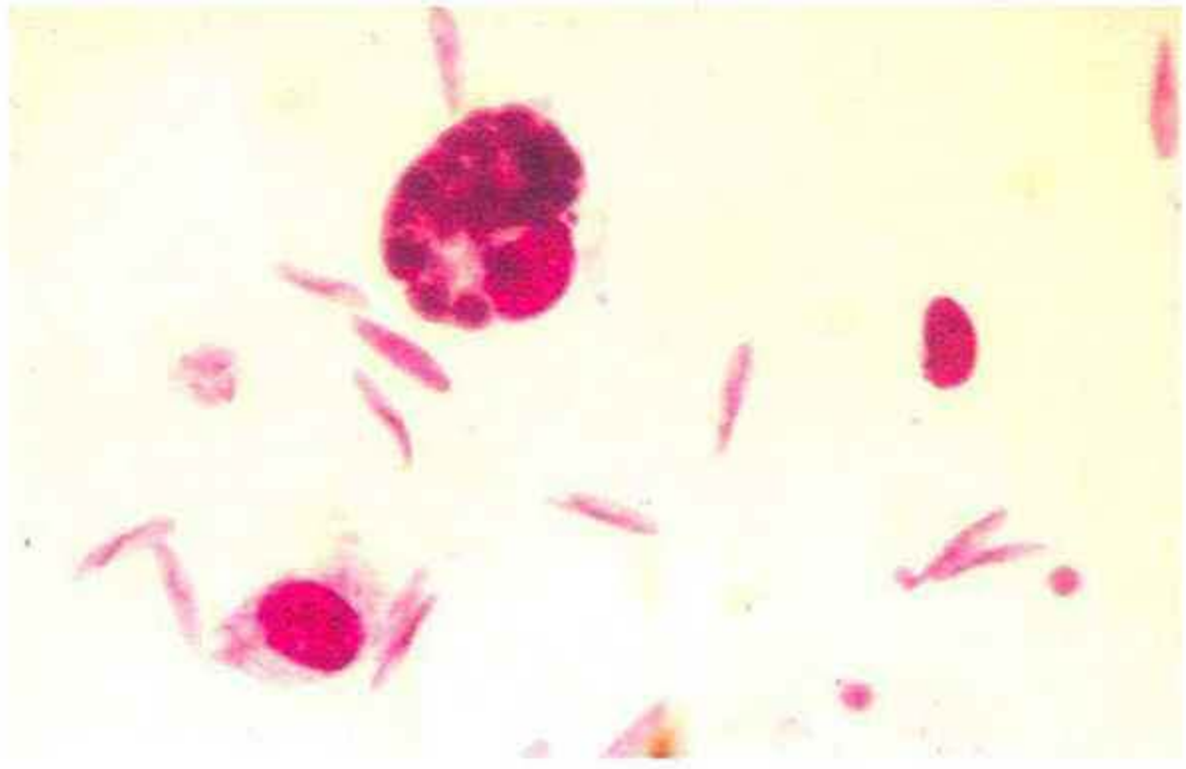


Figure 28.2 Kinetes of *Babesia ovis* in a smear of the haemolymph of a tick



Figure 28.3 Kinetes of *Babesia ovis* in a tick-egg smear

may occur outside the main season of adult *R. bursa* activity, concurrently with the rare appearance of few adult ticks.[80] Relapses of chronic latent cases due to stress factors, such as heat, food shortage and helminthosis, may also occur in the autumn months.

The incidence of babesiosis in 6- to 12-month-old sheep in their first grazing season may be almost double (10 per cent) that of ewes (5.1 per cent).[72, 97] The average rate of parasitaemia in lambs and hoggets (7.5 per cent) may be somewhat higher than in ewes (5.6 per cent).[93] These rates are rather low when compared to those recorded in endemic areas of bovine babesiosis.[16, 65]

It appears that a certain percentage of sheep are not exposed annually to infection and consequently remain susceptible. Enzootic stability in bovine babesiosis depends on regular exposure to the parasite, and is influenced by climatic conditions, animal management, and dipping. It appears that enzootic stability in ovine babesiosis in *R. bursa*-infested areas is rarely established. In general, unstable enzootic situations accompanied by outbreaks of ovine babesiosis depend to a large extent on four factors: transhumance (animals are moved to endemic areas), health management (tick control and chemotherapy), especially unfavourable climatic conditions, and chemoprophylaxis. Intensive tick control should be avoided to maintain enzootic stability.[82]

Recovered animals remain patent or latent carriers of *B. ovis* for varying periods. The parasites persist in the blood of infected sheep for two years,[31] and ticks feeding on the animals during this period may become infected. The presence of

parasites in the circulating blood maintains a state of premunity.[78]

High annual rates of seroconversion have been observed in both hoggets and ewes (84,5 per cent and 89 per cent, respectively). The 'autumn-winter rise' in serological reactions observed in young animals might be caused by larval and nymphal infestations.[97]

In young animals, immunity to babesiosis may be conferred by colostral antibodies and exposure to pre-imaginal and/or adult *R. bursa* ticks. Morbidity in flocks grazing in endemic areas can be attributed to the fact that only 75 per cent of the colostrum samples of the hoggets and 67,5 per cent of those of the ewes may contain maternal antibodies to *B. ovis*, and these may persist for only three months in lambs,[31, 94] as compared to the longer period of resistance known in *B. bovis* in cattle.[75] It therefore appears that lambs must be exposed to *B. ovis* infection up to the age of three months in order to maintain an immune state. Lambs infested with *R. bursa* larvae may develop antibody titres after a relatively long post-infestation period (39 days on average) and may suffer from only mild clinical reactions when subsequently challenged with infected *R. bursa* adult ticks.[94] The delay in the serological reaction and the fact that only 85 per cent of the lambs may become serologically positive are probably due to the low *B. ovis* infection rate of the pre-imaginal stages of the tick.[99]

The relationship between antibody levels and the degree of immunity to babesiosis is not clear. Serologically positive animals, when challenged, may develop clinical babesiosis. A similar situation has been reported in bovine babesiosis.[13, 29] In addition to the climatic and husbandry factors mentioned above, the precarious enzootic stability in ovine babesiosis caused by *B. ovis* might be due to the fact that the active period of *R. bursa* adults is short, resulting in low levels of colostral antibodies, as well as the short lifespan of these antibodies in lambs. The transmission of *B. ovis* by the pre-imaginal stages during the autumn and winter months, causing seroconversion, might play a major role in pre-immunizing and boosting the immunity in sheep against infections which occur during the spring to summer months.[97]

Ovine babesiosis caused by *B. motasi* has not been studied in detail. It has been suggested that Nigerian sheep are resistant to infection with *B. motasi*.[47] Furthermore, certain isolates of *B. motasi* might be of lower pathogenicity, while others are known to cause severe disease and even death in sheep in India[51] and in Somalia.[18] It has been proposed that the virulence of different isolates of *B. motasi* and the severity of the disease might be greatly affected by the tick vector.[51, 18] Although *B. motasi* may be a significant pathogen on its own, it seems more probable that disease in certain regions may be complicated by other tick-borne pathogens, such as *Theileria* spp., *Ehrlichia* spp.,[52] and Nairobi sheep disease virus.[18]

In goats, clinical babesiosis cases are rare.[64] It seems that the local breeds possess a certain degree of natural resistance to babesiosis.

In the absence of non-ovine susceptible hosts, the tick might serve as a reservoir of *B. ovis*.[56] *Babesia ovis* and *B. motasi* have been reported to occur in unusual hosts[20] but the role of these, as asymptomatic carriers, is unknown.

Pathogenesis

Upon infection of a susceptible sheep with *B. ovis* or *B. motasi*, multiplication of the organisms occurs in erythrocytes with the development of a clinically detectable anaemia which results in hypoxia of tissues that may cause the death of the host. Organs, particularly those with high metabolic activity, such as the liver and kidneys,[37] are the most severely affected in ovine babesiosis.[33, 34, 48, 85, 93]

Severe anaemia may occur in sheep harbouring a low parasitaemia, which implies that additional factors are involved in its pathogenesis. Erythrophagocytosis of both parasitized and non-parasitized erythrocytes has been observed in cases of *B. ovis* infection. A similar finding has been recorded in infections with other species of *Babesia*.[37, 53]

The anaemia following infections with *Babesia* spp. is considered to be caused by:

- mechanical damage of red blood cells by invading organisms;
- autoimmune phenomena; and
- adsorption of antigens onto normal erythrocytes followed by the formation of immune complexes and the activation of the classical complement pathway resulting in their destruction.[6]

The pathogenic mechanisms of *B. ovis* are broadly similar to those of *B. bovis*, provoking lesions characteristic of a hypovolaemic and endotoxic shock process. The latter is probably the result of a massive release of catabolites from the lysis of erythrocytes and necrosis of tissues.[33] The lesions observed generally arise from vascular alterations, such as vasodilation and vascular stasis, leading to hypoxia of the tissues.[33] Apart from the changes in the brain, other findings are similar to those reported in *B. bovis* infection of cattle.[91]

Clinical signs

Ovine babesiosis, in general, is an acute disease particularly in susceptible animals. Morbidity and mortality due to ovine babesiosis in flocks in endemic areas occur annually. *Babesia ovis* infections may cause 10 per cent morbidity with 2 per cent mortality,[93] but a mortality rate as high as 25 per cent has been reported.[72]

The clinical signs of ovine babesiosis caused by either *B. ovis* or *B. motasi* are similar. In general, the signs observed are typical of those due to an anaemia. An incubation period, generally ranging from seven to nine days, is followed by a febrile period of several days during which inappetence and depression are evident. The febrile response in babesiosis caused

by *B. ovis* precedes a detectable parasitaemia by at least 24 hours.[99]

Initially, visible mucous membranes are reddened and the urine is normal in colour, but as the disease progresses visible mucous membranes become pale and icteric. Haemoglobinuria is uncommon. Constipation may occur, and faecal pellets may contain blood and be covered by mucus. Respiration and pulse rates are increased. Weakness and prostration develop together with muscular tremors, lachrymation and salivation. The parasitaemia in *B. ovis* infections is usually low (less than 1 per cent).[99]

Death may occur at any time after the onset of the clinical signs depending on the virulence of the *Babesia* strain and the susceptibility of the animal.

Pathology

Infection with either *B. ovis* or *B. motasi* leads to a macrocytic hypochromic anaemia which coincides with the peak of parasitaemia. Reticulocytosis, poikilocytosis and anisocytosis, together with hypochromasia, indicate that haemopoetic centres attempt to compensate for the anaemia. There is an initial leukocytosis, largely due to neutrophilia,[6] and increased total and conjugated bilirubin levels.

The clinico-pathological findings in babesiosis due to *B. motasi* infection are considerably milder than those observed in *B. ovis* infections.[6]

Affected animals show a decrease in the packed cell volume (PCV), which ranges between 30 and 40 per cent.[99] There is an increase in plasma transaminases, particularly aspartate aminotransferase and alanine aminotransferase, dehydrogenase, blood urea nitrogen and creatine, and a decrease in sorbitol dehydrogenase, alkaline phosphatase, total proteins and albumin.[30, 58] A proteinuria occurs in the course of the disease.[30]

In experimental *B. ovis* infection in sheep[33, 99] evidence of anaemia, marked icterus, splenomegaly, a grossly enlarged dark-brown degenerated liver, and nephrosis are noted. Ecchymotic haemorrhages are present in the epicardium, endocardium and subserosa of the viscera.

Erythrocytes parasitized by *B. ovis* have not been observed to block capillaries in the brain,[99] as recorded in *B. bovis* infections in cattle. This explains the absence of neurological signs in acute ovine babesiosis caused by *B. ovis*. A disseminated intravascular coagulation syndrome has been observed in sheep suffering from *B. ovis* infection.[33, 99]

Diagnosis

Acute ovine babesiosis can be confirmed by microscopic examination of stained blood smears made from capillary blood. The parasitaemia in *B. ovis* infection is, however, low. Diagnosis of ovine babesiosis at necropsy is possible if decomposition is not too advanced. *Babesia* parasites deform within a few hours of death, thus making their morphological identification impossible. Thin blood smears from the peripheral circulation, and impression smears of the spleen, liver and kidneys should be made.

A variety of serological tests has been used to demonstrate the presence of antibodies to *B. ovis* and *B. motasi*.[5, 17, 23, 31, 45, 49, 63, 70, 87, 99] Cross-reactions have recently been reported within the genus *Babesia*. A high correlation is found between *B. motasi* and *B. crassa*, and a lower one between these two and *B. ovis*.[63] No cross-reactions have been found between *B. ovis* antigens and *B. motasi*, *B. crassa* or *Theileria ovis*.[32] Serological tests are used mainly in epidemiological surveys. The indirect immunofluorescence test (IFAT) is one of the simplest, most sensitive and effective techniques used for this purpose. However, as in the case with other serological techniques, the specificity of the method is not always satisfactory.[63]

Differential diagnosis

Ovine babesiosis should be differentiated from infections caused by other tick-borne blood parasites such as *Anaplasma ovis*, *Theileria ovis*, *Cytoecetes phagocytophila* and *Eperythrozoon ovis*. Copper poisoning resulting in an acute haemolytic crisis with icterus, should also be considered.

Control

The control of babesiosis in sheep is similar to that used for the control of bovine babesiosis (**see Chapter 25: Bovine babesiosis**) and is based on a combination of strategic tick control, vaccination, chemoprophylaxis, when necessary, and chemotherapy.

Immunization can be achieved by inoculation of a whole blood vaccine containing attenuated parasites.[46, 77, 88] Attenuation is achieved by serial passage of the parasites in splenectomized lambs. A crude extract of *B. ovis* parasites in Freund's complete adjuvant has been also used as a vaccine.[4] There is, however, no ovine babesiosis vaccine available commercially. An effective method for the control of the disease in endemic areas might be the exposure of sheep to pre-imaginal stages of *R. bursa* in October to February before the main adult tick period in April to July.

Animals suffering from clinical babesiosis should be treated with the babesiacidal drugs used in bovine babesiosis. Diminazene aceturate (Berenil®) is used at a dosage rate of 3 to 5 mg/kg body weight subcutaneously.[81, 87] Diminazene can also be used in the chemoprophylaxis of ovine babesiosis with a withdrawal period as recommended by the manufacturers.[28] Imidocarb dipropionate (Imizol®) is used at a dosage rate of 1,2 mg/kg body weight intramuscularly. A second treatment may be necessary in case of recrudescence of the infection.[36] Amicarbalide isethionate (Pirodia®, Diampron®) at 5 mg/kg body weight subcutaneously has also been used with success.[87]

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THEILERIOSES

Synonym: Theileriose (Afrik.)

Theileria is a genus comprising tick-transmitted parasitic protozoa in the family Theileriidae, order Piroplasmida, subclass Piroplasmia, phylum Apicomplexa.[6] It includes parasites previously allocated to the genera *Cytauxzoon*, *Gonderia* and *Haematoxenus*. The taxonomy of the genus at species level is controversial.

Of the species of *Theileria* that infect cattle, the most important in sub-Saharan Africa is *Theileria parva*.[13] Originally described as a single species, it was for a period divided into three subspecies: *T. parva parva* (the cause of classical East Coast fever); *T. parva lawrencei* (the cause of Corridor disease); and *T. parva bovis* (the cause of Zimbabwe theileriosis).[5, 16] This subdivision has now been abandoned.[1, 11] *Theileria annulata*[3] is the only other major cause of bovine theileriosis and occurs in North Africa, southern Europe and Asia. In the course of investigation into the development and implementation of control measures for East Coast fever and *Theileria annulata* theileriosis, a number of other theilerial parasites have been identified in cattle (**see Chapter 29: East Coast fever: Table 29.1**). The first such organism was *Theileria mutans*,[14] a species which is usually non-pathogenic, although it may cause severe or fatal disease in East Africa. *Theileria buffeli* was originally identified in Asiatic buffalo.[9] It, or species closely related to it in the so-called *Theileria buffeli/orientalis* group, is widely distributed around the world in cattle, but rarely causes clinical disease, except in eastern Asia. More recently, two other species of *Theileria* have been described in cattle, both usually non-pathogenic, *Theileria (Cytauxzoon) taurotragi*[7] and *Theileria (Haematoxenus) velifera*.[15]

For the purposes of this book, East Coast fever in its classical form is described as the type example of theilerioses in cattle in southern and eastern Africa. Other diseases caused by members of the *T. parva* complex are considered in comparison with East Coast fever, while the other species of *Theileria* are discussed only to the extent that their presence affects the diagnosis and control of the *T. parva* complex. *Theileria annulata* theileriosis is described as a separate entity.

The extensive literature on the theilerial parasites and the diseases that they cause in cattle have been reviewed comprehensively.[2, 4, 8, 10, 12]

The taxonomy of the species of *Theileria* that infect small ruminants is very confused, but only one species, *Theileria lestoquardi* (*T. hirci*) is of economic significance. It occurs in the Mediterranean basin, North Africa and Asia. The other species are generally regarded as being benign and are widely distributed in Africa, Asia and Europe.

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29

East Coast fever

Synonyms: Cattle-derived *Theileria parva* infection, *Theileria parva parva* infection, Ooskuskoors (Afrik.)

J A LAWRENCE, B D PERRY AND S M WILLIAMSON

Introduction

East Coast fever in its classical form is a usually fatal disease of cattle caused by *Theileria parva*. It is transmitted principally by the brown ear tick *Rhipicephalus appendiculatus* and is characterized by the proliferation of lymphoblasts infected with theilerial schizonts throughout the body, particularly in the lymph nodes, lymphoid aggregates, spleen, kidneys, liver and lungs. The disease occurs widely through the range of its main vector in eastern, central, and southern Africa north of the Zambezi River (**see Chapter 1: Vectors: Ticks**). It was introduced into the region south of the Zambezi River in 1901/02 and spread through most of the range of its vector but was subsequently eradicated (**Figure 29.1**).

The classical disease is seen in cattle of European origin which have been exposed to infected ticks. Cattle of African origin have a very variable response to infection and the disease may be insignificant or subclinical in Zebu calves born from immune dams and raised in endemically infected areas.

The disease was first described as an unusually virulent form of babesiosis by Gray and Robertson[63] in 1902, following its introduction into Zimbabwe. It was initially named Rhodesian redwater. Koch[75] identified the parasite with one that he had seen in 1897 in cattle on the coast of Tanzania. He named the disease African Coast fever, the name being subsequently modified to East Coast fever in most countries in the region other than Zimbabwe. The causal organism was named *Piroplasma kochi* by Stephens and Christophers in 1903, but the name was changed to *Piroplasma parvum* by Theiler[154] and subsequently to *Theileria parva* by Bettencourt, França and Borges in 1907 (as quoted by Henning[64]). Uilenberg[161] proposed that the organism be named *Theileria parva parva* to distinguish it from other subspecies of *T. parva* which cause diseases that can be differentiated from East Coast fever on epidemiological grounds. However, it has since been agreed that classical East Coast fever be known as cattle-derived theileriosis and that it is caused by the same parasite as that causing Corridor disease and January disease.[3, 128]

East Coast fever has long been endemic in eastern Africa. The parasite probably originated from African buffalo (*Syncerus caffer*) populations in eastern Africa and became adapted to cattle following their introduction and dissemination in the region, especially in the Lake Victoria Basin and along the coastal strip.[125] It spread widely through the region during the early part of the nineteenth century as a result of European settlement, which involved changes in patterns of movement, extensive use of ox-drawn transport, and importation of susceptible cattle breeds from overseas. The First World War (1914 to 1918), during which the campaign in what was then German East Africa, now Tanzania, took place, also resulted in unusual cattle movements and further dissemination of the disease.

East Coast fever was introduced into the area south of the Zambezi River in the period 1901 to 1903 by cattle which were imported from Kenya and Tanzania for the purpose of restocking the region after the ravages of the rinderpest epidemic of 1896 and the South African War (1899 to 1902).[77] The disease initially appeared in Zimbabwe, southern Mozambique and the northern parts of South Africa, and then spread southward along the east coast of southern Africa, through Swaziland, KwaZulu-Natal, and the Eastern Cape Province. In the period up to 1914 it is estimated to have killed one and a quarter million of the four million cattle that were present in the affected territories at the beginning of the outbreak. The southward advance was halted in the region of East London in the Eastern Cape Province, and the disease was subsequently eradicated in a prolonged campaign consisting of movement control, tick control, destocking of infected pastures, and slaughter. It was eradicated from southern Mozambique by 1917, from Zimbabwe by 1954, and from South Africa by 1955. The last case south of the Zambezi River occurred in Swaziland in 1960. The disease has persisted in the region north of the Zambezi River to the present day and is a major constraint on the development of cattle production.

East Coast fever, if uncontrolled, may cause over 90 per cent mortality of the susceptible cattle following its

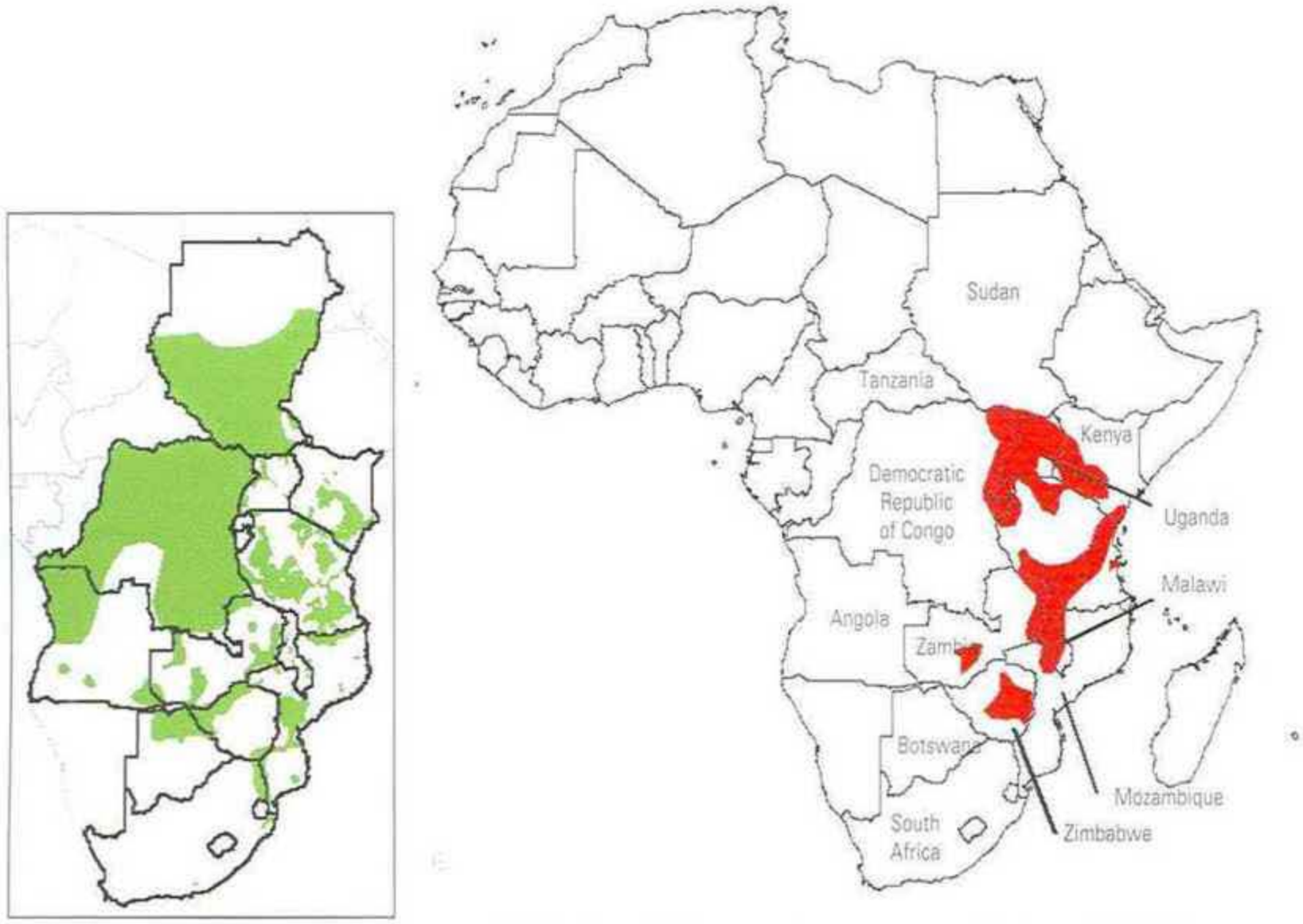


Figure 29.1 The approximate distribution of cattle-associated *Theileria parva* infection, namely East Coast fever and Zimbabwe theileriosis, in eastern and southern Africa, from Lessard *et al.* (1990). Buffalo-associated infection, commonly referred to as Corridor disease, occurs in areas in which cattle and African buffalo (*Syncerus caffer*) have contact. It has a similar but wider distribution that includes South Africa, Botswana and Angola
INSET: The approximate distribution of the African buffalo, modified from Lessard *et al.* (1990)

introduction into a region. In an area where it is endemic, mortality among locally adapted Zebu-type cattle may be negligible but there is evidence that the disease causes a significant reduction in growth and productivity. Susceptible cattle introduced into an endemic area are very vulnerable to infection and the cost of the control measures required is a continuous financial burden on both livestock owners and state veterinary services.[51, 108]

Aetiology and life cycle

Theileria parva, the causal organism of East Coast fever, alternates between ox and tick in its life cycle (**Figure 29.2**). The sexual stage of development occurs in the gut of the tick.[41] Piroplasms in the erythrocytes of the ox are ingested with the blood meal of the tick and develop into thread-like microgametes and globular macrogametes. These fuse to form zygotes which invade the epithelial cells of the gut of the tick. Completion of development occurs in the immature stages of the tick only after they have detached from the host. As the tick moults, a club-shaped motile kinete forms within the zygote. This is liberated into the body cavity and migrates to the salivary glands via the haemolymph. The kinete invades an epithelial cell of the salivary gland, almost exclusively E cells of type III acini,[56] and develops into a large syncytial sporoblast within which many thousands of minute, elongated sporozoites develop during the early part of the next feeding stage of the tick. Acini of salivary glands containing mature sporozoites can be identified easily in whole mount preparations of tick salivary glands stained with methyl green pyronin.[166] A polymerase chain reaction (PCR) and DNA probes have also been used to detect *T. parva* in ticks[36, 168] and to discriminate *T. parva* from *T. taurotragi*.[18]

The sporozoites are liberated into the saliva and inoculated through the skin of the ox as the tick feeds. They enter lymphocytes and develop within them, becoming multinucleate schizonts after a period of about three days.[149] Sporozoite entry is rapid and energy dependent, and is effected by a zippering process between sporozoite and lymphocyte plasma membranes.[57, 143, 169] Differentiation

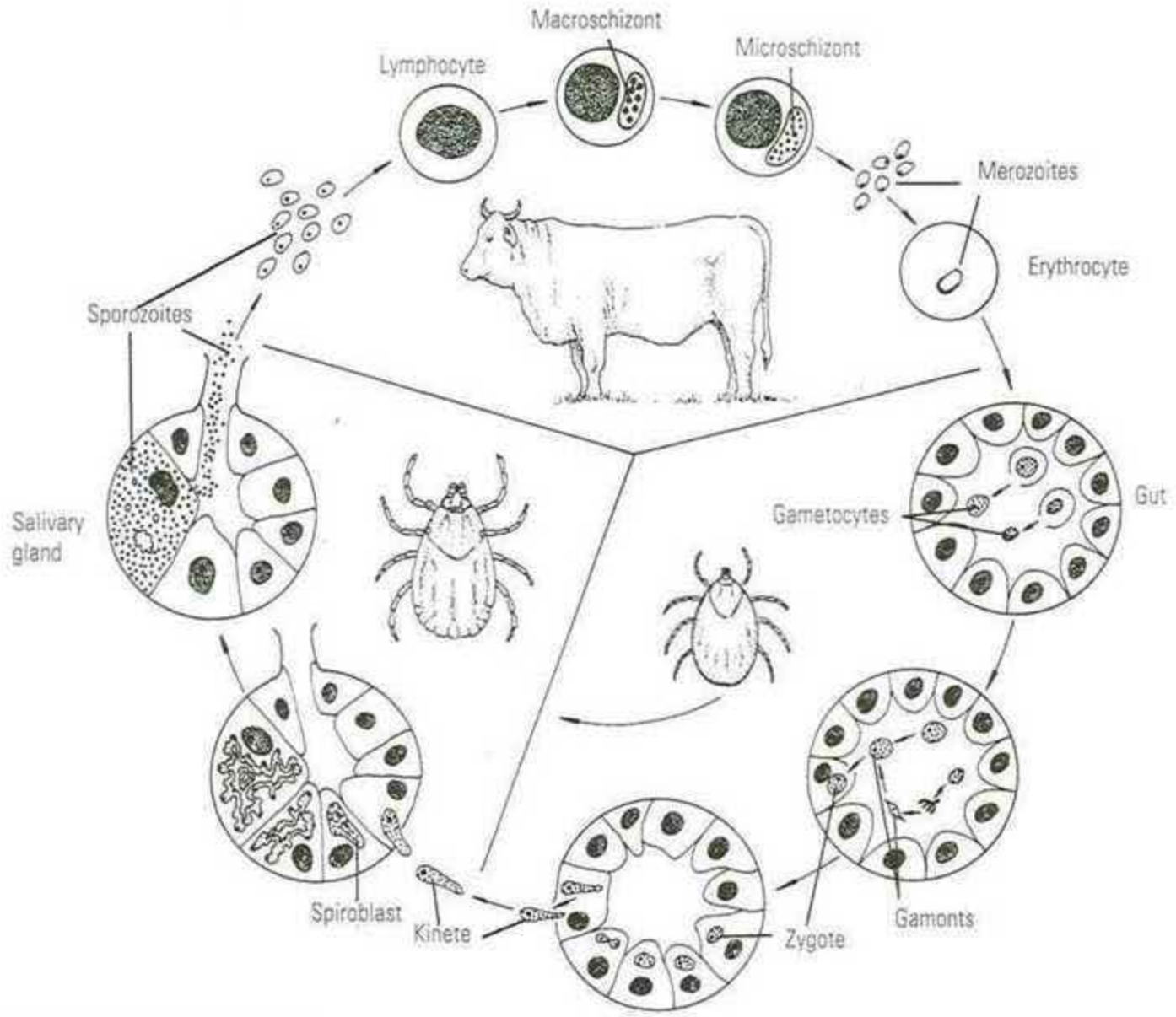


Figure 29.2 Life cycle of *Theileria parva*

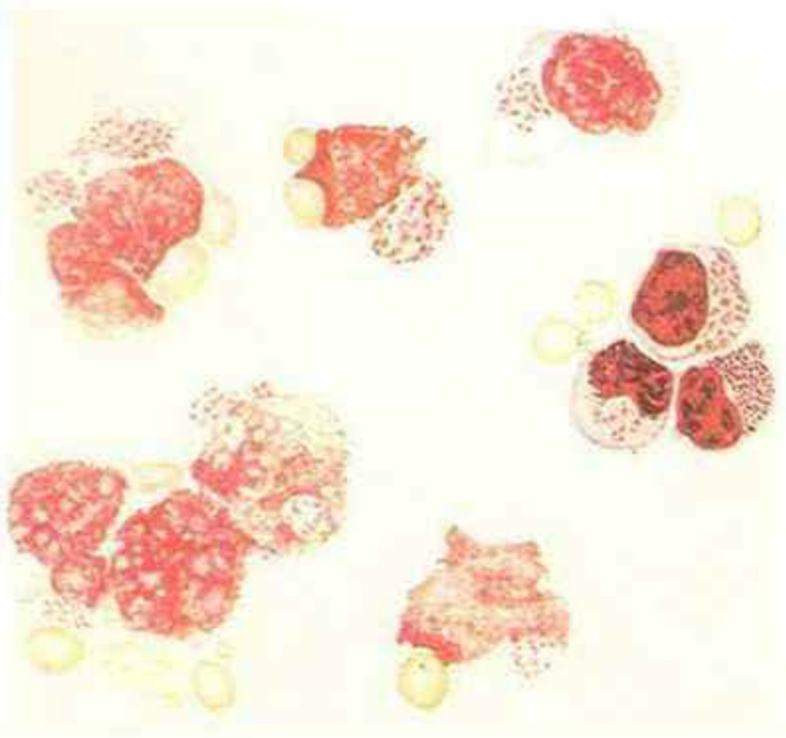
into the schizont stimulates the host lymphocyte to transform into a lymphoblast and thereafter the schizont divides in synchrony with the host cell as it undergoes mitosis.[65] It is believed that schizonts have the ability to transfer from one host cell to another, but the mechanism by which they do so is not known and the frequency with which transfer occurs is probably limited.[104] The kinetics of schizont replication have been described in detail.[102] Schizont-infected cells proliferate exponentially, becoming distributed throughout the lymphoid system, with metastasis of infected cells to non-lymphoid tissues also occurring. Initially the schizonts have large chromatin particles and are called macroschizonts. Later, a generation of schizonts called microschizonts develops with small chromatin particles. Merozoites liberated from the microschizonts invade the erythrocytes, in which they are frequently referred to as piroplasms, thus completing the life cycle. Merozoites have the ability to divide within erythrocytes to produce four daughter merozoites[38] but intra-erythrocytic division is uncommon.

Recent studies on the fine structure of the developmental forms of the organism have been reviewed.[93]

Two stages in the development of *T. parva* are readily identified in the infected ox, namely the schizont in lymphoblasts and the piroplasm in erythrocytes. The schizont is a mass of protoplasm contained within a thin membrane which is situated intracytoplasmically in the infected lymphoblast (**Figures 29.3 to 29.7**). It varies in diameter from about 1 to 16 µm with a mean diameter of 5 µm and contains within it between 1 and 85 chromatin bodies, with a mean of eight.[13] In macroschizonts the chromatin granules average 1 µm in diameter, while in microschizonts they average 0,5 µm.[118] The size of the schizonts and the number of chromatin bodies that they contain increase as the disease progresses.[13] With Romanowsky stains, e.g. Giemsa, the cytoplasm stains pale blue, typically of a lighter shade than that of the host cell, and the chromatin granules stain red (**Figures 29.3 to 29.7**). In haematoxylin and eosin stained histological preparations the chromatin is basophilic and the cytoplasm neutral. The macroschizont was first described by Koch in 1903[75] and is often known as a 'Koch's body' or a 'Koch's blue body', the latter name emphasizing its appearance when stained by a Romanowsky technique.

Schizonts are found intracellularly in lymphoblasts in lymphoid tissues throughout the body (including lymph nodes, spleen, thymus, Peyer's patches and the lymphoid aggregations in parenchymatous organs which are a feature





Figures 29.3 (top) and 29.4 (bottom) Impression smears from prescapular lymph node: drawings of lymphoblasts and lymphocytes containing macro- and microschizonts. Note several schizonts are extracellular after disintegration of lymphocytic cells. (By courtesy of the Editor of the *Onderstepoort Journal of Veterinary Research*. Printed in Volume 27, Number 3, 1957)

of theilerial infections) as well as in the blood. They frequently appear extracellularly in smears as a result of rupture of the host cell. The number of lymphoblasts infected with schizonts is variable and ranges from less than 1 per cent to over 70 per cent (in fatal cases).[13] The infection rate increases as the disease progresses. There may be more than one schizont in a single infected cell.

The intra-erythrocytic piroplasms were first described by Koch[74] in 1898, although he mistook them for a developmental form of *Babesia*. The majority are rod-shaped, but round or oval forms are also seen (**Figures 29.5 to 29.8**). They measure 1 to 2 µm at their greatest dimension, with a

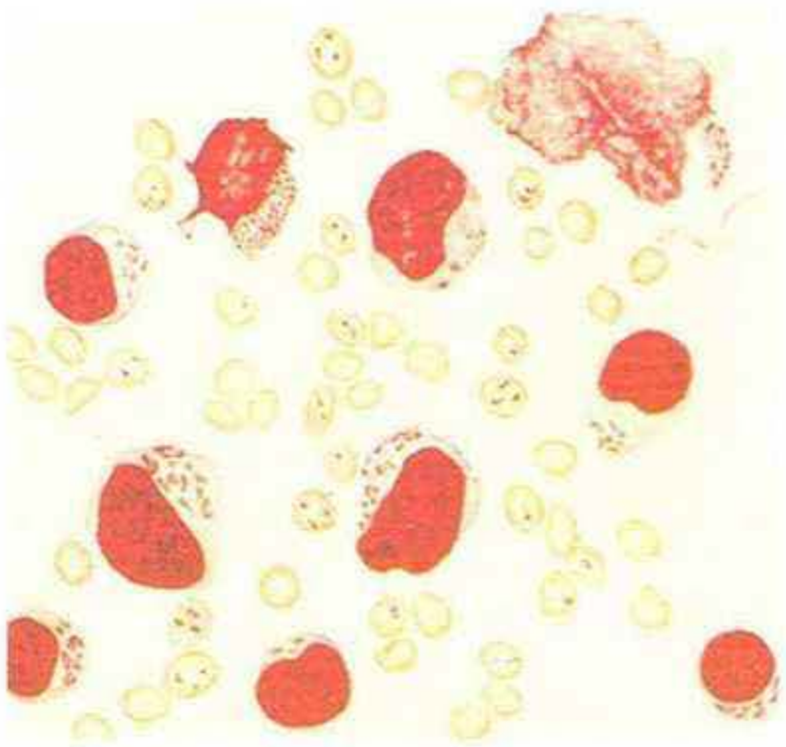


Figure 29.5 Blood smear: drawing of lymphocytes and lymphoblast containing macro- and microschizonts and red blood cells infected with piroplasms. (By courtesy of the Editor of the *Onderstepoort Journal of Veterinary Research*. Printed in Volume 27, Number 3, 1957)

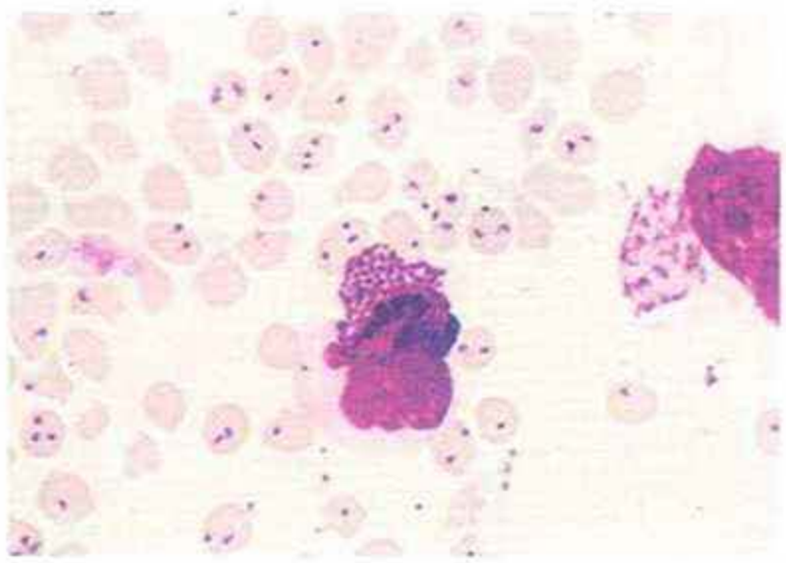


Figure 29.6 Blood smear of a bovine infected with *Theileria parva*

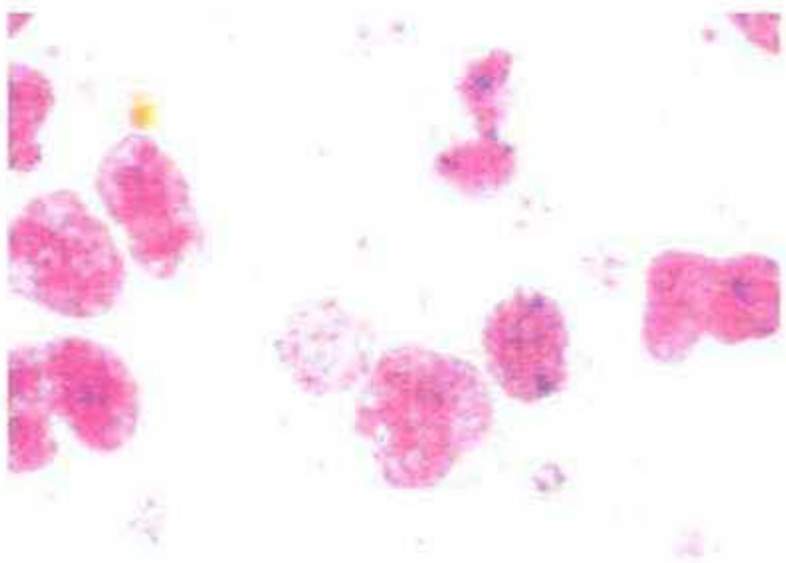


Figure 29.7 Spleen smear of a bovine infected with *Theileria parva*

neutral or pale blue cytoplasm and a red chromatin granule at one pole in Romanowsky-stained blood smears. Although usually single, there may be several parasites in one erythrocyte in heavy infections. Numbers vary considerably and as many as 80 per cent of the erythrocytes may be infected.[118]

Following experimental infection, schizonts may be detected between 5 and 15 days after attachment of the tick,[118] and they persist for a variable length of time. Piroplasms are likely to appear four to five days later and usually disappear about 10 days after final recovery of the animal, but may persist indefinitely.

The schizont of *T. parva* was first cultivated *in vitro* by Hulliger, Wilde, Brown and Turner[65] in 1964 and was subsequently established in bovine lymphoblastoid cell lines by Malmquist, Nyindo and Brown[83] in 1970. Lymphoid tissue or circulating lymphocytes from infected cattle can be cultured directly or seeded onto a feeder layer of bovine embryo spleen cells. Schizont-infected lymphoblasts divide and become established as a self-perpetuating line of cells growing in suspension. The schizonts separate into daughter schizonts in association with the mitotic apparatus of the host cell during division and are distributed one to each daughter cell, with the result that almost all the cells in the culture are infected. Cultures can also be established by the inoculation of uninfected lymphocytes with sporozoites derived from infected ticks.[30] Schizont cultures have proved extremely useful as antigens for use in serological tests to detect serum antibodies in cattle,[33] as antigens for monoclonal antibody characterization of isolates of the parasite,[132] as a source of DNA for molecular studies,[39] as target material for screening chemotherapeutic agents,[88] and as a source of infective material for transmission to cattle.[29] Techniques for the propagation of theilerial parasites in culture are described in detail by Brown.[23, 25]

In the field, transmission of East Coast fever takes place only through the medium of the tick vector. The natural vector is *R. appendiculatus*, a common parasite principally infesting the ears of cattle and other herbivores in the more humid areas of southern and eastern Africa (**see Chapter 1: Vectors: Ticks**).[82] Other members of the genus *Rhipicephalus* and several *Hyalomma* spp. have been shown to be capable of transmitting *T. parva* in laboratory conditions (**Table 29.1**).[21, 22, 78, 118] There is, however, no evidence to suggest that any tick other than *R. appendiculatus* plays any significant role in transmission of the disease in nature.

Rhipicephalus appendiculatus is a three-host tick, and transmission of *T. parva* is achieved only by nymphs infected during the preceding larval stage or by adults infected during the preceding nymphal stage. Transovarial transmission does not occur, nor is there transmission between larva and adult if the intervening nymph feeds on a non-susceptible host. The tick acquires infection when it feeds on an ox at a stage of clinical disease or recovery when piroplasms are present in the blood. The succeeding stage transmits infection as it feeds when the sporozoites in its salivary glands

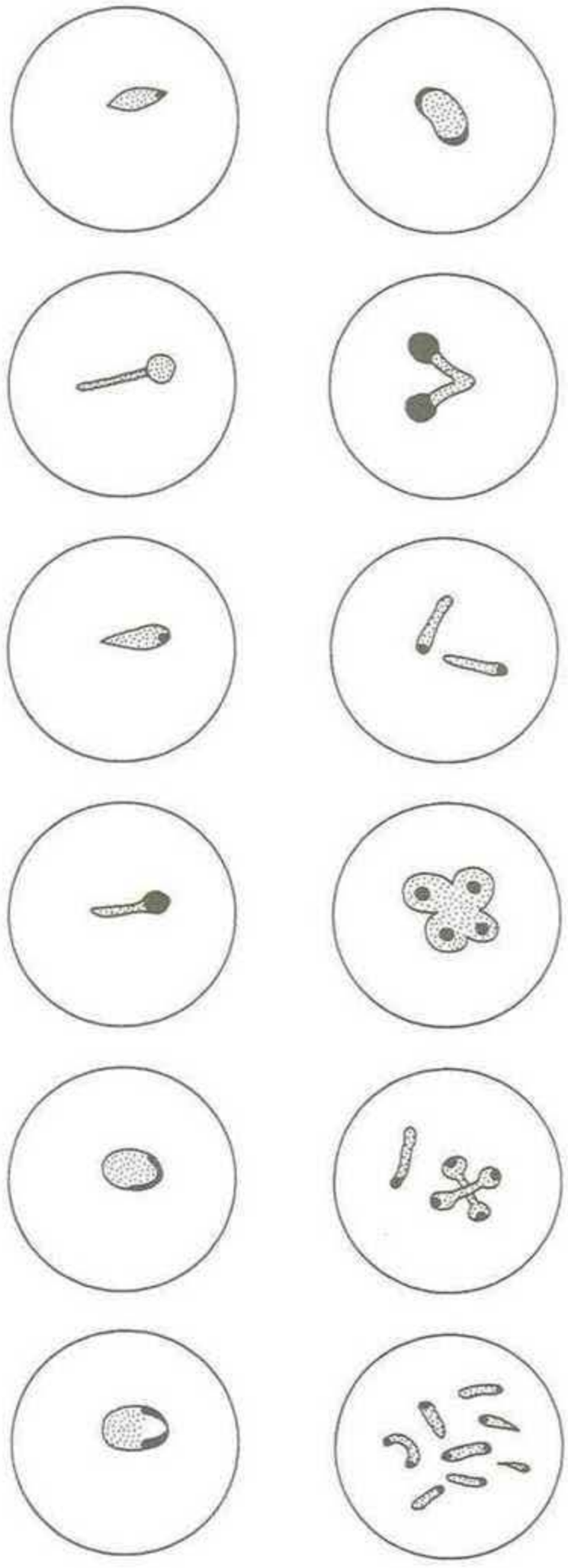


Figure 29.8 Line drawings of piroplasms of *Theileria* spp. other than those which develop veils

have matured. At moderate to low ambient temperatures maturation of sporozoites in adult ticks is not complete until three to four days after feeding commences;[126, 135] maturation in nymphs is more rapid.[133] At ambient temperatures of 37°C or above, sporozoite maturation can occur in the unfed tick,[177] and infection may be transmitted as soon as the tick begins to feed.

Artificial transmission of *T. parva* can be achieved in two ways. The first is by the inoculation of a suspension of sporozoites prepared from homogenized tick salivary glands or whole ticks, the so-called GUTS (ground-up tick supernatant) preparation.[42, 158] This method mimics natural transmission and obviates possible variations in tick attachment and feeding behaviour. By pooling a number of ticks before homogenization it is also possible to eliminate the variation in infectivity between batches of ticks. The method is widely used for the characterization of isolates of *T. parva* in cattle and for the infection and treatment method of immunization **(see Control: Vaccination, below)**. Stabilates of infective sporozoites can be stored in liquid nitrogen and will maintain their viability and biological characteristics for long periods.[43]

The second method is by the inoculation of schizonts. Suspensions of schizonts in spleen and lymph nodes from infected animals were administered intravenously by Theiler[156, 157] to transmit *T. parva* as a method of immunization against East Coast fever, but the technique was crude and the results erratic. Suspensions of infected lymphoblasts in culture provide a far safer and more reliable source of schizonts. Infection can be transmitted regularly to recipient cattle when 10^8 infected donor cells are administered subcutaneously.[24] However, when lower cell doses are used, results are inconsistent and some recipients do not become infected, although an inoculum as small as 10^2 cells will produce infection in the recipient animal if the recipient's own cells infected *in vitro* are used.[101] The critical factor appears to be the ability of the inoculated cells to survive for a sufficient period for the parasite to transfer to the recipient host cells — this depends on the degree of histocompatibility between donor and recipient.

Transmission of *T. parva* by the inoculation of blood can be achieved but the results are very erratic[159] as success depends on the presence of sufficient numbers of infected lymphoblasts in the circulation. Techniques have been described whereby blood from infected animals can be transferred to nymphal ticks as a means of isolating the parasite from blood. This can be achieved either by inoculation into a rabbit on which the ticks are feeding,[134] or by direct inoculation into engorged nymphs.[142]

Epidemiology

The epidemiology of East Coast fever and the other theilerial infections has been reviewed extensively by Norval, Perry and Young.[125] *Theileria parva* is probably originally a parasite of African buffalo which has become adapted to cattle. It has been found to infect waterbuck (*Kobus defassa*)[148] under natural conditions, and the Asiatic buffalo (*Bubalus bubalis*) under experimental conditions.[97] It is not infective to other ungulates, nor to any species of laboratory animal. There is no evidence that wildlife plays any role in the epidemiology of the disease.

Theileria parva depends on its vector tick *R. appendiculatus* for transmission from host to host. The potential distribution of East Coast fever is thus restricted to those areas of eastern, central and southern Africa where ox and tick coexist.[80] In eastern and central Africa, this includes much of Kenya, Uganda, Rwanda, Burundi, the eastern part of the Democratic Republic of Congo, areas of southern Sudan bordering Uganda and much of Tanzania. In southern Africa its range is more limited, and it is confined to the northern and central regions of Malawi, the northern, eastern and central regions of Zambia, and the Tete Province of Mozambique, all lying to the north of the Zambezi River **(Figure 29.1)**. The disease caused by *T. parva* in the Southern Province of Zambia has been historically referred to as Corridor disease, but it seems likely that it is predominantly cattle-associated, rather than buffalo-associated.

The tick vector *R. appendiculatus* is widely distributed throughout the warmer, more humid areas of eastern, central and southern Africa **(see Chapter 1: Vectors: Ticks)**. Within its range its abundance varies considerably, being governed by soil moisture and temperature, presence of suitable habitat and availability of hosts. The distribution of climatic suitability has been plotted on the basis of ecoclimatic indices.[130, 131, 151] Interestingly, areas of ecoclimatic suitability exist where the presence of the tick has yet to be recorded,[124] illustrating that other factors also play a role in determining distribution. Among these is vegetation cover, which influences the microclimate of the habitat of the tick; overgrazing has a marked adverse effect on the tick population.[121, 175] Also important is the livestock production system employed, particularly with respect to how management affects exposure to ticks. Thus in Kenya there is a considerable difference in tick infestation levels and corresponding infection prevalence between open grazing and stall-feeding (zero grazing) management systems.[58] Transmission of East Coast fever may be expected to occur freely in areas where ecological factors permit an average total population of not less than 10 to 15 adult ticks per ox.[153, 174] It is important to note that on the margins of *R. appendiculatus* distribution, the distribution may expand and contract with seasonal or secular changes in climatic suitability.[123]

The seasonality of East Coast fever varies throughout its distribution, but is generally dependent on rainfall and the abundance of adult stages of the tick vector.[145] In the highland and coastal regions of eastern Africa, with its two rainy seasons, seasonality is not very marked, and in areas where all stages occur simultaneously throughout the year, so does East Coast fever. In southern Africa from southern Tanzania

Table 29.1 The theilerioses of livestock in eastern, central and southern Africa

PARASITE	VECTORS	DISEASE	SPECIES AFFECTED	PATHOGENICITY	DISTRIBUTION
Theileria parva	*Rhipicephalus appendiculatus***	East Coast fever	Ox, Asiatic domestic buffalo (experimental)	Usually fatal (benign in resistant Zebu calves)	Endemic throughout most of the range of *R. appendiculatus* north of Zambezi River
	*R. duttoni**				
	*R. zambeziensis**		African buffalo	Benign	
	R. capensis		Waterbuck	Benign	
	R. carnivoralis				
	R. compositus				
	R. evertsi evertsi				
	R. kochi	Corridor disease	Ox	Usually fatal	Localized, in association with African buffalo
	R. pravus		African buffalo	Benign	
	R. pulchellus				
	R. simus				
	Hyalomma anatolicum	Zimbabwe theileriosis	Ox	Variable – often fatal	Zimbabwe highveld
	H. dromedarii				
	H. impressum				
Theileria taurotragi	*R. appendiculatus***		Ox	Usually benign – rarely fatal	Widespread (endemic)
	R. evertsi evertsi				
	R. pulchellus		Eland	Sometimes fatal	
	R. zambeziensis				
Theileria mutans	*Amblyomma hebraeum***		Ox	Usually benign – some strains pathogenic	Widespread (endemic)
	*A. variegatum***				
	A. astrion		African buffalo	Benign	
	A. cohaerens				
	A. gemma				
Theileria velifera	*A. hebraeum***		Ox	Benign	Widespread (endemic)
	*A. variegatum***		African buffalo	Benign	
	A. astrion				
	A. lepidum				
Theileria sp. near *T. buffeli*	*Haemaphysalis* spp.		Ox	Benign	Widespread
Theileria separata	*R. evertsi evertsi*		Sheep	Benign	Widespread (endemic)

* Important local vectors
** Principal natural vectors

southwards, seasonality is much more marked, with clinical disease occurring predominantly during the wet months of January to March, when adult ticks are most active.[172]

Ticks will transmit infection if, during the preceding stage of development, they have fed on an ox with circulating piroplasms. Infective cattle may be clinically ill, recently recovered, or persistent carriers. In general terms, the infective dose the ticks receive will be higher from clinically affected animals than from carriers. For many years it was considered that cattle recovering from infection had a solid, sterile immunity and that ticks could only acquire infection from recently infected animals. There was thought to be no carrier state, and maintenance of infection was thought to depend on a cycle of rapid transmission. This is certainly true of the classical laboratory stocks studied intensively in Kenya (known as 'Muguga') and in South Africa (known as 'Schoonspruit'), and appears to have been true of the disease as it occurred south of the Zambezi River in the first half of the twentieth century.[52] However, investigations in Kenya have demonstrated that the carrier state appears to be widely prevalent in endemic East Coast fever areas.[71, 176]

East Coast fever is maintained by a continuous cycle of transmission between cattle and ticks. Within the infected areas, the incidence of the disease can vary widely depending on numerous factors, including the abundance of the vector and the susceptibility of the host. There is a marked variation in the susceptibility of cattle to infection. Taurine breeds are generally more susceptible than Zebu and Sanga breeds. In addition, there are variations within breeds depending on the epidemiological state of East Coast fever. Under certain specific circumstances, East Coast fever can exist in an endemic form in which it has little or no influence on morbidity and mortality in the herd.[9, 99] This situation was first recognized by Bruce in Uganda,[31] was characterized by Mettam and Carmichael,[95] and is now commonly referred to as endemic stability.[125, 129] However, endemic stability to *T. parva* infection appears to be relatively limited in its distribution and may not be achieved easily. The more common situation seen in the region is that of endemic instability, in which varying degrees of clinical disease are experienced.

Three major epidemiological forms or states of East Coast fever are recognized.[125, 129] These are endemic

stability, endemic instability and epidemic. A state of endemic stability implies an ecological balance between ox, tick and parasite in which regular transmission of *T. parva* occurs in all age groups of the cattle population and high levels of population immunity are achieved, with minimal effects in terms of morbidity and mortality. This is most commonly achieved in areas where indigenous Zebu cattle are kept on pasture in areas highly suitable for the vector, and where major seasonal fluctuations in vector abundance do not occur. Infection prevalence in ticks may be in the region of 1 or 2 per cent,[79] infection intensity in infected ticks is low, infection of calves takes place early in life, and reinfection throughout life is common. Endemic instability is found in two forms, low incidence and high incidence. Low incidence instability is found in areas of very low infection challenge, either in areas marginally suitable for the vector or on properties where acaricides are applied intensively. High incidence instability is characterized by an infection challenge that is insufficient to induce population immunity. This can be as a result of ineffective tick control, or intermediate levels of infection challenge. Epidemic East Coast fever occurs when the disease is introduced to areas previously free of the disease, and often occurs on a seasonal or secular basis at the margins of *R. appendiculatus* distribution.[173, 174]

The distribution of endemic stability and instability is affected by geographical and production system influences. Endemic stability is thus most common in the smallholder systems prevalent in the humid areas surrounding the Lake Victoria basin, where the presence of endemic *T. parva* infection in cattle prohibits or severely limits the keeping of more productive taurine breeds of cattle. Low incidence endemic instability is found on the large-scale commercial beef ranches of the entire region, and in the small-scale dairy systems of Kenya.[58, 59] High incidence instability is characteristic of newer commercial ventures, in which an upgrading of indigenous cattle is undertaken through cross-breeding, and tick control is ineffective. Under such circumstances, and in epidemic East Coast fever, the case fatality may be extremely high.

Pathogenesis

Theileria parva primarily affects lymphoid cells and the course of infection in the ox may be divided into three stages: a prepatent stage; a stage of lymphoid proliferation; and a stage of lymphoid disorganization and depletion.[68]

The prepatent stage covers the period between the inoculation of sporozoites by the tick and the appearance of schizonts in the drainage lymph node. The sporozoites invade lymphocytes and develop into recognizable schizonts after two to three days. There is evidence to suggest that, although sporozoites can infect a similar proportion of both T and B lymphocytes,[7] it is the subsequent rapid multiplication of infected T cells that is responsible for the pathogenicity of the parasite.[104] The schizonts stimulate proliferation of both infected and non-infected lymphocytes, possibly by stimulating production of a T-cell growth factor similar to interleukin-2.[28] Lymphocyte proliferation commences at the site of inoculation, but from five days after infection parasitized cells begin to appear in the draining lymph node.

The appearance of parasitized cells in the lymph node coincides with the onset of fever and stimulates active lymphoid proliferation and the release of large numbers of infected and non-infected lymphoblasts into the efferent lymph.[102] After a further two to three days, more distant lymph nodes in the chain become infected and hyperplastic, and parasitized and non-parasitized lymphoblasts enter the peripheral circulation. These establish in other lymph nodes and lymphoid tissue (thymus, spleen), as well as in many parenchymatous organs (notably liver, kidneys, lungs, myocardium, adrenals), bone marrow, gastrointestinal tract mucosa and sometimes the brain. The distribution of parasites in non-lymphoid organs is variable and may be associated with incidental pre-existing lymphocyte infiltration.

Four to five days after the initial appearance of schizonts, the process of lymphocyte proliferation is succeeded by a process of lymphocyte destruction, and lymphoid organs and lymphoid foci in parenchymatous organs may show evidence of necrosis and depletion of lymphocytes. Both parasitized and non-parasitized lymphocytes are destroyed and this appears to be the result of activation of specific protective cytotoxic T-lymphocytes (CTL) and non-specific CTL ('natural killer' cells).[54] Lymphocyte destruction causes a fall in serum immunoglobulin levels[146] and immunosuppression.[165] This may enhance the progress of the disease and facilitate secondary respiratory infections.

In non-fatal cases, development of specific protective immune responses terminates schizont proliferation. Recovery from infection is thought to depend on the survival of a sufficient number of effector cells over the first 14 days and on their capacity to achieve a specific protective response.[54] A proportion of recovered animals remains unthrifty and unproductive, and foci of infected lymphoid cells may be found persisting in a variety of organs.[48] Immunosuppression may predispose such animals to secondary infections which may eventually prove fatal.

Lymphocyte proliferation and destruction are responsible in a variety of ways for the lesions which characterize East Coast fever. Focal or diffuse infiltration of lymphocytes and lymphoblasts may cause degenerative changes in liver, kidneys and brain, while focal infiltration in the mucosa of the gastrointestinal tract is responsible for necrosis and erosion of the overlying tissue. Activation of the coagulation cascade leading to an accumulation of fibrin degradation products[85, 144] suggests that disseminated intravascular coagulation may be an important component of the terminal stages of the disease. There is also activation of the complement cascade,[144] and vasoactive components are released as a result of lymphocyte disintegration in the lungs. These may be responsible for the pulmonary

oedema which is often the final cause of death.

The invasion of erythrocytes by piroplasms is a striking feature of the disease, but the parasite in this form does not have any consistent pathogenic effect. Non-regenerative anaemia and icterus are sometimes described in East Coast fever, but no evidence of a haemolytic process has been presented.

Immunity to *T. parva* is considered to be principally cell mediated,[127] the most important effector cells responsible for clearance of infection and recovery being CTL, which belong to the $CD8^+$ subset of T cells.[62, 92] These recognize specific *Theileria* antigen combined with major histocompatability complex (MHC) antigens on the surface of schizont-infected lymphoblasts and their cytotoxic activity is thus parasite specific and genetically restricted.[55, 68, 103] There is evidence to suggest that the cytotoxicity is also strain specific, this property correlating with the variable degree of protection to heterologous challenge observed in cross-immunity trials in cattle.[152] Other cellular responses are likely to contribute. For example, $CD4^+$ helper T cells may be involved in the induction of parasite-specific CTL, but their role appears to be secondary to the main CTL immunity.[8] Re-exposure of recovered animals to homologous challenge stimulates activation of memory T lymphocytes which, in turn, stimulate activation of CTL, which eliminate the infection, although some development of schizonts and piroplasms may occur and cause a mild clinical response.

Humoral responses to schizonts are not thought to be important in the immunity of recovered animals, but circulating antibodies which develop against schizonts do provide a useful indication of exposure and are the basis for serological diagnostic tests for *T. parva* (**see Diagnosis, below**). Cattle repeatedly exposed to sporozoites, as occurs in the field in areas of high *T. parva* challenge, develop anti-sporozoite antibodies capable of neutralizing sporozoite infectivity *in vitro*.[111] In these situations, anti-sporozoite immunity may play a role in protection. Significantly, the neutralizing activity of anti-sporozoite antibodies is active against a wide range of *T. parva* isolates, unlike the anti-schizont immunity described above.[112]

Strain differences exist within cattle-derived *T. parva* which are manifest as variations in the degree of cross-protection afforded to field challenge or between different isolates of the parasite. The topic has been reviewed by Irvin.[67] Strains have been identified on the basis of differences in pathogenicity,[11] cross-immunity,[139] antigenic characteristics of the schizonts *in vitro* as detected by monoclonal antibodies[96, 132] and genotypic variation detected with DNA probes.[1, 14, 16, 19] The antigenic diversity is complicated by the fact that most field isolates are composed of mixed parasite populations. Considerable effort has gone into the development of *in vitro* methods of characterizing *T. parva* parasites, particularly in an attempt to find a method giving results which correlate with cross-immunity so as to avoid the need for the use of cattle in expensive and laborious *in vivo* trials. None of the methods studied to date has achieved this aim, although strain specificity of CTL in individual animals has been successfully used to predict the results observed when these cattle were subjected to heterologous challenge.[152] However, as a result of the work done in this area, there are a number of molecular tools available for characterizing strains which can be applied in other important areas, for example, in vaccine production for identifying vaccine stocks, in monitoring changes in epidemiology post-immunization, and in distinguishing possible vaccine breakdown (failure to immunize) from breakthrough (immunization successful but not protective against a field strain).[15]

Clinical signs

East Coast fever in the classical form, as first described by Gray and Robertson[63] in 1902, is characterized by pyrexia, enlargement of the superficial lymph nodes, severe pulmonary oedema and wasting. It usually terminates in death.

The incubation period is generally about 15 days from the time of attachment of the infected tick, but may range from 8 to 25 days. The period and the course of the disease become shorter as the challenge is increased.[69] The first clinical signs are fever and increases in pulse and respiration rates. There may be a sharp decline in milk production. The parotid lymph nodes, which drain the ear to which the infected tick has attached, are enlarged. After a few days the animal becomes depressed and lethargic. The temperature continues to rise, often to 41 or 42 °C. Anorexia may develop but is not inevitable. Lachrymation commonly occurs together with oedema of the eyelids, and may be accompanied by photophobia. The animal is often constipated. There is a generalized enlargement of the superficial lymph nodes: the prescapular and precrural nodes become very prominent.

The disease usually progresses over a period of about 15 days, but may terminate after five days or be prolonged to 25 days. The fever remains high, although in a small proportion of cases there may be a temporary remission for one or two days. Appetite and rumination become increasingly depressed and there is a severe loss of body condition, increasing weakness and ataxia, and frequent recumbency. Constipation is succeeded by diarrhoea and there may be blood and mucus in the faeces. Opacity of the cornea may develop and petechiae may be detected in the mucous membranes, especially those beneath the tongue and in the vulva. Anaemia and icterus have been reported but are inconsistent. Nodular skin lesions have been described in a small number of experimental cases.[164] Pregnant cows may abort.

In the terminal stages of the disease dyspnoea develops, with an increased respiratory rate, a watery cough and the discharge of frothy fluid from the nostrils. The discharge is copious both when the animal is recumbent and immediately before death. Evidence of pulmonary oedema and hydropericardium may be detected on auscultation. Sternal and submandibular oedema may be present. The enlarged superficial lymph nodes begin to regress, the rectal temperature falls to subnormal levels, and the animal

becomes recumbent and dies in a coma.

A small number of animals, usually about 5 per cent, may recover, but convalescence is prolonged and the animals may remain emaciated and unproductive for months.

The disease may assume a less severe form in animals with partial immunity or inherent resistance, but pyrexia and enlargement of superficial lymph nodes remain constant features and in calves may be accompanied by persistent unthriftiness.[100] Mild disease has also been reported occasionally following infection of fully susceptible animals with strains of *T. parva* of reduced virulence.[11] An atypical cerebral form of *T. parva* infection is described in **Chapter 32: Turning sickness**.

In the Asiatic buffalo the disease resembles that in the ox,[76] but in the African buffalo infection is invariably subclinical or mild.[12]

Pathology

East Coast fever is characterized by a severe panleukopenia which coincides with the onset of the febrile reaction.[150, 170] In fatal cases, leukocyte counts fall precipitously until the animal dies. Non-regenerative, normocytic, normochromic anaemia, in which erythrocyte counts may fall to 50 per cent of normal, together with thrombocytopenia and icterus, have also been reported on occasion as prominent features of the disease. There is a moderate hypoproteinaemia, and in the terminal stages there may be clinical pathological changes in serum indicative of renal and hepatic dysfunction.

Schizonts may be present in lymphoblasts and piroplasms in erythrocytes in smears made from peripheral blood, depending on the stage of the disease.

At necropsy the most prominent feature is often severe pulmonary oedema.[63] There is a copious amount of froth in the bronchi and trachea and often also in the nasal passages. Emphysema of the lungs may develop, and a moderate hydrothorax and hydropericardium may also be present.

There is a variable degree of enlargement of peripheral and visceral lymph nodes, which results from hyperplasia and necrosis of cells of the lymphocyte series, as well as oedema, giving the lymph nodes a greyish-white, fleshy appearance. The nodes are sometimes discoloured by hyperaemia and haemorrhage. Splenomegaly may be present but is inconsistent. In young animals the thymus may be enlarged.

The kidneys frequently exhibit one or more raised red or whitish foci or nodules up to 20 mm in diameter within the cortex (**Figure 29.9**). Traditionally, but incorrectly, these are known as 'infarcts' but they are simply aggregations of large lymphoid cells.

The mucosa of the abomasum is generally thickened, due to lymphoid hyperplasia, and congested. Petechiae or ecchymoses may be present and a few ulcers or superficial erosions up to 10 mm in diameter may be found, particularly in the pyloric region. The ulcers are associated with the mucosal lymphoid hyperplasia (**Figure 29.10**). Similar lesions may occur in the intestinal mucosa, particularly in the Peyer's patches of the terminal ileum.

Opacity of the cornea of the eye is often noted, especially at the limbus, and results from infiltrations of lymphocytic cells and from oedema.

Other changes may include emaciation; subcutaneous and intramuscular oedema; ascites; serous atrophy of fat; pallor of the liver as a result of mild degeneration; petechiae in the mucous membranes of the oral cavity, serosal membranes, and the epi- and the endocardium; anaemia; icterus; and greyish-brown foci of hyaline degeneration and necrosis of muscles, particularly in the larger muscle groups of the hindquarters.

Hyperplasia of lymphoid tissue in organs and tissues in which it is normally present, or lymphoid infiltration of other organs, such as the kidneys (**Figure 29.11**), the liver and the eyes, may be recognized macroscopically and confirmed histologically.[150] Microscopic examination will often reveal lymphoid infiltration in other parenchymatous organs, notably in the interstitium and alveoli of the lungs, the portal tracts of the liver, the myocardium, the adrenals, the bone marrow, and the perivascular spaces in the brain. In the early stages of the disease, the lymphoid tissue contains numerous lymphoblasts which are characterized by their large size, a large hyperchromatic nucleus, and relatively extensive hyperchromatic cytoplasm. Mitotic figures are common and germinal centres may be prominent.[10, 44] Schizonts may be seen in some of the cells, the characteristic feature in a haematoxylin-and-eosin-stained section being a cluster of basophilic granules within a lightly staining vacuole in the cytoplasm. In the terminal stages of infection, necrosis of lymphocytes supervenes and the lymphoid tissue becomes rather depleted of cells, with cellular debris being scattered through the tissue.

Piroplasms in erythrocytes and schizonts in lymphoblasts may be recognized with the transmission electron microscope. Ultrastructural pathological changes in lymph nodes have been described by De Martini and Moulton.[44]



Figure 29.9 Multifocal lymphoid hyperplasia in the renal cortex

Diagnosis

The diagnosis of classical East Coast fever is based on the characteristic clinical signs and lesions, and may be confirmed by demonstration of schizonts and, in the later stages of the disease, piroplasms (**Figures 29.3 to 29.8**). The presence of *R. appendiculatus* on the animal provides supportive evidence, but tick infestation may be very light and the ticks may have detached by the time the animal is examined. Theilerial parasites are generally numerous. In the live animal they can be demonstrated in smears prepared from peripheral blood and fine-needle aspirates of superficial lymph nodes (**Figures 29.3 and 29.4**). Parotid, prescapular or precrural lymph nodes may be sampled. In the dead animal, smears prepared from the cut surface of the spleen and any enlarged lymph nodes should be examined. Smears must be of good quality and should be stained with Giemsa or any other Romanowsky stain. The presence of large hyperplastic lymphoblasts should be an incentive to continue the search for schizonts if they are scanty. Care must be taken to differentiate between schizonts and normal azurophilic granules. When smears have not been taken, the diagnosis may still be confirmed if infiltration with infected lymphoid cells can be demonstrated in routine histological preparations. The kidneys, liver, lungs and brain are particularly rewarding tissues for examination.

Intercurrent infection with other haemoparasites may complicate the clinical and pathological picture, and these parasites may be detected microscopically. In those areas where it occurs, East Coast fever is generally the most virulent of the haemoparasitic diseases of cattle, and its possible presence should be given priority over any other disease when making a diagnosis.

Retrospective confirmation of the diagnosis of clinical East Coast fever can be made by demonstration of a rising antibody titre to *T. parva* using serological tests. More usually, serology is used in cross-sectional surveys or longitudinal studies to detect the presence of *T. parva* infection and to determine the prevalence, incidence and seasonality of infection and disease. Circulating antibodies were first demonstrated in recovered animals by the complement fixation test[81] and since then various techniques have been developed using schizonts or piroplasms as antigen.[40] The serological test of choice is a *T. parva*-specific antibody detection enzyme-linked immunosorbent assay (ELISA) based on the use of a recombinant polymorphic immunodominant molecule (PIM).[73, 160] This has superseded an indirect fluorescent antibody test (IFAT), which employs cultured *T. parva* schizont-infected lymphoblasts as the antigen.[6, 33, 61] The IFAT, although extremely useful and still widely used, suffers from subjectivity in interpretation and lack of specificity due to cross-reactivity with some other *Theileria* spp., most importantly *T. taurotragi*.[32, 141] The ELISA does not cross-react with *T. taurotragi*, *T. mutans*, *T. annulata* or *T. buffeli* and shows high sensitivity. It detects antibody of IgG1 isotype and antibody levels remain positive after a single infection with *T. parva* for longer than with the IFAT.[6, 34]

An alternative means of retrospective diagnosis is the detection of infection in recovered carrier cattle (**see Epidemiology, above**). Morphologically, the piroplasms of different *Theileria* spp. are difficult to differentiate especially when present in small numbers. Thus, the examination of Giemsa-stained thin blood smears to detect piroplasms lacks specificity and is also relatively insensitive. Tick transmission (xenodiagnosis) is not practical on a large scale. The need for an improved method of detection of *T. parva* carrier animals has led to the development of a number of PCR techniques using *T. parva*-specific primers based on single copy genes (p104 and PIM antigen genes)[66, 160] or a repetitive gene sequence (TpR).[17] The best of these, based on the sporozoite



Figure 29.10 Ulcerations and lymphoid hyperplasia in the mucosa of the abomasum

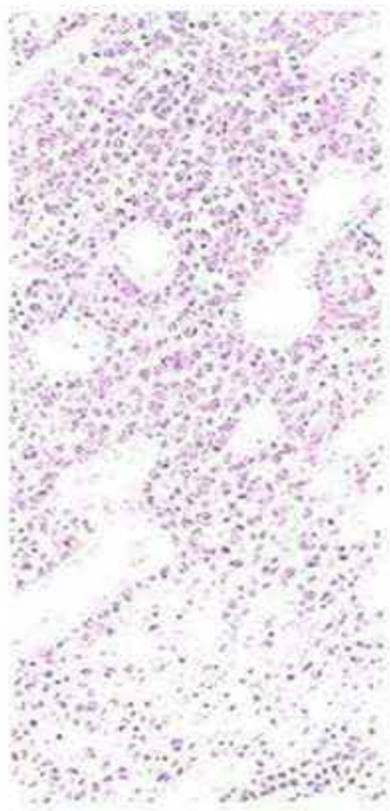


Figure 29.11 Lymphoid infiltration in the kidney

p104 antigen gene, is sensitive and specific; *T. parva*, and not *T. taurotragi*, DNA in the blood of infected cattle is amplified to a detectable level. This assay is currently undergoing validation and comparison with conventional methods of detecting long-term persistent infections in cattle at the International Livestock Research Institute in Nairobi.

Differential diagnosis

Clinically, East Coast fever must be differentiated from other febrile conditions which usually terminate fatally after a course of one to two weeks and which feature progressive emaciation, respiratory distress, diarrhoea, corneal opacity, and lymphoid hyperplasia. Rinderpest, bovine virus diarrhoea/mucosal disease and bovine malignant catarrhal fever may all be distinguished by the presence of ulcerating or necrotizing inflammatory reactions in buccal and nasal mucosae, and conjunctivitis. Contagious bovine pleuropneumonia and other pneumonic conditions are characterized by coughing and evidence of pain on respiration. Acute trypanosomosis does not exhibit diarrhoea or severe respiratory distress. In babesiosis and anaplasmosis, anaemia and icterus are the major presenting signs. The clinical picture in East Coast fever may be complicated, however, by secondary respiratory infections or by concurrent infection with other haemoparasites. It is important for the investigator to look beyond the immediate presenting signs for the primary lesions of lymphoid hyperplasia and subsequent depletion.

A definitive diagnosis of East Coast fever is possible if microscopic examination of blood and lymphoid tissues reveals large numbers of theilerial parasites. If only small numbers of schizonts and piroplasms are present in association with *R. appendiculatus* ticks and severe clinical illness, the possibility exists that the disease under investigation is not classical East Coast fever but is caused by infection with buffalo-derived *T. parva* **(see Chapter 30: Corridor disease)**. Where schizonts are few and a variable number of piroplasms are encountered in mildly affected animals, the presence of another species of *Theileria* should be considered, or it may be that the *T. parva* infection is incidental. For example, Zebu-type calves in areas endemic for East Coast fever tend to undergo moderate, mild or inapparent reactions on infection which do not require treatment. The presence of piroplasms in association with anaemia, minimal lymph node enlargement and few, if any, schizonts is likely to indicate *T. mutans* infection.

To distinguish between the different species of *Theileria* microscopically may be extremely difficult when relatively mild infections are occurring, particularly as mixed infections are frequent in the field,[67] the most common of these being combinations of *T. parva*, *T. taurotragi* and *T. mutans*. Morphologically the piroplasms are difficult to differentiate, except for those of *T. velifera*, and the schizonts are indistinguishable, except for those of *T. mutans*.

Serological examination of recovered animals may permit a retrospective differentiation at the species level with species-specific ELISAs available for *T. parva*[73] and *T. mutans*[72] and standard IFAT[6] for others. Biological transmission of the parasite may also provide a means of differentiation at the species level, but is expensive and requires experimental cattle. *Theileria mutans* and *T. velifera* are readily transmitted by intravenous inoculation of heparinized blood to a susceptible ox — an inoculum of 10 ml should be adequate. *Theileria taurotragi* can sometimes be transmitted in the same way but may require a larger inoculum (100 to 200 ml), while *T. parva* cannot readily be transmitted by blood inoculation. Tick transmission may also be helpful: *T. mutans* and *T. velifera* are transmitted by *Amblyomma* spp. but not by *Rhipicephalus* spp., while the converse is true for *T. parva* and *T. taurotragi*.

A number of molecular, biochemical and immunological tools is available that enables differentiation between *Theileria* spp. Polymerase chain reaction (PCR) combined with oligonucleotide DNA probes detecting ribosomal RNA sequences can be applied to lymph node, blood and culture material, even when only small quantities are available.[2] Where larger amounts of DNA can be purified from schizont-infected cultures or piroplasm-infected blood, two additional methods are useful: restriction fragment length polymorphism (RFLP) patterns obtained when an extrachromosomal fragment is used as the DNA probe;[70, 106] and glucose phosphate isomerase isoenzyme patterns.[3, 94] Species-specific monoclonal antibodies used in the IFAT are also valuable for recognizing piroplasms[60, 72] and, where available, schizonts in cell cultures.[20, 96, 132] More recently, species-specific PCR assays have been developed to distinguish *T. parva*, *T. taurotragi*, *T. mutans* and *T. buffeli*, based on primers from their respective major merozoite/piroplasm surface antigen (mMPSA) genes. These assays are more suitable for routine use on large numbers of samples from the field than the methods described above and are undergoing validation at the International Livestock Research Institute in Nairobi at present.

The differentiation between the various disease manifestations caused by *T. parva* is based on epidemiological, clinical, pathological and parasitological factors, and on the implications of the diagnosis in relation to the animal health control policy of the country. Veterinarians in countries that have eradicated (or are on the verge of eradicating) East Coast fever are far more careful to make a distinction between the forms of disease than those in regions where East Coast fever is endemic.

Control

Treatment

Progress in the chemotherapy of East Coast fever has been reviewed by Dolan.[47] The disease proved refractory to treatment for many years until Neitz,[117] in 1953, found that

certain tetracyclines had a marked suppressive effect on the schizonts if administered during the incubation period. Tetracyclines have been widely used in the treatment of clinical disease, sometimes to good effect, but they have not proved to be completely reliable therapeutic agents. They have been used successfully, however, in immunization against East Coast fever by an infection-treatment technique (**see Vaccination, below**).

An important breakthrough in the control of East Coast fever was achieved in the late 1970s by the development of two highly effective therapeutic agents. Parvaquone, a napthoquinone, known during its development trials as 993C, proved to be a valuable therapeutic agent when administered by intramuscular injection at a rate of 10 mg/kg, repeated after 48 hours.[89] Recovery rates of 90 per cent or better were recorded in field trials.[36, 109] The drug is effective against both schizonts and piroplasms, but treatment does not achieve a parasitological cure, and recovered animals may remain intermittent carriers and some may take several months to return to normal productivity.[48] An analogue of parvaquone, named buparvaquone, has been developed[91] and has replaced parvaquone on the commercial market. It is recommended at a dose of 2,5 mg/kg for the treatment of clinical theileriosis,[87] although, under field conditions where clinical cases are often detected late in the course of the disease, a two-dose regimen is advised with the doses being given 48 hours apart.[49]

A second drug, halofuginone, a quinazoline derived from febrifugine, is effective when administered orally as the hydrobromide or preferably the lactate salt at a rate of 1.2 mg/kg, repeated after 48 hours.[140] It is active against the schizonts but not the piroplasms. The therapeutic index is relatively low and overdosing may cause severe diarrhoea, but the drug has achieved recovery rates of 80 per cent or higher in the field.[35]

These drugs have the disadvantage of being relatively expensive and this reduces their application in the field, but they can be used as a back-up to prevent mortality if other forms of control prove ineffective. Experimental work suggests that buparvaquone could be used chemoprophylactically in areas of intense *T. parva* challenge. The administration of a single dose of buparvaquone at 5 mg/kg seven days after field exposure has commenced may protect susceptible cattle from disease while they become immune.[171]

To prevent the danger of carrier animals, no affected animal may be treated chemotherapeutically in South Africa without the written authorization of the National Director of Animal Health and Production, but there are no such restrictions in other countries in the region.

Vaccination

The development of methods of immunization against East Coast fever has been reviewed by Brown.[26] Protective immunity to *T. parva* persists for at least five years after natural infection.[118] Immunity will develop only in animals in which infection with schizonts has been established. Effective methods of immunization are achieved by controlled infection of cattle using sporozoites derived from ticks, or schizont-infected cells. The infective material is virulent and the method of administration must be carefully regulated to avoid a fatal outcome.

In the early years of the twentieth century, before the introduction of acaricides, crude techniques of controlled infection were developed for immunization. In Kenya, animals from endemic areas which were thought to be immune were exposed to natural infection on heavily infected pastures. The majority survived and could be used thereafter as transport oxen through infected areas.[125] In South Africa, cattle were immunized by the intravenous inoculation of preparations of spleen and lymph node from infected animals, and up to 70 per cent were protected against natural challenge.[157] There was a significant mortality from theilerial infection or from bacterial contamination of the inoculum as a direct result of the immunization procedure, but the technique was used to control the first onslaught of the disease in the Transkei region of the Eastern Cape Province. A total of 275 000 cattle was inoculated and it is estimated that 100 000 survived.[147]

No further progress was made until 1953, when Neitz discovered that the administration of chlortetracycline (Aureomycin) during the incubation period had a marked suppressive effect on the development of infection and permitted the establishment of a solid immunity.[117] His observation was followed up by a team of scientists working in Kenya under the auspices of the Food and Agriculture Organization (FAO) of the United Nations, and an infection and treatment method of immunization was developed.[136] The method is described in detail in FAO and Office International des Epizooties (OIE) manuals.[4, 6] A crude suspension of sporozoites is prepared from adult ticks which have fed as nymphs on infected cattle, have moulted, and have been pre-fed on rabbits for three to four days to allow the sporozoites to mature. The suspension is mixed with glycerol as a cryoprotectant and stored, deep-frozen at –70°C or in liquid nitrogen. Aliquots are titrated in susceptible cattle to determine an optimum dose for immunization. The stabilate is inoculated subcutaneously into the cattle to be immunized and simultaneously an intramuscular injection of 20 mg/kg of a long-acting oxytetracycline preparation is administered. Alternatively, two injections of 10 mg/kg of a standard oxytetracycline preparation on days zero and three or four may be used. Infection develops to a mild or subclinical level and the animals are thereafter resistant to challenge, both experimental and natural, although infection may persist and the animals may remain asymptomatic carriers. The duration of immunity appears to be comparable to that which follows natural recovery. Buparvaquone is also effective as a prophylactic and may be administered at the time of stabilate inoculation.[90] However, there is a risk that this

drug may be so effective that infection fails to establish and the animal is left partially or fully susceptible.[120]

The infection and treatment technique provides good immunity against the homologous immunizing strain, but is not consistently successful against unrelated strains. Therefore efforts have been directed towards finding a 'master' stock or combination of stocks which, when used as the vaccine, give protection against a wide range of antigenically distinct *T. parva* parasites. On the basis of extensive cross-immunity trials, three isolates, identified as cattle-derived *T. parva* Muguga and *T. parva* Kiambu 5 and buffalo-derived *T. parva* Serengeti transformed, have been combined in the so-called Muguga cocktail.[137] This mixture provides protection against most isolates from outbreaks of East Coast fever in eastern Africa and is used in Tanzania, Uganda and Malawi. The use of a vaccine in areas other than those from which the components were isolated carries with it the potential danger of introducing parasites of an antigenic type against which local cattle may have no immunity. To avoid this it may be desirable to isolate local strains to use in the technique, as has been the practice in the Eastern Province of Zambia, where a local isolate known as *T. parva* Katete is used.[84] In Kenya, an isolate from Coast Province, *T. parva* Marikebuni, which has been shown to afford a broad spectrum of immunity to cattle-derived *T. parva*, has been selected for countrywide use.[115] The Muguga component of the 'Muguga cocktail' does not induce carrier status and the capacity of the other two components to do so is not known, while both *T. parva* Marikebuni and *T. parva* Katete do cause carrier status to develop in immunized animals. Most of the vaccine stocks in use have been fully characterized, although unique markers are, so far, only available for *T. parva* Marikebuni and *T. parva* Kiambu 5.

The infection and treatment technique is relatively expensive and cumbersome, but it is being implemented on an increasing scale in a number of countries and has generally proved successful.[105, 107, 138, 163] A liquid nitrogen cold chain is essential in order to maintain parasite viability and, compared to other veterinary immunizations, more training and expertise are required for the vaccine to be delivered safely and effectively. Another important limitation is the risk of occurrence of clinical reactions after immunization.[86] Incorrect drug or vaccine dosing and immunization of cattle under stress from poor nutrition or intercurrent disease are predisposing factors for animals to suffer clinical theileriosis as a result of immunization. Monitoring of animals in the month after immunization is necessary to allow their identification and treatment if they are not to die. Various monitoring strategies are practised in the countries using the infection and treatment method, and in Tanzania, the use of higher chemoprophylactic doses of oxytetracycline (30 to 40 mg/kg) to reduce the reactor rate is proving promising.[36] Serious problems with chronic and cerebral theileriosis in immunized cattle maintained under heavy challenge in traditionally managed herds have been reported in one area of Kenya.[98]

The possibilities of immunization against *T. parva* by inoculation of schizont-infected lymphoblast cultures, which has proved so effective with *T. annulata* infection, have been studied intensively.[24] The technique is possible but impractical, as very large numbers of cells are required to establish infection consistently. Whereas with *T. annulata* inocula of 10^6 cells will regularly establish infection, with *T. parva* it requires 10^8 cells in 100 ml of culture fluid to establish infection reliably in allogeneic animals.[27, 50]

A number of other approaches to immunization is currently under investigation. The presence of sporozoite neutralizing antibodies in cattle repeatedly exposed to field infection, or to sporozoites by stabilate inoculation, provided the rationale for the development of a potential vaccine based on a recombinant sporozoite surface antigen of *T. parva*, known as recombinant p67.[113] This protects two-thirds of immunized animals in laboratory trials[110] and preliminary evidence indicates that it may afford a broad spectrum of immunity against different *T. parva* isolates.[116] Field trials are in progress. The definitive subunit vaccine should ideally contain antigens from both the sporozoite and schizont stages, especially as the latter is known to stimulate the main effector mechanism. Identification of the antigens recognized by CTL is the main focus of research towards a vaccine based on the schizont stage.[114]

Other preventive measures

In the absence of an effective vaccine, control of East Coast fever has depended in the past, and continues to depend, on the prevention of infestation of susceptible cattle with infected ticks. There are three possible approaches:

- **Isolation** Susceptible cattle maintained in a closed herd on properly fenced pastures (which exclude all possible contact with other cattle) will be protected from East Coast fever, as infection can only be introduced by ticks which have dropped from infected cattle during the preceding stage of the life cycle. Where the pastures border an area of high risk, such as a public road on which infected cattle may pass, two fences 5 metres apart will provide an effective barrier. Hay and straw from potentially infected areas may introduce unfed ticks mechanically to the property and must be excluded. Other animals, such as horses, sheep and goats, may transport infected ticks mechanically and must be treated with an acaricide if they are introduced. Wildlife may also transport ticks and should be excluded. In situations where fencing is not practicable, cattle may be housed and fodder transported to them ('zero-grazing'). Isolation as a technique is too prone to breakdown to be relied upon as the sole method of control and is normally used in conjunction with tick control.
- **Tick control** Susceptible cattle can be maintained in an infected environment provided that they are kept completely free of *R. appendiculatus*. Dipping of cattle in arsenical preparations at three-, five- or seven-day

intervals was widely used in the first half of the century to control East Coast fever. In conjunction with clipping of hair from ears and tail-brushes to expose ticks at these sites, and local application ('hand-dressing') of acaricide to ears and the perineal region, short-interval dipping provided very good control of *R. appendiculatus* and prevented ticks feeding to engorgement on infected cattle and thus infecting the pasture.[167] Control was not sufficiently complete to prevent a proportion of susceptible cattle contracting infection when grazed on infected pastures.[53] Arsenic has been superseded by organochlorines, organophosphates, carbamates, amidines and pyrethroids, and techniques of application with spray races, hand-operated sprays, pour-on formulations and impregnated eartags have been developed as alternatives to the dip tank. Cattle must be treated two or three times a week where the challenge is heavy, and even this may not be sufficient to provide absolute protection. If used in conjunction with isolation, it does reduce the risk of infection to a minimum.

Destocking If infected pastures are kept free of cattle for 15 to 18 months, infection will disappear. This period is greater than the limit of survival of the parasite in infected adult ticks in the field.[119, 155] Ticks on the pasture will continue to feed on other species but will not pick up parasites for transmission. Destocking was used effectively in the control of East Coast fever in the early part of the twentieth century. Cattle were moved from infected to clean pasture through a series of 'temperature camps', animals being checked for evidence of fever daily, and those showing a rise in temperature being culled as potential early cases of infection.[64] The infected pasture was fenced and kept cattle-free for 18 months. The technique fell into disuse as the cattle population grew and vacant clean pasture became more difficult to locate. Destocking may also be achieved by the removal of all cattle for slaughter and prohibition of reintroduction of cattle for 18 months. The pasture can be used for sheep or goats in the interim.

Practical approaches to control

Selection of the methods for control of East Coast fever in a given situation will depend largely on the epidemiology of the disease, i.e. whether it is newly established in the area and spreading in an epidemic form, or whether it is endemic.

Where East Coast fever appears in an area for the first time, every effort should be made to eradicate it. This entails the vigorous application of conventional zoosanitary measures, which include an embargo on all cattle movements into, within, or out of the area; enumeration and identification of all cattle in the area; and regular inspection to detect evidence of infection. In addition, the enforcement of short-interval acaricide treatment of all cattle is essential to suppress the tick population and reduce the opportunities for transmission. These measures were applied with considerable success to control the epidemic in southern Africa in the first half of this century.[46] They were supplemented by a programme of intensive surveillance, which involved the examination of blood and spleen smears from every bovine animal which died or was slaughtered other than at a recognized abattoir throughout the infected area, in order to identify residual foci of infection. Infected premises were quarantined for up to two years after the last confirmed or suspected case, and persistent foci of infection were finally cleared by slaughter. Implementation of measures such as these requires a comprehensive infrastructure including effective legislation, adequate and competent veterinary staff, transport, good communications, fencing, dip tanks or spray races, acaricide and, above all, a stable political environment. Failure to achieve rapid control of the epidemic may well result in the disease establishing permanently in an endemic form.

In an endemic area the greater part of the cattle population survives in equilibrium with the disease and there is little incentive for the implementation of vigorous and expensive control measures for the population as a whole. The disease may well cause an increased level of calf mortality and a reduction in productivity, and where intensive regional dipping programmes are introduced, there may be an improvement in the general health of the cattle. However, such programmes are very demanding in terms of finance, organization and effort on the part of owners and veterinary services alike, and in the long run they usually collapse and the cattle revert to their previous stable form. Control can only be justified when East Coast fever is found to be a constraint on attempts to improve production and establish a cattle industry on a commercial basis, particularly where the introduction of improved breeds of cattle is involved. Large production units, provided they are well managed and have adequate financial resources, can control the disease effectively by isolation and intensive acaricide treatment, although the costs of short-interval dipping or spraying are very high, both in terms of acaricide and labour and of reduced production resulting from frequent mustering and treatment. The effects of the occasional breakdowns in control which inevitably occur can be minimized with chemotherapy. Small-scale producers, attempting to run small herds of highly susceptible cattle in the midst of an environment of uncontrolled ticks and infection, have little chance of protecting their animals from the disease by conventional zoosanitary methods.

Control of East Coast fever in endemic areas should ideally be based on an integrated programme aimed at reducing the challenge and increasing the resistance of the cattle. The use of Zebu or Zebu-cross cattle, which have an increased capacity for the development of resistance to ticks, should be encouraged; they will be subjected to reduced disease challenge and less tick worry. Exposure of calves to moderate tick challenge in early life should be allowed to facilitate the development of tick resistance and to permit infection

with tick-borne parasites of all types while the animals are partially resistant. The use of acaricides should be limited to the minimum required to prevent excessive tick loads; this will reduce production costs substantially. Chemotherapy may be used to treat clinical cases. Where such measures fail to limit the prevalence of clinical disease to an acceptable level, immunization by the infection and treatment method should be implemented. The 'Muguga cocktail' of isolates has been well tried and is readily available but may not protect against all strains in the field and may introduce new strains into the environment. Selection of local strains for immunization avoids the latter disadvantage but necessitates the characterization and cross-immunity testing of a number of isolates to establish the most effective combination for use. In the short term, immunization of highly susceptible cattle is the method of choice for the control of the disease until an integrated control programme is developed.

In considering any control programme for East Coast fever it is essential to remember that the disease does not occur in isolation but forms part of a complex of tick-borne diseases and tick worry which may all have an adverse effect on the health and productivity of cattle, especially improved, high-producing breeds. Uilenberg[162] has reviewed the results of field immunization trials in Tanzania and Malawi in which, once East Coast fever was controlled, heartwater, babesiosis, anaplasmosis, *T. mutans* infections and tick toxicosis emerged as significant causes of mortality. In an endemic situation, intensive dipping to control East Coast fever will disrupt the acquisition of immunity to all tick-borne parasites and will lead to an upsurge of tick-borne diseases if the dipping programme is interrupted for financial or political reasons.[122] To be lastingly effective, control programmes must be aimed at the whole complex of ticks and tick-borne diseases.

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30

Corridor disease

Synonyms: Buffalo disease, buffalo-derived *Theileria parva* infection, *Theileria parva lawrencei* infection, buffelsiekte (Afrik.)

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Introduction

Corridor disease is an acute, usually fatal disease of cattle resembling East Coast fever and is caused by infection with buffalo-derived *Theileria parva* strains transmitted by ticks from African buffaloes (*Syncerus caffer*) **(Figure 30.1)**. The disease was first recognized in 1934 in Zimbabwe as a form of pathogenic theilerial infection distinguishable from East Coast fever on clinical, pathological, parasitological and epidemiological grounds.[8] Previous occurrences in the region may well have been obscured by the widespread prevalence of East Coast fever. Twenty years later the disease was recognized in South Africa[15] and the causal organism was then identified as a new species, *Theileria lawrencei*, by Neitz[13] in 1955. It was later proposed as a subspecies of *T. parva*, namely *T. parva lawrencei*.[26] Subsequent investigation has revealed that the parasite is *T. parva* and is the same as that causing East Coast fever and Zimbabwe theileriosis, although the distinction between these disease forms has been retained and remains useful for descriptive purposes.[19] The disease is described by Neitz.[14]

The disease has been named buffalo disease, for obvious reasons, and Corridor disease, because the first outbreak in South Africa occurred in the corridor between the Hluhluwe and Umfolozi game reserves in KwaZulu-Natal. It occurs sporadically throughout eastern, central and southern Africa wherever there is contact between cattle and infected African buffaloes in the presence of the ticks, *Rhipicephalus appendiculatus*, *R. zambeziensis* and *R. duttoni* **(see Chapter 1: Vectors: Ticks and Chapter 29: East Coast fever: Figure 29.1)**. After foot-and-mouth disease it is the most important disease transmitted from the African buffalo to cattle and is a major constraint to the acceptance of the presence of buffaloes in cattle-raising areas for purposes of conservation or recreation.

Aetiology and life cycle

Buffalo-derived *T. parva* is morphologically and serologically indistinguishable from cattle-derived *T. parva* and has an identical life cycle **(see Chapter 29: East Coast fever: Figure 29.2)**. It is mainly transmitted by *R. appendiculatus*, although *R. zambeziensis* replaces *R. appendiculatus* as the vector in the more arid areas of southern Africa.[9, 17] Epidemiological evidence suggests that in Angola the disease is transmitted by *R. duttoni*.[6]

Buffalo-derived *T. parva* can be considered to be a group of *T. parva* strains which are adapted to tick transmission within the African buffalo population, including the red dwarf buffalo (*Syncerus caffer nanus*) of Angola.[6] It is universally distributed in wild buffalo throughout eastern, central and southern Africa, except in the Addo Elephant National Park in South Africa.[20] It is usually non-pathogenic in this species although fatal disease can occur following experimental infection.[2] The parasite persists indefinitely in infected buffaloes in both schizont and piroplasm form and can be recovered consistently by tick transmission or culture of lymphocytes from such animals.[24]

Buffalo-derived *T. parva* is not well adapted to the ox and when transmission from buffalo to cattle occurs it usually fails to complete its development; many cattle die before sufficient time has elapsed for development of piroplasms, and in those that survive, piroplasms are very scanty and recovered animals are not commonly infective for ticks. In southern Africa the disease is self-limiting within a population of cattle once it is removed from the source of infection. Onward transmission from cattle has been demonstrated experimentally and in eastern Africa repeated passage of infection in cattle has resulted in a change of the character of the organism on a number of occasions, the parasite behaviour and the disease that it causes becoming indistinguishable from classical East Coast fever.[1, 10] This change is attributed to a 'transformation' of the parasite as it adapts to the ox, but an alternative explanation that the phenomenon represents selection of a *T. parva* strain from a mixed population in the buffalo must also be considered. There is no evidence that 'transformation' of buffalo-derived *T. parva* occurs in the region south of the Zambezi River, where

classical East Coast fever is absent.[20] This may reflect the infrequent contact between buffalo and cattle in the region. Furthermore, the seasonal appearance of the various tick stages[22] reduces the likelihood of an animal being infected by one stage and infection being acquired by the following stage, since piroplasms are present only transiently and at a low level. In contrast, in eastern Africa, all tick stages may be present on the animal at the same time.[18]

Buffalo-derived *T. parva* has been shown to infect a waterbuck (*Kobus ellipsiprymnus defassa*) from which infection was transmitted to ticks; this was not achieved using cattle-derived *T. parva*.[23] Buffalo-derived *T. parva* will also infect and cause fatal disease in the Asiatic domestic buffalo (*Bubalus bubalis*).[3]

Epidemiology

Buffaloes in *T. parva* endemic areas become infected soon after birth and the vast majority survive to become extremely efficient carriers of *T. parva* and remain so even in the absence of reinfection. Almost all buffaloes in endemic areas in eastern Africa are *T. parva* carriers[25] and ticks feeding on carrier buffaloes become heavily infected. Corridor disease occurs only in cattle that graze pastures on which African buffaloes are or have recently been present. Such situations occur where buffaloes and cattle regularly share the same pasture, where cattle are moved into or through buffalo areas, or where buffaloes stray from their natural habitat or game reserves into farming areas. It is not always easy to detect the presence of wild buffaloes in a farming area. They may travel long distances and remain hidden in wooded areas, and the presence of a single buffalo for a relatively short period may be sufficient to cause a serious outbreak of disease amongst the cattle.

Possibilities for infection occurring in southern Africa are limited as buffaloes do not usually inhabit densely populated or intensively farmed land. Contact is most common in the vicinity of game parks or in extensive cattle-raising areas with a substantial wildlife population. Increasing density of human population and the policy of prohibiting contact between buffalo and cattle as a foot-and-mouth disease control measure are likely to reduce the prevalence of the disease progressively in the future. However, in South Africa game ranching in conjunction with, or in the absence of, cattle is becoming increasingly more popular in certain areas. As one of the so-called Big Five African game trophy animals, the buffalo is amongst the most sought-after game species for introduction to many game farms and game conservation areas in South Africa. Buffaloes that are free from Corridor disease and foot-and-mouth disease are in short supply for stocking purposes and consequently command an exceedingly high commercial value.

In eastern Africa, the absence of game fences, the mode of life of cattle-keeping pastoralist communities and the widespread distribution of the buffalo populations, all contribute to Corridor disease being a more significant problem.

Clinical signs

Corridor disease exhibits the same clinical features as East Coast fever except that the course is usually shorter, death sometimes occurring only three to four days after the onset of the first signs. The emaciation, diarrhoea and regression of lymph nodes, which are features of advanced East Coast fever, are not commonly seen in Corridor disease. Severe pulmonary oedema precedes death. The mortality rate is about 80 per cent.



Figure 30.1 African buffalo (*Syncerus caffer*) (By courtesy of Dr V. de Vos, Kruger National Park, Skukuza, South Africa)

Pathogenesis and pathology

The pathogenesis and pathology of Corridor disease are very similar to those of East Coast fever. Pulmonary oedema is a prominent feature but lymphoid hyperplasia and lymphoid infiltrations are generally not very advanced at death, and macroscopical lymphoid aggregations in the kidneys are uncommon.

Diagnosis

The diagnosis of Corridor disease depends on the recognition of the characteristic clinical signs and pathological changes, and on the microscopic demonstration of schizonts in a situation where contact between buffalo and cattle is known or may be presumed to have occurred. In marked contrast to East Coast fever, the parasites are usually very scanty and a prolonged search of smears of a selection of lymph nodes and spleen may be necessary to demonstrate schizonts. It is usually more rewarding to examine a number of different lymph nodes for a short period than a single lymph node for a long period as the distribution of parasites in the body is very variable. The presence of piroplasms does not provide supportive evidence as these are more likely to result from incidental infection with non-pathogenic species such as *T. taurotragi* or *T. mutans*. The immunofluorescent antibody test (IFAT) and *T. parva*-specific enzyme-linked immunosorbent assay (ELISA) are both applicable for detecting antibody after exposure to buffalo-derived *T. parva* **(see Chapter 29: East Coast fever: Diagnosis)**. The scarcity of piroplasms in recovered animals makes the use of a polymerase chain reaction (PCR) assay an attractive option for detecting persistently infected animals. These tests do not distinguish buffalo-derived from cattle-derived infections. In the limited number of buffalo-derived *T. parva* strains examined, the p67 sporozoite antigen contained an extra insert of 43 amino acid residues; this insert is not present in cattle-derived strains in which the sporozoite antigen is highly conserved.[12] If this holds true for other buffalo-derived *T. parva* parasites, it will be the first genotypic difference found between buffalo- and cattle-derived *T. parva* and could lead to a method for differentiating between the two.

Control

Corridor disease responds to treatment with buparvaquone and halofuginone, but as the course of the disease is usually short it may be difficult to institute treatment in time for it to be effective. In South Africa, the treatment of diseased animals is not permitted by law.

There are no vaccines readily available at present to control buffalo-derived *T. parva*. Cross-protection experiments in cattle,[28] monoclonal antibody studies[5] and genotypic analysis[4] have shown that isolates of *T. parva* from buffalo are antigenically more varied than those from cattle. The 'Muguga cocktail' and other cattle-derived *T. parva* vaccines will thus often fail to protect against natural challenge in situations where buffalo-derived *T. parva* infections are prevalent in cattle **(see Chapter 29: East Coast fever: Control)**.[21] Experiments indicate that the disease is better controlled by the infection and treatment method of immunization using local isolates **(see Chapter 29: East Coast fever: Control)**.[7, 27, 30] Buffalo-derived *T. parva* is more difficult to control using conventional oxytetracycline dosing during immunization and higher reactor rates tend to occur. Buparvaquone controls the post-immunization reactions better than oxytetracycline but can be over-effective leaving cattle partially or fully susceptible.[16] Buffalo-derived *T. parva* transformed by serial passage in cattle, protected cattle against challenge with the original parasite isolated from buffalo in one experiment, and was much easier to handle than the original isolate.[10] Such a parasite might provide a useful source for vaccine material.

In eastern Africa, where susceptible animals are being kept in situations of high risk, and separation of buffalo and cattle is not an option, strict tick control must be practised to prevent disease outbreaks. In indigenous Zebu-type cattle, endemic stability may be established whereby calves born to immune dams are infected while young and most become immune, with few deaths.[11]

In southern Africa control of the disease is based on the prevention of contact between buffaloes and cattle wherever possible. Game reserves in cattle-raising areas which contain buffaloes should be securely fenced and the introduction of buffaloes into farming areas for purposes of game ranching or safari hunting should be strictly prohibited, unless the buffaloes can be certified free from infection. The growing demand for buffaloes that are free from Corridor disease and foot-and-mouth disease, and therefore suitable for non-endemic areas, far exceeds limited supply. In an attempt to help satisfy the demand for such animals in South Africa, Corridor disease-free buffaloes have been bred successfully from Corridor disease-infected parent stock obtained from a foot-and-mouth disease-free area by rearing them in an environment free from tick vectors. Although transmission of infection by tick vectors to the progeny can be eliminated in this way, the calves must be monitored for possible transplacental infection with *T. parva*.

When an outbreak of the disease does occur, the usual procedure is to remove the cattle to uninfected pasture, to institute strict tick control procedures and to attempt to locate the source of contact and thus prevent future recurrences. As the disease is usually self-limiting in cattle, prolonged quarantine of infected herds is rarely indicated, but outbreaks should be monitored carefully for several months to ensure that cattle-to-cattle transmission does not occur.

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Zimbabwe theileriosis

Synonyms: January disease, Fortuna disease, *Theileria parva bovis* infection

J A LAWRENCE, B D PERRY AND S M WILLIAMSON

Introduction

Zimbabwe theileriosis is an acute, frequently fatal disease of cattle caused by *Theileria parva* infection which occurs in Zimbabwe **(see Chapter 29: East Coast fever: Figure 29.1)**. The disease was first recognized and distinguished from classical East Coast fever in 1936,[5] although outbreaks may have occurred previously. It has been variously known as January disease, because of its seasonal occurrence, and Fortuna disease, after the farm on which it was first recognized. It is known officially in Zimbabwe as 'theileriosis'. The disease was described by Neitz[10] under the name Rhodesian malignant bovine gonderiosis, and attributed to a new species, *Theileria (Gonderia) bovis*, but the parasite was declared in a footnote to that paper to be synonymous with *Theileria lawrencei*, the causal organism of Corridor disease. The name *T. parva bovis* was subsequently proposed for the parasite by Lawrence[6] to differentiate the disease which it causes from Corridor disease. The disease resembles East Coast fever but the seasonal occurrence of theileriosis in Zimbabwe and the fact that infections can be milder than in East Coast fever justifies the continued use of the terms January disease and Zimbabwe theileriosis. However, there is at present no good scientific reason to retain the subspecies classification, *T. parva bovis*, as the parasite causing the disease is indistinguishable from cattle-derived *T. parva* from elsewhere in eastern and southern Africa.[12] It is worth noting that *T. parva* isolates have been made in eastern Africa, which show either the lower virulence or parasitological features associated with some *T. parva* strains causing January disease.[1, 13] The disease causes a significant number of deaths each year in Zimbabwe, necessitating the implementation of intensive dipping regulations to control its vector.

Aetiology and life cycle

Theileria parva strains causing January disease are morphologically and serologically indistinguishable from other *T. parva* strains. The life cycle is identical **(see Chapter 29: East Coast fever: Figures 29.2 to 29.8)** and transmission is principally by adults of *Rhipicephalus appendiculatus*. Transmission of the parasite by *Rhipicephalus zambeziensis* has been demonstrated experimentally.[7] Several isolates of the parasite have been studied and differences in virulence and in cross-protective immunity have been demonstrated. Some isolates are relatively benign.[4] The parasite remains stable in regard to its virulence, even after rapid repeated passage by ticks through cattle.[14] Epidemiological and limited experimental evidence indicates that recovered animals are frequently carriers.[3]

Epidemiology

Zimbabwe theileriosis occurs sporadically on the highveld of Zimbabwe **(see Chapter 29: East Coast fever: Figure 29.1)** during the period December to May. The strictly seasonal occurrence of clinical disease coincides with the seasonal distribution of adult *R. appendiculatus*.[8] The development of antibodies to *T. parva* has been demonstrated in cattle in the field during the period September to November, indicating that transmission by nymphs, which has been demonstrated experimentally, may cause subclinical infection.[3]

Most cases occur between January and March and the disease usually affects cattle over the age of one year; it is rarely seen in calves. Primary outbreaks usually occur in herds with a moderate to heavy tick burden and are often associated with new additions to the herd. The disease may appear in the resident cattle (suggesting initiation of infection by introduced carriers) or in the introduced cattle (suggesting introduction of susceptible animals into an endemically stable environment). Morbidity in an outbreak is usually less than 10 per cent. The disease is likely to recur each year on an infected property unless good tick control is achieved, but shows little tendency to spread to neighbouring properties.

Serological evidence suggests that infection with the parasite is widespread in both the highveld and lowveld of

Zimbabwe.[11] It is not yet known whether the limited occurrence of clinical disease, in spite of the widespread prevalence of infection, is evidence of the existence of avirulent strains of the parasite or of an endemically stable balance between virulent strains and cattle. There is no evidence that wildlife plays any role in the epidemiology of the disease.

Clinical signs

Zimbabwe theileriosis exhibits the same clinical features as East Coast fever except that the course is often shorter and death may occur three to four days after the first onset of signs. Sometimes one of the presenting signs is blindness associated with corneal opacity. Clinical disease is often accompanied by evidence of heavy infestation with *R. appendiculatus*. Mortality in the field may reach 90 per cent in severe outbreaks, but many animals recover from experimental infection and serological evidence suggests that subclinical or mild infection may be common.

Pathogenesis and pathology

The pathogenesis and pathology of Zimbabwe theileriosis are very similar to those of East Coast fever **(see Chapter 29: East Coast fever)**. Emaciation and diarrhoea are not a feature of acute cases. Renal lesions are uncommon.

Diagnosis

The diagnosis of Zimbabwe theileriosis depends on the recognition of the characteristic clinical signs, lesions and epidemiological features and on the microscopic demonstration of parasites. In contrast to the situation in East Coast fever, schizonts may be scanty and a prolonged search of lymph node and spleen smears may be necessary to confirm a diagnosis. Piroplasms tend to be present at low levels if they are there at all. The immunofluorescent antibody test (IFAT) and *T. parva*-specific enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) can all be used **(see Chapter 29: East Coast fever: Diagnosis)**.

Control

Zimbabwe theileriosis responds reasonably well to treatment with tetracyclines if it is initiated at the first onset of the febrile reaction. Parvaquone and buparvaquone are more effective therapeutic agents and it may be assumed that halofuginone would also be effective; there is no experience of the use of the latter drug against the disease. Immunization by the infection and treatment method is practised using cattle-derived *T. parva* Boleni as the vaccine. This is a mild stock which has declined rather than increased in virulence on passage.[4, 14] As a result of the mildness of the stock, the vaccine is used without the need for simultaneous treatment with oxytetracycline and without the occurrence of reactions to immunization, which makes the method less cumbersome, cheaper and safer.[9] Vaccination is carried out well before the season of disease and only with the permission of the Director of Veterinary Services.[15] *Theileria parva* Boleni has also been shown to protect cattle against several cattle-derived *T. parva* stocks from Kenya and two buffalo-derived strains.[2] This raises the possibility of using *T. parva* Boleni more widely as a vaccine against East Coast fever, although the fact that it induces the carrier state in immunized animals may make some reluctant to use it. The degree of protection afforded by *T. parva* Boleni to buffalo-derived infections is probably variable.

The disease is notifiable in Zimbabwe, and infected properties are quarantined for a short time and the cattle subjected to intensive acaricide treatment. Where the disease causes severe or recurrent losses, immunization and strategic acaricide use are recommended. It is also recommended that cattle from properties with a history of infection should not be introduced onto a 'clean' farm.

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32

Turning sickness

Synonyms: Cerebral theileriosis, draaisiekte (Afrik.)

J A LAWRENCE AND S M WILLIAMSON

Introduction

Turning sickness is an aberrant form of theilerial infection, characterized by the accumulation of parasitized lymphoblasts in the cerebral blood vessels leading to thrombosis and infarction (**Figures 32.1 to 32.4**). It was first described in Uganda by Mettam and Carmichael[3] in 1936. In East Africa it is usually caused by *Theileria parva*, but in South Africa the condition is attributed to *Theileria taurotragi*.[1] The initial hypothesis that it was caused by *Theileria mutans*[2] reflects the confusion surrounding the identity of that parasite at the time.

Aetiology and life cycle

The life cycles of *T. parva* and *T. taurotragi* are described in **Chapters 29 and 33** respectively.

Epidemiology

Turning sickness occurs sporadically, usually in young cattle less than three years old, in regions which are endemic for *T. parva* or *T. taurotragi*. A very high incidence has been recorded in Zebu cattle which have been immunized by the infection and treatment method and introduced into an East Coast fever endemic area.[4]

Pathogenesis

Proliferation of schizont-infected lymphoblasts occurs in the blood vessels of the meninges, brain, spinal cord and sometimes the spleen,[5] causing thrombosis and infarction. There is usually no evidence of parasite invasion of other organs. This phenomenon is generally considered to result from massive reinfection of a partially immune animal, but the mechanisms which permit the parasite to proliferate in the blood vessels are not understood. It has been suggested that localization of the parasite in the brain may result from intercurrent infection or stress in a chronically infected animal.[4]

Clinical signs

The condition is usually afebrile and is characterized by the sudden onset of a variety of nervous signs, including circling, head pressing, uni- or bilateral blindness, ataxia, opisthotonus and paralysis.[5] Death usually occurs after 2 to 21 days, but occasional cases become chronic and may live for up to six months, the principal signs being blindness and muscular incoordination. In cases in which the spinal cord is involved there is posterior paresis or paralysis.

Pathology

In the acute to subacute form, areas of intense congestion and haemorrhage are present in the meninges and the brain, and are associated with localized softenings and yellowish discoloration of brain tissue.[5] Thrombosed meningeal vessels are often very prominent. There may be extensive haemorrhage into the ventricles (**Figure 32.3**). Histological examination reveals the obstruction of arteries and arterioles in affected areas with large numbers of parasitized lymphoblasts (**Figures 32.5 and 32.6**). The blood vessels are frequently thrombosed and necrotic, with perivascular haemorrhage, and the brain substance which they supply shows a spectrum of lesions ranging from malacia, characterized by spongy change, infiltration of numerous Gitter cells, and proliferation of capillaries and glial cells, to haemorrhage and infarction. Haemosiderin and lipofuscin are widely distributed free or in macrophages through the tissue. In animals which survive for any length of time the necrotic tissue is resorbed, leaving cystic spaces within the brain. Lesions which occur in the spinal cord are similar. In a few cases infarcts may also be present in the spleen (**Figure 32.4**).

Diagnosis

Turning sickness may be suspected when an animal in an area endemic for *T. parva* or *T. taurotragi* develops the characteristic clinical signs and lesions. The presence of



Figure 32.1 Thrombosis of meningeal blood vessels



Figure 32.4 Splenic infarct

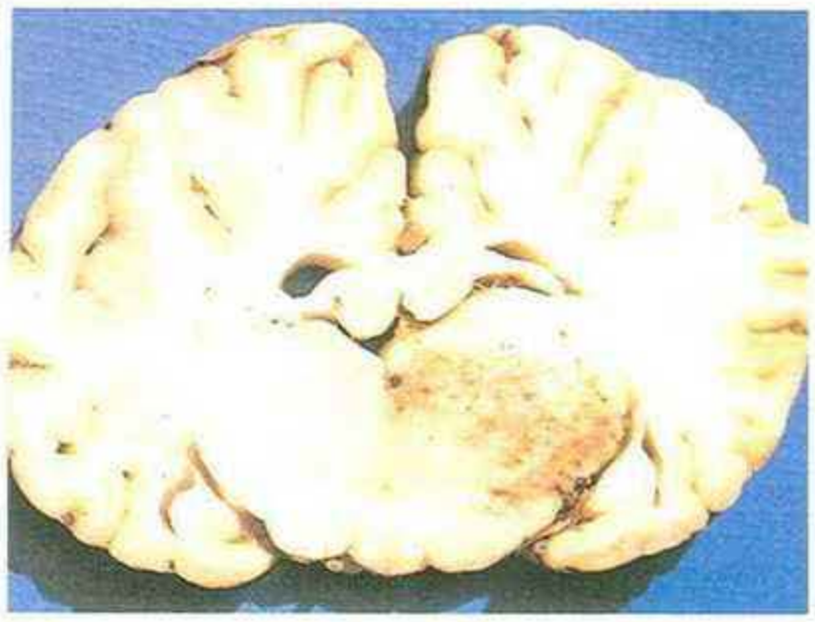


Figure 32.2 Infarct in the thalamus

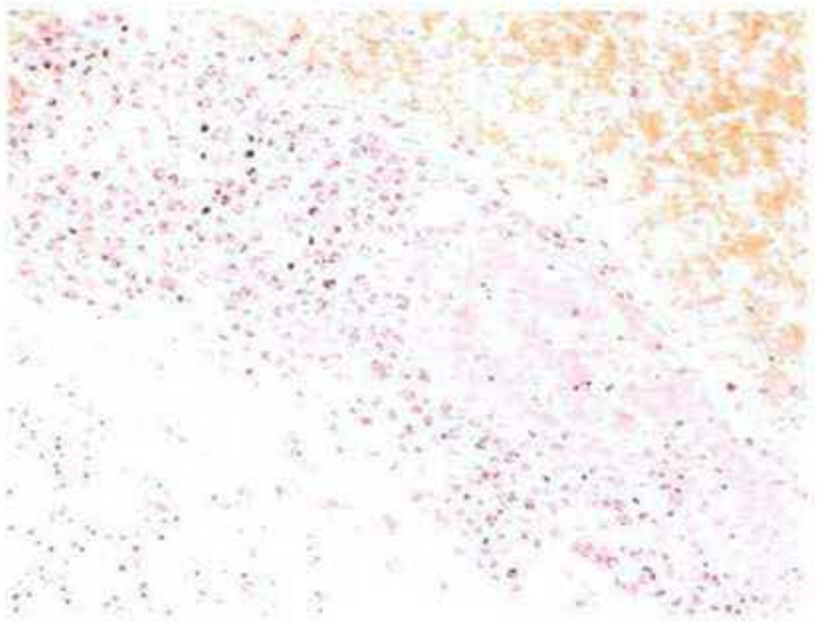


Figure 32.5 Thrombosis and sludging of parasitized lymphoblasts in an area of encephalomalacia

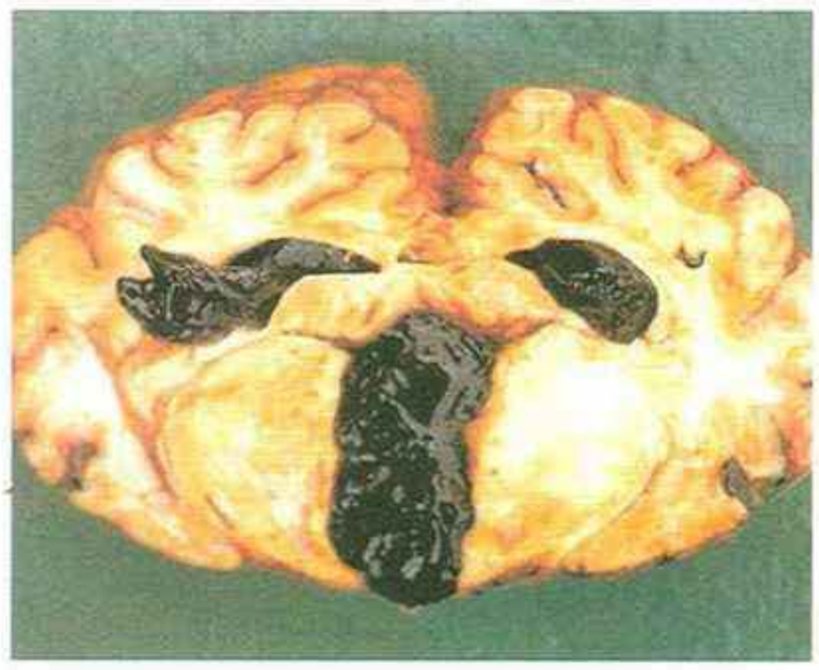


Figure 32.3 Extensive intraventricular haemorrhage

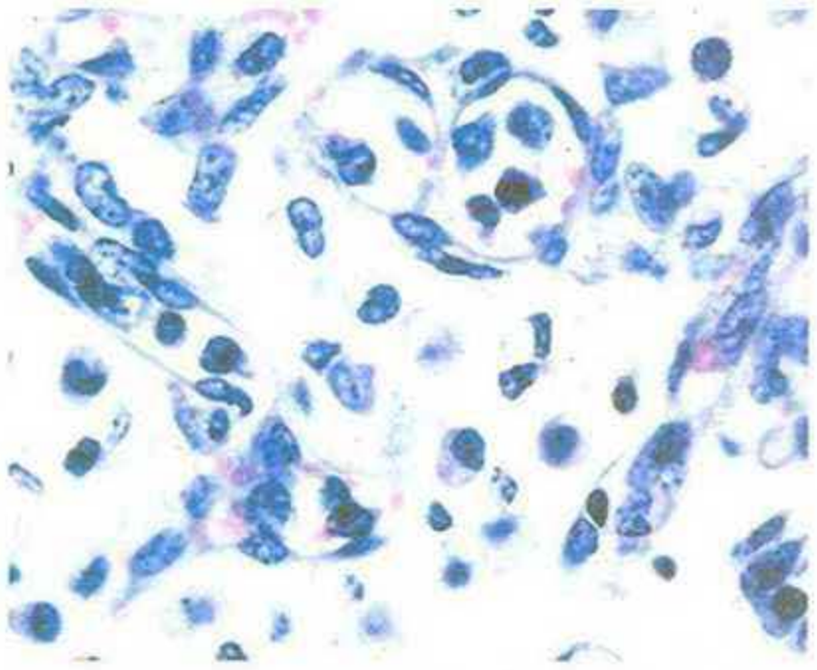


Figure 32.6 Perivascular infiltration of Gitter cells and lymphoblasts in an area of encephalomalacia

circulating antibodies specific to one or other parasite provides supportive evidence. Diagnosis can be confirmed by the demonstration of schizonts in large numbers in smears or histological sections (**Figures 32.5 and 32.6**) prepared from lesions in the meninges or brain. Schizonts may be difficult to find in chronic cases.

Differential diagnosis

Turning sickness must be differentiated from other conditions which present clinically with the sudden onset of nervous signs. Cerebral babesiosis, heartwater, rabies, listeriosis, thromboembolic meningoencephalitis, sporadic bovine encephalomyelitis, lead poisoning, 'stootsiekte', caused by *Matricaria nigellifolia* poisoning, diplodiosis, thiamine-dependent cerebrocortical necrosis, and spinal abscessation ('sitsiekte', **see Chapter 193: *Arcanobacterium pyogenes* infections**) are among the diseases with which it may be confused clinically. Diagnosis is easily confirmed by necropsy but identification of the species of parasite responsible is only possible by immunological or molecular methods.

Control

Cerebral theileriosis caused by *T. parva* may respond to treatment with buparvaquone or halofuginone, but relapses are common and the animal may eventually die after two or three episodes of clinical disease.[4] There is no experience of the use of these drugs against *T. taurotragi*. Prevention should be considered in the context of the control of theileriosis in general. Immunization against *T. parva* by infection and treatment does not appear to provide protection against the condition.

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Diagnosis and differential diagnosis

Theileria taurotragi infection may be suspected when a mild febrile reaction, with enlargement of superficial lymph nodes, occurs in cattle exposed to *Rhipicephalus* infestation, and schizonts are demonstrated in lymph node smears.

Differentiation of infection with *T. taurotragi* from infection with *T. parva* has been discussed previously in relation to East Coast fever **(see Chapter 29)** and turning sickness **(see Chapter 32)**. The *T. parva*-specific enzyme-linked immunosorbent assay (ELISA), unlike the IFAT, does not suffer from cross-reactions with antibodies to *T. taurotragi* and several immunological and molecular tools exist which can distinguish the two parasites, as described fully in **Chapter 29: East Coast fever**. For example, one of the anti-schizont monoclonal antibodies in the monoclonal antibody panel used to characterize cell cultures of isolates of *Theileria* reacts exclusively with *T. taurotragi*.[1]

Control

As *T. taurotragi* is usually a benign parasite, there are no indications for its treatment or control.

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34

Theileria mutans infection

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Introduction

Theileria mutans was first described as a benign parasite of the ox by Theiler,[19] but its identity was inextricably confused with that of other benign species of *Theileria* for many years. It was assumed to be the only benign bovine *Theileria* in Africa until 1977, when it was demonstrated that *Theileria taurotragi* was also capable of causing a mild clinical reaction in cattle.[26] Elsewhere in the world the benign *Theileria* of cattle is considered to be *T. orientalis*.[21] *Theileria mutans* is transmitted by *Amblyomma* ticks and is now known to be confined to eastern, western and southern Africa and to the Caribbean Islands where it was introduced in cattle from Africa. The parasite also infects the African buffalo (*Syncerus caffer*), in which it was first described under the name *Theileria barnetti*.[1] Its only practical significance in southern Africa is the confusion that it causes in the differential diagnosis of *Theileria parva*. In eastern Africa, pathogenic strains of the parasite occur, which may cause severe clinical illness and death.

Aetiology and life cycle

Theileria mutans has a typical theilerial life cycle (**see Chapter 29: East Coast fever: Figure 29.2**) but proliferation in the ox occurs mainly at the stage of the piroplasm rather than at that of the schizont. It is transmitted by several species of *Amblyomma: A. variegatum*,[22] *A. cohaerens*,[25] *A. gemma*,[15] *A. hebraeum*[4, 11] and *A. astrion*.[20] *Amblyomma hebraeum* is the principal vector south of the central watershed of Zimbabwe, and *A. variegatum* is the principal vector to the north (**see Chapter 1: Vectors: Ticks**). Infection occurs transstadially from larva to nymph, or nymph to adult. Recovered animals remain carriers indefinitely. The piroplasm is readily transmitted by blood inoculation.[19]

The piroplasms of *T. mutans* are larger than those of *T. parva* and dividing forms may be seen during the acute phase of infection. The schizonts are also larger and the chromatin granules are more numerous and less uniform in size and shape than those of *T. parva*.[1, 25] It has not yet been possible to culture *T. mutans*-infected lymphocytes *in vitro*.

Epidemiology

Theileria mutans is widespread throughout the range of its tick vectors in sub-Saharan Africa and on some Caribbean islands. It has been shown that in an endemic area 99 per cent of calves have become infected by the time they reach six months of age.[12] *Amblyomma cohaerens*, which is commonly found on African buffalo *(Syncerus caffer)* and develops high infection rates with *T. mutans*, will frequently feed on cattle and is likely to promote high levels of *T. mutans* transmission where buffalo and cattle come into contact.[1] Fatal infections due to *T. mutans* have been described,[7] often in situations where animals have been exposed to *T. mutans* infection after immunization against *T. parva* with a reduction in tick control.[16, 18] It is probable that stress caused by poor nutrition, intercurrent disease or other factors predisposes cattle to anaemia when infected with *T. mutans*. Field and experimental evidence suggests that strains of *T. mutans* derived from buffalo may be more pathogenic to cattle than those derived from cattle.[6, 12, 15]

Clinical signs

Infection with *T. mutans* following tick transmission usually results in a mild febrile reaction and slight swelling of lymph nodes commencing after an incubation period of 20 to 30 days from the time of attachment of the ticks, and lasting for two to five days. Pathogenic strains in eastern Africa cause severe anaemia and icterus and sometimes death.[16, 18] The parasite may also cause a marked anaemia and stunting in young calves.[13] It is unlikely that the clinical disease described in 1937 at Tzaneen in the Limpopo Province of South Africa [2] was, in fact, caused by *T. mutans*. There is no evidence that *T. mutans* causes cerebral theileriosis and reports of cerebral theileriosis in South Africa attributed to *T. mutans* should be ascribed to *T. taurotragi*.[3]

Pathogenesis and pathology

The pathogenic effects of *T. mutans* result from the invasion and proliferation of piroplasms in circulating erythrocytes. Pathogenic strains in eastern Africa are presumed to cause intravascular haemolysis associated with a heavy piroplasm parasitaemia resulting in anaemia and icterus.[7] It has been suggested that this reaction may be associated with an autoimmune response, as with *T. annulata*.[8] The degree of anaemia observed in an animal correlates closely with the level of piroplasm parasitaemia.

Diagnosis

Theileria mutans infection may be suspected when a mild febrile reaction occurs in cattle exposed to *Amblyomma* spp. and when theilerial piroplasms are demonstrated in blood smears. Schizonts, although characteristic, are very scanty and are present for only a short period. They are therefore rarely detected. The presence of large numbers of piroplasms in animals showing anaemia and icterus may rouse suspicion of infection with a pathogenic strain of *T. mutans*, if no other cause can be demonstrated. Confirmation of diagnosis is possible using various methods **(see Chapter 29: East Coast fever)**, the polymerase chain reaction (PCR) being best suited for testing blood samples from the field. Detection of antibodies is possible using an enzyme-linked immunosorbent assay (ELISA) based on a *T. mutans*-specific piroplasm antigen[9, 14] or by indirect immunofluorescence using piroplasm antigen.[10, 17] These tests provide a specific retrospective diagnosis and are also useful in epidemiological studies.

Differential diagnosis

Differentiation of infection with *T. mutans* from infection with *T. parva* has been discussed in relation to East Coast fever **(see Chapter 29: East Coast fever)**. The pathogenic form of *T. mutans* infection seen in eastern Africa must be differentiated from other haemoparasitic infections, notably babesiosis, anaplasmosis and trypanosomosis, by demonstration of the piroplasms in blood smears.

Control

As *T. mutans* is usually a benign parasite, there are seldom indications for its treatment or control under normal conditions. The virulence of field infections with pathogenic strains may be reduced by inoculation of blood containing piroplasms of the local strain, prior to exposure. Parasitaemia is reduced after exposure and the resulting anaemia is less severe.[23, 24] The piroplasms are destroyed by treatment with a number of 8-aminoquinoline compounds (including primaquin), parvaquone[5] and buparvaquone.

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35

Theileria velifera infection

J A LAWRENCE AND S M WILLIAMSON

Introduction

Theileria velifera was first described by Uilenberg[2] in 1964 in Madagascar, under the name *Haematoxenus veliferus*, as a benign parasite of cattle characterized by the presence of a veil-like structure associated with intra-erythrocytic piroplasms (**Figure 35.1**). The parasite is widespread in eastern and southern Africa and also occurs in the Caribbean. It has no practical significance other than its role in confusing the diagnosis of *Theileria parva*. The parasite also infects the African buffalo (*Syncerus caffer*).[1]

Aetiology and life cycle

Theileria velifera is presumed to have a typical theilerial life cycle (**see Chapter 29: East Coast fever: Figure 29.2**) but no detailed studies have yet been undertaken. The parasite is transmitted by ticks of the genus *Amblyomma*, namely *A. variegatum*,[4] *A. hebraeum*, *A. lepidum* and *A. astrion*.[3] The development of *T. velifera* in the gut and haemolymph of *A. variegatum* has been shown to be typical of the *Theileria* genus.[6] The piroplasms are similar to those of *Theileria mutans* except that some are associated with the presence of a veil-like structure in the cytoplasm of the erythrocyte (**Figure 35.1**). This structure is believed to be composed of a crystalline derivative of haemoglobin.[5] The schizonts have not been described. The piroplasms are readily transmitted by inoculation of infected blood.[2]

Epidemiology

Little is known of the epidemiology of *T. velifera*. It is assumed to be widespread throughout the range of its tick vectors.

Clinical signs

No clinical signs have been recorded in intact infected animals. Anaemia occurs in infected splenectomized calves.[4]

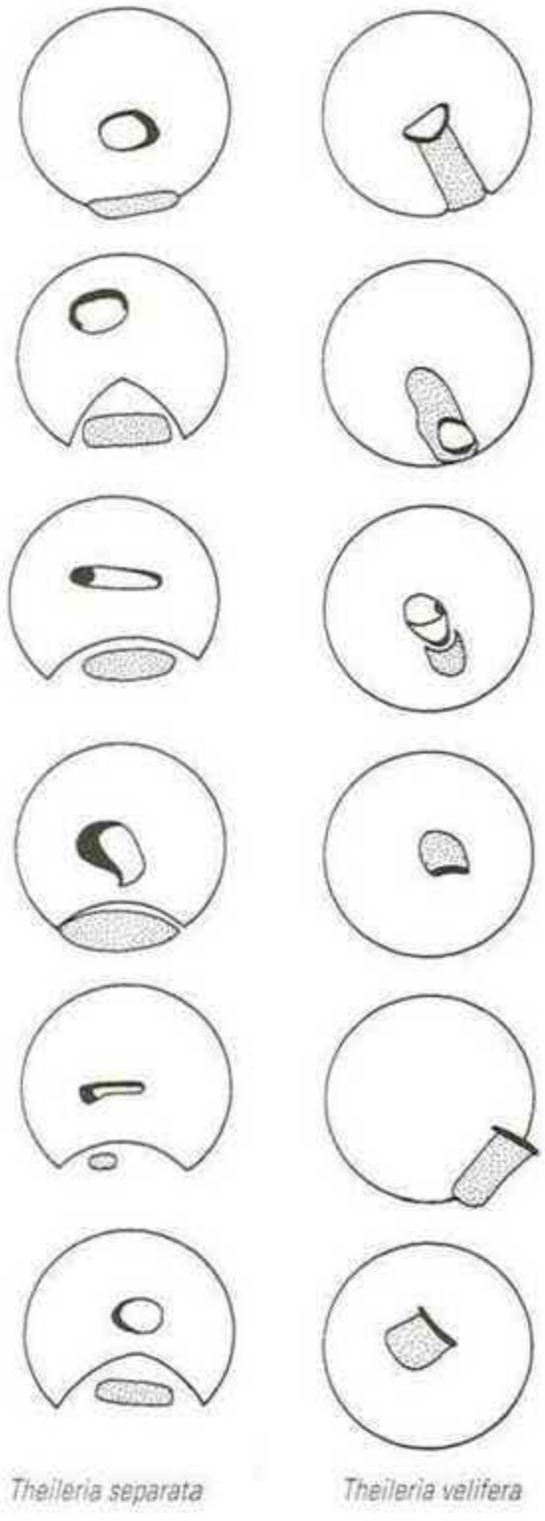


Figure 35.1 Line drawing of piroplasms of *Theileria* spp. which develop veils

Pathogenesis and pathology

No studies have been undertaken on the pathogenesis or pathology of *T. velifera* infection.

Diagnosis and differential diagnosis

Theileria velifera infection can be differentiated from other benign theilerioses if piroplasms associated with veil-like structures are found in erythrocytes. However, as yet, no serological test or species-specific monoclonal antibodies are available.

Control

As *T. velifera* is a benign parasite, there are no indications for its treatment or control.

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36

Theileria separata infection

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Introduction

Theileria separata is a common benign parasite of sheep in the eastern half of southern Africa. It is of no practical significance but piroplasms are encountered occasionally during examination of blood smears. The parasite does not infect goats.

Aetiology and life cycle

Theileria separata shows the characteristic morphological and biological features of a *Theileria* and is transmitted by *Rhipicephalus evertsi evertsi* transstadially from larva to nymph, or nymph to adult.[1, 5]

The piroplasms are characterized by the presence of a clearly defined veil, often situated outside the erythrocyte membrane but lying close to it, opposite a corresponding gap in the erythrocyte cytoplasm (**see Chapter 35: *Theileria velifera* infection: Figure 35.1**). There may also be a small granule, possibly a reduced bar structure of parasitic origin, within the erythrocyte cytoplasm. Veils can be seen associated with a proportion of piroplasms in Giemsa-stained smears but may not be obvious. They are more easily demonstrated in wet preparations by phase-contrast microscopy.[6] The schizonts resemble those of *Theileria parva*.[2]

The piroplasms are readily transmitted by blood inoculation.[4]

Epidemiology

Little is known of the epidemiology of *T. separata*. It is assumed to be widespread throughout the range of its tick vector.

Clinical signs

Infection with *T. separata* results in a mild febrile reaction, which persists for 4 to 16 days, after an incubation period of 9 to 13 days.[1] There is moderate swelling of the superficial lymph nodes. A mild anaemia may be detected.

Pathogenesis and pathology

Enlargement of lymph nodes and mild anaemia are the only lesions to be expected.

Diagnosis and differential diagnosis

Theileria separata infection may be diagnosed when piroplasms or schizonts are found in sheep exposed to *R. evertsi evertsi*. The characteristic features of the piroplasms permit differentiation from other *Theileria* spp. in sheep.

Control

As *T. separata* is a benign parasite, there are no indications for its control.

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37

Theileria annulata theileriosis

Synonyms: Tropical theileriosis, Mediterranean coast fever, Egyptian fever, theileriosis annulata

E PIPANO AND V SHKAP

Introduction

Tropical theileriosis is an infectious disease of cattle caused by the protozoan *Theileria annulata*, which is transmitted by ticks of the genus *Hyalomma*. Invasion by the parasite of cells of the macrophage and lymphoid series results in their replication or destruction, and invasion of erythrocytes results in anaemia. Peracute, acute, subacute, mild and chronic forms of the diseases are recognized. Animals that recover remain latently infected.[110]

Theileria annulata was first described by Dschunkowsky and Luhs in Transcaucasian cattle in 1904;[33] they named it *Piroplasma annulatum*. During the following two decades fatal theileriosis caused by *T. annulata* in the Mediterranean area and India was often mistakenly attributed to *Theileria mutans* infections.[124]

Tropical theileriosis occurs in northern Africa, including the sub-Saharan territories,[54] Sudan and Eritrea, southern Europe, the Near and Middle East, Central Asia, India and northern China. The extent of its distribution in the Far East, where it overlaps with *Theileria sergenti* infection,[56] is not yet clearly delimited.[31] The disease does not occur in southern Africa.

Aetiology and life cycle

Three of the developmental stages of *T. annulata* are infective for cattle: the sporozoites, which develop in the salivary glands of ticks and, in the bovine host, the schizonts in macrophages (monocytes) and the merozoites in erythrocytes. However, in nature, the disease is transmitted only by sporozoites.

Mature sporozoites are oval-shaped, measuring about 1 µm in length. Schizonts are multinucleated, round, varying in diameter from 1 to 15 µm (in some cases up to 27 µm), with an average of about 8 µm. Each nucleus measures about 1.5 µm in diameter.[67, 70] In cultures, schizonts contain an average of 12 nuclei, but some with 80 or more may be found.[89] Schizonts containing micromerozoites (micro-schizonts) are not as common in *T. annulata* infections as they are in those of *Theileria parva*. This is true both in infected cattle and in cultures. Schizonts develop and grow in size as a result of binary fission of their nuclei. The intracellular location of the schizonts stimulates multiplication of the host cells in a manner that results in the distribution of schizont material to each of the newly formed cells (**Figure 37.1**).[89] Some of the schizonts develop into merozoites, which penetrate erythrocytes.[67]

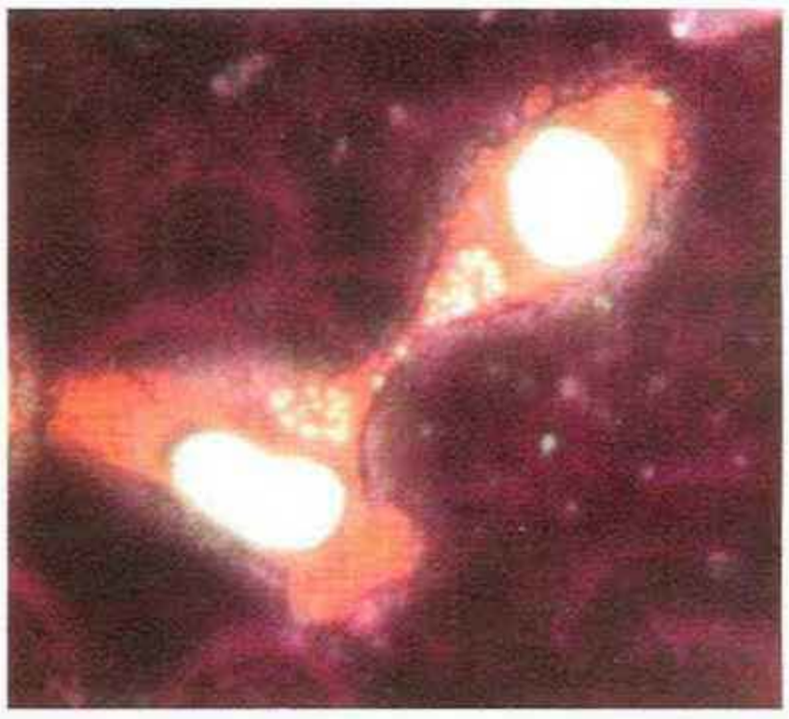


Figure 37.1 *Theileria annulata*-infected cell in culture. The schizont is shared between the new forming cells. Acridine orange staining, ×2 000

Intra-erythrocytic merozoites (also referred to as 'piroplasms') are generally spherical, oval- or comma-shaped, but anaplasma-like forms measuring 0.5 µm may also be encountered; each is bounded by a single-layered cell membrane and possesses an oval nucleus.[67] Spherical forms vary in diameter from 0.5 to 1.5 µm, and oval forms are 0.6 × 2.0 µm and comma-shaped forms 0.5 × 1.6 µm in size.[70] During multiplication of merozoites, nuclear division

precedes cytoplasmic division, in a process characteristic of schizogony.[21]

The life cycle of *T. annulata* is similar to that of *T. parva*[67] (**see Chapter 29: East Coast fever: Figure 29.2**) and only some characteristics specific to *T. annulata* are discussed here.

Theileria annulata is transmitted transstadially by two- and three-host ticks of the genus *Hyalomma*. After the pre-imaginal stages ingest blood containing intra-erythrocytic merozoites, spindle-shaped bodies (considered to be microgamonts, which give rise by fission to microgametes), and spherical forms (which are macrogametes) develop in the lumen of the gut.[67] The zygote-like stages which later develop in the gut wall transform into club-shaped kinetes that migrate to the salivary glands of the adult ticks after the moult. After the moulted ticks have begun to feed, the kinetes become polymorphic and undergo repeated nuclear divisions until sporozoites are formed. Generally, infective sporozoites appear in the salivary glands after the tick has been feeding for 48 to 72 hours.[116] However, 'activation' of ticks by close proximity to cattle or by being warmed to mammalian body temperatures[66, 104] may also trigger the transformation, so that some ticks may be infective before feeding on their host. Ticks can only become infected by ingesting intra-erythrocytic merozoites.[105]

After inoculation into cattle, sporozoites penetrate mononuclear cells and develop into schizonts. These are detectable in smears made from superficial lymph nodes and the liver 7 to 28 days after infection.[110] Merozoites are detected in erythrocytes one to three days after the first appearance of schizonts, and may persist for years in infected cattle. Schizonts also readily infect cattle by needle inoculation. Following their successive passages in cattle or culture, schizonts may lose their ability to produce merozoites, although they retain their infectivity for cattle.[85, 86, 110]

Isolates differing in virulence and cross-protection ability have been reported from various geographical areas.[2, 92, 99, 109] Fifty-three North African isolates of *T. annulata* have been characterized by antiparasitic monoclonal antibody reactivity, isoenzyme electrophoresis and Southern blotting. No identical isolates were detected by these methods and the majority of isolates contained more than one parasite population.[9] It appears, however, that the reported diversity in field *T. annulata* populations is not necessarily related to immunological strain differences and, therefore, it has a limited impact on immunization against tropical theileriosis.

Epidemiology

The occurrence of tropical theileriosis is dependent on the presence of infected ixodid ticks. Fifteen *Hyalomma* spp. have been incriminated as vectors,[101] although some of these may be synonyms for identical species. Among the most important are *H. detritum, H. anatolicum, H. excavatum* and *H. dromedarii*.[70] The infection is generally transmitted transstadially, but infected male ticks that accidentally become detached from one host may reattach to another and, thus, transmit the disease to more than one animal.[110] Not all *Hyalomma* spp. that transmit *T. annulata* under experimental conditions are vectors in nature, since the pre-imaginal stages of some species have a preference for hosts other than cattle.[11, 80] *Theileria annulata* is maintained in nature by a cattle–tick–cattle cycle. The water buffalo (*Bubalus bubalis*) is considered to be the natural host of this parasite and to be the one in which it evolved.[120] *Theileria annulata* usually causes no apparent infection in water buffalo, but the latter may act as a reservoir for infection of ticks. The yak (*Bos grunniens*) is highly susceptible to *T. annulata* theileriosis and suffers severe clinical signs and mortality when infected.[64]

The disease has a seasonal occurrence, which is related to the ecology and biology of its vectors. In most endemic areas, the majority of cases occur between June and September,[11, 35, 80, 110, 112] although sporadic cases may be found throughout the year.

Theileria annulata may complete its life cycle in ticks on pasture lands or in barns. Engorged nymphs of *H. detritum*, one of the main vectors of *T. annulata*, hide in cracks and crevices of clay or stone barn walls and, after moulting, adults infect cattle introduced into these barns.[110] Susceptible cattle on zero grazing in tick-free premises are, in theory, protected from infection, but infected ticks can be introduced on hay or by stray cattle that have grazed on tick-infested pastures.[80]

Engorged adult *Hyalomma* ticks produce large numbers of sporozoites: *c.* 50 000 have been estimated to be present in a single cell of the type III acinus of the tick salivary glands. Since many such cells are generally infected, a bovine host may receive an enormous inoculum from a single tick feeding to repletion.[67] This high infectivity has been demonstrated by infecting cattle experimentally with a 1:100 dilution of a single macerated tick.[88]

In contrast to the situation in some other tick-borne infections, such as babesiosis, where many ticks are needed to infect a single animal,[118] in the case of *T. annulata*, a single tick is capable of transmitting a fatal infection.[88] Examination of the salivary glands of *Hyalomma* ticks showed that a high percentage of ticks harvested in barns or in the field were infected with *T. annulata*.[36, 57, 102, 123] Thus, a small number of infected ticks may cause extensive outbreaks in cattle herds. Calves are considered by some authors to be more resistant than adult cattle, but others consider them equally susceptible.[70, 112] In our experience young calves are highly susceptible to tropical theileriosis, while exacerbation of neonatal diseases may further complicate the course of the infection.

The epidemiology of *T. annulata* theileriosis differs in different geographical regions. In the Mediterranean area, where the two-host tick *H. detritum* plays the major role in the transmission of *T. annulata*, the disease in young calves is relatively rare. During the first tick season that calves are exposed to, the level of infestation with adult ticks is low compared to that in older cattle, and they are therefore

exposed to a limited natural challenge.[11, 37] In contrast, in India where the main vector is *H. anatolicum*, calf morbidity and mortality represent most of the theileriosis cases which occur, since the infestation rate of calves with nymphs is high; and nymphs as well as adults of *H. anatolicum* can transmit *T. annulata* infection.[8, 72]

Antitheilerial antibodies have been detected in colostrum of infected dams,[78] but there is no evidence that colostral immunity can engender significant protection against sporozoite infection in young calves. Endemic (enzootic) stability develops in herds that show a seroconversion rate of 100 per cent in calves in the first season that they are exposed to theileriosis.[25] Older cattle become more heavily infested with ticks[35] and if they escape infection in their first theileriosis season they are more likely than young calves to be infected by *T. annulata*.[11, 37]

Pathogenesis

Sporozoites introduced into susceptible cattle through the saliva of infected ticks infect cells either at the site of inoculation or migrate to the lymph node which drains the infection site. There is no evidence that they pass through the lymph node into the efferent lymph. The rate of lymph flow and total cell output in the lymph node increase 5 to 10 fold from day 6 onwards.[73]

The sporozoites invading host cells transform to trophozoites, which develop into schizonts. *Theileria annulata* predominately infects CD3- and CD 11b+ phagocytic cells (macrophages/monocytes) that express major histocompatibility complex (MHC) class II antigens.[38] Recently, it has been shown[18] that primary infection with *T. annulata* may induce an aberrant T-cell activation, which results in failure of immune responses and which may subsequently result in an acute lethal infection. Schizont-infected cells non-specifically activate naive T cells: within 48 hours following infection, large numbers of macrophages develop in the medulla of the lymph node that drains the site of infection. Within four days of infection, these macrophages activate inappropriately large numbers of CD4 T cells via MHC class II antigens in the medulla, but not in the paracortex of the lymph node. These events lead to diversion of an effective immune response, as T cells are not primed in the normal anatomical site and antigen-driven T-cell stimulation undergoing clonal expansion does not take place.[20]

In culture, *T. annulata* sporozoites invade and transform macrophages, and also B cells characterized by the CD2 lymphoid antigen.[38, 49, 119] Inoculation of schizont-infected cells into cattle results in the transfer of schizonts to cells of recipient animals. This was demonstrated by the fact that when schizont-infected cells from a cow were inoculated into a male calf, the schizonts were later found within the lymphocytes of the recipient.[121] The mechanism by which parasites are transferred from infected cells to host cells is not known. Inoculation with *T. annulata*-infected cells leads to an allogeneic graft rejection response, resulting in release of the schizonts. *In vivo*, the recipient host cells for *T. annulata* are phagocytes, whereas *T. parva* is found largely in T cells. It was, therefore, assumed[13] that efficient transfer of schizonts is associated with their capacity to penetrate into new host cells, and that phagocytic cells actively engulf *T. annulata* schizonts. The proliferating *T. annulata*-infected cells multiply and metastasize in the body,[39] so that the schizont-infected cells become disseminated rapidly through the lymphoid tissues, from the lymph node draining the site of inoculation to more distant lymph nodes, spleen and thymus. Parasitized cells also spread to non-lymphoid organs, such as the liver, kidneys, lungs, abomasum, adrenal glands, and later to the brain and myocardium.[39] Merozoites released from schizonts infect red blood cells, and in severe infections more than 95 per cent of red blood cells are parasitized, each containing one or more merozoites.[70]

The severity of infection (lethal or sublethal) following tick transmission depends upon the number of sporozoites inoculated. The proportions of schizont-infected cells and parasitized erythrocytes, the duration and severity of pyrexia, and the size of enlarged lymph nodes are directly related to the size of the inoculum, while the times to onset of fever and to the appearance of schizonts and erythrocytic merozoites are inversely proportional to the number of sporozoites inoculated.[95, 103]

Significant reductions in erythrocyte count, haemoglobin concentration and packed cell volume, with evidence of severe aplastic anaemia,[62] occur during the course of the disease. Anaemia varies in degree, and icterus develops as a result of erythrocyte destruction and liver damage. It appears that not only merozoites are involved in erythrocyte destruction; cattle infected by needle inoculation with strains of *T. annulata* schizonts that do not produce erythrocytic merozoites may also suffer from a marked anaemia. This suggests that schizonts alone can trigger erythrocyte destruction. It has been suggested that auto-immune mechanisms play a role in the pathogenesis of the anaemia.[48, 53] Leukopenia occurs in lethal and sublethal infections because of a decline in circulating lymphocytes and neutrophils. It coincides with the onset of fever, which is accompanied by initial evidence of schizonts in lymph nodes. An initial brief period of monocytopenia is followed by marked monocytosis in both fatal cases and recovering animals. The monocytosis peaks between three and four weeks after infection.[95, 106]

In general, the pathogenesis of the lesions which occur in organs such as the liver, spleen, lungs and kidneys is poorly understood. It has been suggested that pulmonary congestion and oedema are related to lymphocytolysis of infected cells within the lungs.[53] Toxins have been suspected as causing haemorrhages and lesions in the liver,[62, 70] but the occurrence of such toxins has not yet been demonstrated.

The tumour necrosis factor (TNF)-α is considered to play a major role in the pathogenesis of tropical theileriosis.[16, 39] The level of TNF-α produced spontaneously by macrophages from cattle with either primary or challenge infection increases upon exposure to interferon (INF)-γ.[96] Abnormally high levels of INF-γ that occur in lethal *T. annulata* infections may induce excessive production of TNF-α,[17, 19] and thus contribute to the clinical signs and tissue damage in infected cattle.[4, 16, 39] Excessive production of TNF-α may be related to leukopenia, disseminated haemorrhages and ulcerative lesions.[39] *Theileria annulata*-infected mononuclear cells produce IFN-γ,[96] which may have two antagonistic effects on the host immune response:

- to stimulate production of TNF-α and nitric oxide that protect cattle against infection; and
- to suppress proliferation and dampen the generation of T-cell responses. The suppressive activities of the T-cell responses might overwhelm its beneficial properties.[39]

Increases of calcium, chloride and sodium ions and of total protein in the contents of the abomasum and duodenum during the acute stage of *T. annulata* infection have been reported. It has been suggested that the loss of these ions and proteins from the interstitial fluid into the lumen of the digestive tract may explain the rapid debilitation of diseased cattle.[74]

Latent infections with various agents are often activated during the acute stage of tropical theileriosis. It is possible that the massive destruction of lymphocytes by the schizonts leads to a deficient immune response.[113]

Cell-mediated immunity appears to play a major role in the protective immunity against *T. annulata* infections.[23] The various parasite stages (trophozoites, schizonts, merozoites and erythrocytic merozoites) and the cells they inhabit are affected by different mechanisms.[13, 98] *Theileria annulata* infection stimulates innate (T-cell independent) and adaptive or acquired (T-cell dependent) immune responses. The protective immunity against the infection depends upon the co-operation between the innate and adaptive immune systems,[98] as seen in infections with other parasites of macrophages.[34]

The innate immune responses, stimulated by infection with sublethal doses of *T. annulata* sporozoites or by immunization with schizont-infected cells, include activation of 'cytostatic' macrophages, which suppress the proliferation of schizont-infected cells.[94] These macrophages spontaneously produce TNF-α[96] and nitric oxide,[122] and, in co-operation with natural killer cells, lyse schizont-infected cells.[46, 97] The nitric oxide, implicated as an effective mediator of protective immunity,[122] has also been shown to inhibit the proliferation of schizont-infected cells, thus causing schizonts to disappear and host cells to become apoptopic.[100]

The adaptive immune responses include production of cytotoxic T cells for schizont-infected cells,[3, 22, 51, 98] antigen-sensitized lymphocytes[93] and $CD4^+$ T cells.[12] The cytotoxic cells are known to lyse schizont-infected cells, but may also act directly on trophozoite-infected cells by producing IFN-γ. The cell-line vaccines[81] induce resolution of infection because they generate consistent and prolonged macrophage activity against schizont-infected cells, when only transient cytotoxic T-cell responses are detectable.[51, 94]

The mechanisms that control intra-erythrocytic merozoites are unknown, but since nitric oxide stops invasion of peripheral blood macrophages by sporozoites,[122] it may also prevent invasion of erythrocytes by merozoites.[98] Antibodies to parasite antigens, which develop during primary infection,[44] may inhibit *in vitro* infection of cells by sporozoites,[46] but are thought not to play a role in protection, because they have not been shown to interact with the surfaces of either schizont-infected cells or of merozoite-infected erythrocytes.[98] Recovery from *T. annulata* primary infection results in the development of a persistent carrier state in cattle. This carrier state is of cardinal importance in maintaining immunity, and the accurate identification of carrier animals could be useful in assessing their immune status.[50]

Clinical signs

In natural infections the incubation period varies from 9 to 25 days, with an average of 15 days. However, the prepatent period may be as short as seven days in cattle inoculated with a suspension of macerated ticks. The prepatent period following inoculation of infected blood varies from 12 to 30 days, with an average of 17 days; on rare occasions it may extend to as long as 10 months.[110]

Initially, there is fever, swelling of the superficial lymph nodes and increases in the pulse and respiratory rates. Anorexia and decreased rumen movements are followed by dehydration and constipation. Diarrhoea often occurs in young animals, possibly because of exacerbation of latent infection. Emaciation is rapid. The eyelids may be swollen and hyperaemic, and lachrymation and nasal discharge occur. Anaemia, which becomes progressively more severe during the course of the disease, causes mucous membranes to become pale. Icterus may be present. Petechiae on the visible mucosae usually indicate an unfavourable prognosis.

The urine is dark brown as a result of the presence of bilirubin. In dairy cows there is a sudden and marked drop in milk production. Abortion often occurs. Rarely, nervous signs may appear in acute *T. annulata* infections one to three days before death.[60] Peracute, acute, subacute, chronic and mild forms of the disease have been reported.[70]

The peracute form is most likely to occur in highly susceptible cattle breeds, after a massive infection. The clinical period lasts for between three and five days and ends with the death of the animal. Typical gross pathological changes generally do not have time to develop, but numerous schizonts may be present.

The acute form is the type most frequently seen in susceptible breeds. Clinical signs are well defined, and the disease lasts for one or two weeks. Recovery is slow, especially in adult cattle, and mortality is high.

The subacute form resembles the acute form, but clinical signs are less intense. Most animals recover if complications from secondary infections, such as bacterial enteritis, particularly in calves, do not occur.

The chronic form is rarely encountered. Affected cattle are recumbent, emaciated, and show intermittent fever. In most cases death occurs after two to three weeks, rarely after four weeks.

The mild form is encountered in naturally resistant cattle, in cattle protected by passive immunity, in vaccinated cattle with only partial immunity, or when the infecting strain is of low virulence. Clinical signs are generally unnoticed and cattle recover spontaneously.

In protracted cases, skin lesions consisting of lenticular haemorrhagic spots or skin nodules, which are generated in the perivascular lymphoid tissues of the dermal arterioles, are sometimes observed. Ulceration of the nodules may occur and Giemsa-stained touch preparations reveal numerous schizont-infected cells.[65, 110]

Rarely, infection may occur, even with an otherwise virulent strain of *T. annulata*, in which there are no clinical signs and often no detectable parasites, because the animal is strongly resistant to infection. This condition has been referred to as an immediate latent infection (*infection latente d'emblee*).[108]

In practice it is not always feasible to classify a field case into one of the above categories when it is first diagnosed.

Mortality rates of 20 to 90 per cent have been reported from various geographical areas.[70] This range is apparently due to variations in susceptibility of cattle and in the virulence of the parasites involved. Adult animals which have apparently recovered may die two to three weeks after the disappearance of schizonts as a result of complications, such as liver dysfunction.[63, 115] Growth may be impaired in young calves that recover from the disease.

Pathology

Significant increases in the levels of serum glutamic oxaloacetic transaminase, sorbitol dehydrogenase, isocitric dehydrogenase,[115] alanine aminotransferase, alkaline phosphate, creatinine kinase and γ-glutamyltransferase, as well as increases in the concentrations of uric acid, blood urea nitrogen and bilirubin[106] have been found in cattle suffering from tropical theileriosis. Haematological changes consist of severe anaemia, anisocytosis, and basophilic stippling.

In animals which have died, the severity of lesions is primarily dependent upon the course of the disease. In fulminant cases, lasting only a few days, lesions are limited mainly to hyperplasia of the lymphoid tissues and to disseminated haemorrhages. According to Sergent and collaborators,[110] the lesions in *T. annulata* infection are similar to those observed in haemorrhagic septicaemias, being characterized by haemorrhages and congestion.

In typical cases, the carcass is emaciated, anaemic and icteric, with yellow gelatinous connective tissues. Numerous petechiae or larger haemorrhages are seen in the subcutaneous tissues and in the mucous and serous membranes. All superficial and internal lymph nodes are swollen and often haemorrhagic. The spleen is enlarged, mainly as a result of hyperplasia of lymphoid tissue. The liver is swollen, brownish or orange-yellow in colour, and friable because of parenchymatous degeneration. Subcapsular haemorrhages are often observed. The gall bladder is distended and contains dark-green, thick bile.

Epi- and endocardial petechiae and ecchymoses are found. The myocardium is often degenerated and shows extensive haemorrhagic areas (**Figure 37.2**). Excessive amounts of serous fluids may occur in the pericardial, thoracic and other body cavities. The lungs are frequently oedematous. The kidneys are congested and may show haemorrhages or small (1 to 3 mm), whitish-red foci of lymphoid hyperplasia. Depending upon the duration of the infection before death, red patches or ulcers may be found in the mucous membranes of the abomasum and small and large intestines. The urinary bladder contains urine pigmented dark brown because of the presence of bile pigments.

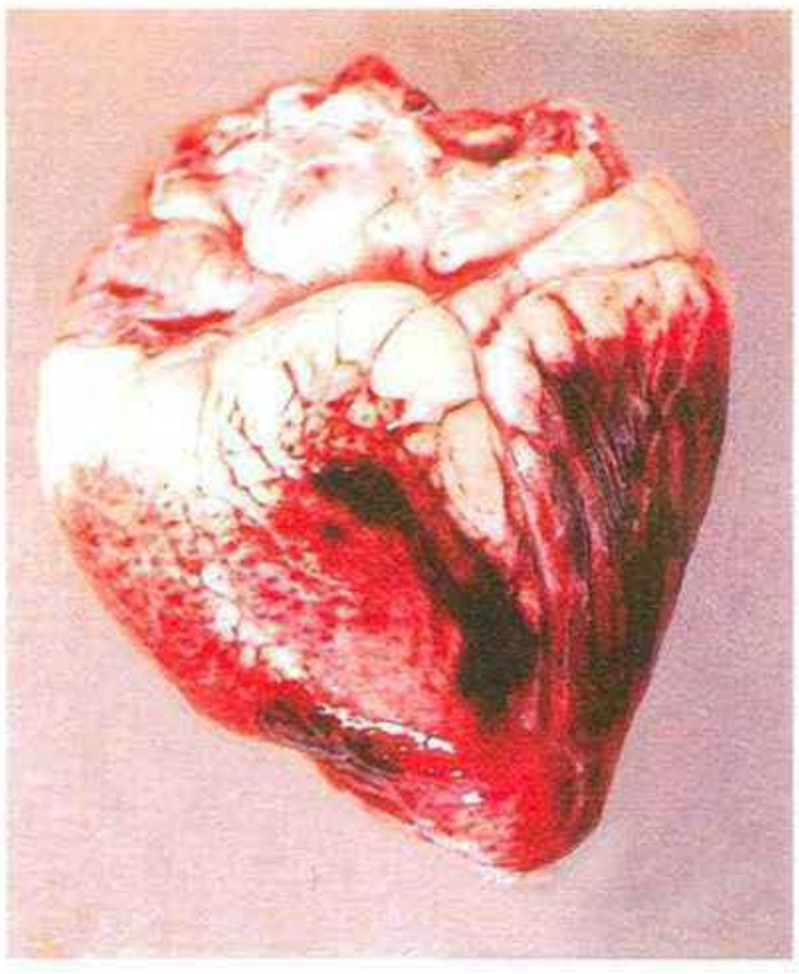


Figure 37.2 Ecchymoses and petechiae in the myocardium. Experimental infection with virulent schizonts

Exceptionally, papular and pustular skin lesions (mainly in the non-pigmented areas) which may be ulcerated or necrotic, may be observed. These lesions are infiltrated by neutrophils and lymphocytes, as well as cells containing schizonts.[65]

The histopathology of tropical theileriosis has been poorly studied.

In cattle that have died after having shown cerebral disturbances, clogging of meningeal and cerebral blood vessels by large numbers of lymphocytes has been observed.[60]

Diagnosis and differential diagnosis

In areas endemic for tropical theileriosis, the non-specific clinical signs of fever, anaemia, icterus and swelling of peripheral lymph nodes justify a presumptive diagnosis of acute *T. annulata* infection.

For laboratory confirmation, thin smears should be prepared from biopsy material of lymph nodes or the liver. Blood films prepared during the initial stage of the disease show rare or no erythrocytic merozoites, a picture also observed in recovered carrier animals. On the other hand, the presence of schizonts in lymph nodes and the liver is often sufficient to support a diagnosis of acute theileriosis. Smears made from liver tissue are considered by Sergent *et al.*[111] to be the most suitable means for the detection of schizonts and for the evaluation of the severity of the infection.

In *T. annulata* infection, schizonts are rare in the peripheral blood, and their presence in blood smears indicates a poor prognosis. The number of parasitized erythrocytes increases progressively during the course of the disease, and as many as 95 per cent of the red blood cells may be infected (**Figure 37.3**), but the severity of the disease does not necessarily correspond to the percentage of parasitized erythrocytes. The Giemsa staining method is considered to be the most suitable one for demonstrating parasites in smears.

To detect schizonts and merozoites in fatal cases, impression smears of the liver, spleen and several lymph nodes should be examined. Both intracellular and free-lying schizonts may be detected, the latter having been released from parasitized cells during preparation of the smears. Most of the merozoites in the acute stage of the infection are spherical or oval-shaped and are smaller (1 to 2 µm) than the merozoites observed in the chronic carrier condition. Merozoites persist for months or even years in recovered cattle, but they are not always detectable by direct microscopic examination of smears. On the other hand, the presence of schizonts is always associated with the acute stage of infection.

The most widely used serological test for detecting latent *T. annulata* infection is the indirect fluorescent antibody test (IFAT). Both schizonts and erythrocytic merozoites are used as antigens.[26, 59, 84] The schizont antigen appears to be significantly better than the merozoite antigen for detecting animals carrying *T. annulata* infection.[26] The IFAT is specific only in areas where *T. annulata* occurs alone, since antibodies to other *Theileria* spp. may cross-react with the *T. annulata* antigen. The IFAT is useful for identifying infected herds containing carrier animals but is not sufficiently accurate to detect all infected individuals. Neither the schizont nor merozoite IFAT detects antibodies in every animal with erythrocytic merozoites in its peripheral blood.[26, 90]

The enzyme-linked immunosorbent assay (ELISA) has been successfully adapted for detection of anti-*T. annulata* antibodies.[45] In contrast to the IFAT, schizont antigen used in ELISA is less sensitive and specific than the antigen prepared from erythrocytic merozoites, since the latter detects antibodies for a longer period.[58, 59] Serological tests are mainly used for epidemiological screening for *T. annulata* infection and assessment of the results of immunization with live vaccines. It appears that immunity to *T. annulata* theileriosis is mainly cell-mediated, but antibodies can be used as an indication for establishment and multiplication of *Theileria* parasites which induce a protective immunity.

DNA techniques based on sequence analysis of genes encoding for particular proteins have enabled the development of molecular methods for the diagnosis of *T. annulata* infection. Specific polymerase chain reaction (PCR) primers, derived from a gene encoding for a 30 kDa merozoite antigen that is used to test samples of whole blood, detects a parasitaemia as low as 0,000048 per cent[32] and can also be used to detect the parasite in *Hyalomma* ticks.[30]

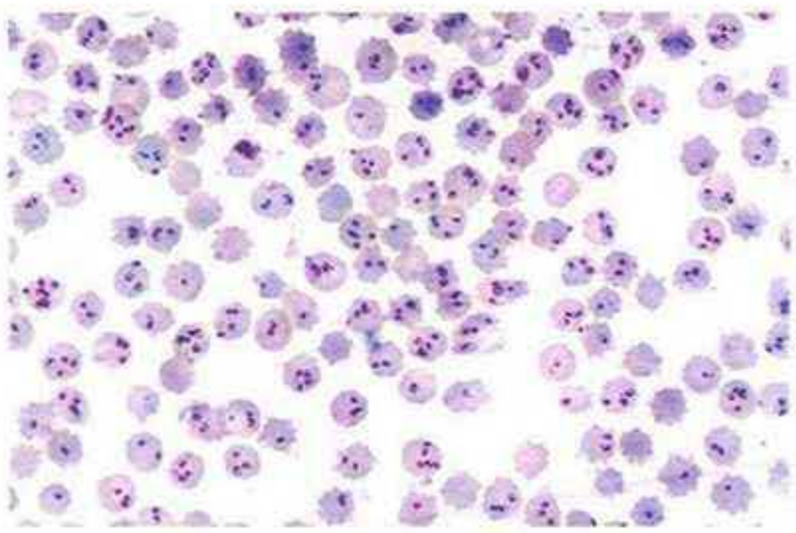


Figure 37.3 Erythrocytic merozoites of *Theileria annulata* following tick-induced infection, × 1 000

Endemic regions of *T. annulata* and *T. parva* infections do not overlap except, perhaps, in East Africa in the proximity of the fourth to eighth parallels north. Schizonts of *T. annulata* are very similar to those of *T. parva*, but those of the former are usually scarce in peripheral blood. Microschizonts are more often observed in East Coast fever than in tropical theileriosis and, in addition, the majority of the erythrocytic merozoites in the former disease are comma- or rod-shaped.

Growth and serial passages in culture result in attenuation of the virulence of the schizonts.[24, 91] At first, cultured schizonts of originally virulent strains cause severe clinical disease and eventually death when inoculated into susceptible cattle. After several weeks or months of cultivation, with passages being performed about twice a week, milder clinical reactions are observed when schizonts are injected into cattle. Microscopically, only a few schizonts are found in internal organs and rare erythrocytic merozoites occur in peripheral blood. Complete attenuation has been achieved when calves inoculated with cultured parasites do not develop clinical signs, and when schizonts or erythrocytic merozoites cannot be demonstrated in smears made from their blood and tissues.[77, 80, 85] Various field isolates of *T. annulata* may require three months to three years of continuous cultivation in order to become completely attenuated, and quality control of the culture-derived schizont vaccine comprises testing for safety, sterility, potency and efficacy.[83] The cell culture vaccine is safe for all breeds and ages of cattle, regardless of their physiological status, such as pregnancy and lactation.[86] No erythrocytic merozoites are detected in vaccinated cattle and they are, therefore, not a source of infection to ticks.[105] Vaccine prepared from fresh suspensions of cultured schizonts has a limited shelf life of only a few days at 4 °C, but storage in a concentrated form in liquid nitrogen extends the effective life of the vaccine to at least two years.[81]

Vaccine containing completely attenuated schizonts induces a marked production of specific, but not necessarily protective, antibodies that can be detected serologically.[40] There are indications that some multiplication of such attenuated schizonts occurs in inoculated animals, since equivalent numbers of killed schizonts inoculated without an adjuvant do not induce the formation of significant serum antibody levels.

Laboratory challenge of schizont-vaccinated cattle with tick-derived sporozoites results in moderate or no clinical manifestation,[42, 75] and immunity lasts for at least three-and-a-half years.[126] The schizont vaccine induces effective immunity against field-transmitted infection in indigenous susceptible cattle as well as in a high-grade exotic stock breed introduced into theileriosis-endemic regions.[81] However, field observations have shown that some vaccinated cattle suffer severe theileriosis four or more years after vaccination.[90] It seems, therefore, that the immunity acquired after a single inoculation of culture-derived schizont vaccine is not life-long, and revaccination might be required for cattle in herds with low tick-infestation rates. The response to primary immunization with anti-theilerial vaccine containing schizont-infected cells is barely influenced by the histocompatibility of the schizont-infected cell and the recipient animals. However, together with the immune response to the *Theileria* schizonts, an allogenic response to the cells carrying the parasites occurs.[51, 52] Upon revaccination the allogenic antibody can block the parasite transfer to the recipient animal and prevent the enhancement of immunity at the time of a second vaccination with the same culture-derived schizont-infected cell line.[71] On the other hand, heterologous schizont-infected cells from low-culture passages considerably boosted the immunity against tick-transmitted infection.[87]

As with other tick-borne diseases, tropical theileriosis can be prevented by control or eradication of the vectors. The sensitivity to acaricides of some *Hyalomma* spp. has been recently evaluated,[6, 61, 127] but adequate treatment regimes to control or eradicate these ticks have not yet been critically evaluated. Since a single infected tick can cause fatal theileriosis,[88] tick control must be highly efficient if it is to be effective. The time schedule for acaricide treatment should take into account the effective residual activity of the acaricide and the very short period required for maturation of *T. annulata* sporozoites in attached ticks.[116] Under some environmental circumstances ticks may carry mature sporozoites even before they commence feeding,[66] and such ticks may transmit the infection within a few hours after attachment. In tick-infested barns, periodic dusting of the floor with acaricide after removal of litter and manure is recommended, in addition to acaricide treatment of the cattle.

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38

Theileriosis of sheep and goats

Synonym: Malignant theileriosis of sheep and goats

J A LAWRENCE

Introduction

The taxonomy of the species of *Theileria* that infect small ruminants is very imperfectly understood. *Theileria lestoquardi* (syn. *T. hirci*) is generally accepted as being the only species of economic significance. The other species currently recognized are *T. ovis* (syn. *T. recondita*) and *T. separata*.[8] It is probable that the parasite generally identified as *T. ovis* may be a complex of several species, judging from the number of genera of ticks that has been implicated as vectors.[7]

Aetiology and life cycle

Theileria lestoquardi is the causal organism of malignant theileriosis of small ruminants and has a life cycle and morphology typical of the genus *Theileria*. It is transmitted by *Hyalomma anatolicum anatolicum*,[3] and possibly by other species of *Hyalomma*.[1] Transmission from nymph to adult has been demonstrated. The parasite infects both sheep and goats. Antigenically it is closely related to *T. annulata* of cattle.[6]

Theileria lestoquardi schizonts can be grown readily in suspension culture in lymphoid cells, in a manner similar to those of *T. parva* and *T. annulata* (**see Chapter 29: East Coast Fever and Chapter 37: *Theileria annulata* theileriosis**).[4] Cultured schizonts are of value in immunization[5] and as antigen for indirect immunofluorescence testing.[6]

Theileria separata, originally described as *Haematoxenus separatus*,[9] can be distinguished morphologically from the other species of *Theileria* in small ruminants by the association with the piroplasms of a clearly defined veil, often situated outside the erythrocyte membrane but lying close to it, opposite a corresponding gap in the erythrocyte cytoplasm (**see Chapter 35: *Theileria velifera* infection: Figure 35.1**). There may also be a small granule, possibly a reduced bar structure of parasitic origin, within the erythrocyte cytoplasm. Veils can be seen associated with a proportion of piroplasms in Giemsa-stained smears but may not be obvious. They are more easily demonstrated in wet preparations by phase-contrast microscopy.[11] The parasite is transmitted by *Rhipicephalus evertsi evertsi* and *R. e. mimeticus*.[8] The piroplasms are readily transmitted by blood inoculation.[9] Infectivity for goats is very low.

The *T. ovis* complex infects both sheep and goats, and its vectors have not been identified with certainty. *Rhipicephalus bursa* is known to transmit one non-pathogenic ovine *Theileria*, and *Amblyomma* spp. are suspected to be the vectors of another,[7] while *Haemaphysalis punctata* has been shown to transmit a third.[8]

Epidemiology

Malignant theileriosis of sheep and goats occurs in the Mediterranean basin, Sudan, western and central Asia and India, and has also been reported from China, although the identity of the parasite involved there has not been confirmed, as a species of *Haemaphysalis* is thought to be the vector. The disease is widespread in some important sheep-breeding areas and causes heavy losses in both indigenous and exotic breeds and their crosses. There is disagreement in the literature as to whether lambs are more or less resistant than adults, and to what extent indigenous breeds are resistant. The development of endemic stability with respect to theileriosis may be hampered by the practice of transhumance in sheep-raising areas, and reported differences in susceptibility of various classes of stock probably reflect differences in the epidemiological status in different situations. Goats are seldom affected clinically. The disease usually occurs over a period of two months at the end of spring, when ticks are active.[2]

Little is known of the epidemiology of the benign species of *Theileria*. They are widespread throughout Africa, Asia and Europe.

Clinical signs

Malignant theileriosis of sheep and goats may be acute, subacute or chronic. In the acute form fever, anaemia and

swelling of superficial lymph nodes are prominent features. Death follows within four or five days, and the mortality rate may reach 100 per cent. Recovery, if it occurs, is slow. Other signs described are listlessness, malaise, nasal discharge, hyperaemia of the conjunctivae, tachycardia, dyspnoea, oedema of the throat, icterus and transitory haemoglobinuria. Clinical signs in subacute and chronic cases are similar but less marked, and anaemia and emaciation may be pronounced.

The benign *Theileria* spp. cause only a mild febrile reaction, moderate swelling of the superficial lymph nodes and possibly mild anaemia.

Pathogenesis and pathology

The pathogenesis and pathology of malignant theileriosis are similar to those of the pathogenic *Theileria* spp. in cattle (**see Chapter 29: East Coast Fever and Chapter 37: *Theileria annulata* theileriosis**). Both schizonts and piroplasms play a role, the former by inducing lymphoid hyperplasia and degeneration, the latter by causing haemolysis, anaemia and icterus. Salient features at post-mortem examination in acute cases include subcutaneous and intramuscular oedema, especially in the ventral parts of the head, neck and abdomen; enlargement of the spleen and lymph nodes; enlargement, pallor and subcapsular haemorrhage of the liver; pulmonary oedema; red or white nodules of lymphoid hyperplasia in the kidney cortex (so-called infarcts) and perirenal oedema; haemorrhages on serosal surfaces and beneath the epicardium and endocardium; haemorrhagic lesions and necrosis of the mucosa of the abomasum and large intestine. In chronic cases, anaemia, emaciation and generalized oedema may be prominent.

Enlargement of lymph nodes and mild anaemia are the only lesions to be expected in benign theileriosis.

Diagnosis and differential diagnosis

The diagnosis of malignant theileriosis of sheep and goats is based on the characteristic clinical signs and lesions and may be confirmed by the demonstration of schizonts in lymph node and liver smears; piroplasms may be present in blood smears, but may be infrequent or even absent in the acute disease.[1] Specific diagnosis can be made retrospectively by means of the indirect immunofluorescent antibody test.[6]

Benign theileriosis may be suspected when piroplasms alone are found in sheep or goats in the absence of severe disease, but it is necessary to exclude the presence of *T. lestoquardi* in a latent or subclinical form by serological examination. The characteristic features of the piroplasms of *T. separata* permit differentiation from other *Theileria* spp. in sheep.

Control

Buparvaquone may be used in the treatment of malignant theileriosis.[1, 2] An attenuated schizont vaccine prepared in lymphoid cell culture has been developed and used successfully in Iran.[5] Tick control in small ruminants is generally impractical as a measure to control the disease.

There are no indications for the control of the benign species of *Theileria*.

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39

Theileria buffeli/orientalis infection

J A LAWRENCE

Introduction

The *Theileria buffeli/orientalis* group of parasites is widely distributed in cattle around the world. The taxonomy of the group is unclear; gene sequencing has revealed close similarities and also differences between isolates from Asiatic buffalo (*Bubalus bubalis*) and cattle in various locations,[4, 5] but the interpretation of these findings has still to be agreed. The name *T. buffeli* was given to a parasite recovered from Asiatic buffalo in Indochina by Neveu-Lemaire in 1912, and that of *T. orientalis* to a parasite recovered from cattle in Siberia by Yakimoff and Soudatschenkoff in 1931.[8] The name *Theileria sergenti* is frequently used to describe a pathogenic member of the *T. buffeli/orientalis* group that occurs in eastern Asia, but this name is invalid as it had been used previously to describe a parasite of sheep.[8] The parasites have been described from Asia, Australia, the Mediterranean basin, western Europe and North America, although they have often been identified mistakenly as *Theileria mutans*. Under the name *T. orientalis* the parasites were first identified in sub-Saharan Africa in Ethiopia in 1983[2] and they have since been described in central Africa[6] and eastern Africa, where they were called *Theileria* sp. (Marula).[9] *Theileria* sp. (Marula) has been shown to be indistinguishable by gene sequencing from stocks of the *T. buffeli/orientalis* group from Australia, Britain, Japan and Russia.[5] The parasites have also been identified by polymerase chain reaction testing in African buffalo (*Syncerus caffer*) in South Africa.[1] In most parts of the world the parasites are usually benign, or cause only mild clinical signs, but in eastern Asia they may pose an economically significant threat to cattle production.

Aetiology, life cycle and epidemiology

The parasites in the *T. buffeli/orientalis* group normally infect cattle, Asiatic domestic buffalo and African buffalo. Persistent subclinical infection of sheep after transmission by blood inoculation has been demonstrated.[15] The morphological and biological characteristics of the group are similar to those of *Theileria parva* (**see Chapter 29: East Coast fever: Figure 29.2**). The macroschizonts are unusually large.[15] The piroplasms are often longer than those of other species, and bars are usually found in the erythrocytes associated with the piroplasms; veils are also commonly found with certain isolates, but not with others.[6, 9, 15]

The main vectors of the group are *Haemaphysalis punctata* in Europe, the Mediterranean basin and western Asia, *H. longicornis*, *H. japonica* and *H. concinna* in eastern Asia,[14] and *H. humerosa* and *H. bancrofti* in Australia.[12] The vectors in North America and sub-Saharan Africa have not been identified and presumably belong to another genus of ticks. No *Haemaphysalis* ticks occur on cattle in North America, while in Africa the only *Haemaphysalis* that occurs on cattle as both immatures and adults is *H. aciculifer*,[13] which is not thought to be sufficiently common to act as a vector.[2] The parasite is readily transmissible between cattle by blood inoculation.[15]

The *Theileria buffeli/orientalis* group is assumed to be widespread throughout the range of its tick vectors and often occurs in mixed infections with other species of *Theileria*. It is rarely associated with clinical disease, except in cattle of exotic breeds in eastern Asia.

Clinical signs

Clinical illness usually occurs in animals subjected to stress, such as intercurrent infection with other parasites or viruses.[7] The main signs described are fever, inappetance, anaemia and reduced milk production; enlargement of superficial lymph nodes may be noted. The condition is occasionally fatal.

Pathogenesis and pathology

The pathogenesis and pathology of *T. buffeli/orientalis* infection have not been described in detail.

Diagnosis and differential diagnosis

Theileria buffeli/orientalis infection may be suspected when a febrile reaction and anaemia occurs in cattle exposed to *Haemaphysalis* infestation, and elongated piroplasms associated with intra-erythrocytic rods, and possibly with veils, are demonstrated in blood smears. Large schizonts may occasionally be demonstrated in lymph smears.

Differentiation of infection with *T. buffeli/orientalis* from infection with other species of *Theileria* can be made by use of the indirect fluorescent antibody test, using a range of *Theileria* antigens, as there is little cross-reaction.[9]

Control

As *T. buffeli/orientalis* is usually a benign parasite there are no indications for its treatment or control. Where it assumes pathogenic characteristics, as in eastern Asia, treatment with the antimalarials primaquine and pamaquine is effective when they are administered intramuscularly at 1,0 to 2,5 mg/kg of the phosphate.[3] Imidocarb has also been used with success at a dose of 1 to 3 mg/kg.[3] Buparvoquone and halofuginone are likely to be effective. Combined treatment with primaquine phosphate (2 mg/kg/day for three days, possibly repeated after one week) and halofuginone lactate at 1 mg/kg or buparvaquone at 2.5 mg/kg on the first and third days is frequently effective in eliminating infection from carrier cattle.[10, 11]

Where necessary, infection can be prevented by appropriate methods to limit exposure of susceptible cattle to infected ticks. It is common practice in eastern Asia to stimulate immunity to the parasites by deliberately exposing cattle to tick infestation and treating them prophylactically.[3]

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SECTION THREE

Rickettsial and Chlamydial Diseases



RICKETTSIAL AND CHLAMYDIAL DISEASES

Diseases caused by bacteria in the orders Rickettsiales and Chlamydiales are considered in this section. Many of these bacteria have recently undergone name changes which may make them unfamiliar to some readers.

Traditional bacterial classification was always difficult, relying heavily on properties where differences were small and hard to quantify. Characteristics such as stain affinity, cellular morphology, metabolic capabilities, and (in the case of intracellular species) host cell type, could often not effectively distinguish between species which had convergently evolved into a common niche, and it had long been realized that a genetically based system would be preferable. From the mid-1970s the usefulness of ribosomal RNA sequences to determine phylogenetic relationships was recognized, and techniques for comparing small-subunit ribosomal RNA (srRNA or 16S) gene sequences began to be applied to bacterial phylogeny.[3] For the first time this allowed the application of statistical phylogenetic techniques to infer the likely paths of bacterial evolutionary divergence. The introduction of the polymerase chain reaction (PCR) technique in 1988[7] allowed the specific amplification of bacterial srRNA genes amid a huge excess of host DNA and the numbers of bacterial 16S sequences available began to increase exponentially. In 1992 the Ribosomal Database Project (RDP) was initiated, and the first public release contained 471 16S rRNA sequences. The current release of the RDP (RDP II version 8.1) is held at Michigan State University: it contains 16 280 bacterial 16S sequences[1] and a phylogeny based on these data. The phylogenetic identities which follow are taken from the RDP II, the URL is *http://rdp.cme.msu.edu/*.

The reclassification of the order Chlamydiales is described in **Chapter 42**. The phylogeny is shown in **Figure 1**:

Phylum: Chlamydiae
Class: Chlamydiae
Order: Chlamydiales
Family: Chlamydiaceae
Genus: *Chlamydia*
Chlamydia trachomatis
Chlamydia suis
Chlamydia muridarum
Genus: *Chlamydophila*
Chlamydophila psittaci
Chlamydophila abortus
Chlamydophila felis
Chlamydophila caviae
Chlamydophila pecorum

Figure 1 Partial phylogeny of the phylum Chlamydiae

Coxiella burnetii, the causative agent of Q fever, is the subject of **Chapter 43**. It is noted there that the previous classification in the order Rickettsiales, family Rickettsiaceae, has been brought into question as a result of srRNA studies, and that reclassification in the order Legionellales has been proposed. This has been confirmed by further phylogenetic work based on the rpoB gene[4] and the currently accepted classification of *Coxiella burnetii* places it in the gamma subdivision of the Proteobacteria (**Figure 2**).

Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Legionellales
Family: Legionellaceae
Family: Coxiellaceae
Genus: *Coxiella*
Coxiella burnetii

Figure 2 Partial phylogeny of the gamma subdivision of the phylum Proteobacteria

Chapters 44 and 45 deal with *Eperythrozoon* and *Haemobartonella* spp. respectively, and there is now good evidence that these organisms are in fact *Mycoplasma* spp.[5, 6] So far only *Eperythrozoon suis*, *Eperythrozoon wenyoni*, *Haemobartonella muris* and *Haemobartonella felis* have been reclassified as *Mycoplasma* spp., their new names being *Mycoplasma haemosuis*, *Mycoplasma wenyoni*, *Mycoplasma haemomuris* and *Mycoplasma haemofelis* respectively. It is likely that the remaining *Eperythrozoon* and *Haemobartonella* spp. will be similarly reclassified in due course. The currently accepted phylogeny of these organisms is shown in **Figure 3**:

Phylum: Firmicutes
Class: Mollicutes
Order: Mycoplasmatales
Family: Mycoplasmataceae
Genus: *Mycoplasma*
Mycoplasma haemomuris
(was *Haemobartonella muris*)
Mycoplasma haemofelis
(was *Haemobartonella felis*)
Mycoplasma haemosuis
(was *Eperythrozoon suis*)
Mycoplasma wenyoni
(was *Eperythrozoon wenyoni*)

Figure 3 Partial phylogeny of the genus *Mycoplasma* showing species previously classified in the genera *Eperythrozoon* and *Haemobartonella*

Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rickettsiales
Family: Anaplasmataceae
Genus: *Anaplasma*
Anaplasma bovis (was *Ehrlichia bovis*)
Anaplasma equi (was *Ehrlichia equi*)
Anaplasma marginale
Anaplasma phagocytophilum (was *Ehrlichia phagocytophila*)
Anaplasma platys (was *Ehrlichia platys*)
Genus: *Ehrlichia*
Ehrlichia canis (unchanged)
Ehrlichia chaffeensis (unchanged)
Ehrlichia ewingi (unchanged)
Ehrlichia muris (unchanged)
Ehrlichia ovina (unchanged)
Ehrlichia ruminantium (was *Cowdria ruminantium*)
Genus: *Neorickettsia*
Neorickettsia helminthoeca (unchanged)
Neorickettsia risticii (was *Ehrlichia risticii*)
Neorickettsia sennetsu (was *Ehrlichia sennetsu*)
Genus: *Wolbachia*
Wolbachia pipientis (unchanged)
Family: Rickettsiaceae
Genus: *Rickettsia*
Rickettsia prowazekii (unchanged)
Rickettsia rickettsii (unchanged)
Rickettsia typhi (unchanged)
Genus: *Orientia*
Orientia tsutsugamushi (was *Rickettsia tsutsugamushi*)

Figure 4 Partial phylogeny of the alpha subdivision of the phylum Proteobacteria showing the order Rickettsiales

Chapters 40, 41 and **46** deal with species in the order Rickettsiales, which has been extensively reorganized as a result of srRNA phylogenetic analysis.[2] The reorganization is described in **Chapter 40**, where the agent of heartwater, previously known as *Cowdria ruminantium*, is now *Ehrlichia ruminantium*. All the *Ehrlichia* spp. described in **Chapters 41** and **46** have been renamed, with the exception of *Ehrlichia canis*. A phylogenetic tree giving the old familiar names is shown in **Figure 40.1** of **Chapter 40: Heartwater**, and the currently accepted phylogeny of all these organisms is shown here, in **Figure 4**. Of interest in particular is that the agent of Potomac horse fever, previously *Ehrlichia risticii*, is now renamed as *Neorickettsia risticii*. An important distinction between the families Anaplasmataceae and Rickettsiaceae should also be noted. The Anaplasmataceae comprises the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia* and *Wolbachia*, which are obligate intracellular bacteria that replicate while enclosed in a vacuole derived from the eukaryotic host cell membrane. The Rickettsiaceae comprises the genera *Rickettsia* and *Orientia*, which are obligate intracellular bacteria that grow freely in the cytoplasm of their eukaryotic host cells.

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40

Heartwater

Synonyms: Cowdriosis, la cowdriose (Fr.), hartwater (Afrik.)

B A ALLSOPP, J D BEZUIDENHOUT AND L PROZESKY

Introduction

Heartwater (cowdriosis) is a tick-borne disease of cattle, sheep, goats and some wild ruminants that is caused by a rickettsia, previously known as *Cowdria ruminantium* but recently reclassified as *Ehrlichia ruminantium*.[92] Typically, the disease is characterized by high fever, nervous signs, hydropericardium, hydrothorax and oedema of the lungs and brain, and death. It is one of the major causes of stock losses in sub-Saharan Africa.

The first reference to what may have been heartwater was made by the Voortrekker pioneer Louis Trichardt in 1838.[164] While trekking through what is today the Limpopo Province of South Africa many of his sheep succumbed to a disease known locally as 'nintas' three weeks after they had suffered massive tick infestation. According to evidence given by a farmer, John Webb, to the Cattle and Sheep Disease Commission of 1876 in Grahamstown, heartwater was observed in 1858 in South Africa in the northern part of the Eastern Cape Province. Because of its confusion with other local diseases of unknown aetiology that were prevalent at that time, some of the earlier information regarding the occurrence of heartwater is unreliable.[108]

The first important experimental findings came in 1898 when both Dixon[70] and Edington[93] showed that heartwater disease could be induced by blood passage from infected to susceptible animals. No organisms could be demonstrated in the blood or other tissues of diseased animals but it was concluded that heartwater was caused by a living micro-organism[112] at that time believed to be a virus.[215] In 1900 Lounsbury published his confirmation of the long-standing suspicion that the bont tick (*Amblyomma hebraeum*) was the vector of heartwater in South Africa,[135] but another quarter of a century elapsed before Cowdry demonstrated that the infectious agent in the tissues of infected animals and ticks was a rickettsia which he named *Rickettsia ruminantium*.[61, 62] The name was later changed to *Cowdria ruminantium*[155] and eventually to *Ehrlichia ruminantium*.[92]

Heartwater occurs wherever ticks capable of transmitting the organism are present (**see Chapter 1: Vectors: Ticks**). The endemic area encompasses most of sub-Saharan Africa, including the islands of Madagascar, Sao Tomé, Réunion, Mauritius, and Zanzibar.[95, 102, 103, 192] The disease is absent from the Kalahari Desert and dry coastal areas of Namibia and South Africa. Heartwater also occurs on the French Antillean islands of Guadeloupe and Antigua in the Caribbean Sea,[34] to which infected *Amblyomma variegatum* ticks were introduced, probably with cattle from Senegal, during the eighteenth century.[22, 145]

The occurrence of heartwater is frequently taken for granted in the endemic areas and definitive diagnoses are not often performed. This leads to the prevalence rates of the disease being under-reported.[55] In the endemic area in South Africa mortalities from heartwater are three times greater than those from babesiosis and anaplasmosis combined.[166, 234] From a livestock census performed by the Directorate of Animal Health, South Africa, in 1996, it was estimated that 17.5 million head of livestock were at risk in the endemic area of the country. Goats are especially threatened, and in some parts of the rural farming sector it is believed that up to 30 per cent of goats become infected with heartwater annually. It is not known how many of these die from the infection, but a substantial proportion must do so.

The economic impact of heartwater is difficult to quantify, both because of the under-reporting noted above and because the actual occurrence of the disease may be partially suppressed by a range of factors. These include the use of acaricides (in 1996 acaricides costing US$ 13 million were purchased in South Africa), antibiotic prophylaxis,[180] immunization by infection and treatment,[247] the resistance of certain animal breeds to the disease,[56] and endemic stability.[223] An estimate of the impact of heartwater was made by the Deputy Director of Veterinary Services of the Eastern Cape Province of South Africa during 1998.[17] His assessment was that 10 per cent of all stock losses, costing up to US$ 30 million annually, were due to heartwater, and this was despite an annual expenditure of between US$ 1 and 5 million for prophylaxis and vaccination. There are estimates from

Zimbabwe of annual losses over a 10-year period amounting to US$ 5.6 million per annum, which includes the cost of acaricides, milk losses and treatment.[157]

Heartwater is a major obstacle to the introduction of high-producing animals into sub-Saharan Africa to upgrade local stock[123, 232] and is of particular importance when susceptible animals are moved from heartwater-free to heartwater-infected areas.[166, 212] Whatever the actual costs may be it is certain that the economic importance of heartwater as a disease of domestic ruminants in Africa is comparable to that of East Coast fever, trypanosomosis, rinderpest, and dermatophilosis.[24, 192]

The possibility of heartwater spreading from the Antillean islands to the American mainland, where a suitable tick vector is present, is an ever-present threat to the livestock industry there.[68, 142, 233, 236] The disease is also a potential danger to countries where the vectors may be introduced and become established.[23, 260] It will be of major importance until an effective and safe vaccine becomes available.

Aetiology

Cowdria ruminantium was traditionally classified as the sole species of the genus *Cowdria*, tribe Ehrlichieae, family Rickettsiaceae, order Rickettsiales.[205] It had long been realized, however, that the organism had a close antigenic relationship with certain *Ehrlichia* spp., and in 1992 the first molecular phylogeny of the organism, based on the 16S ribosomal RNA gene sequence of the Zimbabwean Crystal Springs isolate, revealed that it was related to *Anaplasma marginale*.[65] This was closely followed by an analysis of the (slightly different) 16S sequence of the Senegal isolate, which showed a closer relationship to *Ehrlichia canis* and *Ehrlichia chaffeensis* than to *A. marginale*.[250] As the 16S genes of more organisms of the family Rickettsiaceae were sequenced it became evident that the genus *Ehrlichia* did not constitute a monophyletic group, and that organisms classified as *Anaplasma*, *Cowdria*, and *Neorickettsia* were members of three separate clades, each of which included various organisms classified as *Ehrlichia* spp.[191, 255] These clades became known as genogroups I, II and III *Ehrlichia* (**Figure 40.1**) with *C. ruminantium* being included in genogroup III, together with *E. canis*, *E. chaffeensis*, *E. muris* and *E. ewingi*.[5] As the 16S genes of more isolates of *Cowdria* became available it was found that there were several distinct *Cowdria* 16S genotypes, all of which fell into a tight cluster within the genogroup III *Ehrlichia*.[9]

The most recent revision of the order Rickettsiales[92] abolishes the genus *Cowdria* and the tribe Ehrlichieae. All three *Ehrlichia* genogroups are moved to the family Anaplasmataceae, genogroup I as *Neorickettsia*, genogroup II as *Anaplasma*, genogroup III as *Ehrlichia* (including *Cowdria*). *Ehrlichia ruminantium* is therefore now classified in the family Anaplasmataceae, order Rickettsiales. The question as to whether there should be more than one species of *Ehrlichia* (ex-*Cowdria*) is addressed below.

The type specimen of *E. ruminantium* comb. nov. is the Welgevonden isolate.[79] This was obtained from an *Amblyomma hebraeum* tick collected in the same geographical area as the original *R. ruminantium* which originated close to the Onderstepoort Veterinary Institute north of Pretoria, in Gauteng Province, South Africa.[62] The pathology, morphology[194] and virulence[79] in cattle, sheep and goats appears to be the same as that of the original *R. ruminantium*.[62] The 16S gene sequence[11] is the same as that of the Crystal Springs isolate,[65] which is deposited in GenBank under accession number X61659. The Welgevonden genotype is common in the field[9] and frozen stocks of the isolate, held at the Onderstepoort Veterinary Institute, date back to 1985.

Ehrlichia ruminantium was very early shown to be heat-labile and to lose its viability within 12 to 38 hours at room temperature.[2, 132] The first long-term cryopreservation of infective stabilates in liquid nitrogen was in media containing dimethyl sulphoxide (DMSO).[115, 203] More recently a sucrose-potassium phosphate-glutamate medium (SPG: 0,218 M sucrose, 3,8 mM KH_2PO_4, 7,1 mM K_2HPO_4, 4,9 mM $C_5H_8NO_4K$)[37] has been found to preserve the infectivity of the organism more effectively.[43] The infective half-life of stabilate material frozen in SPG, and then thawed and kept on ice, is only 20 to 30 minutes.[43] Large numbers of stabilates have been preserved from almost all the areas where the disease occurs and these are referred to as 'stocks' or 'isolates' according to the terminology used for trypanosome populations.[13] The names and origins of important stocks which are in current research use around the world are given in **Table 40.1**.

At one time it was thought that *E. ruminantium* was a relatively homogeneous entity, but within the last decade the advent of genetic sequence data has revolutionized this notion. It is now known that there is far more genetic variability among *E. ruminantium* organisms than had ever been suspected.

Currently eight different 16S ribosomal RNA genotypes are known (**Figure 40.2**), all classified at present as *E. ruminantium*. However, 16S sequence data cannot be used as the sole determinant of what constitutes a species; it is also important to be able to grow a stock in tissue culture in order to accurately determine its disease-causing status, and this has not been done for several of these eight genotypes. Some of them are undoubtedly justifiably classified as *E. ruminantium*, but others may need to be reclassified in the future in the light of their infectivity and pathogenicity in ruminant hosts.

It has long been stated that *E. ruminantium* stocks display differing degrees of pathogenicity in different hosts. Unfortunately much of the information in the early literature which relates to pathogenicity was obtained in the days before a tissue culture method had been established, before reliable methods for quantifying the infective dose were

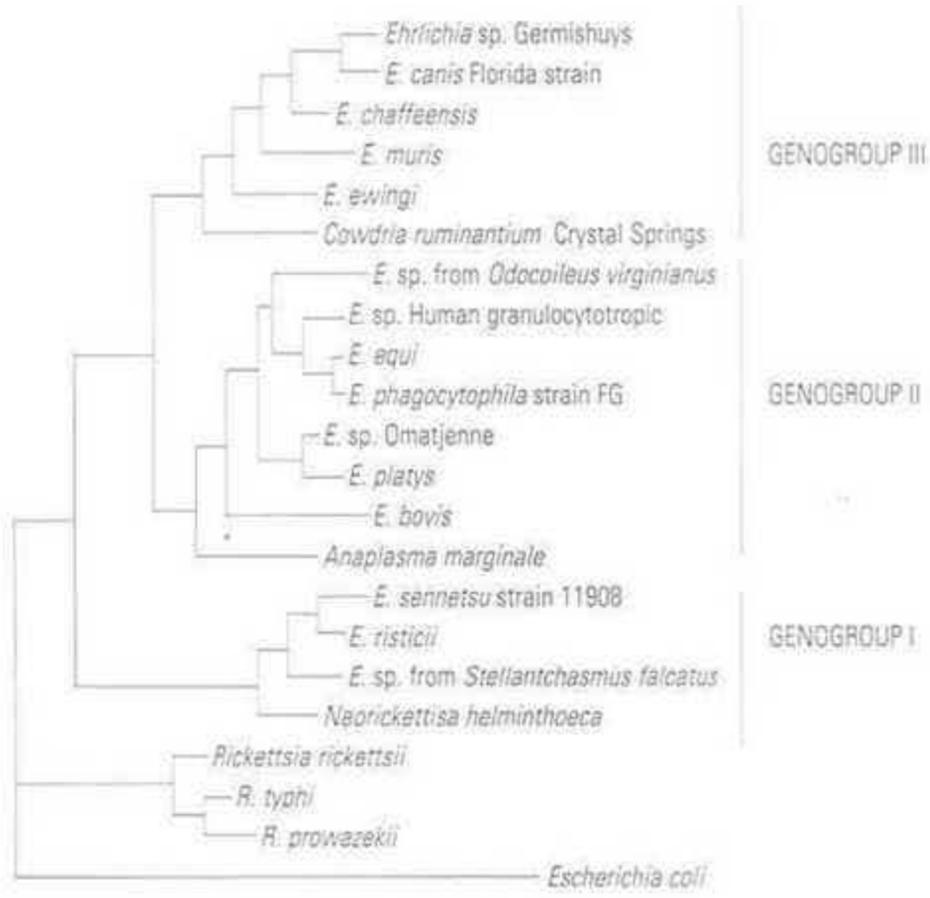


Figure 40.1 Maximum likelihood tree based on 16S ribosomal RNA gene sequences in the order Rickettsiales before reclassification, with *Escherichia coli* as outgroup. Note species classified as *Ehrlichia* occurring in three separate paraphyletic clades (genogroups). *Cowdria ruminantium* is located in genogroup III.

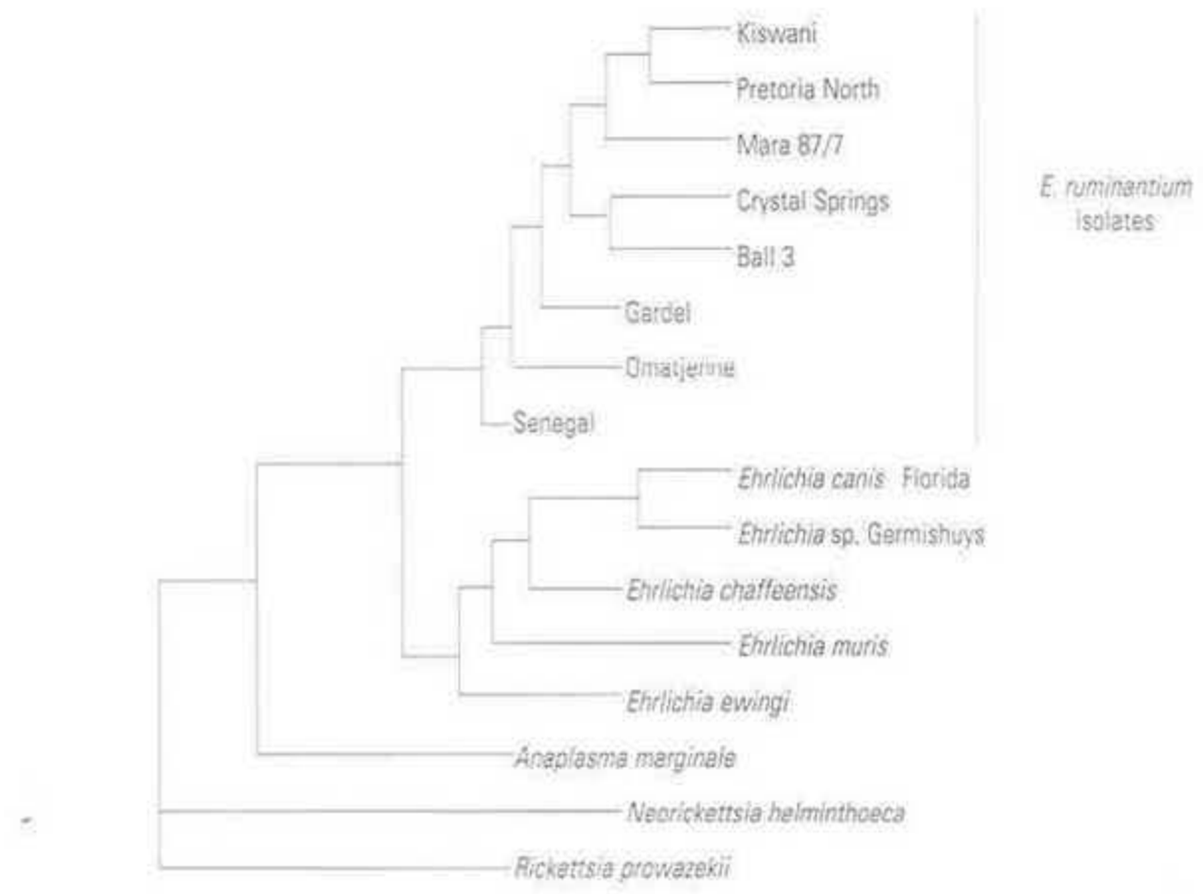


Figure 40.2 Maximum likelihood tree based on 16S ribosomal RNA gene sequences of some species in the family Anaplasmataceae, with *Rickettsia prowazekii* (family Rickettsiaceae) as outgroup. Note the eight isolates of *Ehrlichia ruminantium* which are more closely related to each other than they are to other *Ehrlichia* species.

Table 40.2 Cross-immunity protection engendered in sheep between various *Ehrlichia ruminantium* isolates

STOCK TO WHICH IMMUNE	CHALLENGE STOCK					
	WELGEVONDEN	BALL 3	GARDEL	MARA 87/7	KWANYANGA	BLAAUWKRANS
Welgevonden	+	+	+	+	+	+
Ball 3	–	+	–	+/–	+	+/–
Gardel	–	+/–	+	–	+/–	–
Mara 87/7	–	+	–	+	+/–	+/–
Kwanyanga	–	+/–	+/–	–	+	–
Blaauwkrans	–	+/–	–	+/–	–	+

\+ complete cross-protection
– no cross-protection
+/– partial cross-protection

equally infective by both the intravenous and the intraperitoneal routes, whereas other isolates were much more infective by the intravenous route than by the intraperitoneal route,[136] and it appeared to be non-pathogenic for cattle.[80] The stock also resisted all attempts to establish it in tissue culture over a period of 15 years. Only recently has it been both established in culture and characterized genetically, and the stock is now known to contain two different genotypes, Kümm 1 and Kümm 2, each having a distinct behaviour in culture.[269]

One stock, isolated before any genetic characterization data were available, seems to be the most likely candidate for reclassification. The Germishuys stock was isolated from a naturally infected sheep[92] and the records suggest that it caused disease which was thought to be heartwater. The stock was therefore assumed to be *Ehrlichia* (*Cowdria*) *ruminantium* until 16S gene sequence data showed it to be a previously uncharacterized *Ehrlichia* more closely related to *E. canis* than to *E. ruminantium*.[11] This organism has been provisionally named *Ehrlichia* sp. Germishuys **(Figure 40.2)** and no typical *E. ruminantium* 16S sequences have ever been recovered from it. However, because it has never been grown in tissue culture its ability to cause a disease that resembles heartwater has not yet been properly evaluated.

There are at least two other stocks, currently considered to be *E. ruminantium* but not yet grown in tissue culture, which may need to be classified as new *Ehrlichia* spp. if they are eventually found not to cause heartwater in ruminants. These are the Omatjenne and Pretoria North genotypes. Both are phylogenetically more closely related to *E. ruminantium* at the 16S level than to any other currently known *Ehrlichia* spp. (**Figure 40.2**) so the reclassification question is more difficult than in the case of *Ehrlichia* sp. Germishuys. The Omatjenne genotype was originally derived from a *Hyalomma* tick and it does not appear to cause disease in goats[11] or in sheep.[6] The Pretoria North genotype was originally detected in a dog by DNA hybridization[7] and it is not currently available either as an infective stock or in culture. Both of these unusual organisms must be grown in tissue culture and tested *in vivo* in ruminants before it can be said whether they cause classical heartwater or even whether they pose any disease threat in ruminants.

Ehrlichia ruminantium can be propagated in tissue cultures. In the first successful initiation of *E. ruminantium* cultures a tick-derived stabilate, prepared from *Amblyomma hebraeum* nymphae as the source of infectious organisms, was used.[35] In subsequent improvements to the technique freshly drawn heparinized infective blood and suspensions of liver and spleen prepared from infected mice were employed to initiate cultures.[29] A technique using plasma from heparinized blood was devised which showed that infective *E. ruminantium* is present in the cell-free plasma of infected animals.[49]

Several procedures have been described to facilitate initial infection of the endothelial cell monolayer, such as irradiation and cycloheximide treatment of the endothelial cells[35] or the use of a slow-rocking platform.[29] Although rocking of the culture flasks is still widely employed it has been demonstrated that these treatments are unnecessary for the initiation or propagation of *E. ruminantium*.[225, 264] It has also been shown that continuous propagation of *E. ruminantium* in DH82 (canine macrophage-monocyte) cells was only achievable when cycloheximide was added.[267] *Ehrlichia ruminantium* cultures are normally incubated at around 37 °C and, depending upon the pH of the buffer system used in the medium, either in air or in a carbon dioxide-enriched atmosphere.

The multiplication of *E. ruminantium* within the endothelial cells of infected animals was originally described by Cowdry in 1926,[63] so it is not surprising that the first successful *in vitro* propagation of *E. ruminantium* was achieved using bovine umbilical cord endothelial cells as host cells.[35] Almost all subsequent propagation studies of *E. ruminantium* used endothelial cells from various species and from various anatomical sites, such as bovine aorta and pulmonary artery,[48] ovine aorta,[49] sheep brain,[44] bovine brain,[149] bovine brain microvasculature[225] and caprine jugular vein.[229]

The propagation of *E. ruminantium* in non-ruminant endothelial cells[225] was first carried out using human umbilical and human microvascular endothelial cells.

Subsequently propagation was achieved in endothelial cell lines from a range of wild African species, including those of a bushpig (*Potamochoerus porcus*).[214]

Short-duration cultures, the cells of which are unsuitable for continuous *in vitro* propagation of the organism, have been achieved in caprine neutrophils,[134] in mouse and ruminant macrophages,[74] and in monocyte-macrophage cell lines from mice and dogs.[118] Apart from endothelial cell and DH82 cell[267] lines, continuous propagation of *E. ruminantium* has also been achieved in a tick cell line from *Ixodes scapularis* (IDE8).[25]

Various media, including commercially available synthetic culture media, have been used for the *in vitro* cultivation of *E. ruminantium*. Examples of the latter are the Glasgow modification of Eagle's minimum essential medium (GMEM),[29] Leibovitz L-15,[48] Dulbecco's minimal essential medium (DMEM)[149] and Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham (DME/F-12).[270]

Commonly used supplements for a complete culture medium are foetal bovine serum (FBS), new-born calf serum and bovine serum at various concentrations, usually 10 per cent (v/v), although 3 per cent FBS has been used in one instance.[264] Another supplement frequently used is tryptose phosphate broth. The media mentioned above also contain L-glutamine and antibiotics, and glucose (0,45 per cent) has been added to an L-15 based medium[48] which otherwise only contains D-galactose.

Work to develop a chemically defined culture medium for *E. ruminantium* passed its first milestone when a serum-free culture system was achieved, using a modified HL-1 medium.[270] In subsequent experiments it was found that the serum could be eliminated when DME/F-12 medium supplemented with bovine lipoproteins and bovine transferrin was used.[268] The final step was replacement of bovine lipoproteins by chemically defined lipids, and bovine transferrin by an inorganic source of iron; this resulted in the first formulation of a chemically defined, protein-free medium for the continuous culture of *E. ruminantium*.[266]

Ehrlichia ruminantium is a pleomorphic rickettsia, and colonies containing from one or two to several thousand individual organisms are found in the cytoplasm of endothelial cells (**Figures 40.3 and 40.4**).[63, 187] In general the colonies consist predominantly of small (0,4 µm), medium (0,76 µm), large (1,04 µm) or very large (>1,04 µm) organisms,[187] but a number of smaller organisms are also found in colonies of larger organisms, and *vice versa*. Most organisms are coccoid, except for colonies containing very large organisms in which pleomorphic forms (horseshoe-, ring- and bacillary-shaped) may be seen.[187]

Ehrlichia ruminantium is a Gram-negative bacterium, and stains purplish-blue with Giemsa.[63] Several other staining methods have been used or developed to demonstrate the organism in its vertebrate and invertebrate hosts.[46, 187, 194, 265] In paraffin-sections prepared from tick midgut and stained with Mallory's phloxine-methylene blue stain, *Ehrlichia* colonies and host cell nuclei stain dark blue against a uniformly pink background of tick tissue. This staining method is useful for identifying *Ehrlichia*-infected ticks and is superior to the variations of Mallory's stain which render the colours of colonies and tick tissues various shades of blue.[265]





Figures 40.3 (top) and 40.4 (bottom) Light microscopic morphology of *Ehrlichia ruminantium* in capillary endothelial cells in brain smears stained with Giemsa

The ultrastructural morphology of *E. ruminantium* (**Figures 40.5 to 40.8**) is similar in endothelial cells of the choroid plexus of sheep,[187] endothelial cells in the lungs of mice,[197] endothelial cell cultures,[194, 196] and leukocytes,[134] and in the cells of ticks.[28, 127, 128]

Species in the family Anaplasmataceae, including *E. ruminantium*, grow in an intravacuolar compartment, bounded by a lipid bilayer membrane, within the cytoplasm of infected host cells.[92] This distinguishes them from species in the family Rickettsiaceae which grow freely in the cytoplasm of their eukaryotic host cells. Individual *E. ruminantium* cells, in common with other Gram-negative bacterial cells,[26] are bounded by two membranes, an inner (plasma) membrane and an outer membrane. Internally the cell contains electron-dense and electron-pale

areas, with electron-dense material occupying the greater part of the inner structure of small and intermediate-sized cells, while electron-pale areas dominate the inner structure of large and very large organisms (**Figures 40.5 and 40.8**). The latter are referred to as reticulated bodies, and the smaller organisms are described as elementary (electron-dense) bodies (**Figure 40.7**). Organisms which do not readily fit into one or the other category are referred to as intermediate bodies.[122, 187, 194]

Ehrlichia ruminantium replicates mainly by binary fission, and possibly by endosporulation.[187] It appears that the reticulated bodies are predominantly proliferative[127, 187, 196, 197] while the elementary bodies represent the infective stage.[122]

There is some evidence that *E. ruminantium* undergoes



Figure 40.5 Colony of *Ehrlichia ruminantium* in an endothelial cell

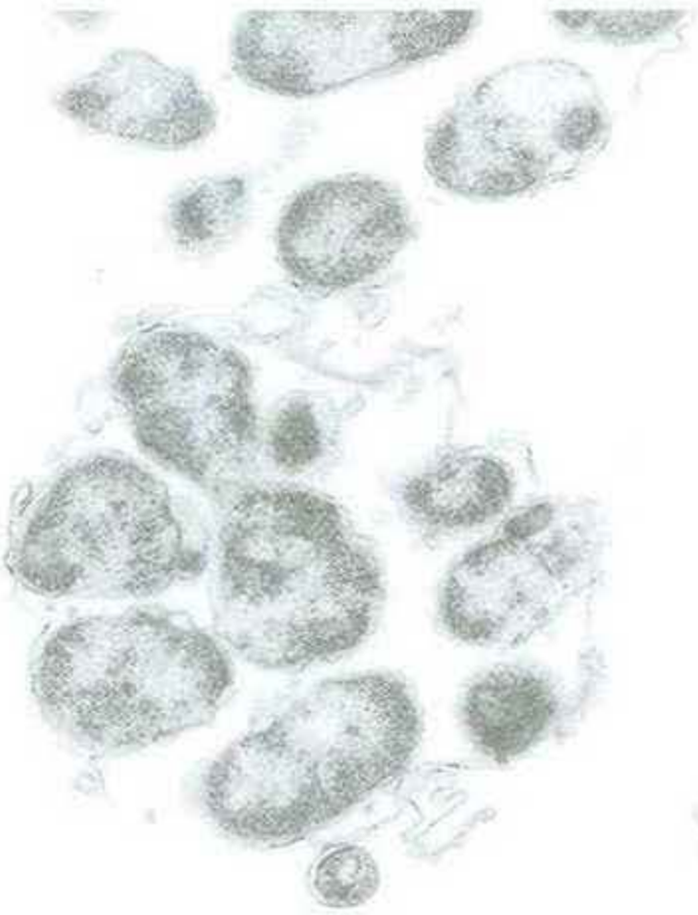


Figure 40.7 Elementary (electron-dense) bodies



Figure 40.6 'Mixed' colony of *Ehrlichia ruminantium*

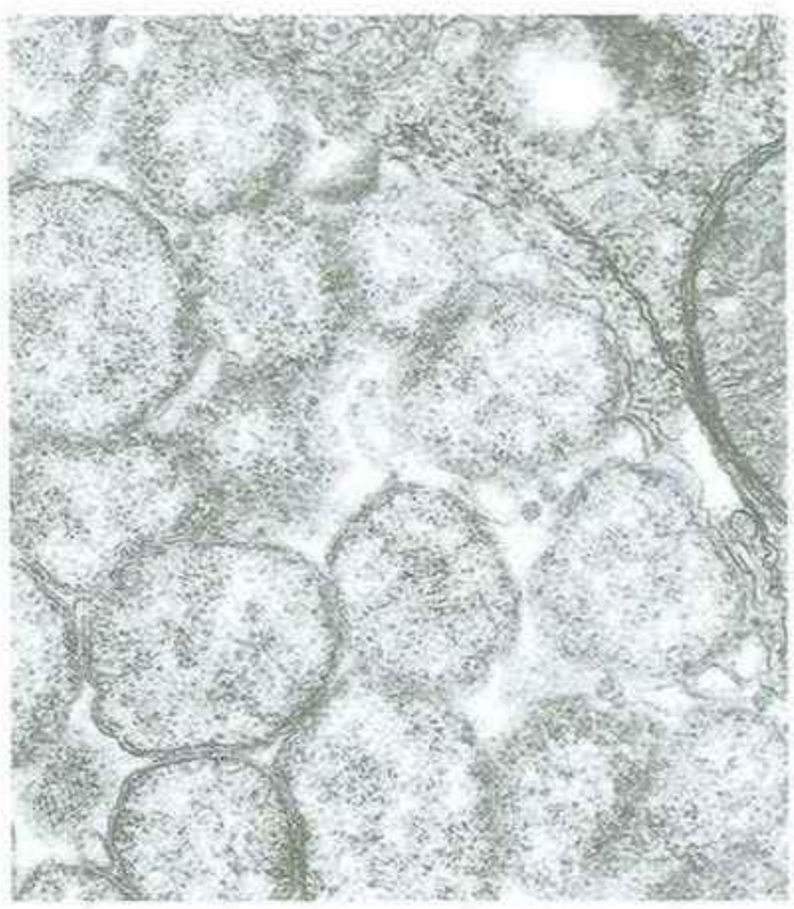


Figure 40.8 Reticulated bodies

a sequential development in both the vertebrate[78] and the invertebrate host.[127] Transmission electron microscopic studies of *in vitro* cultures of *E. ruminantium* in endothelial cells have revealed the presence of intracellular reticulate bodies two to four days post-infection, and intermediate bodies four to five days post-infection.[122] Large numbers of elementary bodies are seen after rupture of the endothelial cells five to six days after culture initiation.[122]

Heartwater is transmitted by ticks of the genus *Amblyomma* (**see Epidemiology, below, and Chapter 1: Vectors: Ticks**). Most *Amblyomma* spp. are three-host ticks. Larvae and nymphs become infected when they feed on domestic and wild ruminants and possibly also on certain game birds and reptiles[31] at a time when *E. ruminantium* is circulating in the blood of these hosts. The immature stages of the tick commonly feed on smaller species of domestic and wild ruminants and game birds, while the adults prefer cattle and the larger game animals, such as African buffalo (*Syncerus caffer*) and giraffe (*Giraffa camelopardus*), as hosts.[186] Nymphs or adult ticks transmit *E. ruminantium* to susceptible hosts without losing the infection. Intrastadial transmission has been demonstrated,[12] and transovarial transmission was once demonstrated in very heavily infected ticks under laboratory conditions[34] but it is unlikely that it occurs in the field.

The developmental cycle of *E. ruminantium* in the tick, and the infectivity of successive stages of the tick, are poorly understood. It is thought that after an infected blood meal, initial replication of organisms takes place in the epithelium of the intestine of the tick and that the salivary glands eventually become parasitized.[127] Colonies of *E. ruminantium* in tick haemolymph[79] may be intermediate forms in transit from intestinal to salivary gland cells. Transmission of the parasites to the vertebrate host probably takes place either by regurgitation or through the saliva of the tick while feeding. The minimum period required for *E. ruminantium* to be transmitted after ticks have attached to susceptible animals is between 27 and 38 hours in nymphae and between 51 and 75 hours in adults.[31]

Epidemiology

The epidemiology of heartwater depends upon factors relating to the tick vector, the causative organism, and the vertebrate hosts. Important considerations relating to the tick vector are infection rates in the ticks, seasonal changes influencing tick abundance and activity, and the intensity of tick control. Significant factors concerning the parasite are differences between genotypes which may affect virulence or the stimulation of immunological cross-protection in ruminants. As far as the vertebrate hosts are concerned, the availability of wild animal reservoirs and the age and genetic resistance of domestic ruminant populations are of importance.[234] Some of these issues are summarized here, while immunological responses to heartwater are dealt with further on in the chapter.

Heartwater occurs only where its vectors are present (**see Chapter 1: Vectors: Ticks and Figure 40.9**), and 10 *Amblyomma* spp. capable of transmitting the organism occur in Africa. The major vectors are *A. variegatum* and *A. hebraeum*, the latter being the main vector of heartwater in southern Africa. *Amblyomma variegatum* has the widest distribution in Africa and is the only originally African *Amblyomma* species that has established itself successfully outside the continent on two islands in the French Antilles.[145] The species *A. sparsum*, *A. tholloni* and *A. marmoreum* were at one time considered to be 'accidental' vectors which do not normally feed on domestic stock,[186] but more recent evidence suggests that this is not true for *A. marmoreum* at least, and that this species can be an effective vector of heartwater.[183] *Amblyomma sparsum* ticks collected in 1999 from leopard tortoises (*Geochelone pardalis*) imported into the USA from Zambia were also found to be carrying *E. ruminantium*,[47] although it was not demonstrated that the genotype detected was infective to ruminants.

Vectors of lesser importance include *A. pomposum* which is prevalent in Angola; *A. lepidum* which occurs in East Africa north of latitude 8 °S; *A. astrion*, which primarily parasitizes African buffalo in Central Africa; and *A. cohaerens* and *A. gemma*, which occur in East Africa.[256]

Among the *Amblyomma* spp. native to the USA, *A. americanum* and *A. cajennense* are only marginally susceptible to infection and apparently do not transmit the parasite,[142] while *A. maculatum*, on the other hand, has long been known to be capable of transmitting the disease[233] and has a vector efficiency in sheep which is similar to that exhibited by *A. variegatum*.[142]

The importance of *Amblyomma* spp. as vectors of heartwater depends not only on their vector efficiency but also on their distribution and adaptation to domestic stock[231] and on their activity and abundance, the latter being profoundly influenced by temperature and humidity.[186] An increased prevalence of heartwater usually occurs when peak numbers of ticks are present, and good rains are often followed by a transient increase in the occurrence of the disease. Its occurrence is not, however, strictly seasonal, a fact which is particularly true in regions like the Caribbean[50] and in parts of Africa which have a temperate climate and a poorly defined rainy season.

Amblyomma hebraeum ticks feeding on *E. ruminantium*-infected sheep have been shown to become infected during a period from two days after the commencement of the temperature reaction to two days after the animals have been treated for heartwater.[31] The transmission of *E. ruminantium* by *A. variegatum* feeding on Creole goats in Guadeloupe is apparently somewhat different, with the organism being transmitted to the ticks after a delay of two to three days for nymphs and four days for adults,[53] and the disease is apparently more often fatal when transmitted by ticks than by needle transmission. Ostensibly healthy ruminant hosts have been shown to remain infective to ticks for long periods, at least 361 days in cattle[12] and 11 months in Creole goats.[51] In the latter case the carrier status could not be detected permanently during the 11-month period, demonstrating the danger which could be

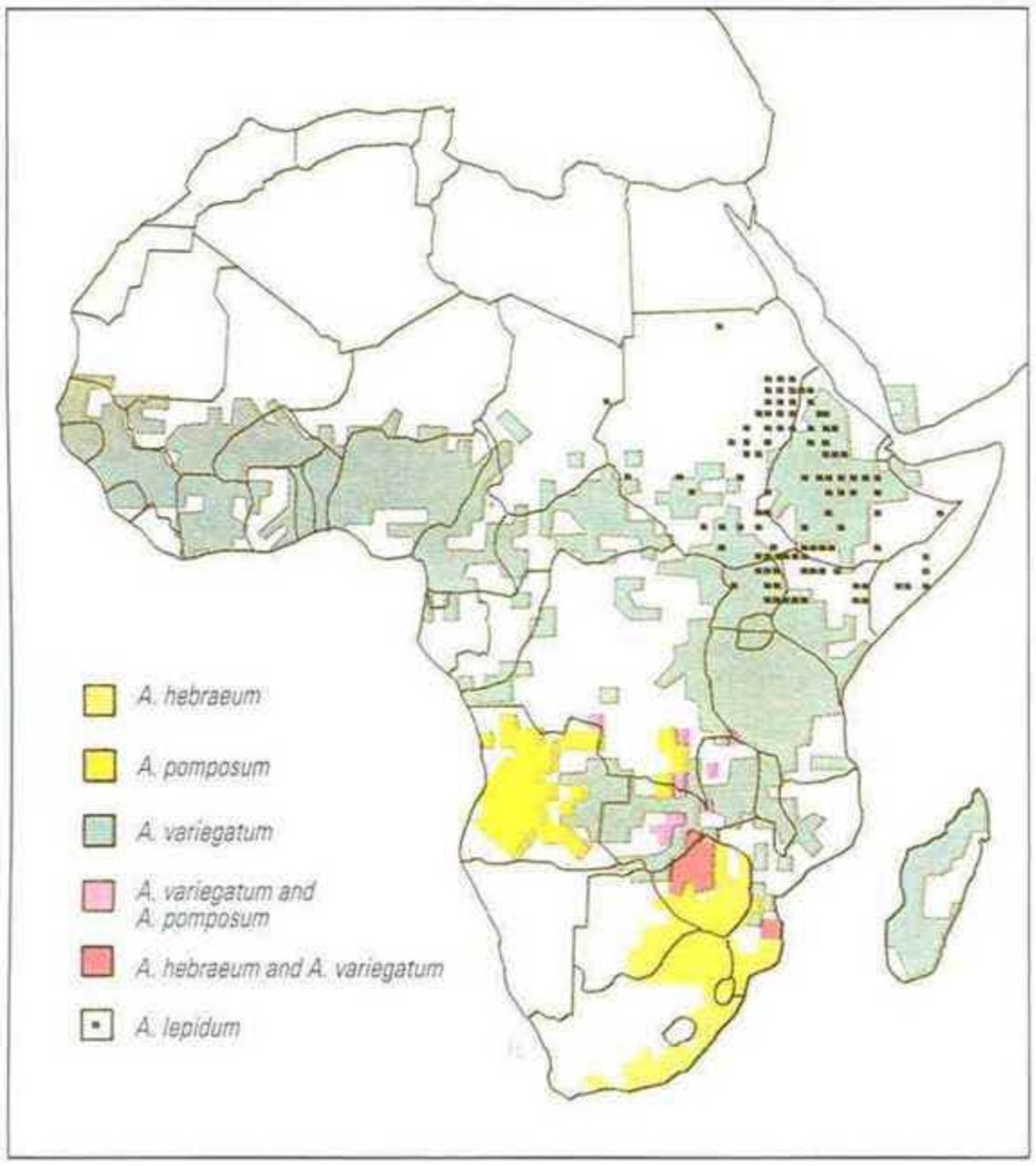


Figure 40.9 Distribution of the major *Amblyomma* spp. vectors of *Ehrlichia ruminantium* in Africa

posed by the movement of apparently negative carrier animals to areas free from the disease.

As little as 2,7 to 5,5 ml of blood collected from a host during the febrile stage can infect *Amblyomma* larvae.[50] Ticks retain their infectivity for life,[53, 113, 166] so a small number of infected ticks could presumably maintain the infection in a particular herd or area. The infection rates of ticks vary according to the season and locality in which they are collected and may be surprisingly low. Surveys in South Africa have found that 1 to 7 per cent of *A. hebraeum* in some parts of the endemic area were infected at any one time.[10, 79, 90] While somewhat higher rates have been determined in Zimbabwe[169, 185] and Senegal,[103] the infection rates are in general low. When infected sheep are used experimentally to feed *A. variegatum* during their larval or nymphal stages, they show infection rates of 100 per cent at the following instar.[103] This suggests that the likely reason for low infection rates is that many ticks feed during the larval or nymphal stages on non-susceptible or non-infected hosts.

Amblyomma variegatum ticks also differ in their infection rates with different genotypes of *E. ruminantium*, and have been shown to be less susceptible to two southern African isolates than to a western African and a Caribbean isolate.[140] On the other hand, a similar level of susceptibility of *A. hebraeum* to infection has been demonstrated in all the isolates tested, which may explain why heartwater is generally a more serious problem in those areas where *A. hebraeum* is the principal vector.

When a pathogenic genotype of *E. ruminantium* infects a susceptible vertebrate host, either inapparent or overt disease may develop depending on the pathogenicity of the organism and on the species, breed, age, degree of natural resistance, and immune status of the host. Young calves, lambs and goat kids possess a reverse age resistance which is independent of the immune status of the dam.[3, 89, 167, 230, 263] This resistance usually lasts for only the first four weeks of life in calves and the first week in lambs and kids,[167] although it may persist for six to eight months in calves.[89] This age resistance is not absolute as infection of some calves less than three weeks of age and of some lambs and kids less than one week old may result in fatal disease.[167, 230]

The susceptibility of different breeds of cattle varies, *Bos indicus* (Zebu) breeds being in general more resistant than are European (*Bos taurus*) breeds.[234] The resistance of local Zebu breeds, such as the Nguni and Sanga, is probably due

regurgitated gut content.[30, 127, 129] Initial replication of the organisms seems to take place in reticulo-endothelial cells and macrophages in the regional lymph nodes, after which they are disseminated via the bloodstream and invade endothelial cells of blood vessels in various organs and tissues, where further multiplication occurs.[73] In domestic ruminants *E. ruminantium* most readily infects endothelial cells of the brain, and this coincides with the onset of the febrile reaction.[73, 75] Genetic variation within both parasite and host populations plays a role in the degree of endothelial cell colonization in the brain and other organs.[45]

Increased vascular permeability with transudation is responsible for effusion into body cavities and tissue oedema, and this is particularly noticeable in the lungs, pericardial sac and pleural cavity. Oedema of the brain is responsible for the nervous signs, hydropericardium contributes to cardiac dysfunction during the terminal stages of the disease, and progressive pulmonary oedema and hydrothorax result in eventual asphyxiation.[178] The effusion of fluid into tissues and body cavities also results in a drastic reduction in blood volume.[58]

The pathogenesis of the vascular permeability remains speculative, and in mice there is no apparent correlation between the number of parasitized endothelial cells in the pulmonary blood vessels and the severity of the pulmonary lesions.[197] It has been suggested that endotoxin[241] and increased cerebrospinal fluid pressure[45] play a role in the development of lung oedema.

It is known that the parasite causes down regulation of interferon (IFN)-γ production and inhibition of major histocompatibility complex (MHC) expression.[238] This would be expected to slow the development of immunocompetent cells and, although no *E. ruminantium* specific cytotoxic $CD8^+$ T cells have been demonstrated, it is known that $CD8^+$ T cells proliferate in animals vaccinated with inactivated *E. ruminantium* and then challenged with virulent organisms.[146] It is possible, therefore, that if T-cell proliferation is slowed until many endothelial cells are infected the subsequent specific killing of these cells could lead to blood vessel permeability. Against this hypothesis, however, is the observation that both infected and uninfected endothelial cells appear to be severely damaged in ruminants which die from the disease.[187]

The earliest work on the immune response to *E. ruminantium* were attempts to transfer immunity from heartwater immune cattle to naive animals by transferring serum.[2, 72, 73] These attempts failed, indicating that antibodies do not control the course of the disease, although antibodies may still play a role in opsonization, complement-mediated killing or antibody-dependent cell-mediated cytotoxicity. As *E. ruminantium* is an intracellular parasite it would be expected that both $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells would be important in the development of protective immunity.

The first confirmation that $CD8^+$ T cells play a major role in protection came from adoptive transfer studies in a mouse model.[78, 81, 84] It has now been shown that cattle immune to heartwater generate lymphocyte responses characterized by a mixture of $CD4^+$, $CD8^+$ and γδ T cells, and the expression of T helper type 1 cytokines.[146, 158, 228] When peripheral blood mononuclear cells (PBMC) from heartwater-immune cattle were exposed *in vitro*, to either autologous-infected endothelial cells or infected monocytes, PBMC proliferation was observed.[158] Under the same conditions, however, *E. ruminantium* elementary bodies did not induce PBMC proliferation.[158] The endothelial cells required pre-treatment with T-cell growth factors to induce class II MHC expression prior to infection and their subsequent use as stimulators of PBMC.[158] Cells from immune cattle have also been shown to respond specifically to recombinant antigens of *E. ruminantium*, $CD4^+$ T cells responding to major outer membrane protein (MAP)1, and γδ T cells responding to MAP2. Both proteins induced a T-helper type 1 immune response.[159]

Peripheral blood mononuclear cells from animals made resistant to challenge by vaccination with inactivated organisms (see below) contain *E. ruminantium*-specific, MHC class II-restricted, IFN-γ-producing, $CD4^+$ T lymphocytes.[229] $CD4^+$ T-cell lines generated by the use of *E. ruminantium* lysates responded strongly to whole lysates but not to recombinant MAP1 or MAP2 proteins.[227] When these cell lines were stimulated with soluble *E. ruminantium* proteins purified by fast performance liquid chromatography (FPLC), a single peak, which included proteins between 20 and 32 kDa, induced proliferation.[224] In the same study only γδ T-cell lines responded specifically to recombinant MAP1 and MAP2. While flow cytometric analysis of PBMC has not shown any significant change in the immune cell population after vaccination with inactivated organisms, significant changes do occur when the animals are challenged. Initially there is a depletion of $CD4^+$, $CD8^+$ and γδ T-cell subsets, and a rise in numbers and activation of monocytes, and only later when the animal is fighting the infection, is there an increase in $CD8^+$ T lymphocytes.[146]

Immune cattle produce IFN-α when subjected to a lethal *E. ruminantium* challenge, while non-immune animals, similarly infected, that died did not produce this cytokine.[226] Even so IFN-α alone could not completely inhibit the *in vitro* growth of *E. ruminantium*, suggesting that other factors are required for complete inhibition. The type 2 cytokine IFN-γ has been shown to be a very potent inhibitor of *E. ruminantium* growth in endothelial cells, but the mechanism of this inhibition is unknown.[224] Another important role of IFN-γ is the upregulation of MHC class I and II expression on various cell types.[60] This effect on monocytes will lead to enhanced presentation of *E. ruminantium* antigens to the immune system. *Ehrlichia ruminantium* has also been shown to elicit *de novo* synthesis of interleukin (IL-1β, IL-6 and IL-8 mRNA in bovine brain endothelial cells *in vitro*, an effect which was potentiated by INF-γ.[36] Since IL-1 and IL-6 can act as co-stimulatory signals for T- and B-cell activation[257] it is possible that by secreting these cytokines, brain endothelial cells may contribute to protective

immune responses. Antigens of *E. ruminantium* capable of inducing the production of these cytokines by immunocompetent cells should be investigated as potential vaccine antigens.

It has been noted that $CD4^+$ T-cell lines from animals immunized with inactivated *E. ruminantium* proliferate in the presence of a fraction of *E. ruminantium* proteins between 20 and 32 kDa.[224] In addition *E. ruminantium* proteins of 11, 12, 14 to 17 and 19 to 23 kDa specifically induced proliferation of PBMC from cattle immunized either by infection and treatment, or with inactivated organisms.[248] These proteins should have potential as vaccine candidates, but none of them has yet been purified to homogeneity for amino acid sequencing, so the genes coding for them have not yet been identified. In fact, relatively few antigens have been characterized to the extent of having their genes sequenced.

The antigen of *E. ruminantium* which is currently the best characterized is the immunodominant MAP1, a protein of about 32 kDa[251] which varies in size from isolate to isolate.[19] The protein appears as a prominent band on Western blots of total *E. ruminantium* proteins[206] and is located on the surface of the organism.[120] The antigen is recognized by immune antisera and by $CD4^+$ T lymphocytes from immune animals,[159] has been used to develop serological assays (see Diagnosis below), and has also been tested as a potential vaccine (see below).

The *map*1 gene appears to be present as a single copy in the genome.[41] It is polymorphic between different *E. ruminantium* isolates and was at one time advocated as a marker for isolates from different geographical areas.[204] As more data have become available, however, it is now known that the variants are not geographically constrained.[8] *Ehrlichia canis* and *E. chaffeensis*, two close relatives of *E. ruminantium*, have orthologous families of immunodominant major surface antigens in the 28 to 30 kDa size range,[176, 262] but it is not known whether these genes are polymorphic between different isolates.

A second immunodominant major outer membrane protein gene (*map*2) has been identified[139] and the protein evaluated for use in serological diagnostic assays.[38] The groES and groEL genes have been sequenced,[130] as well as numbers of clones from genomic libraries which contain open reading frames (ORFs), some having homologues in the public databases, and others currently being unique.[20, 41, 42] The 1576 kb genome of *E. ruminantium* has been mapped[66] and whole genome sequencing is in progress.[4] Once complete this will be an invaluable resource for further gene discovery.

An economic model for the prevalence of heartwater and its impact under various farming systems has been developed as a result of a 10-year study in Zimbabwe.[157] The estimated annual losses in that country amounted to US$ 5,6 million, with economic losses per animal in the commercial production sector being 25 times greater than the losses in the communal and traditional farming sectors, largely as a result of the cost of acaricides. The conclusion was reached that a control strategy based on a vaccine would be the most cost effective way of combatting the disease.[157]

The only commercially available procedure for heartwater immunization is an infection and treatment technique, the use of which is described more fully below (**see Control**). It was developed more than 50 years ago at the Onderstepoort Veterinary Institute, South Africa,[174] but it has a number of drawbacks. The procedure is particularly inappropriate for use in rural areas in Africa or the Caribbean because of the need for continual frozen storage facilities,[247] and both production of the infective material used as antigen and the methods required for its application are expensive. Despite being so outdated nothing better is currently available, so there has been a great deal of research over the last decade aimed at developing an improved vaccine. These research developments are dealt with here.

The Senegal isolate of *E. ruminantium* has been attenuated in culture, and animals immunized with the attenuated organisms are immune to homologous challenge.[117] Unfortunately cross-protection between different isolates is far from complete[101] and it would be necessary to attenuate a number of genotypes to cover the entire antigenic repertoire in any particular area. The fact, however, that some stocks have so far proved to be resistant to attenuation by *in vitro* passage[117] limits the possible usefulness of this method of vaccination.

Inactivated organisms have been successfully used for experimental vaccination against homologous challenge.[138, 148] This type of vaccine consists of inactivated non-viable protein from tissue culture material which can be stored at ambient temperature. Two doses are needed, and large amounts of tissue culture material have to be prepared, which makes production expensive. If production costs can be reduced by industrial-scale culture techniques such a vaccine may be practicable. At present the most important problem to overcome is that, in a field situation where genotypes having differing immunogenicities are in circulation, the protection levels have been disappointing.[143] In one trial the administration of an inactivated vaccine reduced mortality levels in naive sheep and goats from 78 to 54 per cent and in naive cattle from 67 to 19 per cent. Results such as these suggest that the present formulations for inactivated vaccines are not viable as a commercial proposition.

Potentially, it should be feasible to include in an inactivated vaccine as many immunotypes as would be required for effectiveness in a particular area. It would be necessary to isolate organisms from any natural *E. ruminantium* infection appearing in animals immunized with an existing vaccine 'cocktail'. The new isolates would then need to be cultured and added to the cocktail, prior to re-testing in the field situation. This would be costly, but it could be a useful 'stopgap' measure until something better is developed.

The fact that immunization with inactivated *E. ruminantium* organisms is possible implies that the development of a subunit vaccine is feasible. In principle all that is required

is to identify *E. ruminantium* genes which code for antigens which stimulate a protective T-cell response. Unfortunately there are no known directed strategies for doing this.

A naked DNA vaccine expressing the MAP1 antigen of *E. ruminantium* has been shown to protect mice against a lethal homologous challenge.[172] Splenocytes from the immunized mice, but not from control mice, were shown to proliferate *in vitro* when treated with recombinant MAP1 protein or a protein extract of whole *E. ruminantium*. The proliferating cells also secreted enhanced amounts of IFN-γ and IL-2 as compared to cells from control mice. There was one serious concern, however, in that the level of protection was not reproducible, varying from 23 to 88 per cent in different experiments.

In an attempt to overcome the lack of a directed strategy for identifying *E. ruminantium* genes that code for protection-stimulating proteins, a strategy was developed to screen the whole parasite genome, using a *Salmonella* vaccine delivery system to identify protective genes.[42] A cloned segment of *E. ruminantium* DNA, which contained five ORFs, protected 14 per cent of outbred mice against a lethal homologous challenge. In subsequent experiments, using the same delivery and challenge systems, each of the individual ORFs was tested in Balb/C mice, where there were no survivors, and C57BL/6J mice, where the results were not reproducible.[40]

In a comprehensive series of experiments two *E. ruminantium* genomic expression libraries have been isolated from a set of 34 unique clones which reacted with sera from heartwater-immune sheep.[20] These clones were sequenced, giving a total of about 73 kbp of sequence, and were found to include 33 complete and 23 partial ORFs. Selected ORFs were cloned into an expression vector to generate recombinant proteins, which were evaluated for their ability to react with heartwater-immune antisera. Bacterial lysates containing these recombinant proteins were tested for their ability to stimulate proliferation of PBMC from cattle immunized by the infection and treatment method. Many clones were recognized, although the responses between different immune animals differed even for the same recombinant. Recombinants which had both reacted with heartwater-immune antisera and had stimulated proliferation of PBMC from immunized cattle were selected to immunize mice, which were then challenged. Disappointingly, significant protection (58 to 89 per cent survival) was only obtained for two pools of recombinants, but not for any individual recombinant, even those from the protective pools.

Recently, a successful prime-boost strategy has been devised for inducing protective levels of $CD8^+$ T cells against the liver stage of the malaria parasite.[208] A similar strategy has been used in mice with the *map*1 gene and the MAP1 protein of *E. ruminantium*.[171] Priming was carried out with the gene cloned in a DNA vaccine vector and then some of the animals were boosted with the protein, and others were not, prior to virulent challenge. Protection levels were increased from 13 to 27 per cent without the protein boost, and 53 to 67 per cent with the protein boost, but it is disappointing to note that the 88 per cent protection level obtained using only the gene in a DNA vaccine vector in earlier experiments[172] was not attained, even with boosting. This casts doubt on the usefulness of the *map*1 gene as a vaccine candidate and underlines the non-repeatability which characterizes these results.

A disturbing observation common to all the vaccination experiments carried out in mice has been the unpredictable variability of the results. In addition, some experimental immunization results obtained in ruminants have not been repeatable when the mouse model has been used. One example of the latter, as discussed above, was when vaccination with inactivated organisms produced good protection against homologous challenge in cattle, sheep and goats, but attempts to reproduce the findings in mice were unsuccessful.[253] Similarly, encouraging protection levels up to 88 per cent were obtained with the *map*1 gene in a DNA vaccine vector in mice[172] whereas immunization with denatured MAP1 protein appears not to protect goats and sheep at all, despite very high antibody titres being present before challenge.[249] These observations suggest that the murine model may not be suitable for evaluating putative protective proteins or recombinant vaccines which are to be used against heartwater in ruminants.

Clinical signs

Infected domestic ruminants may manifest a wide range of clinical signs. The incubation period, course, severity and outcome of artificially induced disease are influenced by the species, breed and age of animal affected, the route of infection, the virulence of the stock of *E. ruminantium* involved, and the amount and source of infective material administered.[2, 166, 234] Peracute, acute, subacute, and clinically inapparent forms of the disease occur.

In the naturally acquired disease in a herd or flock, the morbidity and mortality rates are influenced by the species, breed and age of the animals affected, the virulence of the *E. ruminantium* stock, the effectiveness of immunization, tick control and specific chemotherapy programmes applied on the farm, and, in some instances, the season.[27, 55, 106, 231] Death usually follows in animals which show clinical signs if they are not specifically treated for heartwater.

Cattle

The incubation period in naturally infected cattle ranges from 9 to 29 days, with an average of 18 days.[2] Cows of *B. taurus* breeds, such as the Jersey, South Devon, Charolais, Limousin and Friesland, especially when in the advanced stages of pregnancy, are particularly prone to develop peracute heartwater, a form of the disease which may sometimes also occur in 6- to 18-month-old animals of different breeds.[108, 246] Peracutely affected animals die within a few hours after the initial development of fever, either without any clinical signs having been manifested, or having shown terminal, paroxysmal convulsions and marked respiratory distress.[2, 166]

Acute heartwater, the most common form of the disease, mainly affects cattle between the ages of 3 and 18 months. It is characterized by a fever of 40 °C or higher, which usually persists for three to six days, showing only small fluctuations before the body temperature falls to subnormal shortly before death.[2, 231] While some observers consider that a mild mucoid diarrhoea is a regular occurrence,[2, 64, 231] others regard it as an infrequent sign.[245] Certain breeds such as the Friesland, Jersey and Simmentaler, and four- to eight-month-old animals of all breeds, apparently develop diarrhoea most commonly. A profuse, often haemorrhagic, diarrhoea may be the most prominent clinical sign in some cases of heartwater, particularly in Jerseys.[2, 245]

During the later stages of acute heartwater, nervous signs occur which range from mild incoordination to pronounced convulsions.[2] The animals are hypersensitive when handled or exposed to sudden noise or bright light. Slight tapping with a finger on the forehead of the animal often evokes an exaggerated blinking reflex. They frequently show a peculiar high stepping gait that is usually more pronounced in the front limbs. Calves may wander around aimlessly and walk into fences, and some, previously unaccustomed to handling by humans, may be approached with ease. Animals may stand with their heads held low, make constant chewing movements, and push against objects. In the later stages they often fall down suddenly, assume a position of lateral recumbency, and show opisthotonus and either have frequent bouts of leg-pedalling movements or the legs may be extended and stiff. In most cases the animals weaken rapidly and death usually follows soon after the commencement of a convulsive attack.

The subacute form of heartwater is characterized by a fever which may remain high for 10 days or longer. The clinical signs are similar to those described for the acute form of heartwater, but less pronounced. During the course of the illness animals may die suddenly, or gradually recover within a few days, and death frequently results from complications such as hypostatic pneumonia.[2]

The prevalence of the clinically inapparent form of heartwater is difficult to determine under field conditions. Apart from fever, apathy and slight tachypnoea, the animals appear to be normal and most recover within a few days.[55] Calves less than three weeks of age,[231] cattle infected with a stock of low virulence,[166] and infected but partially immune animals[2] are particularly prone to develop this mild form of the disease.

Sheep and goats

The incubation period in sheep and goats inoculated intravenously with 10 ml of infected blood varies from 5 to 35 days (average 9 to 10 days), and that of naturally infected animals from 7 to 35 days (average 14 days).[2, 234]

Exotic goat breeds, such as the Angora, and two- to six-month-old Boer goats are commonly affected by the peracute form of the disease. Most animals collapse suddenly and die after a few paroxysmal convulsions without having been observed to be ill.[234] Some, however, are anorectic, appear dull, exhibit nystagmus, bleat, and twitch their tails continuously. They may show forced respiration, frequent urination and defaecation, chewing movements and, when recumbent, leg-pedalling.[124, 216]

As is the case in cattle, acute heartwater is the most common form of the disease in sheep and goats. The majority of animals manifest nervous signs, but these are generally less pronounced than in cattle.[2] Initially, affected animals show fever, a progressive unsteady gait and listlessness, and often stand with their legs wide apart with the head lowered and ears drooping. They eventually become prostrate, assume a position of lateral recumbency and show intermittent leg-pedalling, chewing movements, opisthotonus, licking of the lips, and nystagmus.[2]

Black-headed Persian sheep possess some natural resistance to heartwater, and lambs and kids of all breeds under one week of age have a degree of innate resistance. Such animals, as well as those which are partially immune, may develop the mild form of heartwater and, apart from fever, no clinical signs are generally manifested.[2, 234]

Wild ungulates

Clinical signs of heartwater in susceptible wild ungulates (**Table 40.2**) have not been well studied but are generally similar to those reported in domestic ruminants affected with the acute form of the disease.[97, 99, 261]

Laboratory mice

The incubation period in mice infected intravenously with the Welgevonden stock of *E. ruminantium* ranges from 10 to 14 days.[187] No febrile reaction has been noted in mice experimentally infected with any of the stocks to which they are susceptible.[136] Twenty-four hours prior to death affected animals are lethargic, and develop tachypnoea and a ruffled coat. Incoordination is occasionally seen.[137]

Pathology

A slight reduction in haemoglobin and haematocrit values is observed in sheep, goats and calves. The reductions coincide with, or start shortly after, the commencement of fever reaction and continue throughout the course of the disease, but neither value reaches critically low levels.[1, 58, 178, 242] The anaemia, which is not clinically discernible, is usually normocytic and normochromic,[242] although a microcytic, hypochromic anaemia has also been reported in goats.[1] The pathogenesis of the anaemia is not known.

During the course of the disease the colour of the blood plasma of calves, sheep and goats may change progressively to a dark orange colour[58, 100] as a result of increased levels of bilirubin and/or its conjugates.[154] In calves the bilirubinaemia appears to be associated with anorexia rather than

with haemolysis or liver damage during the initial stages of the disease.[242]

Mild leukopenia, mainly resulting from a decrease in the number of neutrophils, develops in calves and goats prior to the onset of fever and persists throughout the course of the acute form of the disease.[114] An eosinopenia develops before or during the onset of the febrile reaction, and is accompanied by a lymphocytosis which commences shortly after the onset of the febrile reaction.[1, 58, 178, 242]

The total serum protein content is reduced in calves. The commencement of this reduction coincides with the start of the fever reaction and persists throughout the acute stage of the disease.[242] High concentrations of albumin and globulin occur in the oedematous fluid which accumulates in body cavities.[242]

Blood urea nitrogen[58, 98] and creatinine elevations have been detected in the blood shortly after the onset of the febrile reaction in sheep, whereas in calves these are not higher than the upper normal levels, but they do suggest some interference in glomerular function during the acute stage of the disease.[242]

Blood glucose levels rise terminally in the disease and may cause glucosuria in sheep and goats.[58, 98, 114]

Terminal increases in blood pyruvate and lactate levels, and a simultaneous drop in bicarbonate, have been reported in sheep and goats.[58, 114] During the acute stage of the disease in calves respiratory alkalosis and hypoxia occur, as evidenced by a lowered oxygen tension (pO_2).[242] Sheep infected with the Ball 3 stock show retention of carbon dioxide (increased pCO_2) resulting in respiratory acidosis on the twelfth day post-infection. In addition, there is a reduced oxygen diffusion which gives rise to hypoxia (lowered pO_2) that persists until death.[178] In other experiments in sheep changes in blood gas included a decline in arterial pO_2 combined with a respiratory alkalosis. Although the sheep became hypoxaemic, blood-gas changes associated with respiratory failure were not observed.[243]

Increases in both the respiratory rate and tidal volume result in increased minute volume and a simultaneous increase in ventilatory equivalent in sheep.[178] The tendency towards increases in both physiological and alveolar dead space may be associated with lung oedema, and could account for the moderate reduction in pO_2 which occurs during the acute stage of the disease.[242]

In sheep, changes in blood-clotting behaviour, blood calcium and protein levels, haematocrit, and white-cell counts occur. A marked decline in thrombocyte count was recorded during the acute stage of the disease, associated with increases in both prothrombin time and activated partial thromboplastin time. Fibrinogen increased, while there was no detectable increase in fibrinogen degradation products and total serum protein, albumin and globulin dropped very sharply. Total calcium showed a progressive drop but ionized calcium rose initially and was then followed by a terminal decline. The total leukocyte count showed a terminal increase while the haematocrit dropped progressively.[244]

Lesions in cattle, sheep and goats are very similar and differ only in their severity and frequency.[2, 195, 218, 231] Gross lesions may be inconspicuous or even virtually absent in fatal cases.[195]

Severe hydropericardium (**Figure 40.10**) and hydrothorax (**Figure 40.11**), and in some cases a degree of ascites, are striking changes in most fatal cases of the disease. However, hydropericardium is usually more pronounced in sheep and goats than in cattle.[108] The transudate is a transparent to slightly turbid, light yellow fluid which may coagulate on exposure to air. Several litres of it may be present in the thorax in cattle, while in sheep up to 500 ml, and in goats rarely more than 20 ml, may be present.[218]

A moderate to severe oedema of the lungs occurs in most animals that die of the disease (**Figure 40.12**), but it is particularly severe in animals which have suffered from the peracute or acute form.[245] Frothy oedematous fluid oozes from the cut surface of the lungs. The trachea and bronchi are often filled with a frothy serous foam occasionally accompanied by a fibrinous coagulum, and their mucous membranes are often congested and contain petechiae and ecchymoses. The mediastinum and bronchial lymph nodes may also be oedematous. A slight to moderate splenomegaly resulting from congestion and lymphoid hyperplasia is found in most animals, but in sheep and goats it is often not as severe as in cattle.

Oedema of the brain commonly occurs in animals suffering from the peracute and acute forms of heartwater.[188, 245] Occasionally the entire brain, but particularly the gyri of the cerebrum, is prominently swollen, and this results in a partial herniation of the cerebellum through the foramen magnum. Most fatal cases show varied degrees of congestion and oedema of the meninges. The choroid plexus is swollen and dull greyish in appearance. In some animals, petechiae, ecchymoses and sometimes suggilations are evident in the brain substance, particularly of the midbrain, brain stem and cerebellum.[188] Microscopic lesions in the brain are characterized by changes compatible with oedema, such as widened perivascular spaces which in tissue sections sometimes contain material representative of oedematous fluid or protein droplets, swollen and often necrotic astrocytes, swollen axons, and microcavitations, particularly in the white matter. Occasionally small haemorrhages in the neuropil, scant cellular perivascular accumulation of mainly macrophages and lymphocytes, fibrinoid vasculitis, fibrinous choroiditis and multifocal glial nodules (particularly in the white matter around small blood vessels) are encountered.[188] Widespread status spongiosus of varying severity, that mainly affects the larger tracts of white matter in the brain, may be found in animals that have been recumbent for days, and particularly in those that have been specifically but unsuccessfully treated a few days before death.[193]

Nephritis of varying degree, sometimes accompanied by perirenal oedema and petechiae in the renal cortex, occurs



Figure 40.10 Hydropericardium

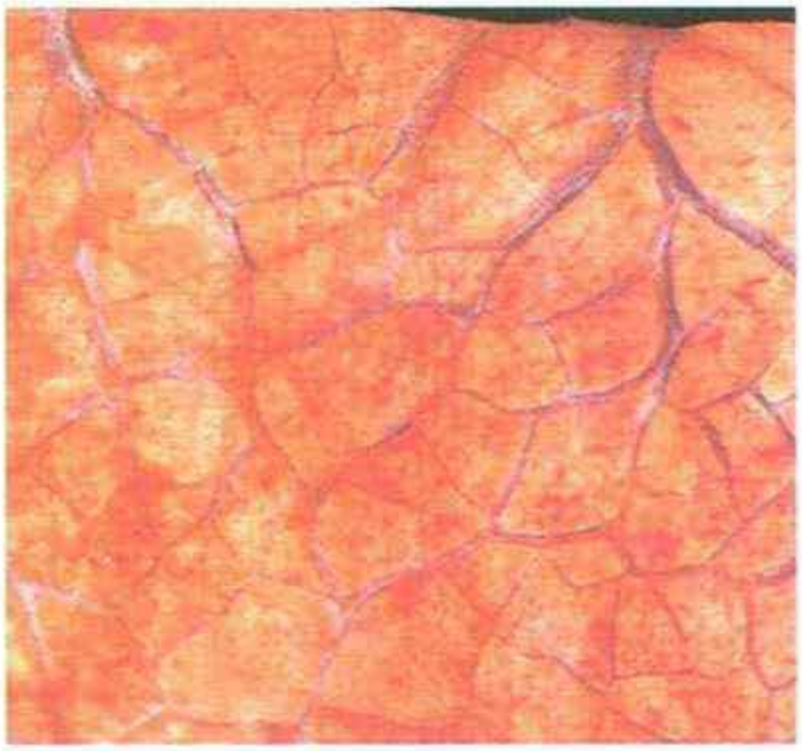


Figure 40.12 Lung oedema



Figure 40.11 Hydrothorax



Figure 40.13 Severe oedema of abomasal folds

in most fatal cases of heartwater.[195] Renal tubular epithelial cells are swollen and occasional tubules may contain hyaline casts. Severe nephritis is particularly obvious in Angora goats that have been unsuccessfully treated for *E. ruminantium* after the first day of the febrile reaction and killed *in extremis* four to seven days after the onset of the febrile reaction, or which have died three to four days after the first evidence of fever.[199]

Congestion and/or oedema of the abomasal folds (**Figure 40.13**) are regular findings in cattle but are not as common in sheep and goats. Petechiae may also be present in the mucosa of the abomasum. Cattle which have shown some clinical evidence of diarrhoea often reveal the presence of a diffuse catarrhal enteritis of varying intensity. In others, particularly Jersey cattle breeds, severe diffuse enterorrhagia, together with intense congestion of the mucosa of particularly the small intestine, may be the most prominent of all the lesions.

Many lymph nodes are moderately swollen, and cut surfaces are oedematous and congested, and may contain small haemorrhages.[2] Petechiae or even larger haemorrhages may be present in serous and/or mucous membranes of tissues or organs such as the epi- and endocardium, urinary bladder, vagina and conjunctivas.

Variable numbers of *E. ruminantium* colonies are discernible in the cytoplasm of endothelial cells, and

particularly those of the brain (**Figures 40.3 and 40.4**), lungs and kidneys but, as a general rule, they are difficult to find in haematoxylin and eosin stained tissue sections.

The lesions in game animals that die of heartwater are very similar to those reported in domestic ruminants.[173, 195]

In mice infected with the Welgevonden stock the lesions closely resemble those in cattle, sheep and goats that have died from heartwater.[197] In tissue sections endothelial cells of the lungs contain the highest concentration of organisms, followed by those of the myocardium. Organisms are only rarely detected in capillaries in tissue sections of the brain of mice.

Transmission electron microscopical studies of the lung lesions in sheep, goats and mice reveal the presence of minor cytopathic changes in endothelial cells. Apart from mild swelling of mitochondria and endoplasmic reticulum, no other changes occur in most parasitized alveolar endothelial cells. Non-parasitized endothelial cells are sometimes swollen, or even necrotic, and are separated from their basement membranes. Oedema of blood vessel walls is infrequently seen.[197, 198]

Diagnosis

The traditional method of making a post-mortem diagnosis of heartwater is the demonstration by light microscopy of *E. ruminantium* in the cytoplasm of endothelial cells of blood vessels in stained smears of brain tissue.[200] Organisms may also be found in tissue sections of the brain, or other organs such as the kidneys.

Brain smears are prepared in such a way that segments of capillaries remain more or less intact and can be examined after staining. A small piece (less than 5 × 5 × 5 mm) of hippocampus or cerebral grey matter is obtained either by opening the skull or by scooping some brain material through the foramen magnum.[209] Smears are made by crushing the sample between two microscope glass slides until the tissue has a soft, pasty consistency. The material is then collected at the end of one of the slides, which is held firmly in a horizontal position. The other slide, angled at about 45 °, is used to make the smear by drawing the tissue along the horizontal slide. While making the smear it is preferable to lift the angled slide slightly, about every 10 mm, so that the smear has alternating thick and thin areas. This procedure stretches the capillaries linearly and facilitates their microscopical detection. Smears should be air-dried before staining.[200, 234]

Various stains may be used to demonstrate heartwater organisms, such as Giemsa or CAM's Quick Stain (C.A. Milsch (Pty) Ltd.),[234] but Giemsa is the method of choice unless large numbers of organisms are present. For Giemsa staining, smears are fixed for about one minute in absolute methanol or ethanol, and are then stained for either 30 minutes in a 10 per cent aqueous solution of Giemsa or 10 minutes in a 50 per cent solution. CAM's Quick Stain yields acceptable results where a high concentration of organisms are present and requires only two to three minutes to complete. Fixed or unfixed smears are still suitable for staining and diagnostic purposes for at least one month after they have been prepared.[234]

Colonies of *E. ruminantium* are generally easy to find in smears of untreated cases of heartwater, but their numbers may vary widely from one animal to another. Organisms can often still be demonstrated in brain smears prepared from animals in an advanced state of putrefaction. The examination of brain biopsies in live animals for the confirmation of a diagnosis of heartwater is useful in experimental animals, but is not practical under field conditions.[52, 219, 239]

For the histopathological diagnosis of heartwater in ruminants by the examination of tissue sections, the kidneys and brain are the preferred organs, *E. ruminantium* particularly being sought in endothelial cells of the renal glomeruli or capillaries of the grey matter of the cerebral cortex.[63] Because of the low numbers of organisms in these tissue sections, it is a time-consuming method of confirming a diagnosis, and may even yield an inconclusive result. Various staining methods may be applied to demonstrate *E. ruminantium* in tissue sections,[46] but those incorporating toluidine blue or Giemsa are preferred.[193]

The confirmation of a diagnosis of heartwater is often difficult in animals that have been treated. A small number of treated animals, particularly those that have received inadequate chemotherapy, do not recover fully, remain recumbent for days, and eventually have, for humane reasons, to be destroyed. Although most of these cases reveal a status spongiosus of varying degrees histologically in the brains,[193] the typical macroscopical lesions of heartwater are usually absent at necropsy, and identification of *E. ruminantium* in brain smears 48 to 60 hours after an animal has been treated is often difficult. In these cases brain smears should be stained for 30 minutes in a 10 per cent aqueous solution of Giemsa. The electron-dense bodies are more severely affected by chemotherapy than are the reticulated bodies, and the organisms are poorly delineated and appear to fuse, making it difficult to distinguish them from phagosomes and chromatin in endothelial cells, and from groups of blood platelets and mast cell granules.[193]

The first serological test for surveys for *E. ruminantium* was the indirect fluorescent antibody test (IFAT).[76] The target antigens were peritoneal macrophages from mice infected with the Kümm stock,[87] or goat neutrophils or cells from endothelial cell cultures.[150] An enzyme-linked immunosorbent assay (ELISA) was also developed.[160] In both of these tests cross-reactions with antibodies against related *Ehrlichia* and *Anaplasma* spp. occur, resulting in the common occurrence of false positive results.[50, 90, 109, 133]

A competitive ELISA using a monoclonal anti-MAP1 antibody was the next development[120] but this too gave false positive reactions[18] with related *Ehrlichia* spp. It is now known that the reason for this problem is the existence of homologous families of immunodominant outer membrane proteins in *Ehrlichia* and *Anaplasma* spp.[176, 179, 262]

In an attempt to overcome the problem the competitive ELISA test has been modified by the use of a fragment of MAP1, designated MAP 1B, in an indirect ELISA format.[252] This has been shown to have a higher specificity for *E. ruminantium* than any other serological test, and it does not cross-react with antibodies against three other organisms in the order Rickettsiales which commonly infect ruminants, namely *Anaplasma bovis*, *Anaplasma ovina* and *Anaplasma phagocytophila*.[252] Note that these three organisms were classified as *Ehrlichia* spp. before the recent reclassification.[92] The MAP 1B ELISA test does, however, detect antibodies to other *Ehrlichia* spp., notably *E. canis*, *E. chaffeensis*, an unidentified *Ehrlichia* sp. infecting white-tailed deer (*Odocoileus virginianus*) in the south-eastern USA,[125] and the organism currently known as *E. ruminantium* (Omatjenne).[11]

Apart from this caveat the MAP 1B ELISA test works well in sheep and goats.[67, 152] In cattle, however, antibody levels against *E. ruminantium* can be very low in heartwater endemic areas, even in cattle that have been vaccinated or are under continuous natural challenge by infected ticks.[67, 210] Antibody levels decline in cattle to such an extent that they become seronegative 14 to 33 weeks after initial exposure owing to a down regulation of MAP1-specific antibody responses post-recovery.[210] Similar results from cattle have been reported using the IFA test[90] and have been confirmed by immunoblotting.[67, 210] Care must therefore be taken when using any serological test in cattle, especially if the animals are being tested in order to decide whether it is safe to move them to a non-endemic area, since it is known that they may be tick infective subclinical carriers of heartwater.[12]

There has been a molecular genetic revolution in diagnostic techniques over the last 10 years and it has become possible to detect the presence of minute amounts of parasite genetic material amid a huge excess of host DNA. The critical factors which allow this are the specificity of DNA-DNA hybridization probes, the sensitivity which follows on target amplification by the polymerase chain reaction (PCR) and the ready availability of public databanks of genetic information. For *E. ruminantium* diagnosis three families of probes are in use, targeting the pCS20 gene, the small subunit ribosomal RNA (16S RNA) gene, and the *map*1 gene.

The pCS20 gene is an open reading frame of unknown function[254] which appears to be specific for *E. ruminantium*, giving no cross-reactions with other *Ehrlichia* spp.[9] It is the most sensitive of the probes for *E. ruminantium* detection and has been widely used to detect *E. ruminantium* in domestic animals, wild game, and ticks.[10, 141, 144, 181, 182, 184]

The 16S RNA gene has been very widely used as a taxonomic and phylogenetic tool for classifying bacteria.[177] There are eight different 16S ribosomal RNA genotypes currently classified as *E. ruminantium* (**Figure 40.2**) and six of these are known to cause virulent heartwater in ruminants, which suggests that they are what is classically understood to be *E. ruminantium*. There are two oddities among them, however, the Omatjenne genotype, which does not appear to be pathogenic to goats,[11] and the Pretoria North genotype, which has been found in dogs.[7] The infectivity for ruminants of this latter organism is not yet known. All eight of these genotypes give a positive reaction with the pCS20 probe and, because of the uncertainty which this may induce, it is routine to use the 16S ribosomal RNA probes as well as the pCS20 probe on animals which are being examined to obtain permits for importation into non-endemic heartwater areas.

The *map*1 gene has also been used as a target for molecular genetic diagnosis of *E. ruminantium*.[9] Because of the extensive polymorphism of this gene it has been used to characterize different genotypes of the parasite.[8] There is no evidence of any immunogenetic or geographic clustering among the variants of the gene, and other gene targets need to be sought in order to better characterize stocks of *E. ruminantium* which are to be incorporated into vaccines for field use.

Differential diagnosis

Nervous signs occur in most animals suffering from heartwater and they must be distinguished from a wide range of infectious and non-infectious conditions that manifest similar signs.

In cattle nervous signs may be caused by other infections such as rabies, the nervous form of malignant catarrhal fever, cerebral babesiosis, cerebral theileriosis, chlamydiosis, meningitis and encephalitis caused by various bacteria, especially *Streptococcus* spp., *Pasteurella* spp., *Arcanobacterium pyogenes*, and *Haemophilus* spp. In sheep and goats meningitis and encephalitis are caused by a wide range of bacteria, and abscessation of the hypophysis occurs, particularly in goats.

In southern Africa nervous signs in cattle may be the result of poisoning with plants such as *Albizia versicolor*, *Albizia tanganyicensis*, *Cynanchum* spp., *Euphorbia mauritanica*, *Sarcostemma viminale* and *Cynodon dactylon*, and fungi, especially *Aspergillus clavatus* and *Claviceps paspali*.[126] Other poisons which may also induce nervous signs are pesticides (e.g. chlorinated hydrocarbons and organic phosphates) and heavy metals (such as lead and mercury).[59, 220, 221] In sheep and goats plant poisoning (especially by *Cynanchum* spp., *Euphorbia mauritanica*, *Sarcostemma viminale* and *Cynodon dactylon*), and heavy metal and pesticide poisoning, induce nervous signs similar to those seen in cattle.

Lung oedema, hydropericardium and hydrothorax are common necropsy findings in cattle, sheep and goats that have died of heartwater but they are also regular findings in the case of gousiekte ('quick disease') caused by the ingestion of the rubiaceous plants *Pachystigma pygmaeum*, *Pachystigma thamnus*, *Pachystigma latifolius*, *Fadogia homblei*,

Pavetta harborii and *Pavetta schumanniana*.[126] Lung oedema is also found in sheep that have succumbed to pulpy kidney disease or bluetongue, and in cattle suffering from Corridor disease or East Coast fever.

The diagnosis in cattle, particularly Jerseys, which have died from the form of the disease in which intestinal changes are the most prominent should be differentiated from poisonings by, for example, heavy metals (arsenic, mercury), plants (*Homeria, Moraea, Urginea, Ornithoglossum* and *Ornithogalum* spp.) and organophosphates.

Chlamydophila pecorum and *E. ruminantium* may be difficult to differentiate morphologically in brain smears or tissue sections made from animals which have died of the respective diseases. Both organisms are pleomorphic, and elementary, intermediate and reticulated bodies are found in both of their multiplication cycles. As a rule, the organisms within a colony of *E. ruminantium* are of the same morphological form — either all elementary bodies or all reticulated bodies.[187] A colony of *Chlamydophila*, however, may contain elementary bodies (0,24 to 0,4 µm in diameter) and reticulated bodies (0,6 to 1,5 µm in diameter).[16] The histopathological lesions in the brain of the two diseases usually differ markedly. Whereas those caused by *C. pecorum* are generally characterized by a moderate to severe multifocal or diffuse lymphocytic meningoencephalitis, vasculitis and, in some instances, also by thrombosis and small foci of necrosis of the neuropil, oedema of varying severity is usually the only lesion that is encountered in the brain of animals that have died of heartwater.

While the characteristic colonies of *E. ruminantium* are easy to detect, if present in sufficient numbers, it is not possible to distinguish between different species of *Ehrlichia* by light microscopy. If one suspects the presence of a species closely related to *E. ruminantium* for other reasons, such as the clinical picture or geographical origin of the case, then the only way to characterize the parasite is to use molecular genetic methods.

Control

Four methods are available for the control of heartwater: tick control, antibiotic treatment of clinical cases, prophylactic use of antibiotics, and immunization.

Tick control

Tick control has been advocated as a means of controlling heartwater from very early days.[2, 71, 222] Even after the infected blood-based vaccine was developed, tick control was still advocated as a supplementary or alternative means of control.[108, 170, 217]

Tick control can be either intensive or strategic (**see Chapter 1: Vectors: Ticks**)[33] but intensive tick control has largely fallen into disuse. The objective was to control all stages of ticks throughout the year and it was a system advocated for marginal areas where *Amblyomma* spp. were only found occasionally. The logistics and expense of such operations are formidable if they are applied over large areas, and acaricide resistance is a widespread problem.[235] The main disadvantage, however, is that animals may lose all immunity to tick-borne diseases because of the lack of a natural challenge. Any breakdown of the intensive control regimen then results in heavy losses from heartwater and other tick-borne diseases.

Strategic tick control implies the control of tick numbers so that natural infection of livestock occurs and high levels of immunity are maintained. The aim is to achieve an epidemiologically stable situation with respect to heartwater by the regulation of the numbers of ticks present so as to prevent the debilitating effects of severe tick infestations. It is usually recommended that animals should be dipped only if they are carrying, on average, more than 10 adult *Amblyomma* ticks each. The animals should be monitored weekly in summer and autumn immediately after or during periods of good rains, and every two to three weeks in winter. It has been shown that this approach can lead to endemic (or enzootic) stability, even when the strategy is somewhat erratically applied.[223] Economic studies have demonstrated that strategic tick control is both a more economical and a more practical option for limiting losses from heartwater and other tick-borne diseases.[153] In fact, the counter-intuitive observation has been made that direct tick-borne disease losses increase with increasing use of acaricides.[57]

Antibiotic treatment

Several drugs have been used to treat animals suffering from heartwater, but the tetracyclines, especially oxytetracycline, are the most widely used.[240] During the early 1950s the minimum recommended therapeutic dose was 5 mg/kg live weight, given either as a single dose or in a divided dose with a 24-hour interval.[107, 258] Some years later it became apparent that this dosage rate was no longer effective and a rate of 10 to 20 mg/kg was recommended. Despite this there is apparently no resistance of *E. ruminantium* to tetracyclines.

Short-acting formulations of oxytetracycline are most commonly used at a dosage rate of 10 to 20 mg/kg body weight, either administered intramuscularly as a single dose, or half the calculated dose is given intravenously and the other half intramuscularly. This treatment is usually repeated 24 hours later. A long-acting oxytetracycline preparation has been shown to be equally effective.[234] Doxycycline has been used successfully at a dose rate of 2 mg/kg body weight in the treatment of experimentally induced heartwater in sheep.[116] This formulation is more lipid-soluble and has a longer serum half-life than has oxytetracycline, and smaller therapeutic doses with longer intervals between treatments can thus be used.

When treatment with tetracycline is instituted during the incubation period of heartwater in cattle (before approximately eight days after infection) the course of the disease is

usually altered; animals usually develop no fever or other clinical signs, except that, in a few, a low-grade transient febrile response will result. In these animals no, or at best only a partial, immunity develops.[88, 190] Treatment of sheep and goats during the incubation period may also give rise to a delayed febrile reaction (it may be as long as 20 to 25 days after infection compared to the more usual 9 to 10 days in untreated animals), and the reaction may be so severe that additional treatment is required.

A wide variety of sulphonomides has been used in the treatment of heartwater;[3, 106, 163] in fact, the treatment of heartwater with uleron by Neitz in 1940 was the first use of any sulphonomide against a rickettsial infection. Sulphadimidine gives good results in severe reactions to heartwater that persist in spite of repeated oxytetracycline treatments[246] but because tetracyclines are normally so effective sulphonomides are not commonly used. Rifamycin has also been used to treat *E. ruminantium* infections in mice and sheep at a dosage rate of 0.2 mg/kg.[178]

Supportive therapy in clinical cases of heartwater is often inefficient because of the poor understanding of the pathogenesis of the disease. Various anti-inflammatory agents have been used but the only published information concerns the use of dimethylsulphoxide[39] and prednisolone.[201] Calves develop halitosis and dark yellowish-brown urine as a consequence of the use of dimethylsulphoxide but no real improvement in the recovery rates occurs.[104] Betamethasone can be given at a dose of 0.1 mg/kg body weight and treatment can be repeated after 12 hours if necessary.

In addition to specific therapy, diuretics have been administered to counteract oedema, and in the past furosemide was used for this purpose.[156] However, the drastic reduction in blood volume which occurs as a consequence of heartwater is exacerbated by the use of diuretics, and the administration of furosemide as a supportive treatment is no longer recommended.[211]

Prophylactic use of antibiotics

Routine oxytetracycline injections may be used to protect susceptible animals against heartwater when they are introduced into an endemic area.[100, 201] In goats it is advocated that short-acting oxytetracyclines be administered at a dosage rate of 3 mg/kg body weight on days 10, 20, 30, 45 and 60 after their introduction, and that the animals should not be dipped until day 60.[100] Injections of a long-acting oxytetracycline in cattle are sufficient to protect them from contracting heartwater, while at the same time allowing them to develop a natural immunity.[201, 202] The dosage is 10 to 20 mg/kg body weight given on days 7, 14 and 21, or days 7, 12 and 17, or even on days 7 and 14. The animals should be kept under close scrutiny and given appropriate treatment if they do develop overt disease. The success of this regimen depends upon the animals becoming naturally infected during the time that they are protected by the drug.

Slow-release treatment with doxycycline has also been advocated, in the form of a tablet implanted behind the ear. In practice this is most often used as part of an immunization-and-treatment regimen.[131] The dosage is critical so the body weight of the treated animals must be known, since an inadequate amount of antibiotic will not prevent fatal heartwater.

Immunization

The economic need for a control system based on vaccination, and the steps being taken to develop a suitable vaccine, are described above (see Pathogenesis).

The only 'vaccine' currently commercially available is a cryopreserved preparation of blood from a sheep infected with virulent *E. ruminantium* organisms of the Ball 3 isolate.[32] The blood is injected intravenously in animals to be immunized, the rectal temperature is monitored daily, and antibiotic treatment is administered at the proper time. The infective blood must be preserved on dry ice or in liquid nitrogen and thawed shortly before inoculation, and the whole procedure must be supervised by trained staff. The duration of immunity is uncertain, and because live organisms are involved the procedure cannot be used in non-endemic areas. The procedure is, however, successfully used to protect susceptible animals against the disease, especially when they are first introduced into endemic areas, or if they are particularly valuable.

The Ball 3 stock was originally isolated in the Limpopo Province of South Africa[105] and was chosen as the vaccine stock because it produces an early temperature rise several days before any other serious clinical signs appear. This makes it relatively easy to decide when to treat. Some stocks, such as the highly virulent Welgevonden isolate, can cause death very shortly after a rapid temperature rise and are therefore not usable for the infection and treatment method of immunization. Unfortunately the Ball 3 vaccine does not protect against all the isolates which circulate in the field. It confers only limited protection against challenge with the Welgevonden[91] and Senegal[121] stocks. It is noted above that the Welgevonden isolate offers a wider spectrum of cross-protection than other stocks, but its virulence makes it difficult to control and therefore unsuitable for an infection and treatment immunization procedure.

Immunization of pregnant cows, especially if their offspring are valuable, is not advisable as it may result in the peracute form of the disease, or abortion.[246] Uncomplicated immunization of ewes that were two to four months pregnant has been reported,[32] but in such instances it is very important to monitor the rectal temperatures carefully and to treat the animals at the first rise in temperature.

Two to five per cent or more of animals, especially cattle that supposedly should have lost their age-related resistance, fail to react after immunization.[82] This could be due to persistence of the innate age resistance in some animals[83] or to high conglutinin levels. Reimmunization of non-reactors sometimes produces the desired effect, but the risk of

anaphylactic shock increases with repeated inoculation of blood or tick suspensions.[21, 231]

In the absence of periodic stimulation of the immune system, resulting from the bites of infected ticks, the duration of immunity after immunization varies greatly between different domestic animal species, and also perhaps between individuals within species. In sheep, the immunity may wane after six months,[77, 162] but in some cases it may remain sufficient to protect animals against a fatal outcome for at least four years.[168]

The duration of immunity in goats following immunization is poorly documented. In Angora goats the degree of immunity seems to depend largely on the time at which the animals are treated therapeutically during the reaction subsequent to the immunization. In one experiment, treatment shortly before the commencement of the febrile reaction resulted in the development of a poor immunity, while animals treated on the second and third days of the reaction were immune to challenge 107 to 205 days later.[85]

The duration of immunity in cattle after immunization in the absence of challenge appears to be approximately two years.[82] It has been suggested that some animals may be susceptible again as soon as two months after immunization[15] but this observation could have been the result of infection with an immunotype against which Ball 3 does not offer protection.

Immunization of very young animals Advantage of the age-related innate resistance of young animals to heartwater is taken in this procedure.[88, 167] Immunization of calves under the age of one month and of lambs and kids younger than seven days does not generally result in clinical disease, but the animals develop immunity. While it is advisable to monitor their rectal temperatures twice daily for a period after immunization, as is advised in the case of older animals, this is still the method with the lowest risk of losses due to fatal heartwater reactions after inoculation.[83]

Practical aspects of the infection and treatment method After the vaccine is administered to the animal a definite fever usually develops and if this is not treated it may be fatal. Inoculated animals are treated early in the course of the disease, but if treatment is effected too early no immunity will result. There is no indication that tetracyclines interfere with the development of immunity to heartwater,[213] unless they are given too early in the incubation period.[88] It is advisable to immunize individuals or small groups of animals because large groups present serious management difficulties.

Starting on the day following vaccination, the rectal temperature of each animal is recorded daily, preferably early in the morning and before the intake of food. In order to identify the first day of the temperature reaction the average normal temperature preceding the reaction must also be determined over a period of about six days for both small stock and cattle. On the first day of the temperature reaction the rectal temperature will usually be 1 °C or more above the average. This can be expected 9 to 14 days after inoculation in the case of small stock and 14 to 18 days in cattle. In exceptional cases it may be as early as seven days in small stock and as long as 25 to 30 days in cattle. A rise in the early morning rectal temperature to above 39,5 °C in cattle and goats, and 40 °C in sheep, is usually regarded as an immunization reaction.[247] The general habitus of all animals should, in addition to the temperature reaction, be closely monitored after vaccination.

It is unnecessary to treat as early as the first day of the reaction, except in rare cases where, after a sudden rise of 1,5 °C or more, a temperature of 41,5 °C is reached. Treatment should be given only if the temperature on the second day of the reaction equals or surpasses that on the first day. Should the temperature on the second day be lower than the previous day the animal is either resistant or partially immune, in which case the temperature on the third day will again be lower than on the first day and treatment would be unnecessary. Occasionally the temperature on the third day is much higher than on the second day and even higher than on the first day. If this occurs the animals should be treated immediately.

Treatment consists of an intramuscular injection of tetracycline at 10 mg/kg body weight, and short- or long-acting formulations can be used. The recording of temperatures must be continued after treatment and if the temperature 48 hours after treatment equals or surpasses that on the day of treatment, a second treatment should be given. A high temperature within 24 hours after treatment can be ignored, unless the animal shows other clinical signs such as listlessness and lack of appetite, in which case it should be treated a second time without delay.

Relapses of vaccine-induced heartwater may occur after treatment. It is therefore advisable to monitor the rectal temperatures, especially of valuable animals, for an additional period of two weeks after the last treatment.[14] A small number of animals may react severely despite the lack of a fever, and other diseases, such as babesiosis, may complicate matters.[167] Blood smears (and possibly other examinations) should be performed when animals do not respond satisfactorily to specific heartwater therapy.

The block method of immunization The block method of immunization,[88, 94, 189, 190] also known as 'systematic treatment',[14, 234] is widely practised in South Africa, especially when immunizing large numbers of kids and lambs. In this method the vaccine is administered and this is followed by treatment as described above on a predetermined day without recording daily rectal temperatures. Treatment is recommended on the following days, assuming that vaccination has taken place on day 0: exotic *Bos taurus* cattle breeds and their crosses, day 14; indigenous *Bos indicus*

cattle breeds and their crosses, day 16; sheep and Angora goats, day 11; Boer and crossbreed goats, day 12.[88]

This procedure makes immunization on a large scale possible and it is particularly suitable for the immunization of large groups of animals being moved from a heartwater-free area into an endemic area. The animals can be immunized on the farm of origin or immediately after their arrival in the heartwater area. Care must be taken, however, as the procedure has some disadvantages. In older animals durable immunity only follows after the animal has developed a febrile reaction.[2, 88] Treatment performed too early in the incubation period results in failure to develop immunity[88] and if treatment is given too late heavy mortalities can result.[85] In exceptional cases some animals may be more susceptible than the rest of the herd and may show clinical signs before they are treated.

A prudent modification which may be applied to the block method is to select 10 animals, representative of the breed and age of the herd, and record their daily rectal temperatures in the usual way. As soon as the temperature of five or more of them is above the average for two consecutive days, the whole herd is treated.

The doxycycline implant method In this method animals are inoculated and at the same time doxycycline tablets are implanted under the skin, usually behind the ear, using a special applicator. Slow release of the active ingredient means that the animal does not become clinically ill, but at the same time develops an immunity.[131] Since the animal is handled only once and daily temperatures are not recorded, this method is suitable for the immunization of large numbers of animals at one time. The dosage is critical, so the body weight of the treated animals must be known, since an inadequate amount of antibiotic will not prevent fatal heartwater, while an excess will prevent the subsequent development of immunity.

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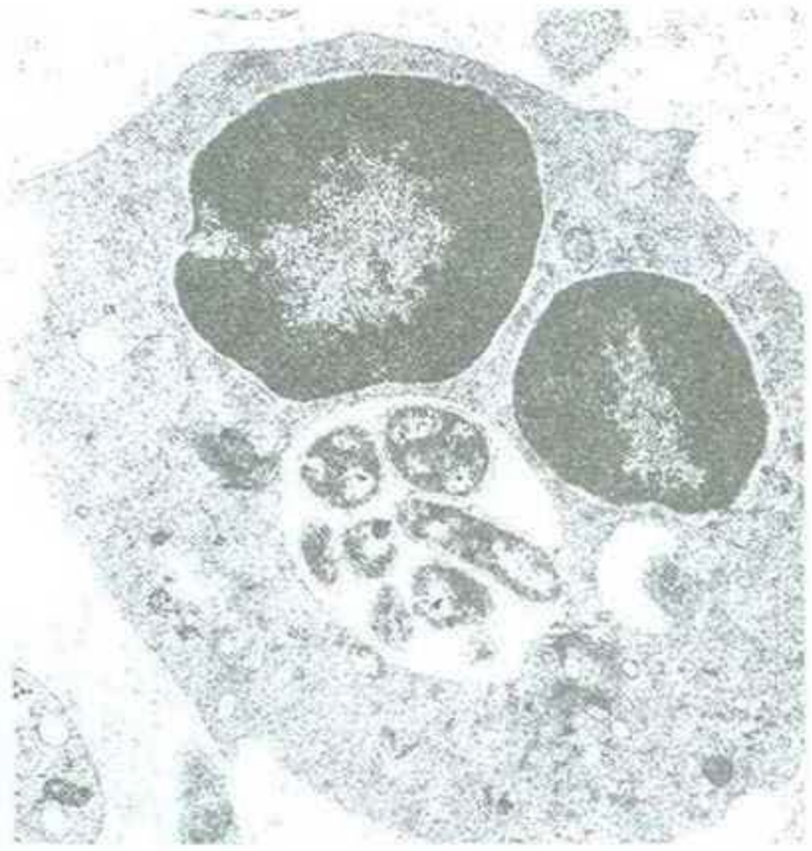


Figure 41.1 Electron micrograph of *Ehrlichia (Cytoecetes) phagocytophila* within an intracytoplasmic vacuole in a granulocyte of a sheep

the clade,[24] but retaining the species name. Under this proposal the three genogroups would become the genera *Neorickettsia*, *Anaplasma* and *Ehrlichia*. Arguably, agents in genogroup III should be *Cowdria* species since the discovery of the agent of heartwater by Cowdry in 1925 predates that of canine ehrlichiosis. In this chapter, the genus names used are those in use by the overwhelming proportion of authors during the period 1995 to 2001. Proposed revisions based on genogroups do not support a genus with a single member (*C. ruminantium*), which would be replaced with *Ehrlichia ruminantium*; but broadly support Foggie's[29] previous proposal for a division of the tick-borne ehrlichias into two, with a genus containing rickettsias parasitizing circulating mononuclear cells, and the other, a *Cytoecetes* genus, comprising rickettsias which primarily parasitize granulocytes.

Considerable attention has been paid since the early 1990s to tick-borne ehrlichioses since the recognition of human infections with the monocytotropic *E. chaffeensis* and with a granulocytotropic agent closely related to *E. phagocytophila*. The unparalleled level of study has provided clear indications for revised concepts of classification, transmission and epidemiology of the lesser-known *Ehrlichia* species. The most obvious message is that a classification of the ehrlichias based on the host species in which it is diagnosed or recognized is misleading, since disease usually occurs only in accidental hosts. Further, the life cycle of each of these *Ehrlichia* 'species' may involve different 'reservoir hosts' according to ecological situations, and for infection of larvae and nymphs of ticks. The extremely close genetic relationship between some European and North American isolates of granulocytotropic ehrlichias further emphasizes the importance of a classification based on a genetic basis rather than the species affected, since apparently the same granulocytotropic agent is associated with disease in dogs, cats, horses and humans,[61] with a role for small mammals in infection of tick larvae.[94] In addition, the recognition that agents closely related to *E. phagocytophila* are found across the temperate broad-leaved and mixed forest zones of the northern hemisphere within ticks of the genus *Ixodes*, resulting in infection in humans and livestock from China to California, further indicates that we should expect to find a very widespread distribution of ehrlichias in nature. All of the major genera of ticks in the family Ixodidae infesting livestock have been shown to harbour ehrlichias, and we should therefore expect that most, if not all, hard tick populations will be involved in the transmission of those ehrlichias with which they may have co-evolved. Available evidence for the well-studied tick-borne ehrlichias (*C. ruminantium*, *E. phagocytophila*, *E. canis* and *E. chaffeensis*) which have a tropism for leukocytes, indicates they are each transmitted by only a single genus of ticks. Although evidence of natural infection in other species of ticks has been reported for some of these agents, only one genus of ticks appears to be competent as a vector of each, for example *Ixodes* spp. rather than *Dermacentor* spp. for agents in the *E. phagocytophila*-group.[104] Evidence of ehrlichial infection in ticks does not therefore indicate that the tick is the natural vector, and care must be taken in interpretation of the finding that inoculation of ground-up ticks can cause infection since this bypasses the salivary glands; transmission by tick feeding is required. The geographical distribution of the lesser-known ehrlichias probably corresponds with that of the vector tick species. Given that rapid discovery of additional ehrlichial agents has occurred since attention has been paid to ticks in temperate regions of the northern hemisphere and the determination that both monocytotropic and granulocytotropic agents can cause human disease, we should expect to find many additional ehrlichias infecting livestock in the tropics, which will require our concepts of ehrlichial infections of livestock to be reviewed.

Studies on the lesser-known ehrlichias of livestock are woefully inadequate: a genetic characterization of the agents involved in bovine and ovine ehrlichiosis in different continents has not been made and is rendered difficult by the extremely limited number of laboratory isolates of these agents. Rather than perpetuate the many doubtful species names, and the potential vector associations of each, a proposed categorization is made below, in the light of a review of the literature and current concepts in the transmission of the ehrlichias. Small and large ruminant ehrlichioses have resulted from transmission experiments, or been associated in natural outbreaks, with *Amblyomma*, *Hyalomma* and *Rhipicephalus* ticks. It is reasonable to suspect that the agents associated with each tick genus are distinct, but those usually identified as *'E. bovis'* and *'Ehrlichia ovina'* in large and small ruminants, respectively, resulting from

Table 41.1 Proven or presumed tick vectors for ehrlichial infections of livestock, excluding *Ehrlichia (Cowdria) ruminantium* infection

IXODID GENUS	IXODID SPECIES	*EHRLICHIA* SPECIES	LEUKOCYTE ASSOCIATION	EVIDENCE (E)	PROBABLE DISTRIBUTION	REFERENCES
Amblyomma	*A. americanum*	*E. chaffeensis*	Monocytic (M)	E1, E5	North America	25
	A. cajennense	*'E. bovis'*	M	E1	Central and South America	59, 60
	A. variegatum	*'E. bovis'*	M	E3	Africa	78
Rhipicephalus	*R. evertsi evertsi*	*'E. ovina'*	M	E1	Sub-Saharan Africa	65
	R. bursa	*'E. ovina'*	M	E4	North Africa, Mediterranean region, Middle East	53
	R. appendiculatus	*'E. bovis'*	M	E2	South and Central Africa	9, 62
	R. haemaphysaloides	*'E. ovina'*	M	E6	Sri Lanka, Indian subcontinent	90
Hyalomma	*H.* species (derived from Iran)	*'E. bovis'*	M	E2*	Iran, Middle East, North Africa	14
	H. excavatum (from Iran)	*'E. bovis'*	M	E2	Iran, Middle East and North Africa	65
	H. anatolicum anatolicum	*'E. bovis'*	M	E1*	India	44
	H. marginatum isaaci	*'E. ovina'*	M	E6	India	97
Ixodes	*Ixodes* species	*E. phagocytophila* genogroup	Granulocytes (G)	E1	North Africa, Europe, paleartic	35
Unknown — possibly *Ixodes*		*?E. phagocytophila* genogroup	G		Habitats in Port Elizabeth/ Humansdorp area, South Africa	
		E. ondiri	G		High altitude habitats in East Africa	

* ticks are presumed to have acquired infection while feeding on *Theileria annulata* infected calves

Key: E1 = biological transmission during feeding of ticks previously fed in the previous stage on experimentally infected animals; E2 = biological transmission during feeding of ticks previously fed in the previous stage on animals in the field; E3 = inoculation of homogenates of ticks collected from the field; E4 = inoculation of homogenates collected of ticks previously fed on infected animals; E5 = identification of agent by molecular tests in ticks collected from the field; E6 = circumstantial evidence exists to associate infection with tick feeding

membrane-lined intracytoplasmic vacuoles in monocytes, producing dense clusters of cocci. Infected monocytes, however, are seldom seen in smears of peripheral blood.[14] Splenectomy,[15] rinderpest[18] and environmental stresses[26] exacerbate *E. bovis* infections. Given the likely occurrence of recrudescent infections, it is possible that the appearance of *E. bovis* in blood smears of cattle following their laboratory infestation with ticks collected in the field as originally reported in South Africa[9] might be related to this phenomenon rather than true transmission. Nevertheless, similar results[62, 68] in Zimbabwe corroborate these findings and do suggest that *R. appendiculatus* is a vector in southern, and probably East Africa.

In one trial, cross-protection between *E. bovis* and *C. ruminantium* did not occur in experimentally infected and challenged calves,[15] but subsequently it has been found that antibodies in sera of cattle believed to have had bovine ehrlichiosis, cross-react with the Kümm strain of *C. ruminantium*.[19]

Epidemiology

Although overt disease is rare, *E. bovis* infections are probably widely distributed in Africa, the Middle East, India and Sri Lanka.[48, 89] In the New World the infection has been suspected in cattle in Guadeloupe[27] (but was later considered to be heartwater[101]) and identified in Brazil.[59] In the USA, natural infection of goats and deer with *E. chaffeensis* has been shown,[22] and cross-reactions in cattle sera with serological tests for *Cowdria* suggest natural infection of cattle with agents closely related to *Cowdria*. Had the latter occurred in other continents, it may well have been recorded as '*E. bovis*' or '*E. ovina*' infections.

Ehrlichia bovis causes a persistent infection of low pathogenicity in healthy cattle reared in tick-infested areas where the parasite is endemic, but when such animals are stressed, either by adverse weather or intercurrent disease, severe clinical disease may be evoked and death may follow.[26] Similarly, overt disease and death may occur in

exotic cattle after their introduction into an endemic area[78] or in naive indigenous cattle following the introduction of persistently infected cattle and their ticks into a previously disease-free area.[71]

Previously infected cattle resist challenge to infection by *E. bovis*.[15] Relapses occur and may be induced by splenectomy.[38]

Reports of the experimental transmission of '*E. bovis*' conducted with different isolates from separate continents in which three different genera (*Amblyomma, Hyalomma* and *Rhipicephalus*) of ticks were implicated, are consistent with the concept of the involvement of distinct agents. Infection with '*E. bovis*' by inoculation of ground-up *Amblyomma variegatum* in West Africa was reported, as was transmission by *A. cajennense* in Brazil.[59] Transstadial transmission through nymphs has been shown to occur experimentally in *A. cajennense*.[59] Monocytotropic ehrlichias may be widely distributed within *Amblyomma* spp. ticks since an agent with genetic identity to *E. chaffeensis* in 16s rRNA gene sequence was detected in adult *A. testudinarum* in China.[5] Transmission by *Hyalomma* ticks has been reported; the original report of Donatien and Lestoquard in 1936 did not specify the species which had originated in Iran; Neitz reported transmission by *Hyalomma excavatum* ticks from Iran[65] but did not state if transmission was by feeding the ticks on susceptible animals or by the inoculation of ground-up ticks. Workers in India have reported transmission by *Hyalomma anatolicum anatolicum* after tick feeding on *Theileria annulata*-infected cattle[44] and by *Hyalomma marginatum isaaci* after tick feeding on *T. annulata*-infected calves.[97] In these experiments the possibility of recrudescence of latent infection brought about by tick feeding or *T. annulata* infection must be borne in mind, as well as the difficulty of discriminating theilerial and ehrlichial infection of monocytes. Similar concerns can be raised regarding the experiments which incidentally recorded the potential role of *R. appendiculatus* in southern Africa.[62] De Kock *et al.*[9] reported that *E. bovis* was detected in cattle after adult *R. appendiculatus* collected from farms in the Limpopo Province had fed on them; since some animals on which the ticks fed had previous exposure to ticks, a recrudescence cannot be ruled out. However, the finding was corroborated by Matson[62] in Zimbabwe who recorded 13 cases of *E. bovis* infection after feeding adult *R. appendiculatus* ticks collected in the field on high-grade dairy cattle: The infection was detected 20 to 41 days after tick infestation, and was accompanied by fever. Norval[68] also reported from Zimbabwe that nymphs of *R. appendiculatus*, collected when engorged, transmit *E. bovis* as adults. Circumstantial evidence also supports the role of *Hyalomma*-associated *'E. bovis'*; in Senegal *E. bovis* was detected in blood smears at the end of the rainy season but not in the dry season in cattle maintained in areas where *Amblyomma* were scarce or absent.[38] but where *Hyalomma marginatum rufipes* was the predominant tick, and *Rhipicephalus evertsi evertsi* common. Furthermore, in the same country Rioche and Bourdin[80] detected infected monocytes in a calf and a pig after they had been inoculated with a pool of ground-up *Rhipicephalus* and (mostly) *Hyalomma* ticks collected from a calf which had been kept in the same pen as affected pigs, which they took as evidence of '*E. bovis*'. The vector associated with the major outbreak reported by Pierre[71] is uncertain, since a heavy infestation with both *Amblyomma* and *Hyalomma* ticks occurred, and transmission may have involved either or both of them.

Clinical signs

It is possible that the difference in severity of the disease recognized as 'nofel' in West Africa and the infection in other parts of the world is related to different genetic types of '*E. bovis*', with the suspicion that the *A. variegatum*-transmitted form can result in more severe disease. Gueye (cited by Uilenberg[101]) considered the disease to be an important one of exotic cattle in Senegal. However, other factors, including breed and effect of intercurrent infections (perhaps trypanosomosis, or dermatophilosis in West Africa), might also be expected to affect the severity of the syndrome.

In most countries *E. bovis* is not considered to cause serious disease in cattle or water buffalo, but in some circumstances might exacerbate other infections. Incubation periods after parenteral inoculation of infected blood or tissue suspensions range from one to two weeks.[15, 78, 80] Feeding infected adult ticks on susceptible cattle induces disease 20 to 41 days later,[62] whereas the injection of a suspension of crushed ticks produces a parasitaemia in 16 to 20 days.[78] Two peracute primary cases in Sahiwal cattle imported to Senegal have been recorded.[78] Both animals exhibited severe nervous signs and died within 12 hours.

In areas where *E. bovis* is endemic, primary infections in indigenous calves are usually not observed. Exacerbation of chronic persistent infections may, however, follow stress, producing clinical signs in 10 to 50 per cent of the animals in the herd, with case mortality rates that range from 5 to 25 per cent.[71] Morbidity and mortality rates are higher in primary infections in imported cattle or in virgin-soil epidemics with case mortality rates ranging from 28 to 50 per cent.[78]

In acute primary cases rectal temperatures fluctuate between 39 and 41 °C for 7 to 10 days. Affected animals are anorectic and have diarrhoea. They are apathetic and the respiration and pulse rates are accelerated. Many develop nervous signs such as hyperexcitability and paralysis of the larynx which disappear on recovery. In others, the nervous signs are very severe, affected animals having bouts of drowsiness interspersed with periods of hyperexcitability and convulsions during which they show galloping movements. Severely affected animals usually die during a convulsion about eight days after the onset of illness.[78]

In some West African countries the disease 'nofel' occurs shortly after the onset of the rains in emaciated cattle

extensively afflicted with dermatophilosis and severely infested with ticks. It is an exacerbation of a persistent *E. bovis* infection, characterized by enlarged superficial lymph nodes and swollen drooping ears.[34] Affected animals have fevers, congested mucous membranes, ocular and nasal discharges, inappetence and constipation. Many collapse and die if not treated. In some animals in which the only signs are lymph node enlargement and inappetence, recovery occurs within a week.[26] In Mali, Rousselot[83] observed a distinct seasonality to cases in 1942 and 1943, occurring during the rainy period, with, for the combined years, six in June, 45 in July, 45 in August, 17 in September, seven in October, and very few at other times when tick numbers were least. Rousselot did not identify the vector, but recorded that sick animals had *A. variegatum*, *R. evertsi* and 'above all' *Hyalomma* infestations, which does not allow us to associate infection with any one of these potential *'E. bovis'* vectors.

It should be noted that monocytotropic ehrlichias can also cause human illness (*E. chaffeensis* infection, *Amblyomma*-transmitted), and therefore some of the *'E. bovis'* agents may also present a risk of zoonotic infection. This may also be the case with *Rhipicephalus*- or *Hyalomma*-transmitted agents, as well as those transmitted by *Amblyomma*, since macaques (*Macaca inua*) reacted with fever and rickettsaemia on inoculation with *'E. bovis'* in Algeria, where *Amblyomma* vectors are absent.[18] The *Rhipicephalus*-transmitted *E. canis* can also cause human infection,[24] apparently with few if any symptoms.

Pathology

Despite the difficulty in detecting *E. bovis* in blood smears during the course of the disease, the haematological changes are of diagnostic significance.[78] The characteristics described for the *Amblyomma*-related *'E. bovis'* in monocytes appear to hold true for the forms of *'E. bovis'* observed in other countries, and transmitted by different tick genera. The cytoplasm of infected monocytes contain several vacuoles — most of which appear empty but some with rickettsias present — and their nuclear membranes are distorted and even shredded. Occasionally, a nucleus contains a vacuole. Lymphocyte numbers are little affected but those of neutrophils and eosinophils decline. Many immature cells enter the circulation and cells in mitosis are usually present.[79] There is, in addition, a thrombocytopenia.[10]

The conspicuous gross lesions observed in West Africa are hydropericardium and severe lung oedema together with hydrothorax and ascites. In some cases the interlobular pulmonary oedema is so severe that it mimics the serofibrinous exudate found in the lungs in acute cases of contagious bovine pleuropneumonia.[78] The lymph nodes are enlarged, oedematous and, sometimes, congested. The spleen is reactive and the kidneys are congested. Rioche[79] considered the kidneys to be the most severely affected organ microscopically. Many of the convoluted tubules are necrotic and are reduced to basal lamina filled with karyorrhectic nuclei and cytoplasmic debris. In contrast, changes in the central nervous system are not marked, and are limited to congestion of the leptomeninges and discrete capillary congestion and rare perivascular haemorrhages arising by diapedesis.

Diagnosis and differential diagnosis

The history, clinical signs and lesions of cattle with primary infections are readily confused with those of heartwater. Haematological examinations will aid differentiation despite the difficulty of detecting *E. bovis* in blood smears because bizarre monocytes do not occur in heartwater.[79] *Ehrlichia bovis* in West Africa is usually detectable in organ smears, particularly of the lung, taken immediately after death. The parasite does not occur in endothelial cells.[78]

The *A. cajennense*-transmitted agent in Brazil and the agent in southern India[93] have been maintained in cultures of monocytes. The agent in India was detected after 10 days of culture of monocytes from a water buffalo. It reacted with *E. canis* antiserum.[93] The results suggest that the agent may be part of the group III ehrlichias, which is characterized by *E. canis*, since cross-reactions by indirect fluorescent antibody test (IFAT) between agents typical of group II and III ehrlichias are weak.[46] The ability to culture the agent in monocytes does suggest fixed monocytes could be evaluated for use as antigens in IFAT, although specificity may prove to be a problem when entire organisms are used as antigens.

Control

Treatment with a tetracycline during the early stages of the disease is usually successful. The essential preventive measure is tick control.[101] The infections probably play a role in complicating other haemoparasite infections, especially if they are immuno-suppressive. Since some of the vectors may also be involved in the transmission of *Theileria parva*, *T. annulata*, or *E. ruminantium*, the use of acaricides for the control of these infections can be expected to affect the exposure and immunity to *E. bovis* agents transmitted by these ticks.

Ovine ehrlichiosis

Introduction

Although *Ehrlichia ovina* was among the first monocytophilic rickettsias to be recognized, and despite the passage of time, there is still not sufficient evidence to consider it as a distinct species from the agent which is involved in bovine ehrlichiosis.[101] There have been a number of serendipitous observations of monocytic ehrlichial infections in sheep, or goats, made in the course of other investigations[15, 52, 66, 75, 85] which

the authors ascribed to *E.* (or previously *Rickettsia*) *ovina*. In addition, the same or a similar rickettsia has been linked to a fatal disease in sheep in Namibia,[85] Algeria and Turkey,[13, 53] Senegal,[41] Mali (Rousselot, 1943, cited by Uilenberg[101]) and Sri Lanka.[90] As in the case of *E. bovis*, there is reason to suspect that according to the agent identified in sheep a species of its own may be misleading, and not indicative of the level of diversity within the species. Since *Rhipicephalus* spp. have been shown to act as vectors, and possibly *Hyalomma*, it is reasonable to suspect that some *Rhipicephalus*-transmitted *E. bovis/ovina* isolates may be genetically homogeneous, and possibly also *Hyalomma*-transmitted *E. bovis/ovina* strains. Since *E. chaffeensis* naturally infects goats,[22] it is possible that *Amblyomma*-transmitted ehrlichias also cause monocytic ehrlichiosis in small ruminants in Africa. *Ehrlichia ovina* is known to cycle in sheep in southern Africa but its economic importance is unknown, but is probably low.

Aetiology

Ehrlichia ovina possesses the morphological characteristics of *E. canis* and *E. bovis* and its mode and site of replication are the same.[16] The host range is not known, but since most of the ruminant infective ehrlichias of ruminants are not species-specific, it is likely that *E. ovina* also infects other ruminants and may be genetically identical to some *E. bovis* strains. Phylogenetic analyses based on 16s rRNA gene sequences, or other genes, have not been reported, and therefore it is unclear to which genogroup of the Ehrlichieae the agent belongs. The antigenic cross-reactivity of a Turkish isolate[46] to *Cowdria* antigens suggests a lesser antigenic similarity to *Cowdria* than occurs between *Cowdria* and *E. canis*, but closer than with *E. phagocytophila*.

Epidemiology

Uilenberg[101] and Neitz[66] have reviewed the scanty literature. *Ehrlichia ovina* has been recorded in Algeria and Turkey,[53] Namibia,[85] South Africa,[85] French Sudan,[66] Iran,[75] Sri Lanka,[90] Nigeria,[52] Senegal (Curasson, 1942, cited by Uilenberg[101]) and Mali (Rousselot, 1943, cited by Uilenberg[101]). It has been identified in blood smears from splenectomized sheep and goats in Senegal,[37] and also in intact goats and sheep in different parts of the same country.

In epidemics in flocks in which mortalities occurred, the animals were frequently suffering from severe helminthosis. In addition, Schulz[85] noted severe tick infestation and malnutrition. In West Africa several reports link the disease to movement of sheep from arid zones with a restricted tick fauna to more humid areas. The low pathogenicity of *E. ovina* for healthy sheep coupled with rare epidemics associated with mortalities in flocks in poor condition in which affected animals develop heartwater-like signs, is strikingly analogous to the epidemiological pattern of bovine ehrlichiosis and its fatal exacerbation known as 'nofel' in some West African countries. The severity of disease in Senegal and Mali suggests that a similar agent, or mechanism of inducing disease, is present in 'nofel' and ovine ehrlichiosis in that region, although concomitant heartwater needs to be considered. Five out of 18 mortalities in the rainy season, and two out of seven mortalities in the dry season, in sheep from an *Amblyomma*-free region of Senegal, vaccinated against heartwater and exposed to natural infection, were recorded as being due to *E. ovina*[41] and the authors suggested that this infection may also exacerbate *Cowdria* infection. The sheep were exposed to heavy infestations of *Amblyomma* sp. and *R. evertsi evertsi* and, given the possible infection of sheep by *E. bovis*, it is possible either or both tick species were involved in the transmission.

Information on transmission is very scant, but what there is suggests that at least one form of *E. ovina* is transmitted by *Rhipicephalus* ticks. Neitz[65] reported that *E. ovina* has been successfully transmitted with adult *R. evertsi* that had been fed as nymphs on infected sheep, and in the original study of Lestoquard and Donatien[53] an emulsion of ground-up *Rhipicephalus bursa* ticks infected sheep, indicating that these ticks were infected by, but not necessarily capable of transmitting, this agent. Several other reports from Africa suggest that *R. evertsi evertsi* may be a vector of sheep-infective ehrlichias. An agent transmitted by *R. evertsi evertsi* from a heartwater-free farm in Zimbabwe induced seroconversion in sheep to *C. ruminantium*;[84] this may potentially be *E. ovina* or a related organism. From the results obtained in another study on cross-reactions to *C. ruminantium* in domestic livestock in heartwater-free regions of South Africa it was concluded that both *Rhipicephalus*- and *Hyalomma*-related ehrlichial infections were present; high proportions of seropositive animals were in areas (parts of Mpumalanga Province) where *R. evertsi evertsi* occurred but not *Hyalomma* spp. or *R. appendiculatus*, and also in areas (such as the Northern Cape Province) where *Hyalomma truncatum* but not the other two species occurred.[21] This is consistent with the probability that both genera of ticks maintain ehrlichial agents, and is substantiated by the report of Du Plessis[20] that he had isolated ehrlichial agents infective to sheep from both *R. evertsi* and *H. truncatum* ticks. In Senegal,[37] *E. ovina* was detected in goats kept in areas where *R. evertsi evertsi* was the predominant tick after they had been splenectomized. In southern India an *E. ovina*-like agent was found in sheep with heavy infestations of *H. marginatum isaaci*.[76] However, it is possible that the appearance of the organism was a recrudescence of a latent infection and that the *Hyalomma* species were not the responsible vector. Recent isolation of ehrlichial agents from *H. truncatum* ticks, and from *R. evertsi* and *R. appendiculatus* ticks is also consistent with the concept that ruminant-infective ehrlichias are maintained by the two-tick genera, although the studies[20] which indicated that pathogenicity of these agents for ruminants increases after transmission via *Amblyomma hebraeum* have not been confirmed by others, and are subject to doubt.

Clinical signs

The incubation period in susceptible sheep following infestation with infected adult ticks ranges from 15 to 18 days.[66] The onset of illness is signalled by a sharp rise in the rectal temperature which persists for 3 to 10 days[66] and in some cases for as long as 17 days.[15] Affected animals are listless and stop eating for three to four days. Towards the end of the febrile stage, anaemia is evident. Recovery is rapid, but the blood picture takes four to six weeks to become normal.[66]

The clinical signs in the outbreak recorded by Schulz[85] were characterized by their sudden onset and short course. Affected animals are febrile and develop signs of ataxia before going into sternal and then lateral recumbency which is followed by opisthotonus, unconsciousness and death.

Pathology

Parasitaemia is at its maximum two to three days after the onset of fever.[15, 66] Relapse parasitaemias may occur several weeks later.[15] A delayed anaemia, eosinopenia and monocytosis have also been reported.[66, 90]

The gross changes in fatal cases of *E. ovina* infection are similar to those of heartwater and include hydropericardium, hydrothorax, ascites, epi- and endocardial haemorrhages, hyperaemia and oedema of the lungs, splenomegaly and nephrosis.[15, 85]

Diagnosis and differential diagnosis

It may be difficult to differentiate ovine ehrlichiosis clinically from other febrile diseases[66] and fatal cases of the disease should not be confused with heartwater clinically and at necropsy. A diagnosis depends on detecting *E. ovina* within monocytes but not in endothelial cells.[15] Antiserum produced to *E. ovina* (of Turkish origin) cross-reacted with *Cowdria* antigens in the IFAT with moderate titres (to 640) but reacted only to the 32 kDa major antigenic protein (MAP1) of *Cowdria*.[46] This supports previous findings from southern Africa that cross-reactions with ovine ehrlichias can give positive reactions in serological tests for heartwater in *Amblyomma*-free areas. A rise in antibody titre, for example using cross-reactive antigens of *C. ruminantium* (IFAT could be used[46]) might indicate a recent exposure but would not be of value in animals with recrudescent infections. Polymerase chain reaction (PCR) and hybridization using the approach of Allsopp *et al.*,[1] and sequencing of the 16s rRNA gene would assist specific identification and placement of the agent within the tribe Ehrlichieae.

Control

Infections with *E. ovina* are either so rare or so ubiquitous that control measures have never been formulated. Treatment with tetracycline drugs is likely to be successful, and should be used together with measures to reduce stress and improve the general health of the animals. Control may become an issue in areas where tick control is relaxed following immunization against heartwater, and pathogenic forms of the infection are encountered.[41]

Porcine ehrlichiosis

Ehrlichia suis was first described in Algeria.[11] It was isolated fortuitously when blood from a sick pig believed to be suffering from African swine fever was inoculated into a healthy pig which subsequently developed an irregular fever and died 12 days later. The lesions mimicked those of heartwater in the sheep.[12] The causative agent was passaged in pigs and at the second passage ehrlichias were allegedly detected in monocytes and in endothelial cells of blood vessels. The onset of the parasitaemias coincided with the onset of fever and the ehrlichias evolved in monocytes in a manner similar to *E. canis* and *E. bovis*. The organisms in endothelial cells are very small.

Rioche and Bourdin[80] observed a fatal outbreak associated with ehrlichial-like organisms in circulating monocytes in pigs. They concluded that it was essentially an '*E. bovis*' infection in pigs, since a calf died 12 days after subinoculation of blood from an affected pig. The studies can be taken as evidence that the agent involved was not host-specific, and further supports the use of genetic groupings rather than host species in the nomenclature of *Ehrlichia* spp. The tick vector was not identified, although the agent was seen in the blood of a pig and a calf after inoculation with a homogenate of *Hyalomma* and *Rhipicephalus* ticks. They suggested that stress played an important role in the manifestation of the outbreak.

Bovine petechial fever

Synonym: Ondiri disease

Introduction

Bovine petechial fever (BPF), or Ondiri disease, occurs in exotic and cross-bred cattle in the highlands of Kenya,[8] Tanzania, and possibly other high-altitude parts of eastern Africa. It was frequently reported in the past and the subject of considerable study in the 1960s and 1970s but is rarely reported now, although individual farmers in endemic locations continue to observe the disease which has been reviewed by Davies,[8] and Scott and Woldehewit.[88] The vector of BPF is not known.

Aetiology

An association of BPF with a rickettsia-like parasite in granulocytes was made in 1962 by Haig and Danskin,[43] but Plowright[72]

and Brocklesby[3] had earlier detected evidence of infection in histopathological specimens and blood smears. Following a subsequent detailed morphological study, the parasite was named *Cytoecetes ondiri*.[50] Phylogenetic analyses have not been conducted and all isolates of the organism appear to have been lost, but available evidence suggests the agent is closely related to the granulocytotropic ehrlichias of the *E. phagocytophila* group, with which it shares antigens, and may tentatively be considered as *Ehrlichia ondiri*. Sera from cattle after experimental infection with *E. ondiri* react to *E. phagocytophila* in ovine neutrophils, and in immunoblots to antigens of *Ehrlichia equi*, another member of the *E. phagocytophila* genetic cluster.[103] Strain variation is recognized,[88] in virulence and in protection against heterologous strains on challenge. In these respects the infection mirrors *E. phagocytophila* infections of ruminants.

Epidemiology

The indicator host is exotic cattle, including Sahiwal breeds, with a sylvatic cycle in wild antelope, particularly bushbuck (*Tragelaphus scriptus*). The disease has only been reported in highly restricted areas of the East African highlands, at altitudes between 1 500 and 2 800 metres. Common features of all sites surveyed by Walker[105] were thick bush or undergrowth giving heavy shade, where the soil had a thick litter layer giving high humidity, and a resident population of bushbuck and duiker (*Sylvicapra grimmia*). The seasonal prevalence is probably related to the use of particular high-risk paddocks at the end of the dry season. The disease sporadically spills over into domestic cattle grazing forest edges or scrub.[92] The vector is unknown, but may possibly be one of the species of *Ixodes* ticks which is found in high-altitude regions of East Africa. Recovered cattle may be carriers for several months, and bushbuck for up to 21 months.[7, 8] The infection has frequently been recovered from the spleens of bushbuck shot in an endemic location.

Clinical signs

Sheep and goats can be experimentally infected, but severe disease is only seen in cattle. It is characterized by a high fluctuating fever, lowered milk yield and widespread petechiation of visible mucous membranes. A few severe cases develop a unilateral conjunctivitis known as a 'poached-egg eye' in which the conjunctival sacs are swollen and everted around a tense and protuberant eyeball containing a pool of blood in the lower part of the aqueous humour. Other clinical signs are absent and affected animals continue to eat. Pregnant cows may abort. The case mortality rate may reach 50 per cent.[6]

Pathology

The conspicuous features at necropsy are widespread haemorrhages, lymphoid hyperplasia, and gelatinous oedema of the intermuscular connective tissues, lymph nodes and the folds of the abomasal mucosa.[70]

Ehrlichia ondiri multiplies initially in the spleen and then, during the clinical reaction, it parasitizes neutrophils, eosinophils and monocytes.[91] Electron microscopic studies show, in addition, that the organism may be free in capillary lumens of the myocardium and in cytoplasmic vacuoles in endothelial and Kupffer cells.[49]

Diagnosis and differential diagnosis

The importance of BPF is small, although should vaccination for East Coast fever (*T. parva* infection) be introduced and tick control subsequently relaxed, cases might occur more frequently. The disease is rarely diagnosed in Kenya, but this may in part be the result of the widespread availability of tetracycline drugs, which are effective in treatment and commonly used to treat disease in cattle of unknown aetiology, as well as the reduction in numbers and habitat for reservoir hosts such as the bushbuck. Differential diagnosis includes other haemorrhagic diseases of cattle such as acute trypanosomosis, acute theileriosis, haemorrhagic septicaemia, bracken fern poisoning, Rift Valley fever and, possibly, heartwater. Diagnosis is by careful examination of Giemsa-stained blood smears for infection in granulocytes, although the ehrlichaemia may be waning when clinical signs are most apparent.[88] Serological diagnosis of exposure to *E. ondiri* can be made by IFAT or Western blotting against antigens of the granulocytotropic ehrlichias such as *E. phagocytophila* or *E. equi*,[103] to which significant cross-reactions occur.

Control

Treatment with tetracycline drugs is effective if given early, since active multiplication occurs before or at the onset of clinical signs,[88] but once marked clinical signs have developed, anti-rickettsial drugs are of little use. The only available reliable prophylactic measure in endemic locations is to avoid grazing cattle in forest edges and in paddocks with patches of thick scrub. The use of acaricides as a preventive measure needs to be investigated, even though the vector is as yet unknown.

Tick-borne fever

Synonym: Pasture fever

Introduction

Tick-borne fever (TBF) is a benign rickettsiosis of cattle, goats, sheep and wild ruminants in Europe in areas infested with the vector tick, *Ixodes ricinus*, whereas pasture fever is a minor epidemic syndrome that occurs when dairy cows are turned out of their indoor winter quarters in the spring and early summer onto tick-infested pastures.[45, 98] The infection

may also be present in South Africa: a single sentence in a paper by Retief *et al.*[77] states that in 1969 Neitz detected tick-borne fever in sheep on farms in the coastal region of the Port Elizabeth and Humansdorp districts of the Eastern Cape Province. This report has, however, not been substantiated and the vector was not determined. Since *Ixodes* ticks are found in South Africa and infest livestock, it would not be too surprising if associated granulocytotropic ehrlichias occur and infections of livestock are occasionally detected.

Aetiology

Ehrlichia (formerly *Cytoecetes*) *phagocytophila* was the first identified agent of the granulocytotropic ehrlichial species,[35] and is considered type-species for the genetic cluster of organisms which now includes *E. equi*, and the agent of human granulocytic ehrlichiosis (HGE), and the burgeoning number of granulocytotropic ehrlichias which are associated with infection (and at times, disease, often called granulocytic ehrlichiosis) in horses, dogs, cats and wild ruminants and small mammals across the northern hemisphere. The infection has been recently reviewed[69] following the discovery of the zoonotic potential of granulocytotropic ehrlichias. The infection of ruminants has also been reviewed.[110, 115] The causative ehrlichia multiplies in membrane-lined vacuoles in the cytoplasm of circulating granulocytes, particularly neutrophils and, less commonly, in monocytes. Its evolution is typical of erlichias of the Ehrlichieae tribe.[114] All European and north American isolates are antigenically related but they are not identical, and considerable antigenic homology occurs within the *E. phagocytophila* group; cross-reactions occur in serological tests with the HGE agent and *E. equi*.[23] and with *E. ondiri*.[103] However, the strains of organism which can cause disease in ruminants cause little if any evidence of infection in horses,[74] and *vice versa*, strains from horses generated very low levels of infection without fever in lambs, and did not protect against challenge with pathogenic *E. phagocytophila*.[96] In addition, there is great genetic similarity in the horse, dog and human pathogenic isolates, whereas ruminant strains are more diverse. This suggests that strains should better be considered as pathotypes, and that those with zoonotic potential are not the same as those commonly causing disease in ruminants; it is not known if the reservoir species are also different.

Epidemiology

As natural transmission is by ixodid ticks, there is in Europe a seasonal prevalence of the disease which is linked to the early summer rise in the numbers of questing ticks.[57] Although *E. phagocytophila* allegedly multiplies in ticks in the ooplasm and mitochondria of developing oocytes,[54] the agents recognized might be true *Rickettsia* spp. since *Rickettsia helveticus* has recently been identified as a common infection of *I. ricinus* ticks in Europe,[67] and only transstadial transmission of *E. phagocytophila* has been demonstrated.[55] There is no transplacental transmission in sheep.[95] The presence of infection in and transmission via a number of *Ixodes* spp. from the temperate regions of Europe, North America, and eastern Asia has been shown, but although there are several reports of infected *Dermacentor* ticks from North America, they do not appear competent for onward transmission.[104] The large number of reports of identification of granulocytic ehrlichias in *Ixodes* ticks from across the northern hemisphere suggests that most if not all *Ixodes* spp. will be shown to have involvement with transmission of ehrlichias to their natural hosts, with occasional zoonotic or animal health consequence.

Recovered animals resist most homologous reinfections but the duration of the acquired immunity varies.[113] Passive protection of the suckling young through the transfer of antibodies in the colostrum does not occur.[63] When previously infected animals react on re-exposure to either a homologous or heterologous isolate, the clinical response and parasitaemia are truncated.

Clinical signs

The natural incubation period ranges from 3 to 13 days.[56] Most primary tick-borne fever infections in young animals in the field are asymptomatic. In older animals the onset of illness is marked by the abrupt onset of a high fluctuating fever of one to two weeks' duration. In non-pregnant and non-lactating animals, clinical signs are mild and non-specific.[57, 102] The prolonged fevers are claimed to impair spermatogenesis transiently in rams[107] and bulls.[77] Naive pregnant animals abort while still parasitaemic two to eight days after the onset of fever.[87] Occasionally, the foetus mummifies and is expelled weeks later.

The dominant clinical signs in the pasture syndrome are high fever and a drop in milk yield.[99] Affected cows are slightly depressed, are partially inappetent and cough infrequently.

The importance of tick-borne fever in non-pregnant animals lies in its ability to exacerbate other concurrent latent infections.[36] The most troublesome sequel to natural infection in lambs is tick pyaemia.[4, 110]

In contrast, *E. phagocytophila* may also mitigate the effects of a concurrent infection, probably via immunosuppressive effects through altered granulocyte[111] and lymphocyte[112] functions. For example, *Eperythrozoon ovis* disappears from the blood of sheep infected with *E. phagocytophila*[30] and, similarly, splenectomized calves infected with both *E. phagocytophila* and *Babesia divergens* have less marked haematological changes than those inoculated with either pathogen separately.[73]

Pathology

The haematological changes are diagnostic: at the onset of fever there is a profound but transient lymphopenia attributable to a significant decrease in peripheral B lymphocytes.[2] In

addition, the reactivity of the lymphocytes is reduced.[112] Two to three days later there is a profound and lasting neutropenia and often a total eosinopenia. Monocytes increase slightly in number towards the end of the overt clinical reaction.[87] Concurrently, there is thrombocytopenia.[31]

After experimental infection in goats, a decrease in serum alkaline phosphatase during the fever, a marked decline in plasma, zinc and iron concentrations, and a slight, steady decrease in haemoglobin and haematocrit values have been detected.[102]

Parasitaemias are patent throughout the fever, being most marked on the second day of the febrile reaction when up to 70 to 80 per cent of granulocytes may be infected. Infected neutrophils are functionally impaired.[32, 111] Monocytes are also parasitized, but more rarely.[28, 45] Most affected animals become carriers and the carrier state persists for weeks, sometimes months, and occasionally, years.[28] Relapses are not uncommon; some relapsing animals develop short fevers but many do not.[87]

Death from uncomplicated tick-borne fever or pasture fever is rare. Splenomegaly, hydropericardium, endocardial haemorrhages, petechiation of the mucosa of the small intestine and haemorrhagic colitis have been recorded.[33, 35, 63] A heifer that died after exposure to ticks had lesions reminiscent of those seen in bovine petechial fever.[109]

It has been reported that the lymphoid follicles in the lymph nodes were drained of lymphocytes.[45] Examination of impression smears made from the cut surfaces of the spleen, liver and lungs will reveal the intracytoplasmic presence of *E. phagocytophila*. Occasional ehrlichias may be observed in alveolar macrophages of the lungs.[64]

Diagnosis

Outbreaks are seasonal and follow the arrival of naive animals onto pastures infested with questing ticks. These factors, together with the clinical signs, warrant a presumptive diagnosis which may be confirmed by the demonstration of *E. phagocytophila* in granulocytes in Giemsa-stained blood smears. Serological evidence of exposure can be made by IFAT with *E. phagocytophila*-infected neutrophils, although antibody levels may rapidly wane.

Control

Short-acting tetracyclines are considered effective[115] for treatment of cases, but a situation of endemic stability should be encouraged wherever possible through exposure of young animals (at least prior to breeding) to infected ticks. Early exposure, during the period when colostrum-derived immunity in young animals is still present, may also assist in control of tick-borne viral infections. In contrast, pregnant animals should never be moved from tick-free to tick-infested areas. If the prevalence of tick pyaemia is high, losses may be reduced by the use of an acaricide and by injecting the animals prophylactically with a long-acting tetracycline.[115] If pastures are re-stocked with adult animals with no prior exposure to ticks, this should occur when they are not pregnant in order to reduce the occurrence of abortion.

Treatment with tetracyclines is not warranted in uncomplicated tick-borne fever in lambs but its use in infected lactating cows reduces losses by abating the clinical signs and the parasitaemia.[98]

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42

Chlamydiosis*

A A ANDERSEN

Introduction

Chlamydiosis in livestock is caused by a number of species or strains of bacteria in the family Chlamydiaceae. This family has recently been divided into two genera, *Chlamydia* and *Chlamydophila*, and nine species.[29] In this chapter, the words chlamydiosis and chlamydia(e) will be used to refer to diseases and strains from both genera, except when a reference to a specific species is necessary to avoid confusion.

The disease chlamydiosis is characterized by the development of a variety of clinical syndromes depending on the species and strain of *Chlamydia* or *Chlamydophila*, the host species, and the affected organ system. A wide range of hosts may be affected by chlamydial diseases, including horses, cows, pigs, sheep and goats. Chlamydial strains are fairly host specific, each primarily infecting a given species or type of animal and causing specific clinical diseases. These diseases may affect the gastrointestinal, respiratory, reproductive and nervous systems, and joints and eyes. Disease severity can vary from life-threatening to asymptomatic, but the usual syndrome is a moderate to mild clinical disease leading to persistent infection. Recurrence of the disease and/or shedding of the organism can occur following stress or physiological changes such as oestrus. What is currently unknown is the extent of synergistic effects these persistent chlamydial infections may have with concurrent infections with other pathogens.

Avian chlamydiosis is the disease caused by *Chlamydophila psittaci* in birds.[4] It has been called psittacosis and ornithosis depending on the type of bird it was infecting or the type of bird from which it was contracted. Psittacosis and ornithosis are now considered to be the same disease, the term avian chlamydiosis being preferred. However, the avian strains have been known to infect humans and other mammals with severe consequences. Transmission of these strains from animal to animal occurs readily. The disease in birds is not dealt with in this chapter; several reviews on the avian disease and its diagnosis in birds and domestic fowl are available.[1, 3, 4, 108]

Chlamydiosis was first recognized in 1879 as a disease that could spread from parrots to humans, when a Swiss physician observed respiratory illness in a household with a sick parrot. Avian chlamydiosis gained world prominence during a pandemic in 1929 to 1930 that involved over 12 countries in Europe and North America. The outbreak led to strict regulations on the importation of parrots from South America. In 1930, Leventhal, A.C. Coles and Lillie independently observed for the first time very small basophilic bodies in the tissues of infected birds and humans, and suggested that they were the causative agent (cited in Pienaar *et al.*[66]). In that year, Bedson and Bland (cited in Meyer[55]) established the aetiological relationship between the basophilic bodies and the disease. It soon became clear that chlamydial infections were not limited to psittacine birds, but that they were widespread in almost all avian species, and that chlamydiae from other birds were transmissible to humans. Chlamydial infection in pigeons was first reported in South Africa in 1940 by J.D.W. Coles after a pigeon fancier who had been experiencing mortalities in his flock sent two affected birds to the Onderstepoort Veterinary Institute.[13]

Enzootic abortion in ewes was first shown to be caused by a chlamydial infection in 1950 by Stamp and his colleagues.[95] Since then chlamydial abortion has been recognized worldwide. It was first seen in South Africa, in 1972, when a severe epidemic of abortions swept through the sheep-raising areas within a single lambing season, resulting in up to 60 per cent abortions in some flocks.[88] In cattle, Schoop and Kauker[86] furnished serological and cytological proof that incriminated chlamydia as the cause of abortions in a dairy herd in 1956. In California, Kennedy and co-workers in 1960[45] described the pathological lesions of aborted

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* For recent changes in the nomenclature of these organisms, refer to the introduction to Section 3, Rickettsial and chlamydial diseases

calves and, from some of them, Storz *et al.* in the same year[100] isolated the chlamydial agent. Because of the sporadic occurrence of outbreaks, the disease was named epizootic bovine abortion. The agent is similar to, or the same as, that causing abortion in sheep, in which the disease is called epizootic abortion of ewes.

Thereafter, chlamydiae in ruminant livestock were also found to be involved in enteritis, pneumonia, arthritis, conjunctivitis, and encephalomyelitis. These diseases are usually caused by the *Chlamydophila pecorum* strains, which are different from the abortion strain, and outbreaks are usually sporadic, primarily affecting young animals. Morbidity may be high, but mortality is usually low.

Aetiology and life cycle

Articles in the historical literature on chlamydia and its taxonomy contain many later-abandoned terms which are, however, still important in order to understand the literature. Chlamydiae were once classified as viruses and grouped with other filterable organisms that required living cells in which to multiply. Some of the names in the literature include the psittacosis-lymphogranuloma-trachoma (PLT) group, miyagawanella, bedsonia, prowazekia, rakeia, and trachoma inclusion conjunctivitis.[4, 66] However, the use of a chlamydia-like name dates back to 1907, when Halbersteadter and Von Prawazek (cited in Storz[98]) described characteristic intracytoplasmic micro-organisms within vacuoles in conjunctival scrap-ings from a human patient with trachoma. They thought that the organisms were protozoa and used the family name Chlamydozoaceae after the Greek word 'chlamys', which means mantle. The term *Chlamydia* was proposed in 1945 and later reaffirmed by Page.[60, 61]

The early classification of the family Chlamydiaceae has been filled with controversy,[60, 61] much of which subsided when Page proposed that all chlamydiae be grouped into one genus (*Chlamydia*)[60] containing two species (*C. trachomatis* and *C. psittaci*).[61] The division was based on susceptibility to sulphadiazine and accumulation of glycogen in the vacuoles when grown in tissues of vertebrate tissues. It also effectively separated the known human strains from the animal strains, with only a few exceptions. At the time it was recognized that there was great diversity in *Chlamydia psittaci*. This classification remained unchanged until 1989 when *Chlamydia pneumoniae* was proposed as a third species.[33] At that time it was thought to be solely a human respiratory pathogen. However, isolates have since been made from koalas (*Phascolarctus cinereus*)[32, 113] and a horse.[96] In 1992, *Chlamydia pecorum* was proposed as a fourth species.[31] It was originally considered to include isolates from cattle and sheep that caused pneumonia, polyarthritis, encephalomyelitis, and enteritis. Since then, *C. pecorum* strains have been isolated or identified from other ruminants and from pigs[85] and koalas.[39, 113]

The recently proposed reclassification of the family Chlamydiaceae in 1999 is based on sequence analysis of its 16S and 23S rRNA genes.[29] Analyses of other genes yield the same results. The resulting division shows a high degree of correlation with host range and disease syndrome, and thus helps in the understanding of the epidemiology of the various strains affecting livestock. The major change in the taxonomy is the formation of two genera which correlate with the former *C. trachomatis* and *C. psittaci*. These genera, *Chlamydia* and *Chlamydophila*, contain three and six species respectively. The species *C. pecorum* and *C. pneumoniae* are included in the genus *Chlamydophila*. This new classification, together with the currently known primary host ranges and disease syndromes, is given in **Table 42.1**.

Table 42.1 Revised classification of the family Chlamydiaceae[29]

GENUS AND SPECIES	PRIMARY HOST(S)	PRIMARY DISEASE(S)
Chlamydia trachomatis	Humans	Trachoma, lymphogranuloma venerium, conjunctivitis, neonatal pneumonia
Chlamydia muridarum	Mice, hamsters	Pneumonia, ileitis
Chlamydia suis	Pigs	Pneumonia, conjunctivitis, enteritis
Chlamydophila pneumoniae	Humans[1]	Pneumonia, systemic disease
Chlamydophila pecorum	Sheep, cattle, swine[2]	Wide range of diseases[3]
Chlamydophila caviae	Guinea pigs	Conjunctivitis, genital tract infection
Chlamydophila felis	Cats	Rhinitis, conjunctivitis, pneu…

Following precedent, there is some controversy about the new classification, primarily concerning the division of the family Chlamydiaceae into two genera and the use of the term *Chlamydophila* for the second genus. It is feared that the new term could create confusion. The two genera and the nine species, however, have merit not only in molecular terms, but also in classifying host ranges and clinical diseases. The terms chlamydiosis and chlamydia(e) are used here as generic terms to refer to members of both and either genera. However, the new scientific names are used when referring to a specific chlamydial species.

Chlamydiae are obligate intracellular bacteria that multiply in cytoplasmic inclusions in the cytoplasm of eukaryotic cells. They have a unique growth cycle consisting of two developmental forms. The elementary body (EB) is a condensed form of 200 to 300 nm in diameter that is suited for survival outside the host. The reticulate body (RB) is the replicating form, ranging in size from 500 to 1 000 nm, and found in a cytoplasmic inclusion within the host cell (**Figures 42.1 and 42.2**). Replication is by binary fission typical of other bacteria, with the exception that chlamydiae rely on the host cell for energy. Intermediate forms between the RB and the EB are usually seen in the inclusion.[111]

The developmental cycle of chlamydia consists of five major phases:

- the attachment to and penetration into the host cell by the EB;
- the transition of the metabolically inert EB into the metabolically active RB;
- multiplication of the RB by binary fission using energy from the host cell;
- maturation of the metabolically active RB into the stable infectious EB; and
- the release of the EB from the host cell.[57, 101]

A critical stage in the chlamydial life cycle is the phase following entry into the host cell. The EB must transform into the metabolically active RB and escape from the host cell defences while transforming. The normal cell processes endosomes containing foreign material by acidification of the endosome and fusion of the endosome with the lysosomes. The digestion and processing of foreign antigens by the lysosome is critical for the processing of antigens during the immune response. It has been shown that live chlamydiae prevent the acidification of the endosomes and the subsequent fusion with lysosomes.[87, 105]

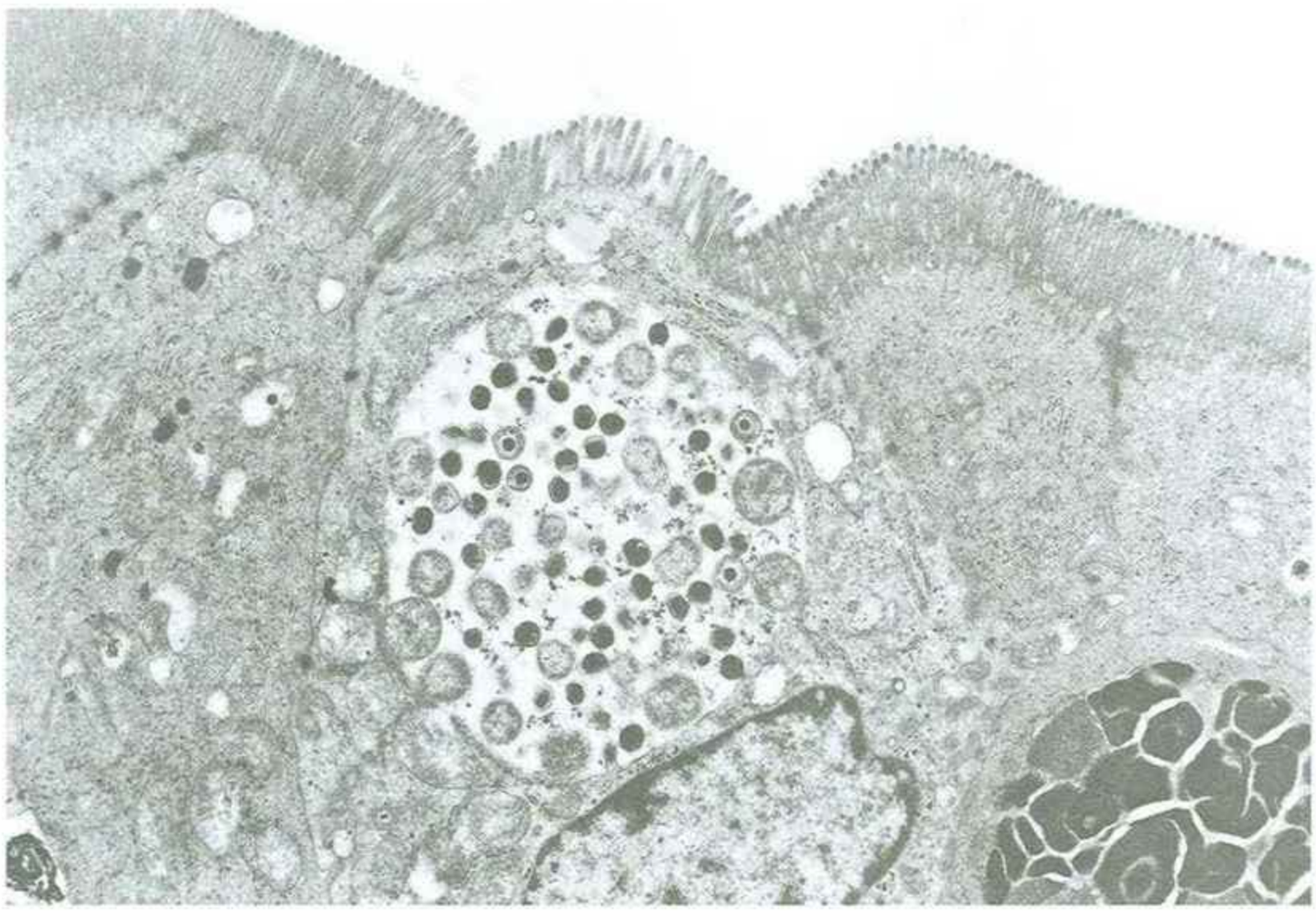


Figure 42.1 Intracytoplasmic vacuole containing chlamydiae in an enterocyte of the ileum of a naturally infected pig. The vacuole contains elementary bodies, reticulate bodies, and a small amount of glycogen. (Courtesy of Douglas Rogers, Lincoln, Nebraska, USA)



Figure 42.2 Intracytoplasmic vacuole containing chlamydiae in a conjunctival epithelial cell of a naturally infected pig. The vacuole contains elementary and reticulate bodies, and a large amount of glycogen. (Courtesy of Douglas Rogers, Lincoln, Nebraska, USA)

Epidemiology

Chlamydiae are among the more successful pathogens known. It was once thought that chlamydial isolates are all similar and that each infected a wide host range. New molecular and serological techniques now show that there are numerous identifiable chlamydial strains and that the isolates from a particular avian or mammalian species tend to be of the same strain. This pattern indicates a triangular relationship among the chlamydial strain, the host and the disease syndrome. Research is increasingly showing that when a strain infects its natural host persistent infections are common.[112] These persistent infections clearly play a role in the maintenance of the chlamydial organism in nature. It should be remembered that a number of the avian and mammalian strains can infect other hosts, including humans, with severe consequences, but secondary spread from these infections appears to be limited.

The role played by wild animals in the transmission of chlamydiae needs to be assessed. Natural infections have been reported in opossums (*Didelphis paraquayensis*), northern fur seals (*Collorhinus ursinus*), muskrats (*Ondatra zibethica*), snowshoe hares (*Lepus americanus*), koalas (*Phascolarctus cinereus*), springbok (*Antidorcas marsupialis*), ibex (*Capra pyrenaica*), and bighorn sheep (*Ovis canadensis*).[66] Molecular and serological typing now make it possible to determine whether these and other wild animals are reservoirs of chlamydiae for domestic animals, or whether these species have their own host-strain relationships. Molecular studies of isolates from koalas have shown these animals to harbour both *Chlamydophila pneumoniae* and *Chlamydophila pecorum* strains.[39, 113] These strains, however, are distinct from those found in other animals and humans.[29] The snowshoe hare and muskrat isolates are identical and similar to avian strains.[29, 93] Conversely, this M56 strain found in snowshoe hares and muskrats has never been isolated from birds. On the other hand, a *C. pecorum* isolate from bighorn sheep appears the same as the strain causing polyarthritis and conjunctivitis in domestic sheep.[2]

Transmission of chlamydiae probably occurs via several routes, as chlamydiae have been found in all body secretions, including faeces. The faecal–oral route may be the most common for most strains.[26, 27] Aerosol and contact transmission may be more common in respiratory tract and eye infections, respectively. Insect transmission,

especially by flies, must be considered. The importance of vertical transmission is unknown, but generally has not been thought to be a major factor.[7] There are, however, increasing reports of persistent infections of the male accessory sex glands and of the presence of chlamydiae in semen.[99] In ewes that have aborted, chlamydiae have been isolated from vaginal swabs at the time of oestrus for over two years.[48, 63]

Pathogenesis

Chlamydial infections are responsible for a wide variety of syndromes in domestic animals, including enteritis, pneumonia, polyserositis, lymphadenopathy, keratoconjunctivitis, mastitis, polyarthritis, meningoencephalomyelitis, epididymitis, orchitis, seminal vesiculitis, and placentitis. The strains of chlamydiae each have a predilection for different tissues and therefore different disease syndromes. The route of infection may play a role in the type of disease syndrome; nevertheless, oral or parenteral inoculation of a given strain usually results in the development of the same disease syndrome from which the strain was originally isolated.

The oral route of exposure is considered the primary means of transmission for most strains.[25, 27] It has been shown that most strains readily infect the epithelial cells of the gastrointestinal mucosa. The organisms localize in the epithelial cells of the mucosa in the jejunum and ileum, and then spread via the blood and lymph vessels of the lamina propria to the mesenteric lymph nodes and the liver. Thereafter, infection may be established in other organs or tissues.

With abortion strains, evidence indicates that infection may be initiated either by the systemic route or by venereal transmission. Systemic infection causes a chlamydaemia that results in spread to the placenta, causing extensive placental damage. The extent of placental damage may determine whether the lamb is born apparently healthy or weak, or is stillborn or aborted.[11, 72] Placental infection can impair maternal–foetal transfer of nutrients, resulting in impaired growth or death of the foetus. Placental infection may also alter placental steroid and prostaglandin release, resulting in premature labour.[48] In some cases, a ewe bearing twins may deliver both a weak and a healthy lamb, as premature labour may be prevented if the healthy placenta produces sufficient progesterone to maintain pregnancy.

Abundant evidence exists that most chlamydial species are capable of producing persistent infection that can endure for months or years in the absence of treatment.[112] The mechanism of persistence is not known. For years it was thought that persistence was associated with incomplete chlamydial development and only sporadic production of EBs. However, the fact that EBs can frequently be recovered from asymptomatic animals using cell culture techniques, and that EBs and chlamydial inclusions are commonly detected in clinical specimens, indicate that productive infections are the norm. Persistent infections with intermittent shedding of viable organisms have been identified following chlamydiosis in humans, animals and birds. Immunosuppression with corticosteroids frequently reactivates shedding in these cases.

Chlamydiae are capable of infecting and multiplying within macrophages. This ability has attracted attention because the chlamydial inclusion is capable of avoiding lysosomal fusion,[87, 105] which is necessary in the normal pathways of processing an antigen in order to evoke an immune response. It is apparent that live chlamydiae are needed to initiate the avoidance mechanism, as inactivated chlamydiae are processed by macrophages.

Clinical signs and pathology

Clinical signs and related pathology in affected animals vary according to the strain of chlamydia and the syndromes from which the animals are suffering. Information on the prevalence, pathogenesis and pathology for many of the disease syndromes is limited. This discussion focuses primarily on those diseases considered to be of major importance.

Sheep, goats and cattle

Chlamydiae in ruminants have been primarily associated with abortions, polyarthritis, conjunctivitis, encephalomyelitis, enteritis, pneumonia, mastitis, and genital infections such as epididymitis and orchitis. Abortions are probably the most dramatic clinical outcome, having both economic and public health significance. They are usually caused by *Chlamydophila abortus*, but an abortion in a cow caused by infection with *Chlamydophila psittaci* serovar B has been documented.[14] This occurred in a dairy herd where pigeons were common in the barnyard. In addition, a bovine abortion caused by a chlamydia-like organism WSU86-1044 has been reported.[22, 46, 80] The *C. abortus* strain may also be involved in mastitis[42] and other forms of genital infection.[102, 109, 116] *Chlamydophila abortus* strains are serologically the same worldwide, with the exception of one isolate from Europe that appears to be serologically distinct.[110] The polyarthritis, conjunctivitis, encephalomyelitis, enteritis and pneumonia disease syndromes are considered to be caused primarily by various strains of *Chlamydophila pecorum*. There is some indication that there are strain differences within the *C. pecorum* group, and that each strain tends to produce a specific type of disease or disease syndrome.

Chlamydial abortions in sheep and goats, also known as epizootic abortion, can occur as an acute outbreak in flocks or herds, or as an endemic infection. In endemically infected flocks and herds, abortions occur year after year at rates of up to 5 to 10 per cent per annum. It is not unusual after three to five years to have an explosive outbreak of abortion in yearlings. When *C. abortus* is newly introduced, abortion rates of up to 30 per cent are common in sheep and sometimes of more than 60 per cent in goats.[72]

A number of human abortions followed by severe

complications have been associated with exposure to infected placentas of ewes and does.[10, 15, 41] The prevalence of such abortions is not known, as infectious causes are often not looked for in humans. Chlamydial infections must, however, be considered an occupational hazard for pregnant women who come into contact with domestic ruminants.

Even ewes exposed to chlamydiae before they have become pregnant may abort.[62, 115] In one study, seven ewes were exposed by rubbing infected placental material on their noses two days after they had lambed.[115] During the following year two of them aborted as a result of chlamydial infection. It has been suggested that in such cases infection is first established in the tonsils, from where it is disseminated haematogenously to other organs. Whether the infection persists in the tonsils or in other organs is not known, but it is thought that a state of persistent infection with intermittent low-grade chlamydaemia eventually results in infection of the placenta. Research has shown that placental infections usually become established between the 60th and 90th day of gestation, and that pathological changes do not develop until the 90th day of gestation.[11, 72]

The method by which chlamydiae cross the placenta is uncertain. After about the 60th day of gestation there is a physiological invasion of carunculae stoma by chorionic villi. This coincides with haemorrhage from maternal vessels, resulting in the formation of haematomas that have been suggested as a means by which chlamydiae in the maternal circulation make direct contact with the chorionic epithelium.[11]

The 30-day period between the formation of the haematomas and the first observation of pathological changes in the placenta raises the question as to whether endocrinological and/or immune-related changes play a role.[11] Chlamydial inclusions and lesions can first be detected in the placenta on about the 90th day of gestation. The lesions first involve the limbus of the placentomas in the hilar region. At this time local necrosis occurs and the infection then spreads to involve the cotyledonary and intercotyledonary placenta and opposing endometrium. A mixed-cell inflammatory infiltrate is present. Underlying the lesions, fibrin deposits and a nonpurulent arteritis are present in the thickened placental mesenchyme. Chlamydial inclusions are present in the epithelial cells of the endometrium where the affected chorion is in opposition to the maternal tissues. Severe necrosis and sloughing of the endometrial epithelium occurs during later stages of pregnancy.

In sheep, infection of the foetus is secondary to the placentitis and is not a major factor in the disease. Focal disseminated necrosis may be evident in the liver and other organs of the foetus and may be embolic in origin.[11] Chlamydial antigen, if present in the foci of necrosis, usually occurs only in small amounts. The mesenteric and popliteal lymph nodes may be enlarged, having a demarcated cortex with several follicles and germinal centres.

The placental lesions may lead to death of the foetus and abortion. If the disease process is not extensive it may lead to the premature birth of a weak lamb that dies shortly after birth or to one that may live but remains unthrifty. Ewes are rarely affected clinically and remain fertile. This is in contrast to the disease in goats and cows in which there is tendency for placentas to be retained and for the development of vaginitis and persistent endometritis. Having once aborted, sheep are solidly immune and will not abort again, even though they may remain persistently infected.[63, 64, 72]

In cattle, chlamydiosis causes abortions, stillbirths, or the birth of weak calves.[19, 20, 24, 34, 37, 98, 100] Abortions are usually sporadic, but may reach 20 per cent in a herd and usually occur during the last trimester, affected cows showing little evidence of disease. The abortion is caused by placentitis, but the aborted foetus may manifest ascites, serofibrinous pleuritis and peritonitis, an enlarged reddish-yellow liver, and hyperplastic lymph nodes. Calves born weak but that survive usually remain unthrifty.

In the UK, a syndrome in cows characterized by a drop in milk production, mucoid/mucopurulent oculonasal discharges, depression and anorexia over an eight-week period has been reported. Recovery is slow but appears to be aided by administration of tetracyclines. Abortion is common weeks to months after the event. The only consistent finding is that chlamydial antigen and/or rise in antibody titre to chlamydiae have been demonstrated in affected herds.[19, 20]

A seminal vesiculitis syndrome (SVS) has been reported in bulls.[99] It is characterized as a chronic inflammation of the accessory sex glands, epididymes, and testicles. Infected bulls have inferior semen quality and may develop testicular atrophy. The syndrome is thought to be widespread in the world. An isolate from a bull suffering from the syndrome was found to be indistinguishable from the EBA59-795 isolate of chlamydia, indicating that the strain is the same as that causing abortion. The SVS can possibly be regarded as the counterpart of the abortion syndrome in cows. Similar diseases or syndromes have been reported in sheep.[50, 66] In open cows, cervicitis and endometritis with a nonputrid discharge have been associated with chlamydial infections.[109, 116]

Polyarthritis in sheep, goats and cattle may be caused by strains of *C. pecorum*.[31] The disease can be readily reproduced experimentally by inoculating lambs or calves by oral, intramuscular, subcutaneous, intravenous or intra-articular routes.[18, 27] In the natural disease, the route of infection is thought to be oral, the organism multiplying in the mucosa of the small intestine. Diarrhoea may be present during this phase. Following multiplication at the primary site, the organism spreads haematogenously to other organs and tissues of which the diarthrodial joints are usually the most severely affected (**Figure 42.3**). Lesions occur in both the peri-articular and articular tissues of the joints,[16, 17, 90, 97] which usually are grossly enlarged because of a localized subcutaneous oedema and an increase in the amount of fluid in the synovial sacs. The fluid is greenish-yellow and turbid. In



Figure 42.3 Serofibrinous synovitis typical of *Chlamydophila pecorum* polyarthritis infection

advanced cases, fibrin plaques may be present in the joints. In severe cases excess fluid of a similar nature may be present in the tendon sheaths. Muscle involvement is primarily limited to mononuclear cells in perivascular areas near the point of tendinous attachment. Histopathological changes in the synovium, tendon sheaths, and subsynovial tissues include infiltration of neutrophils, macrophages and lymphoid cells. Changes in cells in the synovial lining can vary from necrosis to proliferation.[16, 17, 18]

Conjunctivitis frequently accompanies polyarthritis in lambs and may be a prodromal sign of a developing outbreak of polyarthritis. Chlamydial conjunctivitis is usually bilateral, and clinical signs vary from a profuse watery to mucopurulent discharge which, in some, seals the eyelids together, and hyperaemia of the conjunctiva.[38] In severe cases, photophobia and keratitis occur. Secondary bacterial infections of the conjunctiva are common.

Sporadic bovine encephalomyelitis (SBE) is a disease that has been reported worldwide,[35, 54, 65, 67] including in South Africa.[107] It primarily affects young calves, but has been known to occur in adult cattle, and sheep. Generally, the morbidity in cattle is under 10 to 15 per cent, with mortality of up to 30 per cent in affected animals. A generalized chlamydial infection usually precedes, or occurs concurrently with central nervous system (CNS) involvement. The principal clinical signs include moderate fever, stiffness, incoordination and depression. Gross lesions are indicative of a systemic infection and include serofibrinous peritonitis, pleuritis, and sometimes pericarditis, similar to those seen in other *C. pecorum* infections. Gross lesions in the CNS are not as evident and are often limited to meningeal congestion and oedema. Histopathologically, the CNS lesion is a diffuse nonpurulent meningoencephalomyelitis which is especially marked in the cerebellum. The lesions are characterized by perivascular and focal infiltrations of mononuclear cells, focal areas of encephalomalacia and neuronal degeneration.[35] In sheep, the clinical signs and lesions are similar to, but generally milder than those in cattle.

Enteritis and interstitial pneumonia caused by chlamydial infections have been reported in sheep and cattle.[26, 27, 69, 98] The isolates that have been characterized to date from such cases have been identified as *C. pecorum*. There appears to be overlaps in the clinical signs and lesions with those occurring in the polyarthritis, conjunctivitis and SBE syndromes, indicating different tissue predilections for the various strains of *C. pecorum*. Both syndromes primarily affect young animals. The enteritis syndrome is characterized by a mucoid to watery or bloody diarrhoea in young calves and lambs. The onset of the disease is rapid and the severity depends on the virulence of the strain and the degree of protection afforded by colostral antibodies.[25, 26, 66, 98] On necropsy, a focal to generalized peritonitis may be seen. Petechial haemorrhages in the abomasum and intestine occur in infections by highly virulent chlamydial strains. Hyperaemia and congestion of the mucosa are common, and enlargement of the lymph nodes may be seen. The ileum is usually the most severely infected; lesions, however, may occur throughout the intestinal tract.[25, 26, 98]

Histologically, chlamydial inclusions are primarily seen in the villus enterocytes and occasionally in the cryptal enterocytes. Villus atrophy and multifocal necrosis of the villi are often extensive. Peyer's patches may lose their follicular architecture and contain necrotic centres.[25, 26, 66, 98]

Calves and lambs affected with chlamydial pneumonia have fever and a serous to mucopurulent nasal discharge. They are depressed, cough, and have signs of dyspnoea. Older animals will have similar clinical signs. Conjunctivitis may be seen in acute cases. In severe cases, young animals become prostrate, have laboured respiration, and may die. Most cases are of moderate severity, with recovery taking one to two months.[66, 98]

The acute pulmonary lesions are characterized by multifocal greyish-purple consolidated areas, usually located in the region of the hilus and the ventral portions of the cranial and middle lobes (**Figure 42.4**). Serofibrinous pleuritis may accompany these lesions. In severe cases foci of consolidation extend throughout the lungs. The microscopical lesions in acute lesions are characterized by serofibrinous pneumonia and pleuritis, and small haemorrhages are frequently

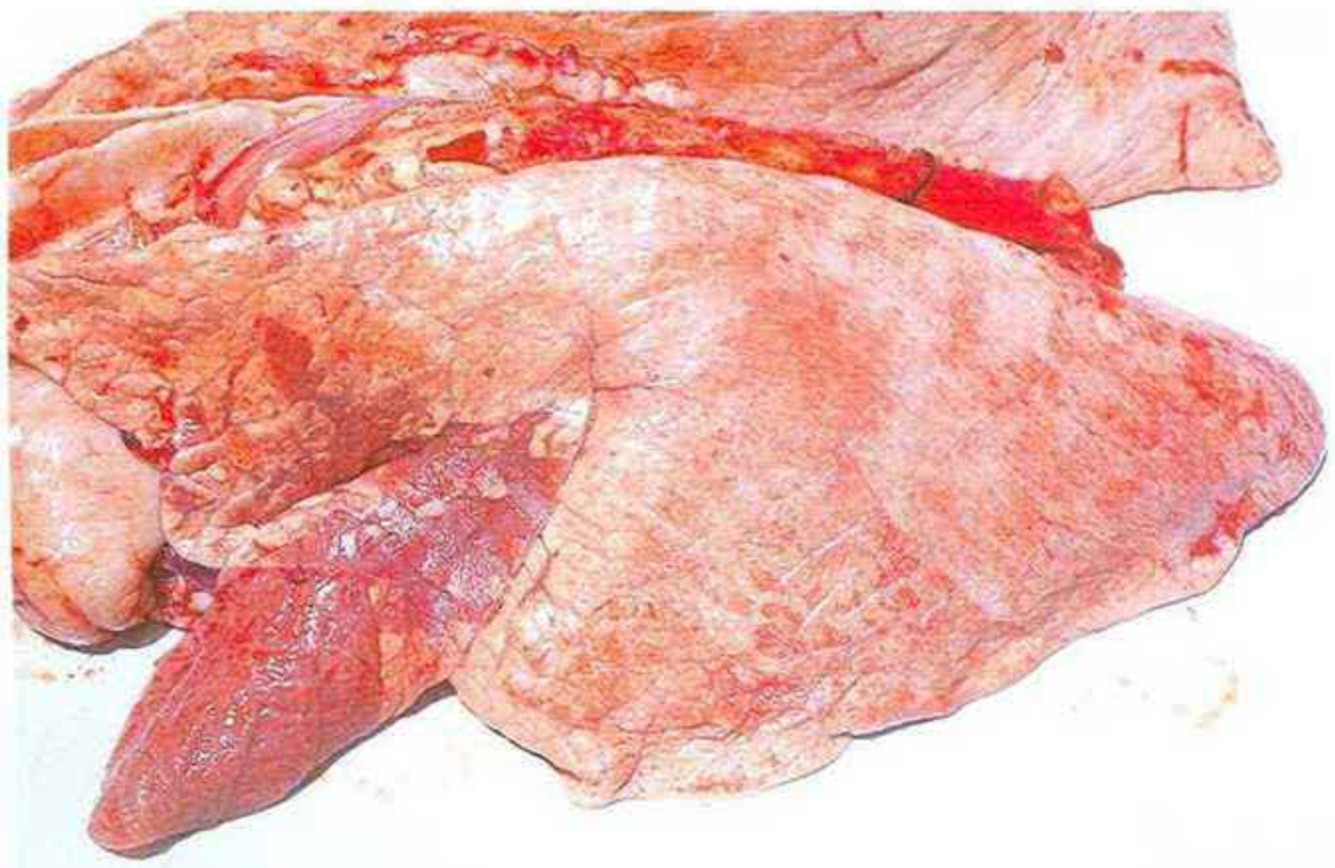


Figure 42.4
Sheep: interstitial pneumonia of the ventral portion of the cranial and middle lobes typical of *Chlamydophila pecorum* infection

present in the parenchyma of the lungs. As the lesions progress, moderate numbers of mononuclear cells, particularly lymphocytes, are found in the alveoli and interstitial tissues. Perivascular and peribronchiolar lymphoid hyperplasia, in which scattered plasma cells, macrophages, and a few neutrophils occur, are also evident.[66]

Chronic pulmonary lesions consist of small greyish-pink areas of pneumonia, depressed slightly below the surface of the pleura. Histopathologically these areas are characterized by atelectasis, infiltration of variable numbers of mononuclear cells and neutrophils, and severe peribronchiolar lymphoid hyperplasia.[66, 98]

Microscopically, chlamydiae are found in monocytes and in the cytoplasm of epithelial cells, mainly of the bronchioli and alveoli. Organisms are frequently difficult to detect, particularly in chronic lesions.[66]

Pigs

Disease syndromes in pigs that are associated with chlamydial infections are those of conjunctivitis, pneumonia, enteritis, reproductive problems, pericarditis, polyarthritis and encephalomyelitis.[5, 106] A number of distinct strains have been identified, including those that belong to *Chlamydia suis*, *Chlamydophila pecorum* and *Chlamydophila abortus*.[29, 43, 44, 84, 85] Of these, *C. suis* appears to be the most common. *Chlamydia suis* strains have been isolated from the conjunctiva and respiratory and intestinal tracts of pigs, where they were associated with conjunctivitis, pneumonia and enteritis.[59, 78] At least 10 distinct strains of *C. suis* have been identified.

Many *C. suis* infections are thought to be mild or asymptomatic. Some studies have indicated that *C. suis* is not a significant pathogen, as it can be isolated from normal and diarrhoeic pigs at the same rate.[59, 103] On the other hand, *C. suis* was the only potential pathogen isolated from four 30-day-old piglets suffering from diarrhoea.[58] In another trial, inoculation of gnotobiotic piglets with *C. suis* resulted in diarrhoea and extensive villous atrophy of the ileum and distal jejunum[75] **(Figures 42.5 and 42.6)**. Inoculation of 21-day-old piglets raised conventionally with the same isolates produced only moderate villous atrophy in the absence of clinical diarrhoea.[77] The role that colostral antibodies to *C. suis* may play in moderating intestinal disease in animals of that age is unknown, and it is not clear what synergistic effect chlamydial infection may have with other pathogens. Recent studies with *Salmonella typhimurium* and *Eimeria scaba* have shown that the diseases produced by those organisms were more severe when chlamydia was present.[47, 68]

The same *C. suis* strains have been isolated from pigs suffering from pneumonia and conjunctivitis although it is difficult to establish their significance as pathogens because they have also been isolated from clinically normal pigs. When a *C. suis* isolate from nursery pigs with pneumonia was inoculated experimentally into gnotobiotic piglets, several of them became moribund or severely dyspnoeic, although the majority exhibited only mild dyspnoea.[79] The gross lung lesions at 17 to 21 days after infection were typical of bronchopneumonia.[103, 117]

Similarly, *C. suis* strains have been isolated both from the eyes of clinically normal pigs and from those of pigs suffering from conjunctivitis.[21, 76, 78] In severe cases, lymphofollicular hyperplasia in the palpebral conjunctiva is often

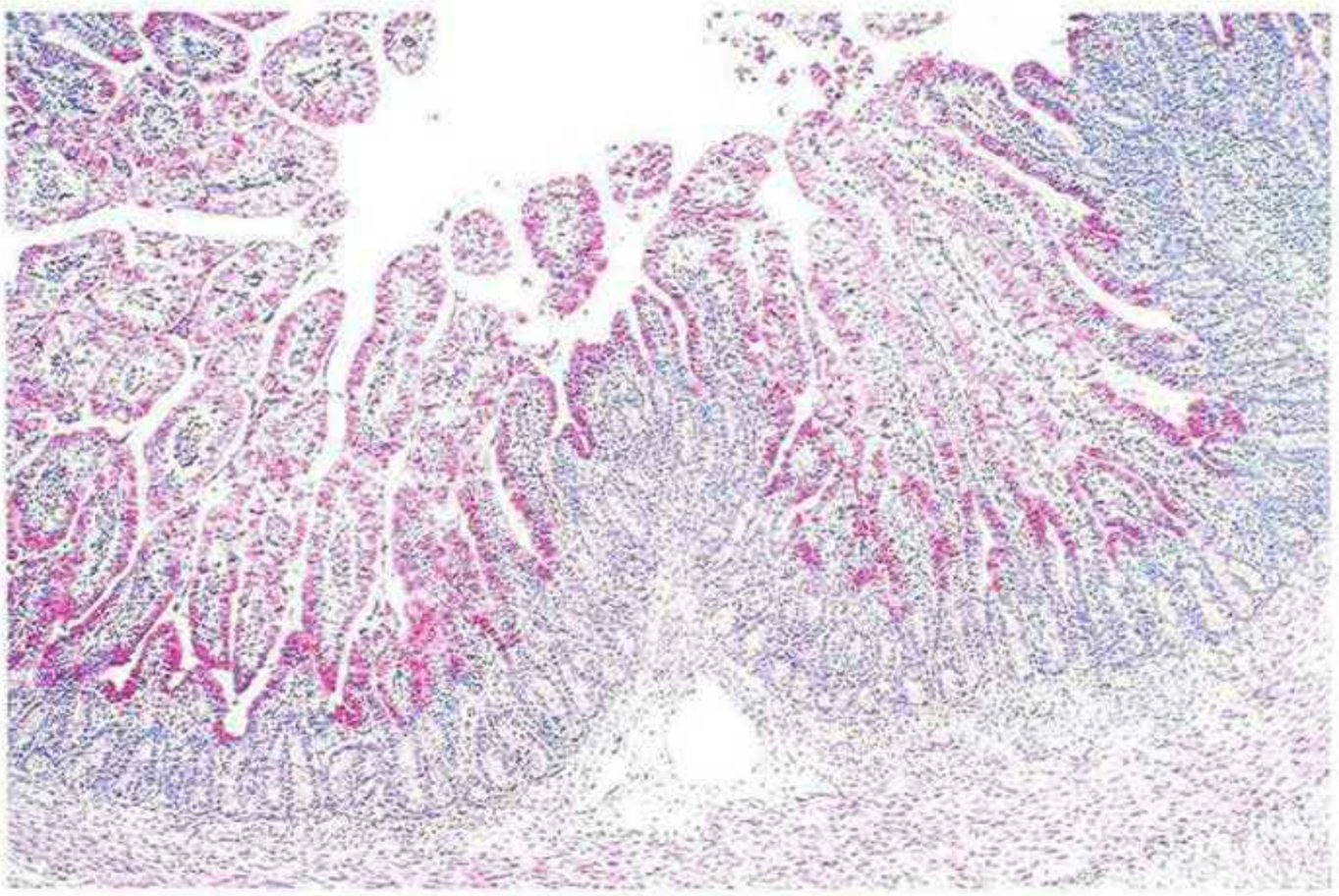


Figure 42.5 Chlamydial antigen in a villus of the ileum (four days post-infection) of a gnotobiotic piglet infected with *Chlamydia suis*. The chlamydiae are stained red (ABC-alkaline phosphatase stain)

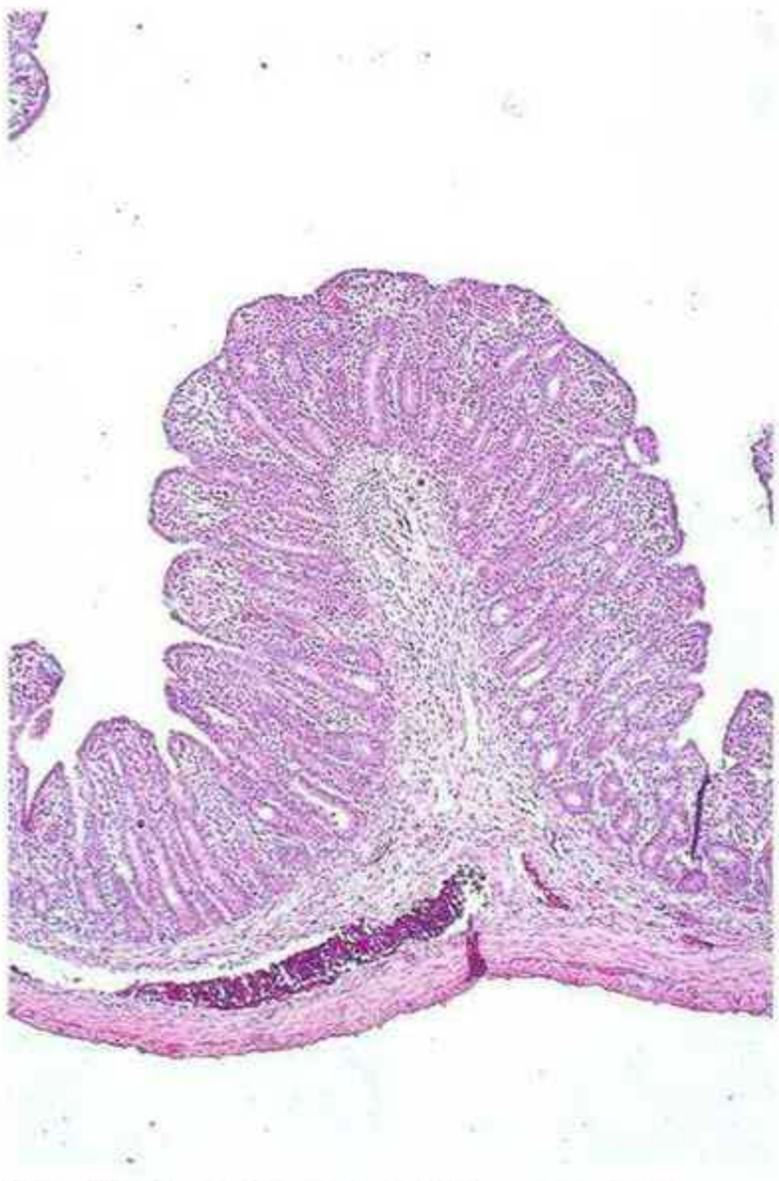


Figure 42.6 Severe villous atrophy in the ileum of a gnotobiotic piglet infected 10 days previously with *Chlamydia suis* (haematoxylin and eosin stain)

present (**Figure 42.7**). When an isolate obtained from the eye was inoculated experimentally into the conjunctiva of normal pigs, they did not develop clinical signs, although histopathological examination revealed mild conjunctivitis. The mild conjunctivitis may have been the result of a first infection, as is the case in humans in whom it is thought that repeated or chronic infections are required for severe cases to develop. The piglets inoculated in the conjunctiva or trachea developed diarrhoea with villous atrophy similar to that seen in the pigs that developed diarrhoea after being inoculated orally.[76, 79]

The epidemiology of *C. suis* strains is of interest because they are ubiquitous in pig populations. Serological and isolation studies indicate that most pigs become infected before six weeks of age, and that virtually all pigs are infected by one or more strains.[89] Infection with one strain may not protect against another. Many infections appear to be persistent, permitting the chlamydiae to be passed on from the sow to her young piglets following farrowing. In the USA, many of the *C. suis* strains are resistant to sulphonamides and tetracyclines, making treatment or control of the disease syndromes very difficult.[6]

Other disease syndromes that have been associated with chlamydiae in pigs include pericarditis, encephalomyelitis, polyarthritis and reproductive problems. These are probably caused by *C. pecorum* strains.[42, 44]

Chlamydophila abortus, of the same strain as that isolated from sheep and cattle, was isolated from a case of abortion in a sow,[2] and has been detected in retrospective studies in other cases of abortion.[84, 85]



Figure 42.7
Lymphofollicular hyperplasia in a section of palpebral conjunctiva from a sow infected with *Chlamydia suis* (haematoxylin and eosin stain).

Horses

Reports of chlamydiosis in horses are limited. However, there is sufficient evidence to indicate that chlamydia should be considered as a cause in the differential diagnosis of such syndromes as abortion, polyarthritis, encephalomyelitis, and respiratory disease. Because many of the isolations of chlamydiae made from horses were obtained before strains could be identified readily, it is not known whether more than one strain of chlamydia infects horses.

An upper respiratory tract/pneumonia syndrome may be one of the more important chlamydial diseases of horses.[9, 51, 53, 56] An isolate (N16) from horses was recently characterized as belonging to *Chlamydophila pneumoniae*.[96] Molecularly, there are enough differences between this isolate and the human strain of *C. pneumoniae* to make it unlikely that transmission of *C. pneumoniae* occurs between horses and humans. Isolations have been made from normal horses, and from those with serous nasal discharges, as well as from the lungs of a mare suffering from pneumonia.[9, 51, 53, 56, 114] Most of the equine strains that have been isolated are difficult to grow in cell cultures and embryonated eggs. Attempts to infect other horses with these strains have resulted only in mild or subclinical disease.[51, 53] This is also characteristic of the *C. pneumoniae* strains occurring naturally in humans and koalas.

Other diseases in horses that may be caused by chlamydiae include a hepatoencephalopathy syndrome reported in Spain.[49] The syndrome is sporadic, appearing mainly in the winter, and affects both very young and older horses. It is systemic, involving most organ systems, including the liver, lungs, and the central nervous system. Polyarthritis in horses caused by a chlamydial infection has been diagnosed in the USA and Europe.[49, 52] During the 1972 to 1973 epidemic of chlamydial abortion in sheep in South Africa, chlamydia was incriminated as the cause of abortion in mares and of keratoconjunctivitis in foals.[66] Abortions in mares due to chlamydial infections have also been reported in Spain[49] and Germany.[8]

Diagnosis

A definitive diagnosis of chlamydiosis requires the isolation or the demonstration of the chlamydial agent from infected tissues, together with typical clinical signs and/or pathological findings. A retrospective diagnosis can also be made serologically if the aetiological agent cannot be demonstrated. Clinical signs of the disease are taken into account together with a four-fold or greater rise in serum antibody titre between acute and convalescent stages of the disease.

Chlamydiae are usually isolated in embryonated chicken eggs or cell cultures. Because of the ease of performing isolations in cell cultures and of demonstrating that inclusions are chlamydia, cell culture is the method of choice. This applies to isolates derived from both birds and mammals. Most isolates will grow in a range of commonly used cell lines, such as Vero, BG and MK,[1, 3] with the exception of *C. pecorum* strains that grow better in embryonated eggs. Conventional culture techniques are adequate but centrifugation (at 1000–2000 Xg for 60 minutes at close to 37 °C) of the inoculum onto the cell monolayer increases efficiency.[1, 3]

Blind passaging at four to six days increases efficiency of isolation. It is important to examine cultures for inclusions at days three to six after infection of cell cultures. It should be borne in mind that chlamydiae are relatively sensitive to freezing; the common practice of freeze–thaw to disrupt the cell monolayers must be avoided.

Collection and handling of specimens is important for the successful isolation or demonstration of chlamydiae. In the case of abortion, the placenta is the preferred tissue. When the placenta is not available, vaginal swabs should be used.[82, 92] Attempts to isolate the organism from the foetus are often not successful. In other chlamydial diseases, swabs from the infected tissues or exudates, or samples of infected tissues can be used. Faecal samples can be used if high levels of antibiotics are added to them. A diluent consisting of sucrose-phosphate-glutamate has proved to be satisfactory as a transport medium for chlamydial field samples[1, 3, 94] and it can also be used as a diluent when freezing cultures for storage. Antibiotics may be added to it to reduce contamination.[1, 3]

Direct staining of chlamydiae or chlamydial inclusions in tissues or smears of exudate, or in cell culture cell monolayers, can be performed using histochemical or immunohistochemical techniques. Some of the more commonly used stains are Giemsa, Giménez, Ziehl-Neelsen and Macchiavello.[1, 3, 66] Many of these are being replaced with specific fluorescein-labelled monoclonal antibodies which have a high sensitivity and specificity.

In recent years, a number of manufacturers have produced enzyme-linked immunosorbent assay (ELISA) kits for the detection of chlamydiae in humans. These can also be used for the detection of other chlamydiae derived from mammals although some may lack sensitivity and specificity. These kits detect the lipopolysaccharide (LPS), and some of the LPS epitopes are shared with other Gram-negative organisms. In addition, the ELISA kits usually require the presence of a few hundred organisms before a positive test is obtained. The general rule to follow is that if the animal concerned is manifesting the clinical signs of one of the chlamydiosis syndromes and the ELISA is strongly positive, the result of the ELISA test can be considered diagnostic. Newer ELISA kits using MABs have fewer cross-reactions; however, these tests still need to be evaluated for specificity and sensitivity with each type of animal specimen.

Polymerase chain reaction (PCR) techniques are relatively new and are being used in a number of diagnostic laboratories, but there are no commercial tests available for use in animals, although there are a number of published primer sets.[30, 36, 42, 104] Most primer sets are designed to react with either the 16S-23S genes or the major outer membrane protein (MOMP) gene. Some of these tests, when combined with restriction fragment length polymorphism (RFLP), will also differentiate the species of chlamydia.[28]

The sensitivity and specificity of these PCR tests still have not been established. In general, tests designed to detect the 16S or 23S rRNA are more prone to give a false-positive result with other Gram-negative micro-organisms. Sensitivity can be increased by targeting a relatively short DNA segment[36] or by the use of a nested procedure.[104] However, the use of the latter procedure will increase the potential for laboratory contamination. Another problem is the need to develop DNA extraction procedures that can be used for all types of samples.

Serology as a diagnostic test for chlamydiosis has never been used extensively as it requires both acute and convalescent phase sera, and is therefore not compatible with the need for rapid diagnosis and treatment. The complement fixation test is the most commonly used.[1, 3, 40] The use of ELISA has been reported.[40] Both of these tests detect antibody to the LPS or group-reactive antigen, but they are prone to provide false-positive results, as some other Gram-negative organisms have the same LPS antigen epitopes and give low-level positive reactions. In recent years, there have been attempts to design serological tests in which chlamydial proteins are used as the antigen.[23] Competitive ELISAs using serovar-specific monoclonal antibodies are also being developed.[81]

The micro-immunofluorescence test (MIFT) is increasingly being employed in human medicine. It is labour intensive and therefore expensive. The MIFT does have the advantage that, if known serotypes are used as antigens, the serotype that infected the animal can be determined. It has been used to show that pigs with antibody to one strain may not have antibody to another strain,[89] and that sheep antibodies in foetal fluids are to the ovine abortion strain.[83]

Differential diagnosis

Many of the clinical signs and lesions caused by chlamydiae are also caused by other bacterial and viral agents. None of the lesions or signs is pathognomic, and therefore laboratory confirmation is almost always needed. In the case of abortion, the other organisms most commonly encountered are *Brucella* spp. In smears or sections of infected tissues, it may be difficult for inexperienced personnel to differentiate between brucella, chlamydia and coxiella morphologically, making the use of immunohistochemical techniques advisable.

Control

Chlamydiae are ubiquitous in the livestock population, which makes vaccination and antibiotic therapy the only effective means of control. Current vaccines are strain-specific, and are only available for the ovine abortion strain and the feline pneumonitis strain. Both attenuated live and inactivated vaccines are produced; availability depends on the country concerned. Only the vaccines produced for the control of ovine chlamydial abortion are discussed below.

Inactivated vaccines against ovine abortion due to

chlamydial infection are usually formalin-inactivated and contain an oil-emulsion adjuvant.[66, 72] To be effective they require large amounts of antigen to be incorporated into the vaccine. Most manufacturers use the level of complement fixing antibodies induced in the sera of rabbits as the standard for judging potency. These antibodies are, however, not strain-specific, and are also not considered protective. Because the strain specificity and antigen incorporated into vaccines are often not controlled, variation in efficacy of this type of vaccine is often a problem. Although most of these vaccines do reduce the abortion rate, they do not prevent infection or shedding.[66, 72] Annual vaccination of ewes is recommended for these inactivated vaccines.

A vaccine containing a live attenuated abortion strain of chlamydia has been developed. The strain is a temperature-sensitive mutant of the *C. abortus* strain AB7 and is designated 1B.[70, 71] It has reduced virulence for ewes, goats and mice. When administered to susceptible ewes or goats before mating, it provides protection against abortion for at least three years and prevents post-parturition shedding of chlamydiae.[71, 73, 74] It can be administered simultaneously with Brucella Rev 1 and the live Salmonella Rv 6 vaccines.[91] When administered with the live Toxoplasma vaccine, it must be given at a different site.[12] The vaccine is currently available only in certain countries.

Some antibiotics are effective both prophylactically and therapeutically in controlling chlamydiosis in most animals, but they must be administered early in the course of the disease and for extended periods to be effective. Tetracyclines are the most widely used antibiotics because of their relatively low cost and ease of administration. They have been used prophylactically with success in ewes under field conditions to prevent abortions. For optimum results they need to be given throughout gestation, which may not be otherwise desirable.[66] Chlortetracycline has been used in feeder lambs to control polyarthritis. Tylosin and long-acting oxytetracycline have been used parenterally.[66] Most of these antimicrobials should be effective in controlling other chlamydial disease syndromes in cattle, sheep, goats and horses, but their use in controlling syndromes in pigs can constitute a problem as many of the current porcine isolates are resistant to tetracycline and sulphadiazine.[6]

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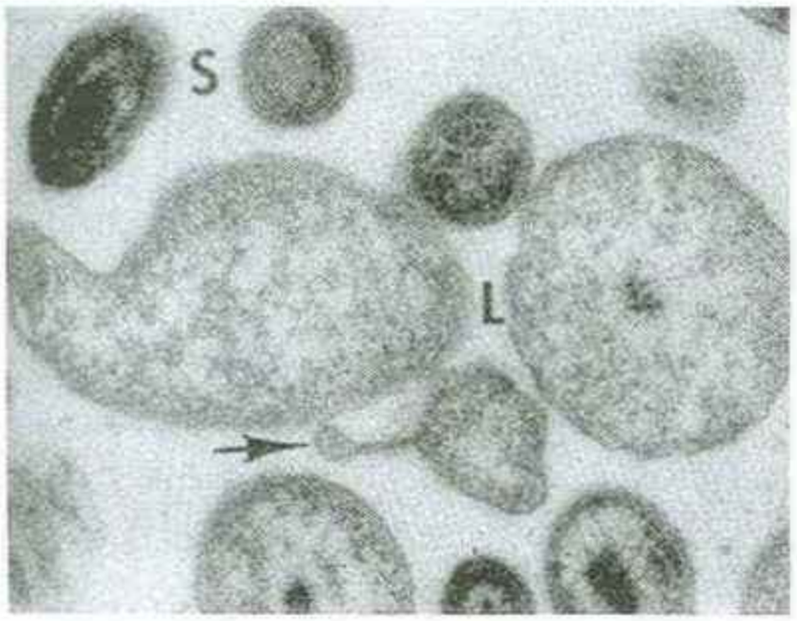


Figure 43.1 Vegetative (L) and small, compact electron-dense (S) forms of *Coxiella burnetii* (McCaul and Williams[53]). (Reproduced by kind permission of McCaul and Williams and the American Society for Microbiology)

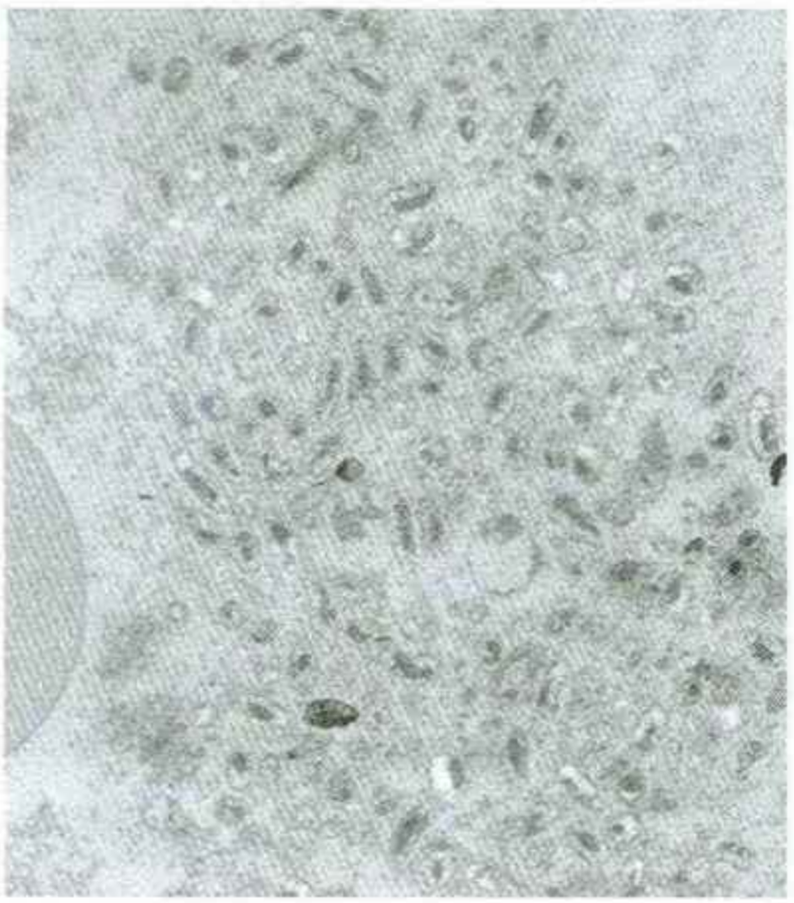


Figure 43.2 Electron micrograph of an intracytoplasmic colony of *Coxiella burnetii*

the bacterium. The cytoplasm of the host cell becomes filled with vacuoles loaded with organisms (**Figure 43.3**). 'Large cell variants' undergo sporogenic differentiation and form resistant, spore-like forms of the bacteria. These develop to SCVs that are released when the cells lyse.

Coxiella burnetii undergoes phase variation,[61] mainly due to variations in the lipopolysaccharide of the organism. Phase I is the natural, highly infectious form of the organism which is found in infected animals, arthropods and humans.

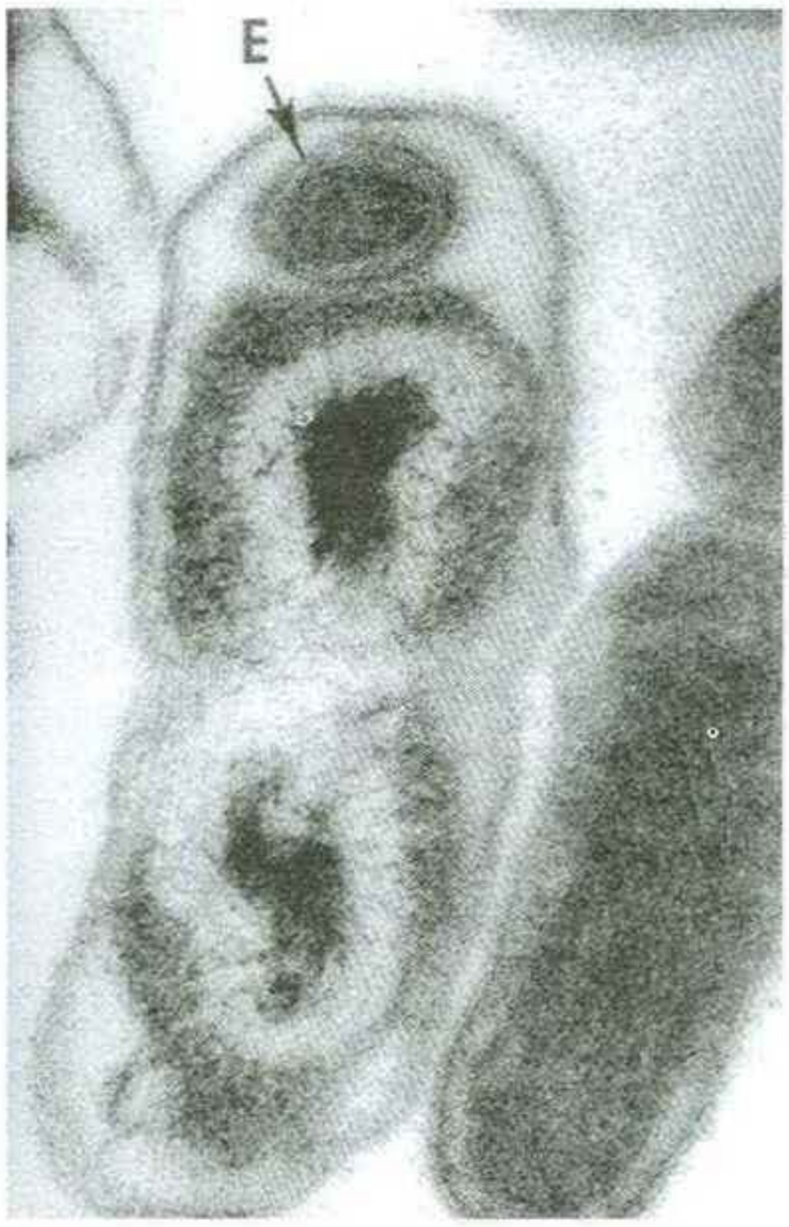


Figure 43.3 Developing polar spore (E) of *Coxiella burnetii* (McCaul and Williams[53]). (Reproduced by kind permission of McCaul and Williams and the American Society for Microbiology)

Phase II organisms are obtained following serial passage of Phase I organisms in laboratory animals or cell culture and are minimally infective. Phase II organisms revert rapidly to Phase I organisms during a single passage in a vertebrate host. Monoclonal antibodies can be used to distinguish the phase variants.[99]

The genome of strains of *C. burnetii* varies from 1,5 to 2,4 Mb and isolates can be placed in genomic groups based on analysis by restriction fragment length polymorphism (RFLP) and pulsed-field gel electrophoresis.[62, 96, 97] Eleven chromosomal genes have been cloned and expressed in *Escherichia coli*[58] and plasmids of 36 to 42 kb have been identified in the genome.[97] Recent studies have indicated that genomic strain variation may be less important than host factors in explaining the occurrence of acute and chronic Q fever in humans.[47]

Epidemiology

Coxiella burnetii has been found worldwide, with the notable exception of New Zealand.[39] It occurs in a large variety of ticks, rodents, birds, and wild and domestic mammals

and anti-*C. burnetii* antibodies have been found in snakes and tortoises in India.[6] Although pigs, horses, camels, Asian buffaloes *(Bubalus bubalis)*, dogs, cats, chickens, ducks, geese and turkeys may be infected, domestic ruminants are the major reservoirs for *C. burnetii* infections in humans.[6]

In southern Africa, serosurveys in cattle have shown that *C. burnetii* is endemic. Antibodies have been reported in cattle in Malawi (2 per cent),[77] South Africa (8 per cent),[35] Zambia (1 to 12 per cent)[31, 63] and Zimbabwe (33 to 75 per cent).[42, 68] Seropositive goats (10 per cent) and dogs (15 per cent) were also found in Zimbabwe and seropositive dogs have also been reported in Senegal (12 per cent) and Ivory Coast (8 per cent).[16]

Prevalences of antibodies to *C. burnetii* in humans have been reported from the Central African Republic (16 per cent),[10] Egypt (16 to 25 per cent),[17] Ethiopia (7 per cent),[1] Sudan (10 per cent),[17] Somalia (37 per cent),[17] Tanzania (5 per cent),[5] Tunisia (26 per cent)[48] and Zimbabwe (37 per cent).[42]

Although cattle, sheep and goats may remain chronically infected for years, the majority of infections are subclinical.[46] Infected animals, however, may shed large numbers of organisms in the amniotic fluid, placenta ($>10^9$/g), foetal membranes, faeces and urine.[82] A major risk factor for humans developing Q fever is contact with infected domestic livestock. Those at particularly high risk are farmers, abattoir workers, hide and fleece processors, and animal health workers. Infections of humans and domestic animals occur principally by inhalation of infected aerosols of the periparturient fluids with only a single organism being sufficient to cause infection.[86] The extracellular *C. burnetii* are extremely resistant to desiccation, low or high pH, disinfectants and ultraviolet radiation.[74] The organisms may remain infective in aerosols for up to two weeks and in the soil for as long as five months.[49] Recent studies have shown that amoebae may be infected with *C. burnetii* and this could further promote their survival in the environment.[58] Humans coming into contact with an infected farm environment are also at high risk of developing Q fever;[85] outbreaks have occurred with exposure to manure.[69] In some outbreaks, however, there is no direct contact with infected farms or animals and their products. In these outbreaks the source has been ascribed to windborne spread of *C. burnetii* from infected areas in the vicinity.[37]

Over 40 tick species have been found to be naturally infected with *C. burnetii*,[6, 58] and transmission of the organism may occur during feeding of ticks. Although tick bites may play a role in the transmission of *C. burnetii* in wild rodents, lagomorphs and birds[6, 46] there is little to indicate they are important in the maintenance of infections in livestock or humans. The organism does, however, multiply in the gut cells of ticks and large numbers of *C. burnetii* are shed in tick faeces. Contaminated hides and wool may be a source of infection for humans[80] either by direct contact or after the faeces have dried and been inhaled as airborne dust particles.[7]

Large numbers of organisms are shed in the milk of dairy cows, particularly at the beginning of lactation. Up to 23 per cent of dairy cows may be shedding organisms in their milk[14] and 21 per cent of bulk milk tanks in England and Wales have been reported to have serological evidence of infection.[64] Suspended in distilled water or sterile milk, the organism survives at room temperature for months and sometimes years.[93] Although people may develop Q fever by drinking infected raw milk[40] or eating infected milk products,[92] in most cases ingestion of infected milk results in seroconversion without disease.[11] Current recommendations for the pasteurization of milk (62,8 °C for 30 minutes or 71,7 °C for 15 seconds) are based on temperatures required to inactivate *C. burnetii*.[27]

In North America, outbreaks of Q fever have resulted from direct and indirect contact with parturient cats.[54] Seropositive cats have been found in Zimbabwe (13 per cent) and South Africa (2 per cent) but they have yet to be implicated in human infections.[57]

Outbreaks have also been reported in humans following exposure to infected pigeon faeces.[78] Person-to-person transmission of *C. burnetii* is rare but occurs transplacentally and during blood transfusions and autopsies.[58] A recent report describes sexual transmission in humans.[60]

Pathogenesis

The target cells of *C. burnetii* are monocytes/macrophages. Most infections occur via the respiratory route and involve the alveolar macrophages.[85] The Kupffer cells of the liver are the primary cells infected in infections that take place via the digestive tract. Subsequently there is a bacteraemia which, in overt clinical cases in humans, coincides with fever.[7] Calves in endemic areas may become infected in the first years of life and either recover completely or become latently infected. Hormonal changes in the final stages of pregnancy in domestic ruminants are thought to trigger massive multiplication of *C. burnetii* in the uterus, foetal tissues and udder. In the pregnant uterus, a mild to severe placentitis may follow the massive multiplication of organisms.[56, 65] About 50 per cent of seropositive heifers shed *C. burnetii* in the placenta, birth fluids and milk at calving, but the percentage drops as they get older. About 50 per cent of chronically infected cows continue to shed organisms intermittently in their milk in successive lactations. After parturition, most infected ewes shed *C. burnetii* in vaginal secretions for up to eight days and some (*c.* 12 per cent) for about two months.[13] They only occasionally shed organisms at subsequent parturitions.[94] Although infected dairy cows may excrete organisms in their milk for many years,[34] ewes only shed organisms in their milk for a few days.[34] It is not clear where *C. burnetii* persists in domestic animals between pregnancies,[14] but in humans the DNA of *C. burnetii* can be detected in the bone marrow for several years after acute infection.[36]

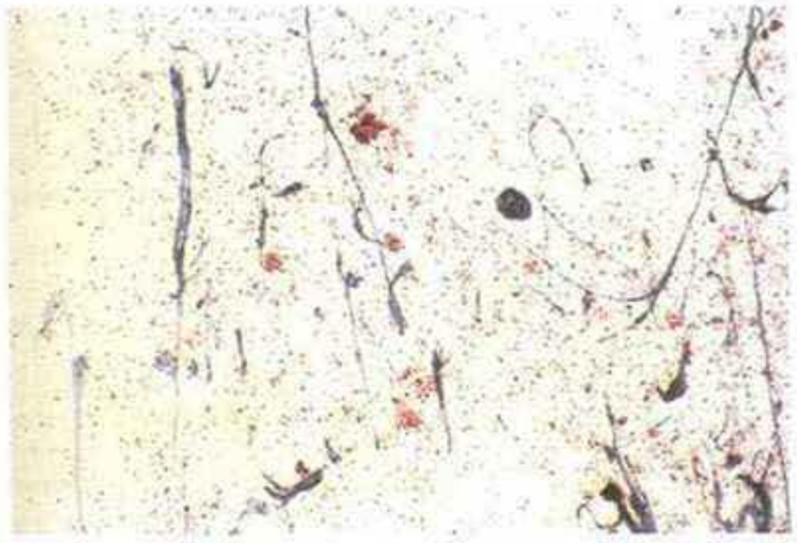


Figure 43.5 Impression smear of the placenta stained with the modified Ziehl-Neelsen method. Note numerous *Coxiella burnetii* organisms

detected with immunoperoxidase assays[19, 89] and capture enzyme-linked immunosorbent assay (ELISA) or ELIFA[84] but these techniques require fresh tissue. Care should be taken in interpreting results as *C. burnetii* may also be found in placentas of healthy sheep and goats.[95]

Recent studies have shown that *C. burnetii* can be detected by DNA probes[29] and a number of polymerase chain reaction (PCR) assays have been described. A PCR with primers derived from the *htpAB*-associated sequence can be used to diagnose Q fever in humans and has been used to detect *C. burnetii* in cow's milk,[52, 88, 98] genital swabs, milk and faecal samples from ewes,[12] cattle sera[88] and dust from barns.[100]

In humans, Q fever is generally diagnosed using serology which enables differentiation of acute and chronic infections based on antibody responses to phase I and phase II organisms.[87] Of the serological techniques available, the indirect fluorescent antibody test is the reference technique for diagnosis in humans; an ELISA has been reported to be useful for seroepidemiological studies.[8, 67] The characteristics and value of other serological techniques such as the microagglutination, complement fixation, radio-immuno assay, indirect haemolysis, ELISA, dot immunoblotting and Western blotting, have been reviewed recently.[43, 58] Serology may be used to detect infected animals but false negative results may occur.[13]

Control

The development of effective communication channels between public health officials and animal health workers is essential for the early diagnosis and control of Q fever outbreaks.

The control of *C. burnetii* infections is difficult as its extreme resistance to physical factors and chemical disinfection makes elimination of the organism from the environment problematic.[3] Appropriate tick control strategies and good hygiene practices can decrease environmental contamination. Infected foetal fluids and membranes, aborted foetuses and contaminated bedding should be incinerated or buried. Infected animals should be removed from herds or provided with separate containment facilities in which to give birth. Detection of infected animals may be difficult, however, as serology is not reliable and the use of PCR is generally impractical.

Inactivated vaccines for domestic animals have been developed. A vaccine containing phase II organisms[44] may be of economic benefit by reducing infertility, abortions and low birth weights. Outbreaks of Q fever in humans have, however, been associated with vaccinated animals[28] as this vaccine does not prevent shedding of *C. burnetii*, particularly in animals naturally infected before vaccination.[18, 71] In Slovakia, large-scale cattle vaccination programmes with a single dose of vaccine containing phase I organisms have been associated with a decrease in the incidence of Q fever in humans.[75]

The need for treatment of infected domesticated animals seldom arises but, if necessary, tetracyclines are the drugs of choice. However, antibiotic treatment of cattle chronically infected with *C. burnetii* does not appear to prevent shedding of organisms.[9] Doxycycline, 100 mg twice a day for 14 to 21 days, is the drug of choice for humans with acute Q fever.[58] Chronic Q fever in humans is difficult to treat: it is recommended that patients be tested with doxycycline (100 mg twice a day) and chloroquine (200 mg three times a day) for 18 months or with doxycycline (100 mg twice a day) and ofloxacin (200 mg three times a day) for three years.[58]

Infections in humans can be minimized by ensuring that workers in the animal industry are fully informed about the risk factors of acquiring Q fever. Laboratories should be provided with appropriate safety facilities and equipment. Vaccination of humans in high-risk occupations should be considered: in Australia, a formalin-inactivated whole cell *C. burnetii* (Henzerling strain, phase 1) vaccine (Q-Vax, CSL) has proven highly effective in reducing Q fever in abattoir workers.[2] Reducing exposure to raw milk and promoting the use of pasteurized milk and its products will also contribute to lowering the prevalence of Q fever.

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44

Eperythrozoonosis*

Synonym: *Eperythrozoon* spp. infection

F T POTGIETER

Introduction

Eperythrozoonosis is caused by *Eperythrozoon* spp., which are rickettsial organisms that occur epicellularly on erythrocytes and thrombocytes, as well as freely in the plasma of a wide variety of animal species including pigs, sheep, cattle, dogs, cats, mice and rats. Most infections are subclinical, but varying degrees of fever, anaemia and icterus occur (particularly in immunocompromised or splenectomized animals). Natural cases of eperythrozoonosis are generally not of great clinical or economic significance in livestock.

The genus *Eperythrozoon* was established in 1928 by Schilling[75] when he named the parasites he observed in mouse blood *Eperythrozoon coccoides*.[5] In 1934 Neitz *et al.*[63] were the first to describe *Eperythrozoon ovis* in sheep in South Africa. In this article a note by Neitz and Quinlan was added, which reported that they had also seen an *Eperythrozoon* sp. in splenectomized calves. In the same year, Adler and Ellenbogen[1] described *Eperythrozoon wenyoni*, which they had observed in blood smears of a splenectomized calf.

Hoyte[36] found and named *Eperythrozoon teganodes*, an organism that occurs free in the blood of cattle. He also suggested that some highly pleomorphic species of *Eperythrozoon* may eventually prove to be mixed infections of more than one species. Although many scientists have recognized *E. teganodes*,[7, 24, 26, 27, 37, 50, 99] this organism is not listed in *Bergey's Manual of Systematic Bacteriology*.[48]

A third species in cattle, which seems to exclusively infect thrombocytes, was first reported as occurring in Madagascar[9] and later in Finland.[7, 98] Uilenberg[100] named this organism *Eperythrozoon tuomii*, and it was subsequently concluded that this parasite is a valid species.[105] All three species in cattle have been identified in Argentina and in Germany.[22, 26] However, *E. tuomii*, which also occurs in South Africa, does not seem to have earned taxonomic status, even after it was shown to have no antigenic relationship to *E. wenyoni*.[97]

Eperythrozoon wenyoni, *E. teganodes* and *E. tuomii* are generally regarded as being non-pathogenic for cattle. There is, however, a report of fatal eperythrozoonosis occurring in five- and six-month-old calves.[6]

Eperythrozoonosis of pigs and sheep are probably the only two diseases of veterinary importance in livestock to be caused by eperythrozoa.[24] According to Jansen,[43] an eperythrozoon had been identified in blood smears from pigs in Zaire by P.J. du Toit of the Onderstepoort Veterinary Institute in South Africa as early as 1942. In the USA it was found that a condition of pigs associated with icterus and anaemia was caused by an eperythrozoon.[86] Splitter[84] named this parasite *Eperythrozoon suis* and also described another non-pathogenic variety, *Eperythrozoon parvum*. The latter parasite was also described in pigs in South Africa by Jansen.[44] Studies in splenectomized pigs revealed that *E. parvum* appears to be more pathogenic than had been originally thought.[3] Although *E. suis* is regarded as an economically significant parasite in pigs, it is considered to be unimportant in the relatively small pig industry in southern Africa.

The aetiology of eperythrozoonosis in sheep in South Africa is well documented.[59, 63] Extensive reviews have been published on eperythrozoonosis and haemobartonellosis comparing the aetiology and other aspects of these infections.[24, 45, 47]

Eperythrozoon ovis may play a role in the poorly understood ill-thrift syndrome (**Chapter 214**) in sheep and goats particularly in the Eastern Cape Province of South Africa.

Eperythrozoon spp. do have a nuisance value in splenectomized donors of live-blood vaccines and in experimental animals. Subclinical *Eperythrozoon* spp. infections in such animals may result in the occurrence of aberrations in experimental data,[21] probably due to the reactivation of latent infections. For example, sheep dosed with *Pachystigma* spp. plants, the cause of 'gousiekte', often develop clinical eperythrozoonosis.[21]

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* For recent changes in the nomenclature of these organisms, refer to the introduction to Section 3, Rickettsial and chlamydial diseases

Aetiology and life cycle

Eperythrozoa are non-motile, prokaryotic, parasitic microorganisms belonging to the order Rickettsiales, family Anaplasmataceae. Morphological differentiation between some *Eperythrozoon* and *Haemobartonella* spp. is difficult.[24, 47] Depending on the species, these parasites occur epicellularly on erythrocytes and thrombocytes, as well as freely in the blood plasma. Their association with the erythrocyte membrane is used to differentiate between some species.

A summary of the morphological characteristics and location in the blood of the different organisms occurring in domestic stock in South Africa is given in **Table 44.1**. The characteristic ring forms of *Eperythrozoon* spp. seen in thin blood smear preparations stained with Giemsa are probably artefacts formed by the spherical organisms during the drying process.[24, 45, 46] The proportions of the morphological types of *E. ovis* apparently change as the parasitaemia increases.[25] Both scanning and transmission electron microscopy have added considerably to our knowledge of their morphology, multiplication and association between them and the cells they parasitize.[25, 45, 52, 81, 98, 102, 105]

There is little difference between the fine structure of eperythrozoa[25, 52, 69, 98, 102, 105] and *Haemobartonella bovis*.[82] The organisms consist of a single membrane enveloping an amorphous matrix containing small ribosome-like granules. Vacuoles and delicate filaments are also sometimes seen within the matrix. Most of these organisms seem to be separated from the host cell membrane by a definite space (**Figures 44.1 and 44.2**).

In the case of *E. ovis*, rod- and spherical-shaped parasites are situated in depressions of the surface of erythrocytes. There are varying degrees of intimacy in the association between the parasite and erythrocyte.[25] *Eperythrozoon suis*, although closely associated with the erythrocyte, is separated from the plasmalemma by a definite space,[102] the interaction between parasites and the cells results in severe deformation of the erythrocyte membrane. *Eperythrozoon ovis* is attached to the red blood cell by a thread-like structure,[39] whereas *E. wenyoni*, which seems to lie in a slight depression on the surface of the erythrocyte, has a loose relationship with the cell and the parasite apparently causes little harm to its host.[45]

It appears that *E. suis* replicates in that small immature forms bud from larger immature and mature forms which seem to correspond to the coccoid, discoid and ring forms of *E. suis*.[102] *Eperythrozoon ovis* also multiplies by budding, and in this way, chains or clumps of organisms are formed in animals with a severe parasitaemia.[25] Scanning electron microscope studies on *E. wenyoni* showed that the parasites occur alone or in chains, clusters, or pairs, which suggests that both budding and binary fission may take place.[45]

It has been suggested that the bone marrow may be a primary site for the multiplication of *E. ovis* and that morphologic differences are due to the stage of multiplication.[39]

The complete life cycle of *Eperythrozoon* spp. is still unknown.

Epidemiology

Eperythrozoon spp. are very successful parasites, being widely distributed throughout the world, causing little disease and surviving indefinitely in the host.

Table 44.1 *Eperythrozoon* spp. that infect comestic stock in southern Africa

PARASITE	HOST	LOCALITY	SIZE/DIAMETER	MORPHOLOGY IN GIEMSA-STAINED SMEARS*
E. wenyoni	Cattle	Epi-erythrocytic and free	0,3–1,5 µm	Rings (typical), discs and coccoids are common forms which stain reddish-purple. Parasites form clusters around circumference of erythrocytes
E. teganodes	Cattle	Free in plasma	Rods and threads 0,3–3,5 µm (length) 0,2–0,3 µm (width)	Slender rods and threads are most common. Rings with rod or thread attached (frying-pan outline) are rare, but characteristic of species. Stain reddish-purple
E. tuomii	Cattle	Attached to thrombocytes, occasionally free in plasma attached to granulocytes (exceptional)	Rings 0,4–0,5 µm	Ring, ovoid and coccoid forms. Darkly stained rods are occasionally seen. Parasites completely surround thrombocytes in severe infections
E. ovis	Sheep, goats	Epi-erythrocytic and free in plasma	0,5–1,0 µm	Rings, rods and irregularly shaped parasites may cover or form clusters around circumference of red blood cells. Stain pale purple or pinkish purple
E. suis	Pigs	Epi-erythrocytic and occasionally free in plasma	Normal 0,8–1,0 µm Large forms 0,3 µm	Ring forms predominate (large ring forms have distorted appearance). Coccoid, disc and rod forms also occur. Stain pale-purple to reddish-purple
E. parvum	Pigs	Epi-erythrocytic and free in plasma	0,5–0,8 µm	Coccoid and occasional ring forms. Parasites accumulate in large numbers on individual red blood cells. Stain pale-purple to reddish-purple

* The colour varies with the pH of the buffer and the batch of stain



Figure 44. 1 *Eperythrozoon wenyoni* in close association with, but separated by a definite space from, the red blood cell membrane. Note parasite free in plasma (× 50 000)



Figure 44. 2 *Eperythrozoon tuomii*: electron micrograph of a section through an infected platelet showing association between the organisms and the platelet

A variety of *Eperythrozoon* spp. infect sheep, goats, cattle and pigs in southern Africa (**Table 44.1**) and are probably very prevalent. However, very little information is available on their epidemiology in the subcontinent. It is believed that heartwater, babesiosis and anaplasmosis live-blood vaccines, produced inadvertently from *Eperythrozoon* carriers, unintentionally caused the widespread distribution of this organism in southern Africa.[70]

In Germany it has been shown in a survey of blood smears from 570 pigs that 5,7 per cent of healthy pigs harboured *E. suis*.[31]

Little is known about the natural transmission of *Eperythrozoon* spp. It is generally believed that they are arthropod-borne; the pig louse (*Haematopinus suis*) is capable of transmitting *E. parvum*[43] and certain mosquitoes (*Culex annulirostris* and *Aedes camptorhynchus*) can transmit *E. ovis* mechanically.[13, 34] Stable flies (*Stomoxys calcitrans*) and lice (*Bovicola bovis* and *Linognathus vituli*)[61] may play an important role in the transmission of infection amongst stabled animals.[70] *Eperythrozoon* spp. can also be easily transmitted iatrogenically by all parenteral routes with infected blood or organ suspensions. *Eperythrozoon suis* is transmitted transplacentally.[4] *In utero* infection of *E. ovis* has not been demonstrated in sheep.[15]

The role that wild animals may play as reservoir hosts would seem to be unimportant as it is believed that *Eperythrozoon* spp. are limited in their host ranges.[24] Their host specificity is poorly defined and this is one aspect which has cast doubt on the taxonomic position of some of them.[48] Neitz[61] showed that *E. ovis* and *E. wenyoni* are both host-specific in domestic stock. Hoyte[37] confirmed this finding and also showed that *E. tagenodes* is not infective to sheep. However, *E. ovis* may be infective to goats,[16] natural infections in goats with *E. ovis* having been reported in both South Africa[95] and Australia.[12] Latent infections have also been produced in two blesbuck (*Damaliscus albifrons*)[60] and an eland (*Taurotragus oryx*) following artificial infection with *E. ovis*.[18]

Neitz[59] reported that in sheep age makes no difference to the susceptibility to infection with *E. ovis* in South Africa. But in contrast, workers in some countries, especially Australia and New Zealand, describe various degrees of susceptibility among lambs, young sheep and adult sheep. Infection may result in subclinical infections, in an ill-thrift syndrome, or in severe clinical disease with high morbidity and mortality.[8]

No detailed studies have been made on eperythrozoonosis of sheep and goats in southern Africa. According to Neitz[59] farmers in South Africa describe an anaemic condition in sheep to which the name 'bleeksiekte' (pallor disease) has been given. The aetiology of this condition remains obscure, and it is unknown to what extent verminosis and malnutrition or other contributory factors may be involved. The epidemiology of *E. ovis* infections in sheep is probably further complicated in endemic areas by other haemoparasitic diseases such as anaplasmosis and theileriosis.

Once animals are infected they remain life-long carriers. Sheep that recover from *E. ovis* infection develop a premunity; artificially infected sheep may be infected for up to ten and a half years.[14] Autosterilization has not been reported, but chemosterilization renders sheep fully susceptible to re-infection.[62, 76] Splenectomy or treatment with dexamethasone results in relapses and a resurgence of parasitaemia.[25, 51, 59] Lambs that suckle *E. ovis*-carrier ewes appear to be protected from patent infection.[15] Serum antibody titres (which are believed to be protective) of uninfected lambs of such ewes apparently decline to zero within three weeks after weaning.[15]

One of the biggest obstacles faced when producing redwater (babesiosis) live-blood vaccine for cattle at Onderstepoort is the interference caused by concurrent *E. wenyoni*, *E. tuomii* and *E. teganodes* infections in splenectomized donor calves. These infections result in prolonged incubation periods and in the suppression of parasitaemias caused by both *Babesia bovis* and *B. bigemina*.[70] Interference between concurrent eperythrozoon and other haemoparasitic infections, such as between *E. wenyoni* and *Anaplasma marginale*[20] and between *E. ovis* and *Babesia ovis*,[23] have also been reported. *Eperythrozoon wenyoni* infection not only causes extension of the incubation period in *B. bigemina* infection,[35] but it also suppresses the parasitaemia of *A. marginale* and *B. bigemina* infections.[57] *Eperythrozoon teganodes* apparently suppressed the development of *A. marginale* in a splenectomized calf.[27] Complement fixation testing has shown that *Anaplasma* and *Eperythrozoon* spp. share antigens.[46] However, the indirect fluorescent antibody test shows no cross-reactivity between *E. ovis* antigen and *Anaplasma ovis* antisera.[40] Even between members of the *Eperythrozoon* spp., such as between *E. tuomii* and *E. wenyoni*, there appears to be no antigenic relationship.[97]

Eperythrozoon suis and *E. parvum* infections result in a subclinical carrier state which may be activated under stress. There is no cross-immunity between these two species,[84] but infection of *E. suis* suppresses the development of parasitaemia of *E. parvum*.[85] *Eperythrozoon parvum* also inhibits the development of parasitaemia of *Babesia trautmanni* infection.[3]

Pathogenesis

Little information is available on the pathogenesis of *Eperythrozoon* spp. infections.

The majority of *Eperythrozoon* spp. infections, including those due to more pathogenic species, are subclinical and may be exacerbated by stress. Diseases produced by *Eperythrozoon* spp. are characterized by the destruction of erythrocytes or, in the case of *E. tuomii*, thrombocytes.

It has been suggested that anaemia in ovine eperythrozoonosis is not directly associated with the parasitaemia, but that it could have what was called an allergic base.[94] *Eperythrozoon ovis* also seems to affect glutathione, which is essential for normal erythrocyte integrity.[89, 92] Heinz bodies in erythrocytes of *E. ovis*-infected sheep[56, 67, 92] have also been implicated in the pathogenesis of anaemia.[56]

Certain aspects of *E. ovis* infections are consistent with those of a haemolytic anaemia.[19, 59] While Sutton[90, 91] believes that intravascular haemolysis occurs, Sheriff[78] concludes that haemoglobinuria, as reported by Overäs,[68] is rarely found in *E. ovis* infection, which supports the contention that extravascular erythrophagocytosis takes place.

The cause of anaemia in eperythrozoonosis in pigs has been ascribed to autoimmune haemolysis cause by 'cold' auto-antibodies.[33] These antibodies are attached to erythrocytes and induce destruction by phagocytosis, primarily in the spleen, but also in lymph nodes.[69] *In vitro* agglutination of sensitized erythrocytes occurs spontaneously at 25 and 4 °C respectively.[103] The agglutination phenomenon correlates with increases of serum antibodies (as measured by the indirect haemagglutination test) and of total serum globulin concentrations associated with the appearance of IgM 'cold' agglutinins.[104] Erythrocytes, lost to the 'cold' agglutinin phenomenon, are replaced by reticulocytes. As the new red blood cells are not readily infected and the altered erythrocytes are phagocytosed, the antigenic stimulus required for the maintenance of indirect haemagglutination titres and globulin concentrations is effectively removed.[103] Haematological findings in splenectomized pigs indicate the development of a normochromic anaemia caused by extravascular erythrolysis.[28]

Natural *E. suis* infections show no signs of penetration and erosion of the red blood cell membrane comparable to those which occur in *Haemobartonella felis* infections.[69] Severe deformation of the red blood cell membrane has, however, been demonstrated in splenectomized pigs infected experimentally with *E. suis*.[103] It is thought that the humoral immune response is directed in part against these parasite-altered erythrocytes.[103] Autoantibodies to erythrocytes, possible mechanisms involved in parasitic immunoregulation, and misdirected immune responses have been implicated in *E. suis* infections.[103] Smith, as cited by Henry,[30] believes that *E. suis* in pigs may affect all age groups and that its clinical manifestation is not limited to the classic disease of anaemia and icterus of feeder pigs under stress.

The only information regarding the pathogenesis of infections by *E. wenyoni*, *E. teganodes* and *E. tuomii* has been obtained from studies using splenectomized animals.[45, 98, 105] In none of these studies were significant ultrastructural changes of infected red blood cells or thrombocytes observed.[45]

Clinical signs

Neitz and his co-workers[60, 62, 64] showed that the incubation period following artificial infection with *E. ovis* could be as short as 4 to 15 days, but could sometimes extend to several weeks. Parasitaemia appears concurrently with the rise in body temperature and increases until the first signs of anaemia are detected five to eight days after the initial detection of parasitaemia. Parasitaemia then wanes abruptly; few parasites are seen when the anaemia is most severe. Recovered animals remain carriers and recrudescent parasitaemias may occur at irregular intervals for six weeks or longer.

The severity of the disease in sheep is extremely variable and seems to depend on the degree of the parasitaemia and its duration.[25] A continuous or intermittent febrile reaction may be followed by variable anaemia and sometimes icterus.[63] Other signs include depression, anorexia, loss of

condition, debility, retardation of growth rate, lowered haemoglobin levels, a drop in wool production and decreased exercise tolerance.[11, 76] The pulse rate becomes rapid and weak and the respiration accelerates. Haemoglobinuria occurs very rarely.[59] Mortality rate is usually very low.[8, 51, 56]

In pigs clinical signs are most likely to be observed in young suckling piglets and sows shortly after farrowing. Most *E. suis* infections result in a subclinical carrier state which may lead to overt disease when animals are stressed.[53] The early phase of the infection is characterized by high fever, but in more advanced cases temperatures may be normal or subnormal, and the mucous membranes may be pale and icteric. Other signs include weakness, depression, loss of appetite, constipation, bile-stained faeces and dyspnoea.[47] Some animals suffering from the disease may die. In animals that recover relapses may occur.

Eperythrozoon wenyoni usually causes only mild fever and anaemia in intact cattle[65] but very occasionally some affected animals manifest severe clinical signs.[71, 73] Severe anaemia and icterus occur in infected splenectomized animals.[61, 93]

Hoyte[36] considered *E. teganodes* to be of similar pathogenicity to *E. wenyoni*. *Eperythrozoon tuomii* have been reported to cause fever and anaemia in splenectomized cattle.[96, 99, 100, 105]

Pathology

The major lesions caused by eperythrozoa (except *E. tuomii*) are the consequence of erythrocyte destruction resulting from erythrophagocytosis or intravascular haemolysis and the stimulation of the immune system, which leads to hyperplasia of the reticuloendothelial cells.

In sheep, lowered haematocrit and haemoglobulin levels, hypochromic anaemia, a rise in leukocyte counts and erythrophagocytosis have been reported.[59, 63] In some animals, lowered haemoglobin and haematocrit levels occur one week after peak parasitaemia,[10] whereas in others, there is a relationship between the severity of anaemia and peak parasitaemia.[55, 67] The main chemical pathological features include an increase in blood lactic acid and a lowered blood pH.[87, 88] Pyruvate and lactate concentrations reduce the blood pH 10 to 14 days after infection.[42] Sutton[87, 88] reported that affected erythrocytes have a high glycolytic activity. On the other hand, Ilemobade and Blotkamp[42] found no effect on the concentration of blood glucose in infected animals.

Variable degrees of anaemia, icterus, and fatty degeneration of the liver have been reported in sheep.[2, 8, 19, 44, 51, 59, 67, 90] The spleen is enlarged and shows a reddish-brown pulp and severe hyperplasia of the Malpighian bodies. There may also be a marked hydropericardium together with subcutaneous oedema along the ventral aspect of the neck. Haemosiderin is present in the kidneys, liver and spleen at peak and late stages of parasitaemia.

Necropsy findings in pigs include anaemia, icterus, a yellowish-brown enlarged liver, and splenomegaly. There may also be hydropericardium, ascites, and epicardial haemorrhages. Microscopic lesions include hyperplasia of the red bone marrow, haemosiderosis in the spleen and liver, and fatty changes and necrosis of hepatocytes.[17, 32, 38, 47] Perivascular oedema and discrete glial mobilization in the brain have been described in *E. suis* carrier pigs that had been splenectomized.[9]

Eperythrozoon wenyoni infections in splenectomized calves result in lowered packed red blood cell volumes, a rise in white blood cell counts and erythrophagocytosis.[61, 71] Changes in splenectomized calves infected with *E. wenyoni* include an increase in lactic acid, lowered blood pH, negative base excess and lowered standard bicarbonate content.[106] Metabolic acidosis has also been reported.[101]

Eperythrozoon tuomii infection results in a prolonged blood-clotting time and severe reduction in the number of thrombocytes and petechiae in the organs of splenectomized calves.[105]

Diagnosis

Eperythrozoon spp. infections should be considered in anaemic animals, especially if they have recently been splenectomized or immunosuppressed. In most cases, eperythrozoonosis cannot be diagnosed clinically until anaemia is well advanced. If the examination of blood smears fails to confirm the diagnosis, subinoculation of blood into susceptible splenectomized hosts may be considered.

Successful diagnosis of eperythrozoonosis is often dependent upon the correct technique being used when making a blood smear. Dirty glass slides and stain deposits can hamper recognition of these delicate parasites. Blood smears should be made from fresh blood preferably collected directly from the live animal, as the organisms tend to lose their structural integrity and may not be identifiable in smears made from stored blood.[7] For example *E. wenyoni* 'disappeared' from the blood of a splenectomized calf within four hours of death.[70] If stored blood is used, blood collected in EDTA is better for diagnostic purposes than heparinized or citrated blood.[8] It has also been demonstrated that *E. ovis* is frequently absent in smears made from the peripheral blood of sheep in which anaemia is advanced.[8, 51, 59, 79]

The thick blood smear technique[54] for *Babesia* spp. identification is extremely useful for making early diagnoses of eperythrozoonosis.[70] Different scoring systems are used to quantify the parasitaemias.[7]

The organisms appear bright orange when stained with acridine orange and viewed under a fluorescent microscope.[24] This staining technique is more reliable for demonstrating low infections than is the Giemsa stain.[25]

Campbell *et al.*[8] believe that *E. ovis* infections result in a characteristic type of anaemia, apparently of diagnostic value, which is predominantly either macro- or normocytic and normochromic in nature. Hoffmann[31] contends that

clinical signs in pigs are non-specific and that the phenomenon of instant agglutination of blood samples is important in the diagnosis of *E. suis*.

A variety of serological techniques can be used to diagnose infection on a flock or herd basis. These tests include direct immunofluorescence,[46] the modified antiglobulin (Coombs) test,[80] indirect haemagglutination,[82, 83] complement fixation,[10] indirect immunofluorescence[40, 41, 64, 66] and enzyme-linked immunosorbent assay (ELISA).[49]

Differential diagnosis

Any condition that results in anaemia and icterus in sheep, goats, cattle and pigs may be confused with eperythrozoonosis. In sheep these include malnutrition, helminthosis, and the syndrome known as ill-thrift (**Chapter 214**). Differentiation between anaemia caused by copper and cobalt imbalances and *E. ovis* infections is difficult.[8, 76] Recent work in Australia has shown that *E. ovis* is the major cause of icterus in lambs and that copper poisoning is much less involved.[2]

The macroscopic lesions caused by *E. ovis* in sheep closely resemble those seen in ovine anaplasmosis[60] or chronic copper poisoning.[2]

In South Africa, concurrent *Anaplasma ovis* and *Theileria ovis* (*T. separata*?) infections may aggravate *E. ovis* infections in sheep.

The poisoning of goats with the pods of *Acacia nilotica braussiana* may result in the development of anaemia, icterus, methaemoglobinaemia and haemoglobinaemia.[95]

Babesia trautmanni infections and leptospirosis should be differentiated from *E. suis* infections in pigs.[29]

Control

Different drugs have been tested for activity against eperythrozoonosis.[24] Arsenicals and tetracyclines appear to be effective against most infections,[27, 99, 100, 105] but in spite of their efficacy, these drugs do not sterilize the infection in most cases.[7, 62, 72] Treatment of sheep infected with *E. ovis* with spirotrypan sterilizes the infection, and such animals become fully susceptible to reinfection.[77]

The arsenical drug, neosalvarsan, is effective in the treatment of *E. ovis* and *E. wenyoni* infections.[61, 63]

Both arsenicals, which are becoming increasingly difficult to obtain commercially, and oxytetracyclines at a dosage rate of 10 mg/kg body weight administered intravenously or intramuscularly, are regularly and successfully used to treat *E. wenyoni*, *E. tuomii*, *E. teganodes* and *E. ovis* infections in splenectomized cattle and sheep at the Onderstepoort Veterinary Institute.[70] In acute cases it may be necessary to repeat the treatment if the febrile reaction and/or parasitaemia persists, and during parasitic relapses. According to Campbell *et al.*,[8] treatment of affected sheep should be based on the reduction of stress by good husbandry rather than by therapy.

Tetracyclines appear to be the drugs of choice for treating *E. suis* infections.[28, 30, 38, 58, 74] *Eperythrozoon suis* infections do not seem to have an appreciable effect on reproductive performance,[104] but chlortetracycline treatment in the diet of sero-positive sows results in increased productivity.[74]

Although not conclusively proven, the possible role of ectoparasites in the transmission of the infections should be considered. It is therefore recommended that animals be treated for ectoparasites during outbreaks. Care should be exercised to prevent iatrogenic transmission of infection.

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45

Bovine haemobartonellosis*

Synonym: *Haemobartonella bovis* infection

F T POTGIETER

Introduction

Bovine haemobartonellosis is caused by *Haemobartonella bovis*, which is a rickettsial-like organism that parasitizes erythrocytes. Most infections are not clinically apparent and disease, characterized by fever and anaemia, is seen almost invariably only in splenectomized cattle.

Haemobartonella bovis was described by Donatien and Lestoquard in 1934, as cited by Gothe and Kreier,[2] and is often encountered in blood smears of splenectomized cattle at the Onderstepoort Veterinary Institute in South Africa.[8] Although the majority of *Haemobartonella* spp. infections which occur in several domestic and wild animal species are non-pathogenic, *Haemobartonella felis*, the cause of feline infectious anaemia in the domestic cat, is of considerable veterinary importance in southern Africa.

Aetiology and life cycle

Haemobartonella spp. are pleomorphic, rod- to coccoid-shaped parasites, 0,2 to 0,45 µm in diameter (**Figures 45.1 and 45.2**), belonging to the order Rickettsiales, family Anaplasmataceae. The organisms occur in indentations of the surface membrane of erythrocytes, and are more closely associated with this membrane than are *Eperythrozoon* spp. *Haemobartonella bovis* is separated from the plasmalemma by a definite space.[9] The parasites are sometimes arranged in chains, groups or pairs and have a limiting membrane, but no cell wall or nucleus. They are rarely encountered free in the plasma.

Haemobartonella stain well with Giemsa stain and are Gram-negative.

The genus *Haemobartonella* was established by Tyzzer and Weinman in 1939. Although 27 species of *Haemobartonella* have been named, and unnamed *Haemobartonella* spp. infections found in a further 11 species of animals,[2] only three species, which infect the mouse, cat and dog respectively, are currently included in the genus *Haemobartonella*.[5] The classification of many of these organisms remains in doubt.[5]

Extensive reviews have been published on eperythrozoonosis and haemobartonellosis in which the aetiology and other aspects of these infections are compared.[2–4]

An elucidation of the complete life cycle of *Haemobartonella* spp. is still awaited.

Epidemiology

Haemobartonella bovis is probably widely distributed in southern Africa. It is believed that live-blood vaccines, such as those for anaplasmosis and babesiosis, produced from clinically inapparent *H. bovis*-infected cattle may be inadvertently responsible for the widespread distribution of *H. bovis* in South Africa.[8]

Haemobartonella bovis infection in cattle is not regarded as of much clinical or economic significance and has only a nuisance value in splenectomized donors of live-blood vaccines and in experimental animals. Inapparent *Haemobartonella* infections may result in aberrations in experimental data and are probably due to the activation of latent infections.

Little is known about the natural transmission of *H. bovis*, but it is thought that the parasite, as is the case with *Eperythrozoon* spp., might be arthropod-borne. Infection can easily be transmitted iatrogenically by inoculation of infected blood or organ suspensions. Unintentional mechanical transmission by all parenteral routes is possible.

Pathogenesis, clinical signs and pathology

No ultrastructural evidence has been found to show that haemolysis of erythrocytes is associated with high parasitaemias of *H. bovis* in splenectomized calves.[9] Packed cell

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* For recent changes in the nomenclature of these organisms, refer to the introduction to Section 3, Rickettsial and chlamydial diseases

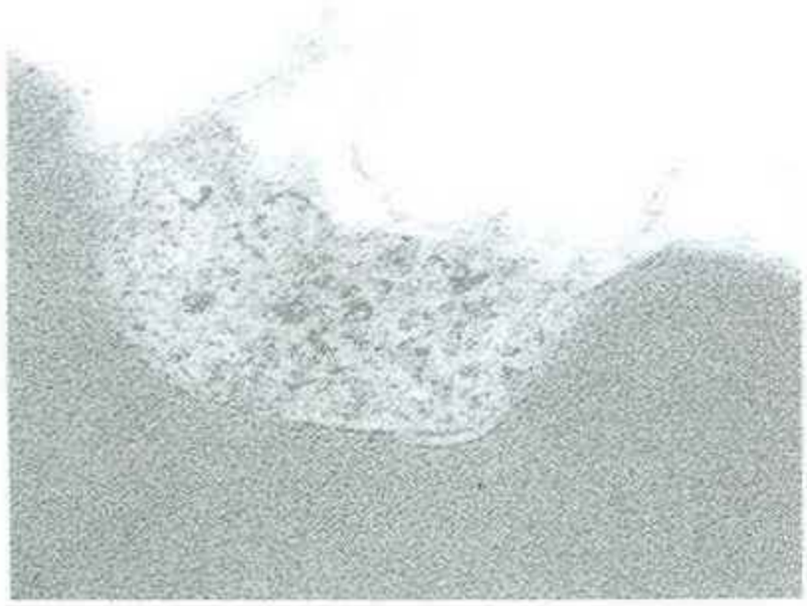


Figure 45.1 Electron micrograph of a section through an erythrocyte infected with *Haemobartonella bovis*. Note the parasite positioned in the indentation in the cell and the intimate association between the parasite and erythrocyte membrane

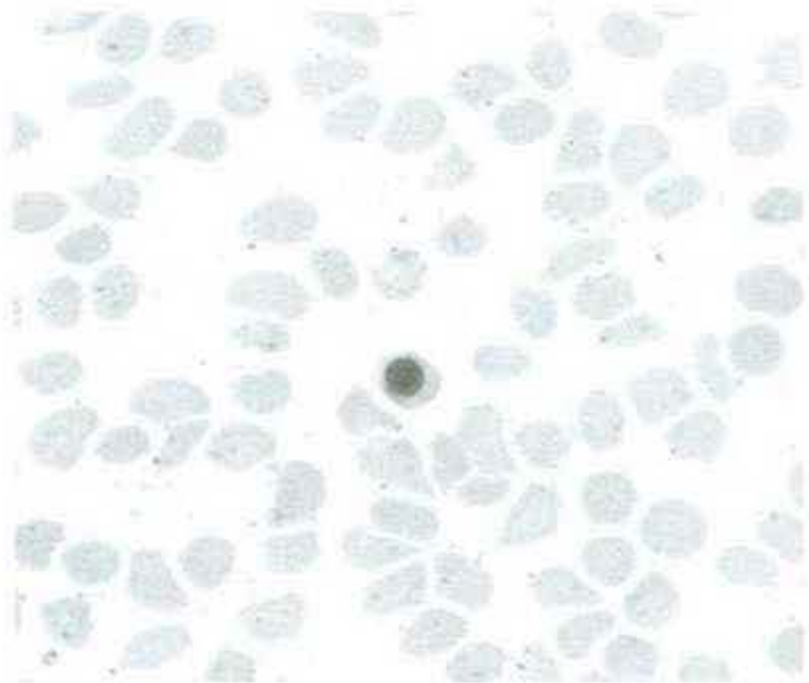


Figure 45.2 Light microscopic photograph of a Giemsa-stained blood smear of a calf infected with *Haemobartonella bovis* (× 1 000)

volumes show a significant decrease after which the parasitaemia subsides, and it is concluded that phagocytosis is responsible for the anaemia. A decrease of blood glucose has been found to occur concomitantly with the parasitaemias in splenectomized calves.[6] Erythrocytes parasitized by an *H. bovis*-like organism show a 3,69-fold increase in glucose metabolism.[7] *Haemobartonella bovis* has not been observed to be pathogenic in Australia[1] or South Africa.[8]

Apart from causing mild anaemia and fever in splenectomized calves, *H. bovis* generally appears to be non-pathogenic.[6, 9] Kreier and Ristic[4] cite two reports of natural outbreaks of severe disease caused by *H. bovis*, but these are regarded as unique.

Little information on the pathology of *H. bovis* infection is available.[1, 6, 9]

Diagnosis and differential diagnosis

A diagnosis of *H. bovis* infection should be considered in anaemic cattle, especially if they have recently been splenectomized or immunosuppressed. Clinical signs are only evident in animals with advanced anaemia. If the examination of blood smears fails to confirm the diagnosis, blood of the suspected animal may be inoculated into susceptible splenectomized animals.

Depending on the level of parasitaemia, *H. bovis* can be confused with *Anaplasma* spp. infections on blood smear examination, particularly of splenectomized animals.

Control

Growth of *Haemobartonella* spp. is inhibited by arsenicals and oxytetracyclines, but not by penicillin or streptomycin.

Gothe and Kreier[2] list drugs which have been tested for activity against eperythrozoonosis and haemobartonellosis. For more information on the specific treatment of haemobartonellosis, refer to **Chapter 44: Eperythrozoonosis**.

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46

Potomac horse fever*

Synonyms: Equine monocytic ehrlichiosis, equine ehrlichial colitis, equine ehrlichial abortion

J E PALMER

Introduction

Potomac horse fever is an acute enterocolitis of equids caused by a group of closely related intracellular-occurring ehrlichias collectively known as *Ehrlichia risticii*, first recognized in 1979 along the Potomac River in Maryland, USA.[29] *Ehrlichia risticii* infection in horses has been referred to as Potomac horse fever, equine monocytic ehrlichiosis, and equine ehrlichial colitis. It has been recognized to occur throughout North America. The original term, 'Potomac horse fever', was coined by a television reporter covering the original epidemic along the Potomac River. Although it is the least descriptive term, its wide acceptance and its high level of recognition ensures that it will remain the most common term for the disease. Equine ehrlichial colitis is a more descriptive term for the originally described syndrome of fever, anorexia, depression and diarrhoea. Even mild cases not manifesting diarrhoea have evidence of colitis. Equine ehrlichial abortion is an appropriate term for the abortion syndrome caused by *E. risticii*.

Aetiology

The causative organism of Potomac horse fever is a member of the tribe Ehrlichieae.[23] Based on nucleotide sequence, the genus *Ehrlichia* is phylogenetically incoherent. *Ehrlichia risticii* is most closely related to *Ehrlichia sennetsu* and *Neorickettsia helminthoeca* (levels of sequence similarity, > 95 per cent).[55] Although only definitively proven for *N. helminthoeca*, it is likely that these three organisms also share the unique property of being the only known obligate intracellular bacteria that are transmitted via a helminth vector.

Although *E. risticii* grows readily in a number of tissue culture lines as long as antimicrobials are not used in the culture media, the continuous murine macrophage cell line P388D1 is frequently used for isolation and propagation. The original Maryland isolate grows readily in this cell line, initially appearing as a cluster of singly occurring elementary bodies followed by the development of initial bodies and later forming morulae (mature inclusions). Heavily infected cells eventually disintegrate, releasing loosely packed groups of organisms held together by cytoplasmic stroma.[69]

There is considerable biological diversity of *E. risticii* isolates from clinical cases of Potomac horse fever. Morphologically, some form large cytoplasmic morulae (inclusions), whereas others form small morulae or are individually dispersed in the cytoplasm in murine P388D1 cells.[11] Similarly, patterns of antigenic proteins may differ considerably between isolates.[11] The sequences of the 16S rRNA genes of isolates may differ between each other more than from the next most closely related *Ehrlichia* sp., *E. sennetsu*. It is evident that Potomac horse fever is not caused by one *Ehrlichia* sp. but rather by several closely related but distinct *Ehrlichia* spp.[83] This antigenic variation of isolates causing the same clinical disease has resulted in incomplete protection from the first generation of vaccines which all originated from the same type strain of *E. risticii*.

Epidemiology

Potomac horse fever is confined to North America where it has been reported in a wide variety of geographic regions in most USA states and Canada. Surveys in the USA have shown that 16 to 33 per cent of horses with no history of illness have antibodies to *E. risticii*[18, 39, 65] and that these are seasonal fluctuations with the highest number of seropositive horses and highest clinical occurrence in July, August and September.[18, 39] Over a five-year period 70 per cent of 900 clinical cases recorded in Maryland occurred during the same months.[53]

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* For recent changes in the nomenclature of these organisms, refer to the introduction to Section 3, Rickettsial and chlamydial diseases

Clinical disease shows an unusual sporadic pattern with a low prevalence rate (< 5 per cent) on any one farm despite its frequent occurrence in an endemic area. In fact, when multiple cases occur on a large farm, the pattern is also temporally (within the season of the disease) and geographically (among the pastures and barns on the farm) sporadic. Occasionally an epidemic form occurs which is characterized by a high attack rate (20 to 50 per cent) on an individual farm. The disease may be concentrated on a particular farm or race track, resulting in an outbreak simultaneously involving a large number of horses. The reason for this epidemiological variation is unknown.[45, 48]

The mode of transmission has been extensively studied but significant questions continue to exist. Direct contact transmission does not occur.[44] The seasonal occurrence leads to early investigation of a possible arthropod vector. Common vectors of other ehrlichial agents are ticks.[67] The only adult tick found feeding on horses along the Potomac River during the original epidemic in the early 1980s was *Dermacentor variabilis*.[9, 72] The larval and nymphal stages of this tick feed on small ground mammals such as the white-footed mouse (*Peromyscus leucopus*).[9] Mice are highly susceptible to experimental *E. risticii* infection.[24] However, attempts to transmit *E. risticii* to horses using field-captured adult ticks (*D. variabilis*) from endemic farms failed.[72] Furthermore, larval and nymphal stages of *D. variabilis* fed on ehrlichaemic mice did not transmit the organism to other mice or to ponies,[20, 39] and white-footed mice infested with immature *D. variabilis* and living in endemic areas of the disease have consistently been found to be seronegative.[9] Dogs, the primary host of adult *D. variabilis*, are uniformly seronegative on endemic farms[53] despite being susceptible to infection.[68] *Dermacentor variabilis* is not the vector of *E. risticii* and attempts to transmit the pathogen with other ticks including *Rhipicephalus sanguineus*, *Ixodes scapularis*, and *Amblyomma americanum* have also failed.[20]

Despite the wide experimental host susceptibility of *E. risticii*,[13, 24, 68, 77] non-equine mammals in endemic areas show a surprisingly low frequency of seroconversion.[9, 19, 53, 71] One investigation revealed that a high percentage of cats on some endemic farms were seropositive,[53] but transmission studies using the cat flea (*Ctenocephalides felis*) and the chigger mite (*Neotrombicula whartoni*), both common ectoparasites of cats on endemic farms, failed when *E. risticii*-infected horses or mice were used as donors and mice were used as recipients.[71]

The ehrlichaemia that occurs during the acute stage of the disease makes transmission by haematophagous flies possible. The role of adult stable flies (*Stomoxys calcitrans*) has been investigated;[8] despite demonstrating ehrlichaemia in donor horses, recipient horses did not seroconvert or develop signs of Potomac horse fever and recovery of *E. risticii* from the flies was not successful.

All the evidence indicates that blood-sucking arthropods are not responsible for transmitting *E. risticii*. Oral infection produces clinical disease which is identical to that seen when the organism is given intravenously.[44] If horses are exposed orally to a sufficient number of viable ehrlichias, infection will occur. Large numbers of *E. risticii* are shed into the lumen of the colon in exfoliating epithelial cells.[64] Thus large numbers of ehrlichias are present in the faeces of infected horses.[6, 38] However, direct contact does not result in transmission,[44] so it seems unlikely that contaminated faeces play a direct role in the transmission of the disease. Indirect oral transmission may occur through concentration of the ehrlichias in a vector. *Neorickettsia helminthoeca* and *Neorickettsia elokominica*, two other members of the tribe Ehrlichieae which share common antigens[59] and DNA homology[55] with *E. risticii*, are transmitted through ingestion of infected helminths.[22, 23] The metacercaria of the salmon fluke (*Nanophyetus salmincola*) carries these pathogens. Dogs are infected by ingestion of fluke-infested salmon or trout. Similarly, *E. risticii* may be concentrated in infected helminths carried by arthropods which are inadvertently ingested. *Ehrlichia sennetsu*, a closely related pathogen of humans, has been suspected of being transmitted by ingestion of a fish nematode.[28] The involvement of an arthropod vector or helminth would be consistent with the seasonality of the disease.

Recently a reservoir of *E. risticii* has been identified in trematode cercariae (virgulate cercariae) parasitizing freshwater stream snails and aquatic insects in two geographically distinct areas of the USA.[2, 10, 25, 56, 58] The trematodes which can become infected with *E. risticii* appear to have a broad intermediate host range, having been found in abundance in adult and immature forms of the following aquatic insects: caddisflies (*Trichoptera*), mayflies (*Ephemeroptera*), damselflies (*Odonata, Zygoptera*), dragonflies (*Odonata, Anisoptera*), and stoneflies (*Plecoptera*).[10] In one study, the prevalence of *E. risticii* was 32 per cent in 13 of 17 aquatic species of insects acting as hosts for the trematodes.[10]

The discovery of an aquatic reservoir is not surprising considering the early observations that the disease outbreaks centred along major rivers.[48] This finding could easily explain the observation that the disease appears to be associated with areas and not horses in the areas. One of the original index farms along the Potomac River was abandoned and left without horses for several years because of the high prevalence of the disease. It was then repopulated with a new herd. Potomac horse fever re-emerged on the farm during the first spring thereafter, a high attack rate being recorded in the newly introduced horses.

The connection between the aquatic reservoir and transmission to horses has not been clearly characterized. Transmission attempts using naturally infected trematodes through skin contact with water harbouring *E. risticii*-infected cercariae, through ingestion of water harbouring *E. risticii*-infected cercariae or through ingestion of different aquatic insects — caddisfly larvae (Limnephilidae), adult

caddisflies (Leptoceridae, Limnephilidae), nymph and adult mayflies (Heptageniidea) and stonefly nymphs (Perlodidae) — harbouring *E. risticii*-infected metacercariae were only successful in one horse which received large numbers of adult caddisflies. Two other horses receiving fewer adult caddisflies did not develop disease, seroconvert, develop ehrlichaemia or shed ehrlichias in their faeces.[33] Considering the susceptibility of horses to oral infections, it is surprising that it was not easier to transmit the organism through ingestion of the trematodes.[44] The failure to do so is probably associated with lack of sufficient viable ehrlichias.

How horses would ingest sufficient numbers of these aquatic insects to result in infection has not been explored. Caddisflies spend their life primarily on the water or in vegetation near water. They may be attracted by lights at night. When the ecosystem of horses and caddisflies, or other aquatic insects, would allow for transmission is unclear. Another issue that needs resolution is the observation that cases of Potomac horse fever can occur in horses at significant distances from major waterways as well as in horses that have no apparent access to streams or other aquatic environments.[42, 45, 48]

The faecal shedding of *E. risticii*, which appears to be a consistent finding in infected horses,[6] is an ideal way to seed aquatic environments with the organism, and introduction of recently infected horses could conceivably convert a previously disease-free area into one potentially endemic for the disease, if aquatic environments do prove to harbour the source of endemic disease in horses. It is apparent that, although a freshwater reservoir is probably important in the epidemiology of *E. risticii*, many questions have been left unanswered.

Pathogenesis

Although the mode of transmission is unknown, it is clear that within hours of infection *E. risticii* can be found in a subpopulation of blood monocytes. Although the pathogen is readily phagocytized by monocytes,[84] it appears to elude the host's defence mechanisms by inhibiting lysosomal fusion with phagosomes.[82] The ehrlichaemia persists throughout and beyond the clinical period. The pathogen has a predilection for the mucosa of the caecum and large colon but is occasionally found in the mucosa of the jejunum and small colon.[64] Intestinal epithelial cells, mast cells and macrophages are the targets of infection. Lesions are confined to the intestinal tract.[12] The inflammatory response to *E. risticii* infection, which appears to vary in intensity, is mediated through cytokines. Preliminary studies in mice suggest that increased macrophage production of interleukin-1 alpha, but not tumour necrosis factor-alpha, interleukin-6 or prostaglandin E2, may be primarily involved in the pathogenesis of the disease.[79]

Ehrlichia risticii causes significant immune depression in mice and measurable alterations of the equine immune system, probably through modification by inflammatory cytokines. Whether or not the immune depression is clinically important in horses is unclear. Infected mice show evidence of significant depression of both humoral and cellular immunity[62] and develop lymphoid depletion in lymphoid tissues.[53] Although infected horses have a transient decrease in antibody production[16] and lymphocyte function,[62] no histologic lesions develop in the lymphoid system.[12, 63] In rickettsial infections antibodies can be protective when they block the pathogen's attachment to or penetration of host cells.[21] Anti-*E. risticii* equine IgG inhibits *E. risticii* internalization and interferes with the metabolic activity of *E. risticii*, rendering them incapable of proliferation in host cells and resulting in the eventual destruction of the organisms.[36] Opsonization of *E. risticii* with anti-*E. risticii* serum renders *E. risticii* more susceptible to macrophage destruction.[84] Mice can be passively protected from *E. risticii* infection by antibody transfer.[26] However, ehrlichias can be isolated from animals in the presence of a rising antibody titre,[14] and presence of antibodies does not always correlate with clearance of ehrlichias or with presence of protective immunity.[60] Effective neutralizing antibodies are the important subset of the general antibody response to ehrlichial infections. In horses, clinical disease occurs in the face of rising antibody titres. Modulation of the immune response by the pathogen so that the initial antibodies are not protective may be an important aspect of the pathogen's virulence.

Clinical signs

There is considerable variation in clinical manifestations of Potomac horse fever. The clinical signs include fever, depression, anorexia, ileus, colic, diarrhoea and laminitis.[40, 45, 48, 51, 52, 69] It is a common misconception that typical cases show most of these signs. On the contrary, any combination of these signs may be present. Common to all cases are the clinical manifestations of colitis which do not always result in colic or diarrhoea. Mild colitis can result in depression and anorexia, with or without fever. Normally formed faeces may be passed without signs of colic being present. Cases with equivocal signs of gastrointestinal tract involvement typically have, on careful auscultation of the abdomen, a remarkable decrease in borborygmal sounds, confirming gastrointestinal disease and long periods of silence broken by short bursts of loud, high-pitched sounds produced by gas passing through a tense intestine with a gas-fluid interface will be revealed. Simultaneous auscultation and finger percussion often result in a high-pitched resonance in the dorsal abdomen. Simultaneous auscultation and ballottement of the abdomen reveal splashing sounds confirming a gas-fluid interface. These signs of ileus are the most consistent clinical findings in all cases, and take on added significance when they occur in the face of an absence of clinical signs of colic, and the passage of normally formed faeces.

The fever is frequently biphasic, although the first phase is often not detected. The initial increased rectal temperature ranges from 39,4 to 41,1 °C but, since it is not accompanied by other signs, the presence of this initial fever will not be realized unless the horse's temperature is measured frequently. It typically resolves within hours and is followed in three to seven days by a more persistent fever accompanied by other clinical signs.

Although diarrhoea is often thought of as a cardinal sign, it develops in less than 60 per cent of cases.[40] When present, faecal consistency can vary from cow-like to watery, pipestream, but the diarrhoea is not as profuse as that associated with enteric salmonellosis in horses. The duration of diarrhoea may be as short as one day and rarely lasts as long as 10 days. Chronic diarrhoea is not a manifestation of *E. risticii* infection.

Decreased feed intake may be a prominent sign. Some horses may have complete anorexia for up to 10 days in the absence of other signs. The major manifestation in other cases may be a profound ileus evident by severe colic, severe toxaemia and dehydration. In some outbreaks severe laminitis in all four hooves may occur in up to 40 per cent of affected horses, but in most areas it is a rare sign.[40, 45, 48] The laminitis, when present, may be the major manifestation of disease and result in humane euthanasia of the horse within days of onset. More often, however, it occurs together with other signs, is mild and completely resolves within three to five days. A few cases develop chronic laminitis.

The course of disease without therapeutic intervention is usually 5 to 10 days. Chronic manifestations, with the exception of laminitis, do not occur. The case fatality rate of untreated clinical cases varies from 5 to 30 per cent. Some fatalities are related to an apparent uncontrolled sepsis, the horse suddenly dying despite not being severely dehydrated or having extreme acid/base or electrolyte imbalances. This may be an extreme inflammatory response orchestrated by a modified systemic inflammatory response syndrome response. Other horses may have to be euthanased because of severe laminitis.[40]

Ehrlichia risticii can cause abortion. Experimentally, naive pregnant mares infected between 100 and 160 days of gestation aborted between 190 and 250 days of gestation. The mares developed typical signs of Potomac horse fever and appeared to have recovered fully before aborting. Abortion is accompanied by placentitis, detectable histologically, and a retained placenta in most cases. Foals which are carried to term may or may not have a presuck titre of serum antibodies to *E. risticii*. In either case full-term foals are healthy and are not ehrlichaemic.[15, 31, 32]

Pathology

The leukocyte count during the early phases of clinical signs may be variable. Leukopenia with a neutropenia and lymphopenia producing a haemogram similar to that typically seen in equine salmonellosis may be present, but many horses have normal leukocyte counts despite other signs of enteritis. A marked leukocytosis can occur within a few days of onset of clinical signs in some cases. A total white blood cell count of 20 000 to 30 000 cells/µl may be found after an initial leukopenia. Although this profound leukocytosis does not occur consistently with *E. risticii* infection, it is a rare finding in mature horses suffering from most other diseases. If it is detected in a horse with consistent clinical signs, suspicion of Potomac horse fever should be high.

Gross necropsy, even in fatal cases of Potomac horse fever, is generally unimpressive. The most consistent postmortem findings are increased amounts of fluid contents in the caecum and large colon, and focal areas of mild hyperaemia primarily in the mucosa of the caecum and large colon but occasionally also in that of the small intestine. The intestinal wall is not oedematous and the mucosa is intact.[12] By light microscopy a colitis is usually evident and ehrlichias can be identified in tissue sections stained with a silver stain.[76] Ehrlichias may also be detected by electron microscopy, and by a tissue polymerase chain reaction (PCR) assay.[41] The histologic appearance is somewhat variable but generally includes multifocal necrosis limited to the mucosa with a mixed inflammatory infiltrate. The histologic lesion often appears mild in relation to the severe clinical disease which has led to a fatal outcome.

In addition to the placentitis, histologic examination of foetal tissues reveals a remarkable colitis, periportal hepatitis and lymphoid hyperplasia of the mesenteric lymph nodes and spleen.

Diagnosis

Although the epidemiology, clinical presentation, haematology findings and response to treatment may all be suggestive of Potomac horse fever, its diagnosis is based on serology, PCR and/or isolation of *E. risticii* in cultures. Although the organism does parasitize circulating monocytes and can be seen by light microscopy of blood smears stained with common haematology stains, only a small percentage of circulating monocytes are infected; this renders the examination of peripheral smears a not very rewarding diagnostic procedure.

Ehrlichia risticii can be isolated from monocyte cultures of blood within days of experimental infection and consistently until 30 days after infection.[44] *Ehrlichia risticii* may also be cultured from affected foetal tissues, especially when the period between infection and abortion is long. The placenta is usually retained and bacterial contamination of it interferes with the isolation of *E. risticii* from it. However, isolation and growth of *E. risticii* in antibiotic-free tissue culture (co-cultivation of tissue or blood monocytes with murine P388D1 cells) are research techniques not easily adapted to diagnostic laboratories. Although a definitive diagnosis of Potomac horse fever requires that *E. risticii* be isolated, in practice

reliance is placed on serology or PCR for its diagnosis. Serology is not a straightforward diagnostic technique because of the somewhat unique kinetics of antibody production stimulated by *E. risticii*. Infected horses have a rapid rise in fluorescent antibody titre, which level out within a few days. In the absence of a blood sample for serology taken in the early stages of the disease, detecting a significant rise in antibody titre may be impossible. Even samples taken within hours of the onset of clinical signs can show fluorescent antibody titres of 1:2 560 or higher. As a result, a decrease in titre is as common as a rise in titre and must be taken into account when antibody titres in acute and convalescent serum samples are compared. Thus a four-fold or greater increase or decrease in fluorescent antibody titre can be considered diagnostic. Approximately half of acutely affected clinical cases will have no significant change in titre because the antibody level has reached a plateau before the first sample is drawn.[35]

The determination of antibody titres in single serum samples serves no purpose. Although clinical cases will have titres greater than 1:80, there is no correlation between height of titre and likelihood of acute disease. Antibody titres in horses that have been exposed to Potomac horse fever for longer than a year before antibody determinations are done may be as high as 1:5 120. On the other hand, if a horse is found to have a negative titre after clinical signs develop, it is probable that it is not suffering from the disease and other diagnoses should be pursued. Treatment with oxytetracycline early in the clinical course of the disease does not affect the development of high antibody levels. Horses which have been vaccinated with a Potomac horse fever vaccine may have titres as high as 1:640. In general, titres consistently decrease after vaccination and after six to nine months have disappeared. When vaccinated animals develop the clinical disease (e.g. when a vaccine 'break' occurs) antibody titres may rise dramatically. In such cases antibody titres are often over 1:100 000 despite the presence of clinical signs of the disease.[35, 40, 70]

The first blood sample for diagnostic serum antibody determinations should be taken early in the course of the clinical disease and the second five to seven days after the first to maximize the chance of seeing a significant change. This short time interval between the first and second antibody determinations is contrary to what is generally the case for most other infectious diseases in which, for diagnostic purposes, reliance is also placed on the differences in antibody levels in acute and convalescent phase sera, the latter being determined two to three weeks or longer after the former. In Potomac horse fever this longer time interval has no advantage. It is of utmost importance to have the antibody levels in both the acute and chronic phase sera determined simultaneously in the same laboratory.

Great care must be taken in choosing a laboratory to assay serum samples. The most common technique used is an indirect fluorescent antibody test. Laboratories adept at running other indirect fluorescent antibody assays may still have difficulty with the procedure for Potomac horse fever. Inconsistent antigen preparations, improper use of positive and negative controls, confusing nonspecific fluorescence, and inexperience with identifying specific fluorescence can all lead to erroneous results. The most consistent results will be obtained from laboratories which frequently run the assay and take great care in running both negative and positive controls.

A recent study has shown that even when performed by experienced research laboratories, the serologic test results may not be accurate. Serum samples from suspect cases and a sample from an experimentally infected horse were sent blindly to several university and private laboratories. Most laboratories reported high titres in many of the samples. Close scrutiny of the samples by Western blot analysis showed that the only true positive sample came from the experimentally infected horse. The rest were false positives.[34] This suggests that caution should be used in interpreting the results of serological tests when used for diagnostic purposes. Serology should be abandoned in favour of more accurate diagnostic methods, as they become available.

A PCR technique has been adapted to detect *E. risticii*.[3, 5, 6, 38, 42, 57] The organism can be identified in blood samples and tissues[41] without the need for culturing, and it takes less than a day. Although false negative and false positive results[54] can occur, with proper controls the PCR assay should be a rapid, definitive diagnostic test and should replace serology as the preferred clinical diagnostic method. This testing procedure should not be viewed as a 'gold' standard since doing so may lead to unnecessary morbidity and mortality from unnecessary antimicrobial therapy resulting in antimicrobial-induced complications, such as antimicrobial-associated enteritis and renal disease, as has recently been reported in human medicine associated with the diagnosis of Lyme borreliosis.[37, 50]

Ehrlichial abortion can be tentatively diagnosed by histologic examination of the foetal colon. In fact, the colonic lesions are as helpful in establishing a diagnosis of *E. risticii* abortion as the foetal liver lesions are in equid herpesvirus abortion. Confirmation is possible through a PCR assay of foetal tissues.[41]

Differential diagnosis

The differential diagnosis of Potomac horse fever includes salmonellosis, intestinal clostridiosis, nonsteroidal anti-inflammatory drug toxicity, antimicrobial-associated enteritis, colitis x, fungal colitis, and toxic enteritis.

Control

Ehrlichia risticii is sensitive *in vitro* to demeclocycline, doxycycline, oxytetracycline and minocycline.[61] Oxytetracycline is the drug of choice for treating *E. risticii* infection in horses. The organism is an obligate intracellular parasite

that survives within phagosomes in macrophages by inhibiting phagosome-lysosome fusion.[82] This inhibition is reversed *in vitro* by oxytetracycline, resulting in destruction of the ehrlichias by the host cell.[82] Oxytetracycline may interfere with the production of a cell wall protein that is responsible for the phagosome-lysosome fusion inhibition.

Oxytetracycline administered intravenously at the dosage rate of 6,6 mg/kg once a day for five days is a very effective treatment for Potomac horse fever when given early in the course of the disease. A response to treatment is seen within 12 hours as noted by a decrease in rectal temperature, followed by an improvement in appetite, habitus and borborygmal sounds.[47] Diarrhoea, if present, does not respond to oxytetracycline therapy.[47] Since destroying the pathogen would be expected to take longer than 12 hours, the rapid clinical response to treatment implies that metabolic by-products acting through the production of cytokines result in the systemic inflammatory response syndrome which may be responsible for many of the clinical signs. Once treatment is begun, progression of the disease stops. The response to oxytetracycline therapy is so dramatic that it can be used to strengthen a clinical diagnosis. If the diagnosis is correct, a response to therapy should be clearly seen within 12 hours (with the exception of a change in diarrhoea). If no improvement occurs within 24 hours, the diagnosis should be reconsidered. If therapy is begun early in the course of the clinical disease, clinical signs resolve by the third day of treatment and no more than five days of therapy are needed.[47] If a relapse occurs after discontinuing therapy, a second course of oxytetracycline is usually as successful as the first.

When treatment is begun early in the course of some rickettsial diseases, relapses may occur.[27, 75, 78] Relapses are thought to be due to lack of sufficient time for adequate immune stimulation of the host resulting in a lack of protection.[75] In experimental *E. risticii* infection, relapses do not occur when therapy is initiated early in the clinical course.[47] However, treatment during the incubation period merely prolongs the incubation period but does not prevent clinical disease from developing.[49] Early treatment does not interfere with the serologic response or the development of protection.[47]

A combination of erythromycin (25 mg/kg) and rifampin (10 mg/kg) administered orally twice a day is also effective in treating Potomac horse fever when given early in the course of clinical disease.[43] The response to this treatment is similar to that to oxytetracycline, with the exception that there is a slightly longer delay before the animal becomes afebrile (24 hours vs. 12 hours).[43, 47] Such a delay in the induction of apyrexia has also been observed when rifampin has been used to treat rickettsial infections in humans. The time period between initiation of therapy and return to normal of other physical findings is similar for both treatment regimes.[43, 47] Although no experimental evidence is available in horses, it is considered that doxycycline administered orally should also be effective in the therapy of the disease.[62]

As in any horse suffering from colitis, the administration of fluids is an important consideration. Fluid and electrolyte replacement therapy should be tailored to suit the individual case. Many of the clinical signs in acute colitis cases can be attributed to inflammatory cytokine responses. These cases may benefit from therapy with anti-inflammatory mediators. When choosing such therapy, it should be borne in mind that experimental evidence suggests interleukin-1 alpha may be much more important than tumour necrosis factor alpha, interleukin-6 or prostaglandins in the inflammatory response caused by *E. risticii*.[79] Secondary salmonellosis is not uncommon in horses suffering from Potomac horse fever and this possibility should not be overlooked. For this reason, and because a definitive diagnosis is often difficult, it is felt that horses suspected of having Potomac horse fever should be isolated despite the lack of direct contact transmission.

Until the mode of transmission is clarified and the vector unequivocally identified, attempts to prevent exposure are unlikely to succeed. Although aquatic environments appear to be a location of one reservoir for the organism, there is no current evidence that the avoidance of such areas will prevent infection since the disease also occurs in horses which have had no direct contact with them.

After infection with *E. risticii*, protective immunity develops to the infecting strain as evidenced by clinical protection to reinfection for as long as 20 months.[46] Protective immunity can be passively transferred,[26] suggesting that it is primarily humoral. Neutralizing antibodies to specific protective antigens appears to be an important aspect in the induction of an effective immunity.[17, 66, 74, 81] These antibodies appear both to block internalization of *E. risticii* and to interfere with its metabolic activity in host cells, rendering it incapable of intracellular proliferation, and result in its eventual destruction.[36] Thus it should be possible to induce immunity by the administration of a vaccine.

A number of inactivated, partially purified, whole cell vaccines are commercially available in the USA. Vaccination has been reported to protect 78 per cent of experimentally infected horses from all clinical manifestations of Potomac horse fever except, in most cases, the fever (22 per cent of them had no clinical signs, 56 per cent developed fever only, 22 per cent developed fever and other signs of disease).[70] Vaccination-induced protection is incomplete at best, which may be associated with poor stimulation of neutralizing antibodies by the vaccine.[17] The protection induced by vaccination wanes much more rapidly than that induced by experimental infection. Only 50 per cent of vaccinates are fully protected at six months after vaccination,[73] and protection decreases to 33 per cent after nine months.[4] Based on this evidence, revaccination at four-month intervals appears to be necessary to maintain a reasonable likelihood of protection. In most endemic areas the disease occurs seasonally. In these areas, if any of the currently available inactivated vaccines are used, horses should

be vaccinated in the spring one month before the first cases are expected and again four months later, if cases are still occurring in the area.

During the past few years, protection by vaccination seems to have become less efficacious. Initially, after the introduction of the vaccines, vaccinated animals which did develop disease appeared to have a mild form of it. More recently, however, some vaccinated horses have developed the severe natural disease from which some died despite the fact that they were apparently adequately vaccinated. In some areas, the prevalence rate of the disease and treatment expenses are not decreased by vaccination.[1] The reason for these vaccine failures may be associated with inconsistent antigen preparation or, more likely, it is associated with the appearance of several genetically distinct strains of *E. risticii* which vary in their ability to induce the formation of protective antibodies.[7, 17, 80] All vaccines currently available contain only the strain that was originally isolated. Polyvalent recombinant vaccines containing antigens which elicit protective antibody formation may, in future, become available. These should increase the efficacy of protection afforded by vaccination.

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ANAPLASMOSES

Anaplasmosis is an arthropod-borne disease of cattle, sheep, goats and some wild ruminant species caused by obligate intra-erythrocytic rickettsial organisms of the genus *Anaplasma* in the family Anaplasmataceae*, order Rickettsiales. The parasites are biologically transmitted by ticks, but mechanical transmission by haematophagous insects and iatrogenic means also occur. Clinically, *Anaplasma* spp. infections in mammalian hosts may range from inapparent infection to severe disease and mortality. Generally, the disease is characterized by fever, progressive anaemia and icterus. Following subclinical infection with *Anaplasma*, or recovery from the disease, infected animals usually remain carriers of the parasite for life.

Differentiation of species within the genus *Anaplasma* was traditionally based on one or more of the following criteria: differences in host range; location within erythrocytes; morphology of the inclusion bodies; and, to some extent, pathogenicity of the organism and immunological differences.[9] Under the traditional system only four species of *Anaplasma* were recognized, three of which (*A. marginale*, *A. centrale* and *A. caudatum*[2]) occur in cattle, while the other (*A. ovis*) occurs in sheep and goats. The changes which have occurred in bacterial classification over the last decade, as a result of the application of phylogenetic techniques, have been noted in the Introduction to the section on diseases caused by Rickettsiales and Chlamydiales. As a result of these changes the genus *Anaplasma* now contains several species, previously included in the genus *Ehrlichia*, which are not intra-erythrocytic.[3] The obligately intra-erythrocytic *Anaplasma* species are now known as the erythrocytic *Anaplasma* species.[7] The name *Anaplasma mesaeterum* was proposed for an apparently distinct erythrocytic *Anaplasma* infective for sheep and goats[6] but as yet there are no phylogenetic data for this organism so its provenance is uncertain.[10]

A variety of African and North American wild ruminant species have been found to be susceptible to *A. marginale* and/or *A. ovis* infection, but anaplasmosis in these hosts is usually mild or subclinical, and very little is known about the role that wild ruminants may play in the epidemiology of anaplasmosis in domestic animals. Uncharacterized *Anaplasma* spp. have been observed in, and in some instances isolated from, a number of wild ruminant species in Africa, and there is serological evidence of *Anaplasma* infections in an even wider host range.[6, 8] Giraffe (*Giraffa camelopardalis*) is the only wild ruminant in which anaplasmosis has been associated with serious disease and mortality.[2, 8] *Anaplasma marginale*[4] and also uncharacterized *Anaplasma* infections[1] have been found in North American white-tailed deer (*Odocoileus virginianus*) but there is no evidence that these animals are an important disease reservoir for cattle.[4]

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* For a classification refer to the introduction to Section 3, Rickettsial and chlamydial diseases

47

Bovine anaplasmosis

Synonyms: Gallsickness, galsiekte (Afrik.)

F T POTGIETER AND W H STOLTSZ

Introduction

Anaplasmosis in cattle is an arthropod-borne disease caused predominantly by *Anaplasma marginale*, and is generally characterized by fever, progressive anaemia and icterus. Two species of *Anaplasma* — *A. marginale* and *A. centrale* — are known to infect cattle in southern Africa. *Anaplasma centrale* generally produces mild disease, and, as cross-immunity between it and *A. marginale* exists, *A. centrale* is used in a live-blood vaccine to protect cattle against the more virulent *A. marginale*. Colostral and non-specific immunity largely protects calves up to the age of about nine months from the clinical effects of infection. Susceptibility to severe disease increases with age.

'Peripheral coccus-like bodies' and 'marginal points' were observed in erythrocytes of cattle by Theiler in 1910, which was long before he identified these inclusion bodies as being the causative organism of the specific disease known by the local farmers at the time as gallsickness.[228–230] Mixed infections of *Babesia bigemina* and *A. marginale*, which frequently occur, led earlier workers to believe that these 'coccus-like bodies' represented a stage in the life cycle of *B. bigemina*.[218] Theiler in 1911[231] referred to the following serendipitous discovery as a 'lucky coincidence'. In 1908, cattle due to be imported from England to South Africa were vaccinated against *B. bigemina* with a South African isolate of this parasite. On their arrival in the then Transvaal, Theiler challenged them with blood from immune local cattle. Fifty per cent of these animals died, having shown fever, icterus, severe anaemia, 'marginal bodies or points' in the erythrocytes but no haemoglobinuria, and this led him to believe that these deaths were due to a different cause. He concluded that the 'marginal points' were independent organisms responsible for the acute signs observed and called them anaplasmata because of their lack of cytoplasm, and the disease caused by them anaplasmosis. The specific organisms were called *Anaplasma marginale* because of the marginal position (**Figure 47.1**) most of them occupied in the erythrocytes.[77]

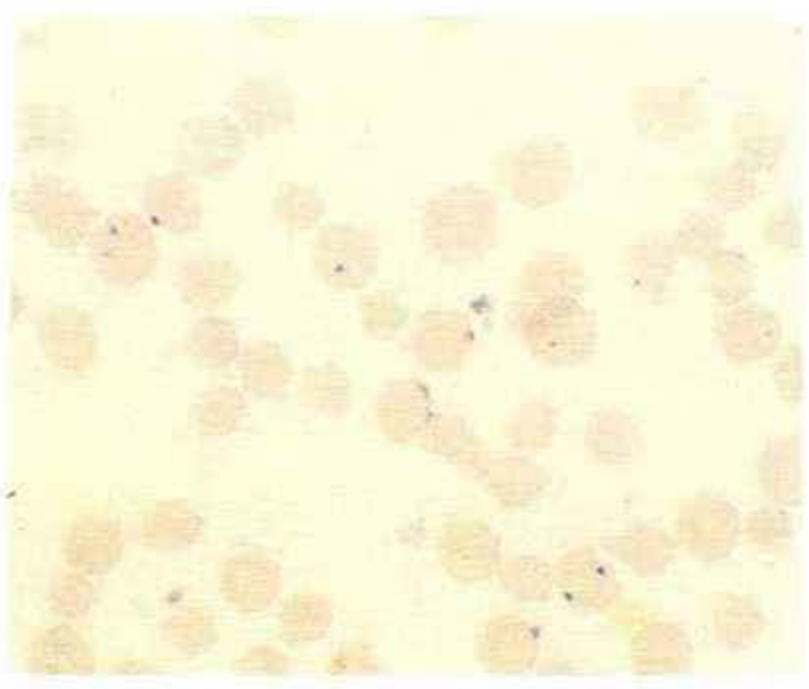


Figure 47.1 *Anaplasma marginale*: blood smear

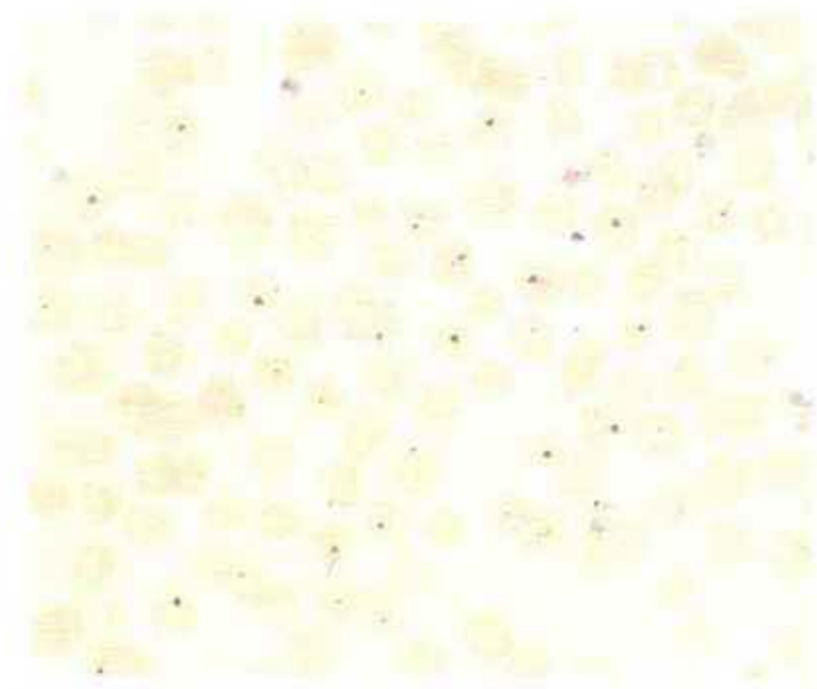


Figure 47.2 *Anaplasma centrale*: blood smear

Theiler[231] in 1911 discovered what proved to be invaluable in the control of anaplasmosis. This was a different variety of the organism, which he called *A. marginale* (var. *centrale*) and which was subsequently named *A. centrale* (**Figure 47.2**). He based the distinction between *A. marginale* and *A. centrale* on:

> the different position taken up by the two parasites within the erythrocyte, the presence of slightly smaller sized organisms in *A. centrale* infections, the lower virulence of *A. centrale*, and the incomplete cross-immunity to *A. marginale* demonstrated in animals recovered from *A. centrale* infections.

Theiler immediately realized the tremendous potential of this organism of lower virulence for use in a vaccine, and the difference in the lengths of the incubation periods of *B. bigemina* and *A. centrale* infections made it possible and practical to vaccinate imported cattle with a combined redwater and anaplasmosis vaccine, which was sold for a shilling.[232]

The first *A. centrale* isolate made by Theiler was probably unique. Since this isolation was made it has been, and still is, extensively used in a live-blood vaccine in southern Africa, as well as in other parts of the world, including Australia, Israel and South America. Therefore, the distinct possibility exists that any subsequent isolations of *A. centrale* in these countries may represent reisolation of the original isolate of Theiler.

A more detailed account of the history of anaplasmosis in the southern African region and elsewhere is given by Henning.[77]

An analysis of the sales records of the *A. centrale* vaccine produced at the Onderstepoort Veterinary Institute[176] serves as a rough indirect parameter of the prevalence and importance of clinical anaplasmosis in South Africa. Judging by the demand for the vaccine and the wide distribution of the tick vectors of the disease identified thus far,[174] bovine anaplasmosis is the most widespread, economically important tick-borne disease of cattle in South Africa. The fact that the disease is also transmitted mechanically increases its potential distribution to areas which are free of the tick vectors. No studies, however, have been undertaken in southern Africa to determine its relative economic importance in the cattle industry. The severity of the disease is, amongst other factors, probably strain-dependent and is not regarded as being of equal importance in different countries. Contrary to the situation in southern Africa, certain South American countries and Asia, anaplasmosis in Australia is a relatively mild disease.[36]

Aetiology and life cycle

Of the three *Anaplasma* spp. that are recognized in cattle,[198] *A. marginale* is pathogenic in cattle, *A. centrale* causes a relatively mild form of the disease, and *A. caudatum*,[103] which has been isolated from mixed infections with *A. marginale*, produces mild to severe anaplasmosis in North America.

There is no small laboratory animal known to be susceptible to *Anaplasma*, and the absence of a suitable laboratory animal model, as well as the lack of a long-term *in vitro* culture system, have placed severe constraints on the progress of anaplasmosis research. Natural infection in the African giant rat (*Cricetomys gambianus*) in Nigeria has been reported,[55] but could not be confirmed in giant rats of the same species trapped in the Mpumalanga Province lowveld in South Africa.[170] In addition, all attempts to infect intact and splenectomized giant rats by inoculation of *A. centrale*- and *A. marginale*-infected bovine blood, and by tick transmission, using *A. marginale*-infected *R. simus* nymphae, have failed.

In Giemsa-stained blood smears, the intra-erythrocytic organisms appear as dense, deeply purple, roundish inclusions 0.3 to 1 µm in diameter (**Figures 47.1 and 47.2**). The organisms of the three bovine *Anaplasma* spp., as well as *A. ovis* in sheep, are morphologically indistinguishable in Giemsa-stained blood smears and are located predominantly marginally in the erythrocyte, except for *A. centrale* where the majority of organisms are located in a more central position, as the name indicates. *Anaplasma caudatum* and some *A. marginale* isolates have so-called inclusion body appendages. These are thought to be a product of bovine host erythrocytes as they do not occur in parasitized erythrocytes of deer.[40] The appendages in North American isolates of *A. marginale* are antigenic[101] and their presence is thought to be linked to tick transmissibility of the isolate.[95] Similar inclusion body appendages occur in anaplasma parasites in the midgut tissues of *Dermacentor andersoni*, which is one of the vectors of *A. marginale*. Their attachment to midgut epithelial cells and intimate association with certain forms of the parasite known as small particles, has led to the belief that these structures may be involved in the infection of tick midgut cells. Nevertheless, some isolates from the USA that possess appendages are not transmissible by ticks.[249]

Ultrastructurally, initial bodies have outer and inner cell membranes with a matrix in between. A filamentous protoplasmic network is attached to the organismic membrane and is continuous with dense chromatin clumps and aggregations.[100]

The development of *A. marginale* in the vertebrate host was first studied in detail by Ristic and Watrach in 1963.[200] Its complete developmental cycle occurs in mature erythrocytes. Initial bodies released from parasitized erythrocytes enter other erythrocytes by invagination of their cytoplasmic membranes to form vacuoles containing the organism.[65, 197] The initial bodies then undergo a series of binary fissions to form structures known as inclusion bodies, which are made up of between four and eight initial bodies (**Figure 47.3**). When erythrocytes containing inclusion bodies are disrupted, initial bodies are released which invade other erythrocytes.

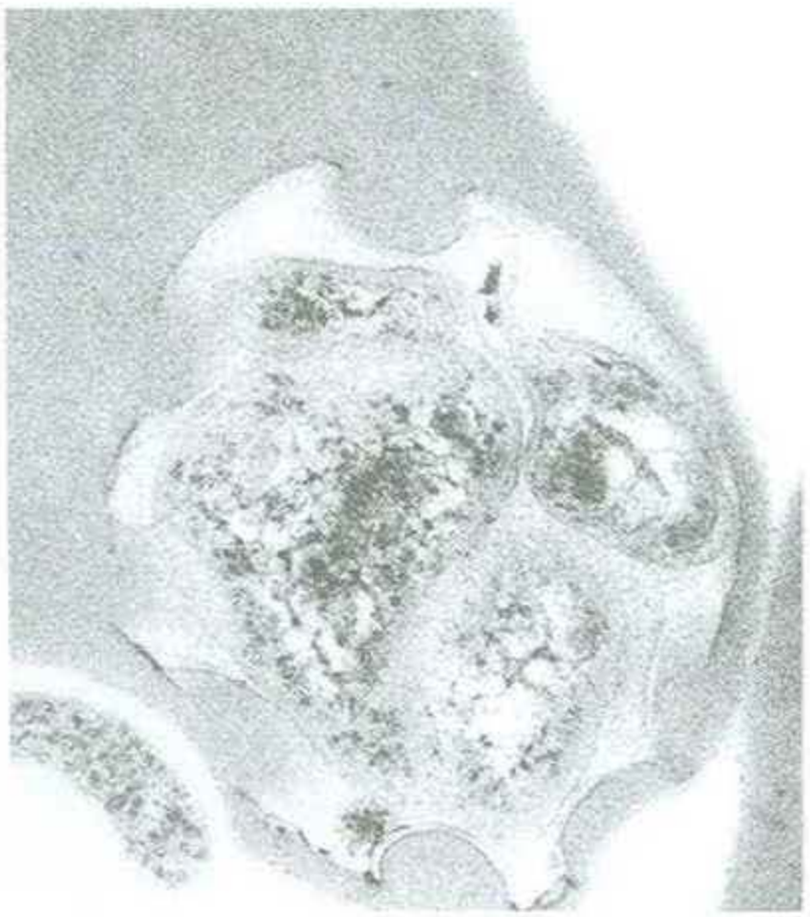


Figure 47.3 Ultrastructural morphology of *Anaplasma marginale*

Ixodid ticks are the principal biological vectors of anaplasmosis, but the argasid tick *Ornithodoros savignyi*, can also transmit *A. marginale*.[188] Several studies have been undertaken to determine the developmental stages and morphological characteristics of *A. marginale* in adult *D. andersoni* ticks.[97, 161, 220] Small electron-dense forms, larger reticulated forms, pleomorphic forms and small particles have been identified in five morphologically different colony types in the midgut of these ticks.[96] Similar developmental stages have been recognized in *Rhipicephalus simus* infected with a South African isolate of *A. marginale*[179] and, although the complete life cycle of the parasite and its mechanism of transmission by ticks are not fully understood, a complex developmental cycle, based on these studies, involving several morphological stages has been proposed.[93]

Theiler in 1911[231] was the first to report on tick transmission of bovine anaplasmosis. He showed that transovarial transmission of the infection by one-host and three-host ticks could take place, although subsequently, the majority of attempts to substantiate this observation, including more recent attempts involving *Boophilus microplus* in Australia, *D. andersoni* in the USA, and five different South African tick species (*Boophilus decoloratus, B. microplus, Rhipicephalus evertsi evertsi, R. simus* and *Hyalomma marginatum rufipes*) have failed.[41, 98, 174, 175, 186] The few successful attempts reported in the literature are generally regarded as being exceptional.[10] It is believed that transmission mainly takes place transstadially, although intrastadial transmission by interhost transfer (i.e., normal feeding being interrupted on one host and continued on another) has also been shown to take place in the one-host tick *Dermacentor albipictus*.[222] This has also been shown experimentally in the five different tick species (*B. decoloratus, B. microplus, R. e. evertsi, R. simus* and *H. m. rufipes*) commonly found on cattle in South Africa[175] and in male *D. andersoni* ticks.[257]

Not all *A. marginale* isolates are equally virulent. Kuttler,[106] using Theiler's *A. centrale* isolate in a study of the comparative virulence of *A. marginale* and *A. centrale*, observed only minor differences between the latter organism and two American isolates of *A. marginale*.

In vitro cultivation of *Anaplasma* has been the objective of numerous studies.[79] Only short-term erythrocyte cultures have been successful, and in these it was shown that cell-to-cell transmission does occur.[90] Successful cultivation of the organism in a tick cell line derived from *Dermacentor variabilis* embryos has been reported.[80] More recently continuous cultivation of the organism *in vitro* was achieved using a tick cell line derived from *Ixodes scapularis*.[153] The morphology and development cycle of *A. marginale* in a tick cell line culture were found to be similar to those observed in naturally infected *Dermacentor* spp. ticks.[24, 93] Such a system offers considerable advantages to future investigations into the biology of *Anaplasma* spp.

Epidemiology

Anaplasmosis has a very wide distribution in the world and appears to be endemic in most of the cattle-farming areas in southern Africa. The occurrence of anaplasmosis is still spreading, and in countries such as France it is a notifiable disease. It is not endemic in Canada but occasional outbreaks do occur, following which it is apparently successfully eradicated, at high cost, by the slaughter of serologically positive cattle.[105]

In South Africa, the role that specific ticks play in the transmission of the disease has not been extensively studied. The one-host tick *B. decoloratus* has been incriminated circumstantially as being the most important vector,[21] simply because occurrence of the disease is thought to correlate with the distribution of this tick. However, four other tick species (*B. microplus, R. simus, R. e. evertsi* and *H. m. rufipes*) have been shown experimentally to be capable of transmitting the infection,[174, 175] and their distribution largely overlaps that of *B. decoloratus*.[81] These ticks are therefore also considered to be involved in the epidemiology of anaplasmosis in South Africa.

In Australia, the epidemiology of the disease is less complex; only a single vector, *B. microplus*, is recognized.

The transmission of anaplasmosis in southern Africa is apparently complex in the sense that some of the tick vectors studied, such as the three-host tick *R. simus*, which has been identified as being an efficient transstadial vector of both *A. marginale* and *A. centrale* in the laboratory, do not feed on cattle under natural conditions except in the adult stage. *Hyalomma m. rufipes* larvae and nymphae will not feed on cattle at all, yet intrastadial transmission by adults

has been demonstrated. Attempts to prove that transstadial transmission occurs in the two-host tick, *R. e. evertsi*, have failed, but again intrastadial transmission with adult ticks removed from clinical field cases has been successfully accomplished in the laboratory. The two one-host ticks *B. decoloratus* and *B. microplus*, contrary to what would be expected, are incapable of transovarial transmission, but both transstadial and intrastadial transmission are possible.[42, 174, 175, 186, 242]

The transmission of anaplasmosis by the interhost transfer of larvae and males of *B. microplus* in the field may be important[139] because *B. microplus* males may survive on cattle for periods exceeding two months.[36] *Rhipicephalus simus* males (infected as nymphae) were shown to have retained the infection after having fed for nine days on a bovine.[183] Male ticks of *D. andersoni*, fed on a chronic carrier cow, subsequently transmitted the infection intrastadially and biologically to susceptible cattle.[257]

The time required by feeding ticks to transmit the infection seems to vary between species. Adult *D. andersoni* have to feed at least six or seven days before transmission takes place,[94] while adult *R. simus* may transmit the infection within 24 hours after infestation.[185]

Not all isolates of *A. marginale* are readily tick transmissible. The *A. centrale* isolate contained in the commercial vaccine produced by the Onderstepoort Veterinary Institute can be transmitted by *R. simus*, but not by *B. microplus* or *B. decoloratus*,[186] whereas both the latter species are capable of transmitting *A. marginale*.

Extracts of ground-up infected ticks from a variety of tick species and salivary gland extracts, but not salivary gland secretions, are infective when inoculated into susceptible animals.[99]

Neither the precise mechanism involved in the transmission of the infection by ticks nor the infective stage of the parasite has been identified with certainty. Developmental colonies of *A. marginale* have been identified in the salivary glands of three *Dermacentor* spp. by fluorescent antibody techniques and electron microscopy.[99, 102, 223]

Anaplasmosis is relatively easily transmitted mechanically by needle passage of infected blood, and, in such cases, the prepatent period of the infection is probably dose-related.[195] Circumstantial evidence indicates that some anaplasmosis outbreaks have followed vaccination programmes in South Africa, incriminating needle transmission between animals. Instruments used in veterinary procedures, such as castration, dehorning and ear-tagging operations, which are contaminated with fresh blood, may also be responsible for the mechanical transfer of the infection.[53]

Reviews on the mechanical transmission of anaplasmosis by blood-sucking arthropods have been published.[53, 172, 234, 256] It is believed that successful mechanical transmission can only be achieved where there is minimal time lapse (a few minutes) between consecutive feeds, although the parasites may remain viable and infective in arthropods for at least two to three days after ingestion.[201]

Some insects that have been incriminated as mechanical vectors include biting fly species of the genera *Tabanus*, *Chrysops*, and *Siphora* and mosquitoes of the genus *Psorophora*.[195] It has also been shown experimentally that calves can be infected with *A. marginale* via the ocular route,[48] and it has been suggested that eye gnats (*Hippelates* spp.) may transmit *Anaplasma*-infected blood to the eye of a susceptible animal after they have visited bloody horse fly wounds.[48] In parts of southern Africa bleeding skin wounds caused by the filarial worm *Parafilaria bovicola* and the behaviour of face flies, such as *Musca xanthomeles*, *M. lusoria* and *M. nevilli*, which lap their meals, and also possibly other *Musca* spp., may be significant in this regard (**see Chapter 3: Vectors: Muscidae**).

In the high semi-arid region of eastern Oregon in the USA, *D. andersoni* has been shown to be the only vector of anaplasmosis. The disease was not transmitted from carrier cattle by any of the haematophagous insects which are present there.[171] In Mississippi, however, Culicidae and Tabanidae were shown to be potential vectors in the absence of ticks.[202] Observations made on a dairy farm in Tanzania incriminated *Tabanus taeniola* as the principal mechanical vector,[251] but, in addition, frequent outbreaks of anaplasmosis were apparently caused by needle transmission. There is little evidence to suggest that anaplasmosis is commonly transmitted by biting flies in Australia.[36]

In southern Africa, the mechanical transmission of *Anaplasma* spp. has not been studied in any detail. The seasonal occurrence of the disease and the prevalence of the cattle louse fly (*Hippobosca rufipes*) in Namibia suggests that this species may be a mechanical transmitter of anaplasmosis. However, while a laboratory investigation failed to demonstrate that this fly can transmit the infection, a similar investigation with the stable fly (*Stomoxys calcitrans*) succeeded.[182] In Zimbabwe it is believed that mechanical transmission of anaplasmosis, after its introduction by infected ticks, may play a significant role in the epidemiology of this disease.[125] In South Africa it is believed that close social association of cattle, as in dairy herds and feedlots, promotes mechanical transmission — especially by stable flies once a tick-transmitted outbreak has occurred. Other haematophagous insects may be involved in this region, but all of them probably play a lesser role than do ticks.

Losses as a result of anaplasmosis occur under both extensive and intensive farming conditions, but dairy cattle seem to be more prone to serious disease outbreaks. This may be due to the close daily observation of dairy cattle which makes this difference more apparent than real.

Cattle reared in endemic areas develop a naturally acquired immunity to the disease. Calves under the age of six months, irrespective of the immune status of their dams, are resistant to severe clinical disease.[157] Cross-bred cattle infected during the first year of life suffer none of the clinical

effects of infection,[169] but the risk of clinical anaplasmosis increases with age.[85] Cattle raised in endemic areas therefore have the opportunity to develop immunity to the disease before the age when clinical effects become severe.

Bos indicus cattle breeds appear to possess a greater resistance to *A. marginale* infection than *Bos taurus* breeds, but variation of resistance of individuals within breeds of both species may well occur.[36] Cattle of the Boron breed have been found to be more resistant than Ayrshires maintained under the same experimental conditions.[130] although there is a natural variation in individual resistance between animals, even amongst those of the same breed.[163, 195]

The nutritional status of animals may play a role in the severity of the clinical disease. Cattle on a reduced energy intake apparently show less severe clinical reactions than do those receiving a normal plane of nutrition,[254] while those on a higher plane may, in general, be most severely affected.[4]

In one laboratory study, a 15,6 per cent prevalence of *in utero* transmission of *A. marginale* and *A. centrale* infections occurred in 77 calves born of 75 infected cows.[187] No clinical signs of disease were observed in any of the calves prior to splenectomy, which was performed when they were a few months old. It is probable that the frequency of *in utero* transmission is underestimated.

Animals which recover from anaplasmosis usually become life-long carriers of the parasite.[38, 39, 172] Autosterilization renders animals susceptible to infection again within several months,[77] because the clearance of parasites from the bloodstream is followed by the disappearance of antibodies to the parasite.[82]

The level of immunity which develops after primary infection apparently depends on the degree of the antigenic stimulus during the primary reaction;[253] the response is, however, inconsistent.[184, 245] *Anaplasma centrale* does not afford complete protection against *A. marginale*,[107, 184, 210, 253] and there are antigenic differences between some isolates of *A. marginale*.[103]

Natural infection involving all animals during calfhood in endemic areas will ensure an 'endemic stable disease situation'. However, low or fluctuating vector populations and limited parasite reservoirs cause varying numbers of susceptible individuals in different age groups to be present within a herd, resulting in 'disease or endemic instability'. Disease stability under these conditions can be achieved by a long-term commitment to the vaccination of all calves born in the herd as well as any cattle that are introduced.

In geographically defined areas that are marginal for vector-tick survival, changing climatic conditions that enable successful, but often only temporary, establishment of vectors may lead to disease outbreaks. Exceptionally wet weather conditions may lead to epidemic spread of anaplasmosis and other tick-borne diseases.[181]

The use of the *A. centrale* live-blood vaccine in South Africa since 1912[232] is believed to have played a major role in the countrywide distribution of this organism. It was also recently shown that this isolate is readily transmissible by ticks, even after 70 years of regular needle passage in the laboratory for the purpose of vaccine production.[186] This mildly pathogenic organism competes with the more virulent *A. marginale* in invertebrate and vertebrate hosts, and possibly plays a significant role in the epidemiology of anaplasmosis.

In endemic areas, clinical disease in herds generally develops only in the older animals that, for whatever reason, escaped infection as calves. Furthermore, the movement of cattle often leads to the unintentional introduction of susceptible cattle onto properties where, because of the existence of a stable or near-stable disease situation, clinical anaplasmosis is never observed or diagnosed, and the properties are therefore thought to be anaplasmosis-free.

It is frequently the case that cattle of unknown immune status introduced into a 'clean' herd can be a source of infected ticks or can act as reservoirs of the infection.

The role that factors such as differences in pathogenicity and immunogenicity of different strains of *Anaplasma* spp., stress, and concurrent infections play in the development and manifestations of clinical disease has not been adequately studied.

Investigation into the role that possible reservoir hosts, other than cattle, play in the epidemiology, has a greater potential in subtropical regions with abundant game, as in parts of southern Africa, than elsewhere. It has been shown that blesbok (*Damaliscus dorcas phillipsi*), common duiker (*Sylvicapra grimmia* L), and black wildebeest (*Connochaetes gnou*) are susceptible to experimental infection with *A. marginale*, but that infections are subclinical.[155, 158] Blesbok are also susceptible to *A. centrale* infection.[156, 158] Based on these observations, it was concluded that anaplasmosis is primarily a disease of antelope, which serve as reservoir hosts for *Anaplasma* spp.

Anaplasma spp. have been recorded in a variety of African game species, such as giraffe (*Giraffa camelopardalis*),[13, 31, 129] sable antelope (*Hippotragus niger*),[233] buffalo (*Syncerus caffer*), and black wildebeest.[177]

Pathogenesis

After primary infection, there is a prepatent period that terminates when the first infected erythrocytes are detected in blood smears. This is followed by a phase during which there is increasing parasitaemia and anaemia, and that ends either in death (preceded by evidence of severe anaemia and other clinical signs) or convalescence and the development of a carrier state.

Neither the minimal infective dose nor the location of the parasite during the incubation or prepatent period has been determined. The duration of the prepatent period seems to be directly related to the number of organisms in

the inoculum, body weight of the host and route of infection. Generally, prepatent periods are shortest after intravenous inoculation, longer after intramuscular inoculation, and even longer following subcutaneous injection. Artificial needle-induced intravenous infections using large volumes (4 litres) of infected blood can result in parasitaemias of up to 88 per cent within four days. The prepatent period can be as long as three to five weeks[197] and, in exceptional cases, it may exceed three months. The course of the infection is not affected by the size of the inoculum,[106] but prepatency may vary even between animals which receive the same infective doses.[175] In spite of this, the number of *Anaplasma* organisms in live-blood vaccines is manipulated in such a way that the desired prepatency is achieved so that a reasonable synchronization of reactions results in those animals that will react clinically to the vaccine. Similarly, in the manufacture of frozen vaccine, adjustments can be made to the number of organisms per vaccine dose to compensate for loss in viability after freezing and thawing of the infected blood.[173] Prepatent periods may be prolonged after administration of tetracyclines.[84]

The intensity and duration of parasitaemia and the resulting anaemia are directly related to the age of the host.[85] Increasing anaemia may develop over 4 to 15 days, and animals may lose up to 70 per cent of their circulating erythrocytes. Maximal anaemia usually occurs one to six days after the peak parasitaemia. In animals that survive the patent phase, convalescence begins with evidence of erythropoiesis. Convalescence usually lasts one to two months, but in aged cattle as well as in splenectomized animals it may be prolonged (three or more months) due to recrudescence of parasitaemia.

Although the manner by which erythrocytes become infected is not clear, an electron microscopic study has revealed how initial bodies penetrate the erythrocytic membrane.[200] It has been suggested that a direct transfer of initial bodies between erythrocytes takes place.[200] Increased osmotic fragility, preceded by a decrease in erythrocytic acetylcholinesterase has been observed in bovine anaplasmosis.[216, 247] This indicates that changes in the acetylcholinesterase activity may be associated with increased cell membrane permeability, which in turn facilitates the entry of *A. marginale* organisms into red blood cells.[216]

The pathogenesis of the anaemia in anaplasmosis was originally thought to be due to destruction of the erythrocytes by the parasites. However, the absence of haemoglobinaemia and haemoglobinuria indicates that intravascular haemolysis is not a feature of the disease.[84] The anaemia is caused by extensive erythrophagocytosis initiated by parasite-induced red blood cell damage and the role of anti-erythrocytic autoantibodies.[14, 27, 104] Erythrocytes that become physically and chemically altered during the course of the disease[54] are removed by the reticuloendothelial (RE) cells in the spleen, liver, lungs and associated lymph nodes.[215] In addition, antibodies produced against the infected and altered erythrocytes sensitize them, thus aiding their removal by the RE system.[104, 194] Autohaemagglutination in blood specimens of *Anaplasma*-infected cattle is effected by immunoglobulins, which react with erythrocytes but not with *Anaplasma* antigens.[138] Anti-erythrocyte antibodies are present in the circulation as both erythrocyte-bound and free antibodies.[211] Apparently, bound autoantibodies are able to attach to normal uninfected erythrocytes.[46, 211, 212] The discrepancy between the number of infected erythrocytes lost and the total loss of erythrocytes can probably be explained by an autoimmune mechanism whereby normal erythrocytes are also removed.[211] Free antibodies developing during the course of the disease do not opsonize erythrocytes and their significance remains unclear in the pathogenesis of anaemia.[213] A factor that stimulates phagocytosis nonspecifically, and that is not produced in the spleen, has been detected in cattle. This factor increases five- to ten-fold at the time of the anaemic crisis in anaplasmosis.[62] It has been suggested that the phagocytosis of infected erythrocytes is limited by the clearance capacity of the RE system.[14, 85]

The rate of erythrocyte loss and the level of parasitaemia during the period of increasing anaemia prior to the detection of reticulocytes in the circulation are usually closely related. Evidence of a haemopoietic response in the peripheral circulation, which is a favourable prognostic sign, is first apparent just before the occurrence of maximum anaemia.[85]

Anaplasma marginale-infected bovine erythrocytes have altered phosphofructokinase activity and very low levels of adenosine triphosphate (ATP),[137] although the glycolytic pathway does not seem to be affected. These phenomena may indicate that *Anaplasma* is reliant on erythrocyte ATP for its survival and multiplication. As ATP is critical in the physiology of erythrocytes, its depletion would cause severe disturbances to their functions.

The membranes of infected erythrocytes are altered with regard to their protein and glycoprotein components.[159] which further indicates that the metabolism of infected erythrocytes is impaired. Lectins form cross-linkages with receptor molecules of cell membranes leading to agglutination of infected as well as non-infected cells, but agglutination is greater with the former.[73] This observation supports the fact that the erythrocyte membrane is altered by the interaction with the parasite.

The nature of the resistance of calves to anaplasmosis is unknown, but it can be overcome by splenectomy.[50] It appears that the competence of the RE system and immunological competence of individual animals are the factors that govern the outcome of the patent stage of anaplasmosis in adult animals.[197] Greater immune competence and the presence of foetal haemoglobulin have been suggested as possible factors associated with higher resistance in young cattle.[9] Age-associated resistance in *Bos indicus* cattle is similar to that described for *Bos taurus* breeds.[163]

Cattle infected with *A. marginale*,[38, 39] and those to which an inactivated vaccine has been administered,[19, 26] develop both humoral and cell-mediated immunity (CMI). As CMI is probably of particular importance in the protective immune response,[19, 38, 39] and as chemically modified *Anaplasma* antigens stimulate mainly CMI, this latter method of producing inactivated vaccine has been pursued in countries where the use of live vaccines is prohibited.

Primary exposure of the host's RE system to replicating parasites provides the antigenic stimulus for the simultaneous induction of both CMI and humoral responses.[197] Subsequent low-level parasitaemia of the infection ensures continuous antigenic stimulation. In the case of re-exposure to the parasite, the memory T-lymphocytes are stimulated to proliferate and afford a specific protective response.

The current model for vaccine-induced immunity against *A. marginale*[167] proposes that clearance of the organism is effected by antibody against surface epitopes in combination with macrophage activation for enhanced phagocytosis and killing. Central to this model is the $CD4^+$ T-cell expression of interferon gamma (IFN-γ), which enhances synthesis of the predominant opsonizing bovine IgG subclass, IgG2, concomitantly activating macrophages to increase receptor expression, phagocytosis, phagolysosomal fusion and release of rickettsiacidal nitric oxide.

Carrier animals cleared of the infection by chemotherapy are apparently resistant to clinical disease following challenge exposure for periods of variable duration, which may be as long as 30 months.[58, 133]

Humoral immunity may contribute to the maintenance of the state of equilibrium in the host–parasite relationship, and suppression of the humoral immune response alters the course and outcome of infection in *Anaplasma* carriers.[45] Recrudescence of clinical anaplasmosis is well recognized, especially in dairy cattle; age, condition of the animal, environmental stressors and the effects of hormones during pregnancy and lactation may be important in this regard. Dexamethazone, a synthetic glucocorticoid, suppresses acquired resistance to anaplasmosis and causes recrudescence in carrier calves.[118] It also suppresses antibody production, and treated animals show more pronounced parasitaemia and anaemia than do controls.[59]

Recrudescent parasitaemias are accompanied by an anamnestic response in both IgM and IgG fractions, suggesting that the maintenance of carrier infections is primarily controlled by antibody responses.[246] Paradoxically, such persistence of infection occurs in the face of a protective immune response, suggesting a mechanism by which the parasite evades the immune system. Low parasitaemias in chronically infected cattle are known to fluctuate in sequential cycles[69, 90] and recent evidence favours the mechanism of antigenic variation,[60, 69] rather than any significant waning of immunity, in explaining rickettsaemic cycles in persistent infections.[167]

Although *A. marginale* may cause abortions and neonatal anaplasmosis,[23, 225] these are rare.[187] Foetuses between 100 and 140 days of gestation are capable of producing anti-*Anaplasma* complement fixation antibody.[239]

Clinical signs

The severity of anaplasmosis is directly related to the age of the animal: in animals less than one year old it is usually subclinical; in yearlings and two-year-olds it is moderately severe; and in older cattle it is severe and often fatal.[84] Differences in virulence between *Anaplasma* strains and the level and duration of the parasitaemia also play a role in the severity of the clinical manifestations.[8] Generally, cases are presented for treatment when the disease has advanced to the stage of the anaemic crisis. Anaemia is usually not clinically apparent until a loss of about 40 to 50 per cent of red blood cells has occurred.[86]

After a natural prepatent period, which generally varies between 15 and 36 days (with an average of 26 days), although it may be as long as 100 days, peracute, acute or chronic anaplasmosis may follow.

Peracute anaplasmosis is the most severe form of the disease. It is rare but usually fatal, and it occurs most frequently in purebred animals and high-producing dairy cows.[195] Animals succumb within a few hours of the onset of clinical signs. In addition to anaemia, milk production ceases, and there is excessive salivation, rapid respiration, irrational behaviour and signs of nervousness.

The most outstanding features of the acute form include pallor of mucous membranes, depression, inappetence, a decrease in milk production, general weakness, and a rapidly rising parasitaemia. In some animals, fever provides initial and persistent clinical evidence of anaplasmosis,[4, 86] but this is not consistent,[163] as in others the rectal temperature may remain below 40 °C throughout a fatal attack.[36] As the disease progresses, constipation, rumen stasis, weight loss, dehydration, icterus, laboured respiration, a thumping heartbeat and muscle tremors are observed in severely affected animals. The faeces are frequently bile-stained. Diarrhoea may be manifested in the initial stages of the disease.[86] Abortions may occur in severe cases.[23, 43, 64, 164, 248] Abnormally aggressive behaviour, probably resulting from the anaemia and resultant cerebral hypoxia, is also manifested by some animals. The appearance of icterus indicates that the acute phase has passed and that convalescence has begun,[36] but not all icteric animals recover. The course of the acute disease is generally protracted and may last for two weeks or more before there is any evidence of improvement in the animal's condition.

Chronic anaplasmosis is manifested by slow recovery following acute disease, and it may persist for a period of between two weeks and three months.[195] It is characterized by poor appetite, loss of weight and varying degrees of dehydration, anaemia and icterus which are usually milder than in the acute disease.

Convalescence following an attack of anaplasmosis is always slow, and a recovered animal may take several months to regain its previous condition. Relapses associated with anaemic changes and fever may occur a few weeks after apparent recovery.[77]

Anaplasma marginale infections may induce temporary infertility in bulls,[86] anoestrus in heifers,[227] and abortions and neonatal anaplasmosis following foetal infection.[23, 160]

The mortality rate, particularly in adult cattle of exotic breeds, may exceed 50 per cent.[77]

Pathology

Increases in levels of serum urea nitrogen (SUN), aspartate aminotransferase (AST), total bilirubin (TBILI) and alkaline phosphatase (ALP) occur at the onset of parasitaemia in acute infections, and these may be related to the destruction of erythocytes by the RE system and cellular damage in the liver and kidneys.[5, 7, 8, 75] On the other hand, it is thought that the increases of AST, ALP and direct bilirubin (DBILI) that occur or continue after peak parasitaemia has been reached indicate liver parenchymal cell damage, and that large quantities of erythrocyte catabolites, probably in addition to hypoxia, lead to liver cell degeneration and other hepatic changes.[8] A study on *A. marginale*- infected calves has shown that the rapid destruction of erythrocytes, and the concomitant rapid release of their contents could lead to imbalances in blood pH and electrolytes.[6] Dramatic reductions in pH and concentration of HCO_3 during the anaemic crisis have been observed in calves that have died of the disease.

Little has been added to the comprehensive description of the lesions made by Theiler in 1910.[228] The lesions are typical of those occurring in animals that have suffered from anaemia due to erythrophagocytosis. Prominent changes in fatal cases include severe anaemia, icterus, splenomegaly and hepatomegaly. Lymph nodes may be enlarged, the carcass may be dehydrated and, depending on the duration of the course of the disease, also emaciated with serous atrophy of perirenal and cardiac fat. The splenic pulp is dark red and has a meaty consistency, while the cut surface of the liver varies in colour from orange-brown to mottled yellow and shows evidence of centrilobular degeneration. The gall bladder is distended and contains inspissated thick brown or yellowish-brown bile. There are serous effusions in body cavities, pulmonary oedema, petechial haemorrhages in the epi- and endocardium, and often evidence of severe gastrointestinal stasis especially of the rumen, omasum and colon. The omasal contents are dry and impacted, and the colon contains hard, dry, often bile-stained faecal balls. A variable degree of red marrow hyperplasia may be present in long bones. The urine is yellow to dark brown due to the presence of bilirubin.

There are few reports on the histopathology of anaplasmosis. Histologically there is evidence of hepatic and renal damage, haemosiderosis, erythrophagocytosis by the RE system and hyperplasia of erythropoietic elements in the bone marrow. The latter may show depletion in chronic cases.[84] The hepatic lesions consist of fatty infiltration, centrilobular and paracentral degeneration and necrosis of hepatocytes, bile stasis, and proliferation and haemosiderin pigmentation of Kupffer cells. Haemosiderin may also be present in macrophages of the lungs and lymph nodes.[240] Varying degrees of degeneration, necrosis and pigmentation (bilirubin and haemosiderin) of tubular cells in the kidneys may be present.[215, 240] It is believed that the parenchymal cell damage in the liver and kidneys is attributable to tissue hypoxia resulting from the severe anaemia.[5, 215]

The spleen shows congestion, hyperplasia of the red pulp, and an increase in the number of plasma cells and haemosiderin-bearing RE cells.[215, 240]

Diagnosis

The diagnosis of anaplasmosis requires a comprehension of not only the pathogenesis of the disease, but also its clinical, pathological and epidemiological features.

A variety of diagnostic methods is available for the confirmation of the clinical diagnosis. In most cases confirmation of a diagnosis depends on the microscopic examination of conventionally stained thin blood smears in order to demonstrate *Anaplasma* organisms in the erythrocytes of an affected animal. However, microscopic examination of blood smears from suspected cases of anaplasmosis is usually performed at a time when the clinical signs are most pronounced (i.e. at the height of the anaemic crisis) and by this time the parasitaemia may be very low because of the removal of most of the infected erythrocytes from the circulation. In addition, the presence of a regenerative anaemia requires careful examination of blood smears to distinguish *Anaplasma* inclusion bodies from basophilic stippling in reticulocytes, Howell-Jolly bodies, and possibly other stained particulate matter in erythrocytes. A positive diagnosis can be made if morphologically distinct organisms are observed in the mature erythrocytes which are still present. Subclinical carriers are difficult to identify by this method because of their low or, more frequently, undetectable levels of parasitaemia.

In a light microscopic demonstration of the intraerythrocytic parasites by the examination of stained blood films, personal preference and experience play a role in the choice of stain and staining procedure. Giemsa stain is very reliable and is commonly used in laboratories. The commercially available Diff-Quick stain set has been found to be superior to Giemsa and Wright-Giemsa staining methods in that it takes only a very short time to stain a blood smear and apparently allows more accurate parasite counts.[56] Other stains that may be used include toluidine blue,[209] new methylene blue,[113] and acridine orange (which requires the use of an ultraviolet-light microscope).[70]

A direct fluorescent antibody staining technique has been found to be superior to the Giemsa staining method for the diagnosis of anaplasmosis at necropsy.[83]

Several serological tests have been developed for the study of *A. marginale* infections. Their principal objective has been to identify carrier cattle, mainly for regulatory reasons, but also for disease control, especially in chemotherapeutic eradication programmes (**see Control**). Serology is also widely used in epidemiological studies and to screen experimental animals and potential live-blood vaccine donors for susceptibility to *Anaplasma* infections. In South Africa the complement fixation (CF) test, and subsequently the rapid card agglutination (CA) test, have been used in anaplasmosis studies, while in Namibia a modified indirect immunofluorescent antibody test (IFAT) was adopted in studies on the epidemiology of the disease.[22, 72]

Both soluble and particulate *Anaplasma* antigens have been isolated from infected animals and used in various serological tests. Despite problems of non-specific cross-reactivity and lack of sensitivity, reasonably reliable tests are available to diagnose anaplasmosis.[113, 131] A high degree of correlation exists between results of the CF and the capillary tube (CT) agglutination tests, as well as between those of the CF and CA tests. Both the CF and CA tests are recognized in the USA as official tests for the interstate movement of cattle where regulations require a pre-shipment negative test.

Enzyme-linked immunosorbent assay (ELISA) is accurate and can differentiate cattle free of the haemoparasite from those with *A. marginale* infections.[18] False positive results, however, have been obtained from cattle that have been inoculated twice with *Babesia bovis*-infected erythrocytes. The cross-reactions are attributed to antibodies produced against erythrocyte antigens.[18]

More recently, a competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) based on recombinant major surface protein 5 (MSP 5) which is conserved among *Anaplasma* species[244] has been developed and shown to reliably detect both acute and chronic infections of *A. marginale* in cattle.[49, 92, 151, 189] Results suggested that the sensitivity and specificity of the test was high[49, 92, 151] and highly comparable to those obtained with the CA test.[151]

The IFAT, adapted for use with microfluorometry, has been successfully used for the diagnosis of *A. marginale* infections.[72] Its accuracy exceeds visual determinations and is more sensitive than both the CF and CA tests in identifying infected cattle.

The simple latex agglutination (LA) test, suitable for practical field use, shows a high degree of specificity and sensitivity when compared to the CA test.[152] Culture-derived soluble LA test antigens are highly specific when compared to antigens derived from infected cattle. In tests conducted with the latter a relatively large proportion of non-specific reactions, which are probably due to the presence of bovine erythrocytic stroma, are encountered.[152]

In a comparison of the CA, IFAT, CF and ELISA tests for the detection of humoral antibodies to *Anaplasma*, an agreement of only 86 per cent was obtained, which was considered inadequate.[255] It is believed by some that the CA test is the simplest and most reliable test, whereas the CF remains useful in experimental situations.[236] The CF test detects antibodies sooner than the CA test, but it does not detect carriers for as long.

Radioimmunoassay (RIA) for the detection of *A. marginale* antibodies has been used.[214] Optimal sensitivity and specificity are obtained in this test by the use of an *A. marginale* antigen and an erythrocyte antigen originating from an uninfected calf. In this test cross-reactions may occur in cattle that have suffered from a *B. bovis* infection, but if the serum samples to be tested are pre-absorbed with an extract of *B. bovis*-infected erythrocytes, the number of cross-reactions is reduced. The use of the two-antigen test system is the major factor in reducing problems associated with cross-reactivity. When purified *A. marginale* antigens become available, cross-reactions of this nature should no longer occur.

Species-specific DNA probes for *A. marginale* and *A. centrale* have been developed[243] for the detection of *A. marginale* infections in carrier cattle[61] and in ticks,[71] but have thus far been used mainly for experimental purposes.

Differential diagnosis

The clinical signs of anaplasmosis should be differentiated from infectious and non-infectious conditions presenting with fever, anaemia, icterus, depression, inappetance, and a reduction in milk yield. Similarly, on post-mortem examination, anaplasmosis should be differentiated from conditions which primarily cause lesions attributable to anaemia resulting from erythrophagocytosis or to icterus.

In southern Africa, anaplasmosis is most commonly confused clinically and pathologically with bovine babesiosis (redwater) caused by *Babesia bigemina* or *B. bovis*. Many of the epidemiological factors of the two diseases are very similar — both diseases are tick-borne, the respective causative organisms share common tick vectors, and animals of different age groups show a similar variation in susceptibility to both diseases. Although the distribution of anaplasmosis in southern Africa largely corresponds to that of babesiosis, anaplasmosis generally enjoys a far wider distribution. One of the main distinguishing clinical features in babesiosis, which is absent in anaplasmosis, is the presence of haemoglobinuria in many affected animals. Haemoglobinuria, however, is not a constant feature of babesiosis, particularly during the early stage of *B. bovis* infection. Although diarrhoea, which commonly occurs in babesiosis, may be present in acute anaplasmosis, constipation and rumen stasis are more regular findings. The course of the clinical disease in anaplasmosis is often longer than that of babesiosis. Care should be taken as outbreaks of anaplasmosis and babesiosis may occur concurrently.

Anaemia and progressive weight loss are also clinical signs often associated with *Trypanosoma* spp. infections. Trypanosomosis (nagana), however, generally occurs within well-defined tsetse-infested areas.

Some forms of theileriosis should, in some circumstances, be differentiated from anaplasmosis. Theileriosis can usually be distinguished by the presence of enlarged lymph nodes and the microscopic demonstration of schizonts in lymph node biopsy smears. High piroplasm parasitaemias and anaemia are most commonly associated with clinical disease caused by *Theileria mutans*.

Anaemia and icterus may also be encountered in cattle suffering from leptospirosis, post-parturient haemoglobinuria, bacilliary haemoglobinuria,[89] chronic copper poisoning and intoxications with *Brassica* and *Allium* spp.[88, 89] In these conditions, haemoglobinaemia and haemoglobinuria are usually also present.[89] Causes of hepatotoxicity, such as *Lantana camara* and *Microcystis aeroginosa* poisonings, in which icterus is usually one of the principal signs, should also be considered.

Changes in behaviour, such as aggression, occasionally manifested by cattle suffering from anaplasmosis may also be part of the clinical signs associated with several other infectious diseases (e.g. heartwater, cerebral babesiosis, cerebral theileriosis and rabies) and non-infectious conditions (e.g. lead poisoning).

Control

Control strategies vary greatly in different countries and geographic regions. Generally, the effectiveness of a strategy in a specific region depends on a thorough knowledge of the epidemiology of the disease, but it is frequently the case that the scientifically based measures are constrained by such factors as legislation, available infrastructure, funding, and the level of management. For these reasons no universal control programme can be advocated.

Following the introduction of anaplasmosis, whether by infected vector ticks, cattle or other reservoir hosts, into regions completely free of the disease and where an epidemic amongst the susceptible resident cattle populations could be expected, eradication of the disease is indicated. This is achieved by identifying infected cattle by microscopic examination of blood smears or serologically. Such animals are then either sterilized by intensive drug treatment, or are slaughtered. Concurrent stringent vector control measures to ensure eradication of infected ticks and to prevent mechanical spread of the infection must be implemented. In order to eradicate the disease successfully, a sophisticated veterinary infrastructure, the support of relevant legislation and the availability of ample funding are essential. Regulatory measures should also be implemented to prevent the subsequent reintroduction of the disease.

In southern Africa, where anaplasmosis is endemic in many of the important cattle farming areas, its eradication followed by the implementation and long-term maintenance of strict tick control programmes (to control it and other endemic tick-borne diseases, such as heartwater, babesiosis and anaplasmosis) is not generally recommended. Furthermore, eradication of anaplasmosis on the subcontinent and most other parts of Africa is not practically possible for obvious reasons. Stringent tick control would render the cattle population susceptible to several other tick-borne diseases with potentially catastrophic consequences (**see Chapter 1: Vectors: Ticks**), as has been experienced in Zimbabwe.[125] The development of acaricide resistance by ticks is common and the cost involved in high-frequency dipping programmes cannot be afforded by many farmers in the region. Further complicating factors are that populations of wild ruminants sustain tick populations and that some are probably reservoir hosts, but much pertinent information concerning these aspects is still lacking.

For these reasons it is generally recommended that a stable disease situation be pursued in the southern African region by allowing natural exposure of calves to tick-borne diseases, including anaplasmosis, during the period when they are naturally resistant or protected by passively acquired maternal antibody. As far as anaplasmosis is concerned, the existence of epidemiological factors which inhibit the exposure of calves to infected ticks within the relevant period can be compensated for by the administration of the *A. centrale* live-blood vaccine. If this control strategy is followed, natural or artificial manipulation of tick populations, or any other factor which may lead to an unstable disease situation, will generally not result in disease outbreaks.

Vaccination strategies differ in accordance with veterinary policies and availability of vaccines. In some states in the USA, for example, the use of live-blood vaccines is prohibited, but inactivated vaccine is available as an alternative. Certain other states, however, have been granted special licences to use live attenuated *A. marginale* vaccines for the control of anaplasmosis.

Immunization

The oldest and probably still the most effective and widely used method of prophylaxis against anaplasmosis is vaccination with Theiler's *A. centrale* isolate.[184, 208, 253] However, *A. centrale*, although generally a mild pathogen, can cause severe clinical reactions following vaccination.[20]

In South Africa the original isolate of *A. centrale*, identified and used by Theiler as a vaccine for the first time, is still used to produce a live-blood vaccine which is extensively used. For many decades this vaccine was only available as a chilled (4 °C) vaccine of which the dose was 2 ml per animal regardless of age and was administered subcutaneously. However, this chilled vaccine has a limited shelf-life,[174] which prohibits its use in countries or regions that lack an efficient cold-chain distribution network.

The limited shelf-life of a live *Anaplasma*-blood vaccine has prompted the development of frozen vaccines (stored at temperatures lower than –80 °C on dry-ice or in liquid nitrogen) with extended periods of usefulness.[145, 173] This offers a practical method of ensuring vaccine availability in regions or countries where continuous production of the chilled product is labour intensive and not cost effective. Infected blood cryopreserved with glycerol remains highly infective after thawing, despite dilution and incubation for eight hours at 30 °C.[87]

In South Africa the use of the chilled vaccine has been replaced by the use of a deep frozen vaccine that is distributed on dry-ice and administered intramuscularly at a dose of 1 ml irrespective of the age of the animal. This deep frozen vaccine may be used for up to four hours after slow thawing in melting ice.

A lyophilized, experimental live-blood vaccine has been developed in South Africa, which is more stable than the frozen vaccine and has the advantage that it can be stored at –20 °C for extended periods without loss of effectivity.[178] For practical purposes this vaccine unfortunately still has to be distributed on dry-ice or in liquid nitrogen.

As young calves do not react severely, it is generally advised that they are vaccinated before or at approximately six months of age. In older animals more severe reactions can be expected and close supervision of them is indicated. Pregnant cows should preferably not be vaccinated as they may abort. The incubation period following vaccination usually varies from five to seven weeks. Vaccinated animals should be closely observed for clinical signs of anaplasmosis during this period, and should, if necessary, be specifically and symptomatically treated. In view of the possible reaction to vaccination in older animals, only a limited number of animals should be immunized at any given time.

The use of an inactivated *A. marginale* vaccine prevents serious clinical disease.[26] However, the need for two initial inoculations, followed by periodic booster injections, may result in fatal neonatal isoerythrolysis in calves born to vaccinated cows.[51] It has been suggested that the use of an inactivated vaccine of sheep origin may avoid eliciting the neonatal isoerythrolysis syndrome.[37] Cattle vaccinated with this vaccine are still highly susceptible to challenge with heterologous strains.[124] Such vaccines are not available in southern Africa.

A method by which a minimal infective dose of *A. marginale* (obtained by diluting heavily infected frozen blood stabilates after thawing and prior to inoculation) is used to reduce the severity of the clinical reaction following vaccination has been successfully applied under experimental conditions.[67, 74] In field trials it was established that the minimum infective dose for *A. marginale* is 10^6 parasites following intravenous inoculation; the same dose is, however, not infective when administered subcutaneously.

The attenuation of *A. marginale* by irradiation of a virulent strain and its subsequent serial passage in deer and sheep has been accomplished.[199] This strain, which has a virulence similar to that of *A. centrale* and produces a higher level of immunity against virulent *A. marginale* challenge than does *A. centrale*,[111] has been successfully used for vaccination purposes in cattle in Mexico,[162] Colombia[44, 245] and California.[78] However, in Argentina[11] *A. centrale* is preferred because of severe vaccine reactions experienced with the attenuated *A. marginale* vaccine.

The prolonged maintenance of a virulent *A. marginale* field isolate in a splenectomized white-tail deer (*Odocoileus virginianus*) has apparently been sufficient to induce attenuation of the organism for cattle.[123] Vaccination of cattle with this attenuated parasite protects against a virulent heterologous *A. marginale* challenge.

Similarly, some *Anaplasma* isolates from other wild ruminants may also produce only mild infections in cattle, suggesting a loss of virulence after prolonged maintenance of *A. marginale* in the wild hosts.[117, 129] There is some evidence to suggest that such attenuated *A. marginale* isolates may regain virulence upon passage in cattle.[129] On the other hand, cattle are frequently not susceptible to infection with anaplasms from wild ruminants, or they develop only subclinical infections that do not provide immunity to virulent *A. marginale* challenge, indicating the possible existence of unidentified *Anaplasma* spp. that are host-specific or that are possibly more closely related to *A. ovis*.[117]

Subsequent to the establishment of long-term continuous *in vitro* cultivation of *A. marginale* in a tick cell line,[153] it was shown that culture-derived organisms retained all current candidate diagnostic and vaccine antigens as well as infectivity for cattle, thus providing a valuable alternative source of organisms for the development of diagnostic tests and vaccines.[15]

The current anaplasmosis vaccines rate poorly against the concept of an ideal vaccine.[154] By definition the latter should be a so-called dead vaccine, have no attendant risk of inducing disease, afford life-long protection, neutralize natural infection, and prevent a carrier state from developing, thereby removing the source of infection for vectors. In addition, ease of production, long shelf- and storage-life, and affordable cost are important elements which will also determine the success of such a vaccine.

Advances in molecular biological techniques have stimulated attempts to produce a subunit vaccine for anaplasmosis based on the presence of an antigen common to all *Anaplasma* isolates. Such a common antigen, referred to as the major surface protein complex MSP-1 (previously designated AM105), composed of two proteins, AMF105 and AMF100 (previously designated AM105U and AM105L, respectively), has been shown to immunize cattle against homologous and heterologous *A. marginale* challenge.[141, 165] The genes coding for both proteins have been cloned and expressed in *Escherichia coli* and are being characterized for possible future construction of a recombinant or synthetic replica of MSP-1 which could be used as a

vaccine.[16, 141] A second potential subunit vaccine candidate, MSP-2 (previously designated AM36), has also been identified and this similarly protects cattle against both homologous and heterologous challenge.[166]

Treatment

The treatment of clinical anaplasmosis in cattle requires, firstly, the suppression or elimination of the parasite (specific treatment) and, secondly, the alleviation or prevention of secondary complications and the hastening of recovery (symptomatic or supportive treatment).[84]

Secondary complications, generally the result of tissue hypoxia, are a frequent sequel to anaemia and in acute anaplasmosis the severity of this anaemia is, to some extent, proportional to the extent of the parasitaemia.[30, 84, 228] However, even in animals with relatively low-peak parasitaemias (below 10 per cent), or in animals with persistent low parasitaemias (0.1 to 2 per cent), a significant anaemia may develop if they are not treated.[32, 237] Therefore, specific treatment early in the course of infection is desirable,[32, 47, 63, 84, 115, 146, 147, 149, 196, 197] and such treatment prior to the development of a high parasitaemia or the onset of severe anaemia is considered essential for a favourable outcome.[115, 250] Often, however, by the time that acute anaplasmosis is diagnosed the level of parasitaemia is already declining as a result of the phagocytic removal of parasitized erythrocytes, and the animals are suffering from severe anaemia.[30] Nevertheless, even when treatment is slightly delayed, specific treatment is more beneficial than supportive treatment.[147]

Specific treatment

Prior to the development of the tetracycline drugs, a variety of chemotherapeutic agents, including arsenicals, antimalarials, antimony derivatives and dyes, were used to treat acute anaplasmosis, but these had little, if any, chemotherapeutic effect on *Anaplasma* infections.[66, 77, 147, 150, 168, 197]

In many countries the tetracyclines (chlortetracycline, tetracycline, oxytetracycline, rolitetracycline, doxycycline and minocycline) are the only effective chemotherapeutic agents approved for the treatment of anaplasmosis.[114, 115, 134, 196, 197] Of these, chlortetracycline, tetracycline and oxytetracycline are the most commonly used drugs for the treatment of acute and chronic carrier infections.[112, 114, 115, 208] *Anaplasma* infections in cattle are not sterilized at the usual recommended therapeutic doses of the tetracycline drugs.[120, 147, 221]

Gloxazone (alpha-ethoxyethylglyoxal dithiosemicarbazone) and two derivatives of carbanilide, amicarbalide (amicarbalide isethionate) and imidocarb [3,3'bis-(2-imidazolin-2-yl)-carbanilide dihydrochloride (or dipropionate)] also have specific chemotherapeutic effects on *Anaplasma* infections.[17, 32, 52, 108-110, 112, 121, 143, 144, 203] However, gloxazone has not been made commercially available because of its unacceptably severe toxic effects in lactating cows.[142] The carbanilides, particularly amicarbalide and imidocarb, on the other hand, have been approved for commercial sale in some countries. These two drugs are primarily used as antibabesials.[2, 3, 115, 218, 238] The carbanilides are usually administered parenterally, and at normally recommended therapeutic dosage levels do not sterilize *Anaplasma* infections in cattle.[2, 3, 32, 52, 109, 112, 143]

There is some controversy about the desirability of treating acute anaplasmosis at the time of maximum anaemia.[30] Much of this controversy is related to whether or not an animal will recover spontaneously, and the conflicting opinions which have been expressed range from those who consider treatment to be beneficial at this time to those who feel that the excitement and exertion incident to treatment are more likely to be detrimental to the animal. At the time when increased erythropoiesis becomes evident, specific treatment probably has little, if any, effect on the course of the disease, since at this stage the parasitaemia is in any case rapidly decreasing as a result of the phagocytic removal of infected erythrocytes, and spontaneous recovery is frequent.[30] Specific treatment with chemotherapeutic drugs is therefore probably only useful when the parasitaemia is increasing.

The tetracycline drugs effectively inhibit the multiplication of *Anaplasma* in erythrocytes and have been widely used for the treatment of anaplasmosis.[28-30, 47, 63, 140, 147, 196, 197] Single intravenous administrations of chlortetracycline at a dosage rate of 11 mg/kg body weight, or single parenteral (subcutaneous, intramuscular, or intravenous) administrations of oxytetracycline at dosage rates ranging from 2.2 to 6.6 mg/kg body weight have proved effective in reducing *Anaplasma* parasitaemia and alleviating clinical signs.[12, 147, 148] However, when employing a standard or short-acting (SA) oxytetracycline formulation (containing 50 to 150 mg/ml concentrations of the drug), tetracycline or chlortetracycline for the treatment of clinical anaplasmosis, two to three injections administered intravenously or intramuscularly at the rate of 6.6 to 11 mg/kg at 24-hour intervals are usually recommended.[84, 115] The usual dosage rate of oxytetracycline (SA) used under field conditions is 10 mg/kg.[208, 221]

The chemotherapeutic effect of doxycycline, rolitetracycline and minocycline in the treatment of acute anaplasmosis is similar to that of oxytetracycline (SA), with two to three administrations at a rate of 4 to 10 mg/kg being recommended at 24-hour intervals.[120, 140, 250]

Single intramuscular administrations of long-acting (LA) formulations of oxytetracycline (200 mg/ml concentration), at the rate of 20 mg/kg body weight appear to be equally effective in moderating the course of *A. marginale* infections in cattle.[120, 221, 235] The use of a single treatment of oxytetracycline (LA) for acute anaplasmosis has an obvious practical advantage in that cattle receiving multiple injections of oxytetracycline (SA), or any of the other tetracycline drugs, need to be restrained repeatedly and receive larger (two to six times) total volumes of the drug formulation.[122, 135, 235]

Imidocarb hydrochloride[76] and its dipropionate salt[143]

both have a marked inhibitory effect on *A. marginale*. Therapeutic dosage levels of imidocarb for the treatment of acute anaplasmosis in cattle vary from 2 to 5 mg/kg body weight[208] and should be administered subcutaneously or intramuscularly.[112, 114] It has been found that a single subcutaneous administration of imidocarb at a dosage rate of 2,5 mg/kg body weight is as effective as higher rates varying from 5 to 10 mg/kg in the control of acute anaplasmosis,[203] and that a dosage rate of 2,5 mg/kg body weight is the most efficacious under practical field conditions.[2, 144]

Administration of imidocarb at recommended therapeutic dosage rates usually causes transient side-effects.[2, 3, 144, 203] These include a localized swelling at the injection site (following subcutaneous administration) and systemic effects, characterized by increased salivation, lachrymation and nasal discharge.[2, 142, 203] The severity of these signs is dose-related, and when doses are repeated or increased to eliminate infections (**see Control: Chemosterilization**), more severe clinical signs of toxicity including diarrhoea, dyspnoea and depression frequently occur.[1] Since intravenous inoculation similarly results in more severe signs of toxicity[112, 114, 144, 238] and even mortality at rates of 3 mg/kg or higher,[112, 238] this route of administration is not recommended.

It has been found that imidocarb administered once at a dosage rate of 3 mg/kg body weight is a more effective chemotherapeutic agent than oxytetracycline, tetracycline or chlortetracycline, even when multiple administrations of the latter drugs are used.[150] Apart from only a single dose being required, imidocarb has the added advantage of being highly effective against *Babesia* spp.,[2, 3, 115] and the neccesity of making a differential diagnosis in areas in which both anaplasmosis and babesiosis occur is obviated.[114, 208] However, this latter practice is not generally recommended since a specific diagnosis is essential for the implementation of appropriate control measures for the two diseases. Furthermore, the dosage level of imidocarb required for the treatment of anaplasmosis in cattle results in the sterilization of *Babesia* infections, and possibly renders the animals susceptible to babesiosis on re-exposure.[2, 3]

One or two subcutaneous administrations of amicarbalide at a dosage rate of 10 mg/kg body weight have a therapeutic effect on *A. marginale* infections comparable to two intravenous administrations of oxytetracycline (SA) at a dosage rate of 10 mg/kg.[52]

Following successful treatment of a primary *Anaplasma* infection with any of the above-mentioned drugs, the parasitaemia decreases to 1 per cent or lower within four to seven days,[203, 250] but there are frequent relapses (recrudescences of parasitaemia) of varying intensity at variable intervals thereafter.[2, 32, 52, 112, 144, 221] The severity of this relapse parasitaemia appears to depend on the virulence of the parasite strain and the susceptibility of individual animals. In non-splenectomized cattle, the relapse reaction is generally much less severe than was the primary reaction and clinical signs of anaplasmosis are seldom observed. These mild relapse reactions usually do not require treatment.[237] Relapse reactions in splenectomized cattle, on the other hand, are frequently as severe as, or only slightly less severe than, the primary reactions[32, 52, 120] and frequently require treatment.

The duration of the relapse interval may reflect the efficacy of the drug and the treatment regimen used, as a longer interval between relapses indicates greater suppression of the parasite.[221] In one investigation it was found that the mean duration of the relapse interval in untreated control cattle was approximately 18 days, compared to 36 days in animals receiving two treatments of oxytetracycline SA (at a rate of 10 mg/kg, 24 hours apart), and 56 days in animals receiving a single treatment of oxytetracycline LA (at a rate of 20 mg/kg).[221] Similarly, three treatments with oxytetracycline SA (11 mg/kg), combined treatments of oxytetracycline and gloxazone, or three treatments of gloxazone also resulted in longer relapse intervals.[108–110]

After a single administration of imidocarb (2,5 to 3,5 mg/kg), relapses occur approximately three to four weeks after treatment,[108–110] while at lower dosage rates, treated animals relapse earlier. A second treatment with imidocarb administered at the onset of a relapse delays and reduces the severity of the relapse. However, when the second dose is administered before a relapse occurs, its onset is delayed but its severity is not reduced.[2, 108–110, 144]

Supportive treatment

Following treatment with an effective chemotherapeutic compound, the *Anaplasma* parasitaemia declines over a period of several days, reaching levels of 1 per cent or lower only after approximately one week. The decline in parasitaemia is usually accompanied by a reduction in packed cell volume over a similar period of time. It is therefore important to bear in mind that the clinical condition of an animal may deteriorate considerably as a result of progressive anaemia notwithstanding successful specific chemotherapy, and that supportive symptomatic treatment may be required to prevent mortality.[30]

The administration of blood transfusions to cattle suffering from acute anaemia is advocated in the treatment of anaplasmosis,[66, 77, 132, 135] but the recommendations for the actual volume of blood to be used for this purpose vary greatly. To be effective, it would appear that large volumes (2 to 10 litres or more, depending on the size of the animal and the extent of the erythrocyte loss) need to be transfused.[84, 132] As a general guide, 15 ml of whole blood per kilogram body weight has been recommended.[136] However, the results from such administrations are often disappointing.[84] For reasons that are not quite clear, blood transfusions may also lead to a rapid rise of *A. marginale* parasitaemia in cattle,[84, 252] but generally they lead to an improvement in the condition of anaemic animals, particularly those animals with severe anaemia in which increased haematopoiesis is not yet

evident.[132] Despite the possible benefits to be derived from blood transfusion, the technique does not lend itself to large-scale application under field conditions and should probably be reserved for very valuable animals. Where blood transfusions are indicated, blood should be administered slowly, keeping in mind the risks of vascular overload and the possibility of anaphylaxis due to incompatible blood groups, although the latter apparently seldom occurs. The donor animal should also be carefully selected so as to prevent the possible transmission of other blood-borne parasites.[77, 132] Because of the accelerated removal of erythrocytes in cattle with anaplasmosis,[14, 232] the benefits to be derived from blood transfusion are of short duration (18 to 48 hours), and transfusions should therefore be repeated, preferably within 24 hours.[84] Blood transfusion is contra-indicated in animals that are restless and difficult to restrain, or in those with icterus.[84] The transfusion and excitement incident to treatment may cause stress and the death of the animal.[30, 84]

Because of the risk of mechanical transmission of *Anaplasma*, affected cattle (especially those with high parasitaemias) are a major source of infection to uninfected susceptible cattle in the herd and should preferably be separated from the healthy animals. Discretion should be applied in deciding whether to remove the healthy animals or the diseased ones, as severely affected animals could succumb as a result of exertion. When isolating diseased animals, consideration must be given to the provision of adequate protection against the elements, easy access to palatable feed and water, as well as facilities for continuing observation of the animals and for the administration of any additional chemotherapy, which would require the minimum amount of exertion on the part of the animals.[77]

Additional symptomatic treatment may be beneficial and is usually recommended. Although haematinics such as cobalt and iron probably do not have adequate time to stimulate erythropoiesis sufficiently to influence whether or not an animal will recover,[30] these and liver-supportive drugs may reduce the time required for convalescence. Furthermore, correction of any gastrointestinal disturbances such as ruminal stasis, constipation or lack of appetite by the oral or parenteral administration of rumenotorics, ruminal fluid, mild laxatives and appetite stimulants, may help prevent additional secondary complications and promote a more rapid recovery.

Longer-term control of anaplasmosis can be achieved by a variety of methods, which may include one or more of the following that involve the use of chemotherapeutic drugs:

- the use of chemotherapy to control primary vaccine reactions following the administration of live vaccines (infection and treatment method of vaccination);
- prevention of clinical disease in susceptible cattle by the prolonged administration of chemotherapeutic drugs during exposure (chemoprophylaxis); and
- elimination of carrier infections (chemosterilization).

Control of vaccine reactions

Following the administration of live-blood vaccines, some animals may develop clinical anaplasmosis. For all practical purposes these cases should be appropriately treated specifically and symptomatically as though they were naturally acquired clinical infections.

Chemoprophylaxis

No chemoprophylactic drugs, such as those used for the control of bovine babesiosis, are available at present for the control of anaplasmosis in cattle. The chemoprophylaxis of anaplasmosis is, therefore, largely based on the constant or repeated administration of chemotherapeutic drugs to control *Anaplasma* parasitaemias in naturally infected cattle.

Generally, chemotherapy during the prepatent period merely results in the prolongation of the prepatent period and delays the onset of clinical signs.[57, 63, 127] With repeated administrations of oxytetracycline LA (at a rate of 20 mg/kg at seven-day intervals), commencing seven days after infection, the prepatent period is extended by approximately the same time-span as the duration of treatment.[57, 127] Although all animals thus treated remain infected, fewer animals develop clinical signs of anaplasmosis as the number of treatments increases.

For animals exposed to natural infection, a treatment regimen consisting of intramuscular administrations of oxytetracycline SA (at a rate of 6,6 to 11 mg/kg or LA, at a rate of 20 mg/kg at 21- to 28-day intervals) to all susceptible cattle throughout the season when vectors are most active and abundant, and continued for a further one or two months after its end, have been employed or recommended.[84, 114, 128, 190] This treatment regimen should prevent clinical disease in most animals that become infected, but will not sterilize *Anaplasma* infections. Thus, infected animals become carriers and subsequently develop premunity.

Similar protection against clinical disease is afforded by prolonged oral administration of chlortetracycline throughout the vector season, or throughout the year.[114, 190, 193]

The use of sustained-release oxytetracycline boluses has also shown considerable promise in the chemoprophylaxis of bovine anaplasmosis.[33–35] This method should impose fewer management and labour constraints relating to parenteral administrations of tetracyclines, and should provide more predictable and consistent drug levels than is possible with oral administration of chlortetracycline in medicated feed or licks.

When tetracyclines have been used to prevent clinical anaplasmosis, animals should continue to be closely observed for clinical signs of the disease after withdrawal of medication.

Chemosterilization

Anaplasma infections in cattle can be sterilized by a variety of drugs and treatment regimens (**Tables 47.1 and 47.2**). The methods used to determine whether successful sterilization

Table 47.1 Successful treatment regimens for the sterilization of *Anaplasma* infections with the tetracycline drugs. (Modified from Kuttler, 1979[114])

DRUG	RATE OF ADMINISTRATION	ROUTE	NUMBER OF TREATMENTS	INTERVAL
Tetracycline	11 mg/kg	IV or IM	10	Daily
Tetracycline	11 mg/kg	IV or IM	10	Daily*
Tetracycline	11 mg/kg	IV or IM	5	Daily
Oxytetracycline	11 mg/kg	IV or IM	10–14	Daily
Chlortetracycline	33 mg/kg	IV	16	Daily
Chlortetracycline	2,2 mg/kg	Orally	41	Daily
Chlortetracycline	5,5 mg/kg	Orally	45	Daily
Chlortetracycline	1,1 mg/kg	Orally	120	Daily**
Chlortetracycline	11 mg/kg	Orally	30–60	Daily
Chlortetracycline	3,3–5,5 mg/kg	Orally	60	Daily
Chlortetracycline	11 mg/kg	Orally	30–60	Daily
Minocycline	20 mg/kg	IV	2	7 days
Minocycline	10 mg/kg	IV	6	2–3 days***
Oxytetracycline	22 mg/kg	IV or IM	5	Daily
Oxytetracycline (LA)	20 mg/kg	IM	2–4	7 days
Oxytetracycline (LA)	20 mg/kg	IM	4	3 days

IV = Intravenous; IM = Intramuscular; LA = Long-acting

* Divided schedule of two five-day treatment periods with an intervening period of 10 days

** Negative status determined by serological procedures

*** Divided within 12 days

or elimination of carrier infections has been accomplished include haematological and serological examination of treated animals, the subinoculation of blood from treated animals into susceptible splenectomized calves, and splenectomy of the treated animals.[207]

However, treatment to eliminate *Anaplasma* infections in most endemic areas is contra-indicated because of the high risk of reinfection and the relative susceptibility of the cattle population in the absence of any long-term immunity which accompanies low-level persistent infections (premunity).[114]

With the advent of sensitive serological tests, a method has been developed whereby the source of infection (chronic carrier cattle) can be eliminated from a herd. It involves testing the herd in order to identify the carriers which may then be slaughtered,[25, 105] segregated and kept isolated from the rest of the herd,[190] or sterilized of the infection by chemotherapy.[84, 190, 196] This approach has successfully been used in parts of the USA and Canada, but is usually only feasible in areas with a low or moderate prevalence of anaplasmosis.[114, 196]

The disappearance of antibodies to *Anaplasma* spp. from the serum of treated cattle, determined serologically, is most often used under field conditions to indicate successful sterilization of infection.[196, 197] However, serum from animals in which the infection has been cleared may still react positively in these tests for periods varying between two and six months following treatment,[134, 206] or even longer, particularly when using the CF test.[68, 82, 170, 205] Both the CT and CA test titres subside more rapidly than do CF test titres,[82, 197] indicating that the former tests may be more reliable indicators of successful elimination.[134] On the other hand, it has been found experimentally that consistently negative CF and CA test reactions in animals following the elimination of their infections are only obtained after 12 months have elapsed.[205] Serological testing to determine the success of elimination should thus only be conducted after sufficient time has elapsed to allow animals to revert to sero-negativity. Any animals serologically positive after this period should be regarded as treatment failures and either retreated or separated from the rest of the herd.[114]

Because of the possibility of transplacental infection, calves should also be screened serologically, and positive cases treated or eliminated in order to prevent them from becoming a future source of infection to adult cattle that have been cleared of infection. It must, nevertheless, be borne in mind that the serum of uninfected calves born of infected dams may also react positively in serological tests for periods of up to three months after birth, due to the presence of colostral antibodies.[187]

Sterilization regimens of *Anaplasma* infections with the tetracycline drugs are summarized in **Table 47.1** and include the parenteral administration of tetracycline,[30] oxytetracycline (SA),[82, 134, 170, 207, 219] oxytetracycline (LA),[132, 207] minocycline[140] and chlortetracycline,[219] as well as the oral administration of chlortetracycline.[68, 191, 226, 241] It would appear that the duration of an effective level of antibiotic in animals is an important factor in the successful elimination of infection. Treatment failures frequently occur at the lower dosage levels and when divided treatments are given.[140, 170] Furthermore, reportedly successful treatment regimens have not always yielded consistent results. For example, although two administrations of oxytetracycline LA (at 20 mg/kg body weight) at an interval of seven days reportedly eliminated carrier infections in non-splenectomized cattle,[207] three administrations of oxytetracycline LA (at 20 mg/kg body weight) at seven-day intervals during the incubation period failed to

Table 47.2 Treatment regimens for the sterilization of *Anaplasma* infections. (Modified from Kuttler, 1979[114])

DRUG	RATE OF ADMINISTRATION	ROUTE	NUMBER OF TREATMENTS	INTERVAL
Oxytetracycline	11 mg/kg	IV	3	1 or 2 days
+				
Gloxazone	5–10 mg/kg	IV	3	1 or 2 days
Oxytetracycline	20 mg/kg	IM	4	7 days
+				
Imidocarb	5 mg/kg	IM or SC	2	14 days
Oxytetracycline (LA)	20 mg/kg	IM	4	7 days
+				
Imidocarb	5 mg/kg	IM or SC	2	7, 14 or 21 days
Imidocarb	4–6 mg/kg	IM or SC	3	Daily
Imidocarb	2 mg/kg	IM or SC	3	Daily
+				
Gloxazone	5 mg/kg	IV	3	Daily
Imidocarb	4–5 mg/kg	IM or SC	2	14 days

IV = Intravenous; IM = Intramuscular; SC = Subcutaneous; LA = Long-acting

eliminate *Anaplasma* infections.[57, 127] The observed difference between the efficacy of the drug in eliminating infections in the carrier stage and that in the incubation period, and the finding that four intramuscular administrations of oxytetracycline (LA, at a rate of 20 mg/kg, or SA, at a rate of 20 mg/kg) at seven-day intervals, also failed to eliminate carrier infections in splenectomized cattle,[224] seem to suggest that the host immune response probably plays an important contributory role in the success of treatment.

Oral administration of chlortetracycline for the elimination of *Anaplasma* infections may have advantages in that treatment on a herd basis (in the form of feed premixes incorporating the drug) is possible,[190] but the consistent elimination of carrier infections by this method is often difficult to attain because of differences in amounts of medicated feed or salt-mineral mixes consumed by individual animals.[112, 196, 197]

Sustained-release oxytetracycline boluses administered to *A. marginale* carrier cattle result in a reduction or elimination of CF test titres in most animals but fail to eliminate the infections.[33, 34]

Alternative treatment regimens to eliminate *Anaplasma* infections in cattle involve the use of imidocarb and/or gloxazone, as well as combinations of these drugs with oxytetracycline (**Table 47.2**). As with the treatment regimens that involve the use of tetracycline drugs alone (**Table 47.1**), variable degrees of success have been achieved.[3, 204, 206, 224]

There appears to be considerable individual variation in tolerance to imidocarb[112] and severe toxicity and even mortality may result at the dosages recommended for chemosterilization. For example, although it has been reported that the administration of imidocarb at the rate of 4 to 6 mg/kg body weight on three consecutive days is a safe treatment for the elimination of *A. marginale* carrier infections in cattle,[109] mortality may occur in calves treated with three doses of imidocarb at a rate of 5 mg/kg body weight at 24-hour intervals.[225]

High, but safe, dosage levels of amicarbalide fail to eliminate *A. marginale* infection,[52] and it would appear that the repeated high doses probably required for successful elimination result in such severe hepato- and nephrotoxicity that the use of this drug for chemosterilization of *Anaplasma* is precluded. The high dosage rates of gloxazone which apparently eliminate *A. marginale* infections[108, 109] also resulted in fatal toxicosis in all treated animals in one trial. However, combined treatments with gloxazone and oxytetracycline, or gloxazone and imidocarb, are more efficient in eliminating carrier infections than is treatment with either drug alone.[108–110, 112] Attempts to eliminate infections with a combination of oxytetracycline and imidocarb have apparently been unsuccessful.[112] On the other hand, the combination of four oxytetracycline treatments (LA or SA at a rate of 20 mg/kg body weight at seven-day intervals) and two imidocarb treatments (5 mg/kg body weight at 14-day intervals) (either of which treatments have individually been reported to eliminate carrier infections), appears to eliminate infections more consistently than does either drug used alone.[224]

Cattle in which infections have been eliminated shortly after primary infection exhibit no residual immunity upon challenge with *A. marginale*.[140, 195] In contrast, cattle cleared of carrier infections are susceptible to reinfection but show resistance to clinical anaplasmosis for periods ranging from a few to 30 months following parasite elimination.[58, 133, 190–192, 205]

Furthermore, re-exposure of carrier animals to *Anaplasma* infection shortly before or during the period of treatment may result in reinfection and failure to eliminate the infections, even when treated with parenterally administered drugs at dosage rates in excess of those required to eliminate infections under laboratory conditions.[116, 119] This augments the recommendation that programmes aimed at the elimination of the carrier state are best conducted during the period of lowest vector activity and

should be accompanied by the implementation or maintenance of vector control measures.[114, 115] Long-term oral administration of chlortetracycline for 45 to 60 days or longer has been proposed as being a possibly more suitable method for the elimination of carrier infections in herds where re-exposure cannot be prevented.[116]

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Ovine and caprine anaplasmosis

W H STOLTSZ

Introduction

Anaplasmosis of sheep and goats is an arthropod-borne disease caused by the intra-erythrocytic rickettsial organism, *Anaplasma ovis*. It is usually a subclinical or mild condition, but moderate to severe clinical disease is generally characterized by fever and a variable degree of anaemia and icterus that may occasionally lead to death. Recovered animals remain carriers of the organism, resulting in premunity, which persists for life.

Anaplasmosis in sheep was first reported in 1912 in Zimbabwe.[4] Although a number of reports of *Anaplasma* infections in sheep and goats in Africa soon followed,[51, 52, 62] the report by Di Domizio in 1919[8] is considered to be the first authentic description of anaplasmosis in non-bovine hosts. In 1924 Sergent *et al.*[49] reported an *Anaplasma* infection in sheep that was not transmissible to cattle, and Lestoquard[22] differentiated the causative organism from the *Anaplasma* spp. of cattle, describing and naming it *A. ovis*.

Ovine and caprine anaplasmosis occurs in many parts of Africa, North and South America, Asia (including the Middle and Far East), the southern and central parts of eastern and western Europe, and the former USSR.[2, 13, 19, 28, 32, 35, 43, 53, 54, 56, 66, 69] However, the disease may be more widely distributed than present data indicate.

The condition in southern Africa is of relatively little economic importance. Because of its often insidious nature, the deleterious effects caused by *A. ovis* infection, such as weight loss and unthriftiness, are probably erroneously ascribed to other causes. Although mortality due to anaplasmosis in sheep and goats is seldom observed in southern Africa, it would appear that under certain circumstances, overt disease, abortion and mortality may occur.[1, 3, 35]

Aetiology and life cycle

Anaplasma ovis is the main cause of ovine and caprine anaplasmosis. It is more pathogenic for goats than for sheep[56, 63, 69] and only very rarely is it infective to cattle.[20, 48] *Anaplasma mesaeterum*, the name proposed for a hitherto unrecognized anaplasm of sheep and goats,[63] is closely related to *A. ovis* and may also cause clinical disease in sheep and goats, but, unlike *A. ovis*, it appears to be less pathogenic for goats than for sheep and is not infective for cattle.[63] *Anaplasma marginale*, a species pathogenic for cattle, may also, under certain circumstances, cause latent infection in sheep and goats.[9, 16, 24, 29, 34, 36]

In Giemsa-stained blood smears *A. ovis* is morphologically indistinguishable from *A. marginale*,[56, 57] and appears as irregularly shaped, almost spherical, intra-erythrocytic granules staining a deep purple colour. The organisms (inclusion bodies) vary in diameter from approximately 0,4 to 0,8 µm,[22, 35, 57] with an average of 0,5 µm.[35] Within erythrocytes, 60 to 70 per cent of the inclusion bodies are located marginally and 30 to 40 per cent submarginally or centrally.[22, 34, 56, 57] In comparison, up to 90 per cent of the inclusion bodies of *A. marginale* are situated marginally.[34] Differences between *A. ovis* and *A. marginale* with regard to the intra-erythrocytic location of organisms have been found to be statistically significant.[34]

The ultrastructure of the marginal bodies of *A. ovis* in sheep erythrocytes is very similar to that of *A. marginale* in bovine erythrocytes.[15, 30, 44] Development of *A. ovis* in sheep and goat erythrocytes, and in the tick vector, is presumably similar to the cycle of development proposed for *A. marginale*.[16, 17, 45, 57] The inclusion bodies of *A. mesaeterum* are morphologically indistinguishable by both light and electron microscopy from *A. ovis*, but a distinctive feature is that fewer than 30 per cent of the inclusion bodies are situated marginally in erythrocytes.

Apart from the morphological similarities, complete serological cross-reactivity has been demonstrated between *A. ovis* and *A. marginale*.[57] They are nevertheless immunologically distinct as infection with one does not provide immunity against the other.[56] *Anaplasma ovis* and *A. mesaeterum*, on the other hand, appear to be immunologically more closely related, but cross-immunity is nevertheless incomplete, a situation somewhat reminiscent of the relationship between *A. centrale* and *A. marginale* in cattle.[63]

Epidemiology

Anaplasmosis in sheep and goats has been reported from most tropical and subtropical parts of the world.[2, 19, 28, 35, 43, 53, 54, 56, 66, 69] With the exception of Namibia, *A. ovis* has been reported from all the countries in southern Africa and is believed to be prevalent in most sheep- and goat-farming areas. In these areas, small numbers of parasites may frequently be detected during routine microscopic examination of blood smears from sheep and goats. Considering that microscopic examination of blood smears and serological techniques fail to distinguish between *A. ovis* and *A. marginale*,[20, 56] the fact that sheep and goats exposed to *A. marginale* may yield serologically positive results without being immune to *A. ovis* may be of epidemiologic significance. Similarly, calves infected with *A. ovis* have been noted to react positively to *A. marginale* antigens in the complement fixation and rapid card agglutination tests, and yet were fully susceptible to *A. marginale* infection.[20] Thus far *A. mesaeterum* has only been recorded on the Dutch island, Ameland, but circumstantial evidence seems to indicate a possible wider occurrence in northern and north-eastern Europe.[63]

The prevalence rates of *A. ovis* infection in sheep or goats vary considerably within endemic areas, and may be influenced by management factors such as the control measures employed and the movement of livestock from non-endemic to endemic areas.[35] Although no extensive surveys have been conducted to determine the prevalence of *A. ovis* in South Africa, limited microscopic observations in sheep have revealed a prevalence ranging from 33 to 90 per cent in endemic areas.[59, 64] Similarly, in Mozambique, its prevalence ranges from 0 to 85 per cent in sheep and from 0 to 90 per cent in goats.[2] In India it has been shown that approximately 60 per cent of sheep and 70 per cent of goats are infected,[27] while in Kenya the prevalence of infection in goats from different localities ranges from 22 to 87 per cent.[54]

Although sheep and goats of all ages are susceptible to *A. ovis* infection,[1, 54, 56, 60] older animals suffer from a greater reduction in haematocrit values.[56, 69]

Overt clinical disease and mortality are rare in non-splenectomized animals,[11, 35] but splenectomy drastically increases the susceptibility of animals and usually results in a recrudescence of parasitaemia (relapse) in carrier animals.[6, 7, 19, 20, 28, 34, 47, 56] It may thus be assumed that any condition that results in suppression of immunity or decreased resistance to disease in animals, such as debilitating diseases, concurrent infections (particularly of other arthropod-borne rickettsial and protozoal infections such as *Babesia* spp., *Theileria* spp., *Trypanosoma* spp. and *Eperythrozoon ovis*,[2, 5, 34, 57, 59, 60] heavy helminth or tick parasitism,[31] malnutrition,[13] and other stress factors (such as pregnancy), as well as variations in the pathogenicity of different parasite strains and individual susceptibility, could lead to a higher prevalence of clinical disease and mortality.

The tick vectors of *A. ovis* in southern Africa have not been determined. Based on the observations elsewhere that *A. ovis* and *A. marginale* may share common tick vectors,[48, 58] one or more of the tick vectors of *A. marginale* or *A. centrale* reported from South Africa[38, 39] may also prove to be vectors of *A. ovis*. However, during experimental studies on the tick transmission of *Theileria separata*,[59] *Rhipicephalus evertsi evertsi* and *Rhipicephalus evertsi mimeticus* transmitted only *T. separata* from splenectomized sheep infected with both *T. separata* and *A. ovis*.

Experimental studies have indicated transstadial transmission of *A. ovis* by *Ornithodoros lahorensis*,[41] *Rhipicephalus bursa*[40] and *Rhipicephalus turanicus*.[56] Transovarial transmission in *Dermacentor silvarum* has also been reported.[42] Adults of *Dermacentor andersoni* have been found to be naturally infected,[48] and intrastadial transmission by adults of this tick species has been demonstrated.[58] Other tick species that have been incriminated as vectors include *Hyalomma plumbeum*,[37] *Haemaphysalis otophila*,[37] *Dermacentor albipictus*, *D. occidentalis* and *D. variabilis*.[58] *Haemaphysalis punctata* and *Ixodes ricinus* are suspected of being vectors of *A. mesaeterum*.[63]

Considering the ease with which *A. ovis* can be transmitted by the subinoculation of blood, transmission by iatrogenic means or mechanical transmission by biting flies would appear to be distinct possibilities. However, experimental attempts to transmit the parasite by means of bites of the sheep ked, *Melophagus ovinus*, a likely candidate as a mechanical vector of *A. ovis* in sheep, have failed, even when sheep with high parasitaemias were used as the source of the infection.[68]

Intra-uterine transmission has been reported in sheep[10] and in goats.[3] In sheep, transplacental infection has been found to occur during the second and third trimester of pregnancy, but no lesions have been observed in foetuses or neonatal lambs.[67] In contrast, a high percentage of artificially infected female goats aborted or resorbed their foetuses following acute infection.[3]

Sheep and goats that have recovered from *A. ovis* infection remain carriers of the parasite, and in areas where both sheep and goats occur, sheep may be particularly important as reservoir hosts because of the lower pathogenicity of *A. ovis* in this host.[55]

White-tailed deer (*Odocoileus virginianus*), eland (*Taurotragus oryx*) and blesbok (*Damaliscus dorcas phillipsi*) are susceptible to experimental infection with *A. ovis*,[12, 18, 34, 36] and these hosts could act as potential reservoir hosts of infection for sheep and goats.[12, 34] Although wild ruminants usually develop only mild or subclinical infection, severe disease may occur after splenectomy.[18, 34]

A variety of North American, Asian and African wild ruminant species has been found to be susceptible to *A. marginale* infection (**see Chapter 47: Bovine anaplasmosis**). Naturally acquired *Anaplasma* infections isolated from blue wildebeest (*Connochaetes taurinus*), Coke's hartebeest (*Alcelaphus buselaphus cokii*) and Thompson's gazelle

(*Gazella thompsonii*) resulted in mild infections in calves and provided little or no immunity to subsequent *A. marginale* challenge.[25] Positive serological reactions to *A. marginale* antigens have also been recorded in impala (*Aepyceros melampus*), waterbuck (*Kobus ellipsiprymnus*) Grant's gazelle (*Gazella grantii*) and eland,[25, 26] but attempts to recover *Anaplasma* spp. from impala and Grant's gazelle by inoculation of their blood into cattle have failed. These results seem to indicate the possible existence of hitherto unrecognized *Anaplasma* spp. in wild ruminants, some of which may be more closely related to *A. ovis*,[21] as has been demonstrated in the case of a sable antelope (*Hippotragus niger*) in South Africa.[61] Circumstantial evidence would similarly seem to indicate the susceptibility of wild ruminant hosts for *A. mesaeterum*.[63]

Naturally occurring infections with *A. marginale* in sheep and goats have not been reported although these species may become latently infected with *A. marginale* after experimental infection.[30] Prolonged repeated passage by subinoculation of blood in sheep may lead to the gradual adaptation of *A. marginale* to this host, which results in a higher parasitaemia during infection and an attenuation of the organism for cattle.[9, 14, 16, 20, 24, 36, 43, 46]

Sheep and goats cannot be consistently infected experimentally with field isolates of *A. marginale*,[7, 16, 29] indicating that there are possible strain differences of *A. marginale* with regard to its ability to infect these hosts. Furthermore, the carrier state of *A. marginale* in sheep and goats may be of short duration.[11, 29] In splenectomized goats this transient carrier state has been found not to exceed 208 days.[29]

Anaplasma ovis has only rarely been shown to be infective for cattle. Numerous attempts to transmit it to non-splenectomized[48] and splenectomized cattle have failed,[7, 9, 30, 34, 47, 56] and this characteristic of *A. ovis* is often used to differentiate it from *A. marginale*. This difference would seem to indicate a greater host specificity in *A. ovis* than in *A. marginale*. However, short-term survival of *A. ovis* in splenectomized calves has been demonstrated,[48] but confirmation of infection requires the subinoculation of blood into splenectomized sheep. In one study,[20] *A. ovis* could not be recovered from a splenectomized calf at 17 days post-infection, but the animal was subsequently found to have retained the infection for up to 262 days. No loss of virulence of *A. ovis* for sheep has been observed following maintenance in splenectomized calves.[20, 48]

Pathogenesis

The pathogenesis of ovine and caprine anaplasmosis appears to be similar to that of *A. marginale* infection in cattle **(see Chapter 47: Bovine anaplasmosis)** and, as in bovine anaplasmosis, anaemia results from erythrophagocytosis.[37] Unlike the situation in bovine anaplasmosis, there appears to be no marked variation in age susceptibility to *A. ovis* infection in sheep and goats, and both young and adult animals usually develop only a mild form of the disease.[35, 56] Various stress factors and splenectomy increase individual susceptibility and predispose to acute disease.[5, 13, 34, 48, 56]

Splenectomy of chronically infected (carrier) animals is frequently followed by a severe recrudescence of parasitaemia and manifestation of clinical disease, indicating the importance of the spleen in controlling the infection and maintaining immunity.[5–7, 19, 20, 47, 56, 59]

In experimental transmission studies, in which *A. ovis*-infected blood is used as the inoculum, the prepatent period may vary from 5 to 40 days, with an average of about two to three weeks.[20, 22, 35, 56, 57] Generally, the duration of the prepatent period is inversely proportional to the number of parasites in the infective inoculum.[23] Similarly, following transmission by a single pair of adult *Dermacentor andersoni*, a prepatent period of slightly more than six weeks has been recorded in a splenectomized sheep,[48] whereas the prepatent periods are shorter when there are larger numbers of ticks.[58]

The parasitaemia gradually increases to a maximum level one or two weeks after the parasites have first become microscopically detectable.[3, 19, 56, 57] In non-splenectomized animals, the maximum parasitaemia may range from approximately 0,1 to 4 per cent in sheep, and approximately 1 to 12 per cent in goats, but in splenectomized animals the maximum parasitaemia is more variable and may range from 2 to 90 per cent.[19, 20, 28] The course of *A. ovis* infection in splenectomized sheep is identical to that of *A. marginale* in cattle, with the number of parasites doubling approximately every 24 hours.[57]

During the period of rising parasitaemia, parasites are removed from the circulation by phagocytosis of infected erythrocytes. This results in a gradual decline in the number of parasites and a concomitant development of a progressive anaemia. The haematocrit value gradually declines and reaches its lowest level a few days after the peak of parasitaemia, when parasite numbers are already reduced.[19, 69]

The severity of the anaemia is usually greater than can be accounted for by simple removal of parasitized erythrocytes alone. Severe anaemia in the absence of a high parasitaemia[31, 63] suggests an autoimmune response which results in the phagocytosis of infected as well as uninfected erythrocytes by the reticuloendothelial system.[63] In non-splenectomized sheep and goats, with average maximum parasitaemias of 0.8 and 2 per cent respectively, the corresponding average reductions in haematocrit values were approximately 40 and 50 per cent.[56] However, in individual animals, the reduction in haematocrit values may be considerably greater. Similar reductions in haematocrit values were observed in *A. mesaeterum* infection, despite the presence of a low parasitaemia.[63] In splenectomized animals, haematocrit values may be reduced to well below 10 per cent.[30]

The corresponding drop in the haemoglobin concentration,[3, 31, 48, 56, 69] if severe, will lead to hypoxic damage to various organs, which may ultimately result in the death of the animal.

Sheep and goats that recover from *A. ovis* infection remain carriers of the parasite.[22, 34, 35]

Clinical signs

The clinical or patent phase of the disease lasts approximately one to two weeks.[19, 30] In animals that survive this phase, convalescence begins with increased erythropoiesis, which is characterized by reticulocytosis, anisocytosis, polychromatophilia, basophilic 'stippling' of immature erythrocytes and the presence of Howell-Jolly bodies. Convalescence is usually slow and may last from several weeks to a few months.[69] During this time, the haematological and clinical signs gradually return to normal.

Anaplasma ovis infection in sheep and goats usually results in subclinical or mild disease, but occasionally severe clinical disease and mortality may occur. When mortality does occur, its rate is seldom very high and may be due, in part, to concurrent infections with other haemoparasites.[11] Because of the greater pathogenicity of *A. ovis* for goats, clinical signs of infection are more frequently observed in them than in sheep.[3, 31, 56, 69]

Fever is not always present and temperature reactions are quite variable. A slight rise in body temperature may occur at the onset of patent parasitaemia, followed by a return to normal for a few days before it again becomes elevated.[3] The maximum rise in body temperature usually coincides with the peak of parasitaemia,[22, 56] and may range from 40 to 42 °C. In some animals the temperature may fluctuate during the clinical phase of the disease,[22] while in others it may remain elevated for more than a week, before returning to normal.[3, 35]

The clinical signs are usually most pronounced at the height of anaemia, which occurs a few days after the parasitaemic peak and approximately one to two weeks after parasites first become microscopically detectable. The severity of clinical disease is to some extent dependent on the degree of the maximum parasitaemia,[22] but severe anaemia may occur in the absence of a high parasitaemia.[31, 56] However, even in animals with low haematocrit values, no obvious clinical signs of disease may be observed,[1, 3, 56] but signs of rapid fatigue and severe respiratory distress, indicative of lung oedema, may become evident when they are herded or forcefully exercised.[3, 56] Occasionally, animals may die acutely upon exertion.[1]

Generally, only slight listlessness and weakness,[22, 56, 69] or a loss of condition[5] are noticeable. The appetite usually remains unaffected, but reduced appetite or anorexia, rumen stasis and constipation may be observed in some severely affected animals.[3, 56, 60, 69]

On closer clinical examination of affected animals signs of anaemia, such as pale mucous membranes, thin watery blood, and increased heart and respiration rates are evident.[5, 22, 69]

Icterus is not consistently observed, even in cases which end fatally.[35, 69] Submandibular oedema of the jowl and ventral part of the neck may be noticed.[35] In milch goats, a marked drop in milk yield may occur, and this may persist for several weeks.[35]

The return of normal appetite and an improvement in the general condition of the animal may serve to indicate recovery.[31] However, recovery from clinical disease is usually slow,[67] and anaemia and emaciation may persist for several weeks when the nutrition of the animals is inadequate.[35]

Abortion and resorption of embryos have been reported in domestic goats infected experimentally with *A. ovis*.[3] These phenomena occurred particularly in those animals which had developed a high fever and severe anaemia. *Anaplasma ovis* was transmitted transplacentally and frequently caused anaemia and death of the foetus *in utero*. In foetuses and live or stillborn kids, the *A. ovis*-parasitaemia ranged from 1 to 12 per cent.

Pathology

The severity of the lesions vary according to the duration and the severity of the disease.[35] Numerous reports include descriptions of necropsy findings.[1, 3, 22, 28, 35, 56, 60, 69]

The gross lesions to a large extent resemble those found in cattle that die of anaplasmosis. The carcass generally appears pale because of the anaemia, and may be slightly or moderately icteric. The blood is thin and watery, and emaciation, serous atrophy of fatty tissues, and anasarca or dehydration may also be apparent. Increased amounts of straw-coloured fluid may be present in body cavities. The lungs are pale or congested, and oedematous. The liver is enlarged and yellowish-brown and the gall bladder may be enlarged and filled with viscous yellow-green bile. Nephrosis and enlargement of lymph nodes and adrenals may also be evident.

Microscopically in blood smears, evidence of a regenerative anaemia, characterized by the presence of reticulocytosis, anisocytosis, polychromasia, basophilic stippling and Howell-Jolly bodies, is found. In animals suffering from severe anaemia a monocytosis with erythrophagocytosis (as evidenced by the presence of parasitized erythrocytes, or their remnants, in macrophages) and eosinophilia (after a number of remissions of severe anaemia) may be observed.[5]

Changes in parenchymal organs characteristic of anaemic and hypoxic states may be seen in various organs (e.g. centrilobular degeneration and necrosis of varying intensity in the liver, and degeneration especially in cells of the convoluted tubules of the kidneys). The canaliculi in the liver may be distended as a result of the bile stasis. The red bone marrow is usually hyperplastic, but in chronic cases

may show evidence of depletion. Haemosiderin accumulation in reticuloendothelial cells is usually most extensive in the spleen.

Diagnosis

The possibility of anaplasmosis should be considered in all cases of anaemia in sheep and goats in the tropics and subtropics.

During the acute stage of the disease, a diagnosis in the individual animal is usually made on the basis of clinical signs, the presence of the organism in blood smears and haematological evidence of infection. However, when clinical signs are most pronounced the degree of parasitaemia may have already declined considerably and confirmation of the diagnosis may be difficult.[56] In addition, the presence of a regenerative anaemia during this stage may complicate the microscopic detection of parasites (*Anaplasma* inclusions at times being difficult to differentiate from basophilic stippling in reticulocytes and Howell-Jolly bodies or other stained particulate matter in erythrocytes). Microscopic detection of infection during the chronic or carrier stage is also often extremely difficult because of the exceedingly low parasitaemia. In doubtful clinical cases and during epidemiological surveys, serological tests, subinoculation of blood into susceptible animals, and DNA hybridization techniques may provide additional confirmation.

The subinoculation of blood from suspected carrier animals into susceptible splenectomized sheep or goats is an effective way of detecting *A. ovis* infection, but the technique is too expensive and time-consuming for routine application.[54] Various serological techniques including the complement fixation (CF) test,[30, 56, 67] capillary tube agglutination (CT) test,[27, 31] direct- and indirect fluorescent antibody (FA and IFA) tests[18] and the rapid card agglutination (CA) test[30, 67] have been used. Due to the high degree of serological cross-reactivity between *A. ovis* and *A. marginale*,[30, 56] either of these may be used as antigen, but *A. marginale* antigen is most frequently employed because of its greater availability.

Sheep and goats, on average, develop positive reactions in the CF test from a few days before the onset of patent parasitaemia.[48, 56] Antibody titres are highest during patent parasitaemia and lowest during the carrier phase but, generally, sheep and goats develop lower antibody titres than do cattle infected with *A. marginale*. The majority of sheep and goats remain serologically positive after one year, but some known carriers have been observed to revert to negativity against *A. marginale* and *A. ovis* antigen.[48, 56] The CA test similarly seems to lack the sensitivity to detect all carrier infections in sheep and goats.[30, 59]

Uninfected lambs born of infected ewes may react positively in the CA test and the CF test for variable periods, but antibody levels diminish or disappear at the age of approximately three or four months.[67]

The CT test has been found to be a reliable technique for the detection of infection in goats.[31]

Similarly, a competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) based on a major surface protein 5 (MSP 5) B-cell epitope which is conserved among *Anaplasma* spp.[65] was shown to reliably detect different isolates of *A. ovis* in both acute and chronically infected goats.[33] Results suggested that the sensitivity and specificity of the test was high.

Serological techniques fail to distinguish between *A. ovis* and *A. marginale* and such differentiation may be of epidemiologic significance. DNA hybridization techniques, using either radio-labelled or biotin-labelled species-specific probes,[54, 64] can differentiate between these species and appear to be more sensitive than the examination of blood smears by light microscopy for the detection of *A. ovis* infection in sheep and goats.

Differential diagnosis

The clinical signs of anaplasmosis in sheep and goats are non-specific and the disease, therefore, should be differentiated from other infectious and non-infectious causes of such signs as fever, anaemia, icterus, listlessness, inappetance, loss of condition, constipation, and submandibular oedema of the ventral region of the neck.

Fever, listlessness, inappetance, and loss of condition may be caused by a variety of infectious and non-infectious diseases including babesiosis, eperythrozoonosis, ehrlichiosis [*Ehrlichia ovina* and *Ehrlichia (Cytoecetes) phagocytophila* infections], theileriosis, trypanosomosis and severe internal or external parasitism. Of these, eperythrozoonosis and verminosis (e.g. haemonchosis) are probably the most likely to be confused with anaplasmosis in southern Africa, and either may occur concurrently in the same host. *Eperythrozoon ovis* frequently occurs as part of a mixed infection with *A. ovis*,[12, 18, 34, 57] but the former is distinguishable by its generally smaller size and extracellular location.

Malnutrition, plant (e.g. *Brassica* spp.) or mineral (e.g. copper) intoxications, or deficiencies (e.g. cobalt and copper) may also cause anaemia. Icterus may to a greater or lesser extent also occur in certain plant and mineral (e.g. copper) intoxications. In addition to icterus, inappetance and weakness, chronic copper poisoning is also characterized by haemoglobinuria,[47] which is not a sign associated with anaplasmosis.

It should also be borne in mind that the usually mild clinical signs of anaplasmosis may be exacerbated by concurrent infections or non-infectious conditions.

Control

Both specific and supportive therapy should be initiated as soon as possible, preferably before the onset of severe anaemia, in order to avoid mortality.[31]

Chlortetracycline and oxytetracycline are effective for the treatment of *A. ovis* infections.[35] Anaplasmosis can be successfully treated by the intramuscular administration of oxytetracycline at a rate of 8 to 10 mg/kg body weight daily, administered in equally divided doses at 12-hour intervals for four to five days.[31] The administration of divided doses at 12-hourly intervals is desirable in order to maintain higher blood levels throughout the period of treatment. However, two to three daily intravenous or intramuscular administrations of oxytetracycline at a rate of 10 mg/kg have also proved to be effective for the treatment of acute anaplasmosis in sheep.[59] A single intramuscular administration of a long-acting formulation of oxytetracycline, at a rate of 20 mg/kg body weight, is also effective in preventing mortality in sheep.[67]

Symptomatic treatment and rest are beneficial.[35] Supportive therapy may consist of the administration of haematinics, tonics and fluid.[31] Gastrointestinal disturbances such as inappetence, constipation and rumenal stasis may complicate recovery and, where indicated, attempts should be made to correct such disorders by the administration of appetite stimulants, rumenotorics and mild laxatives. In valuable animals suffering from severe anaemia, blood transfusion may be indicated.

Adequate protection against the elements and easy access to palatable feed and water should be provided. In addition, anaemic animals should not be subjected to unnecessary exertion and rams should be rested before being used for breeding.[35]

Judicious control of external and internal parasites should be practised.[35] Since intercurrent viral, bacterial, rickettsial and protozoal infections may precipitate disease,[35, 56] these should also be controlled wherever possible.

Neither oxytetracycline (12 daily intravenous administrations at a rate of 10 mg/kg body weight) nor a combination of oxytetracycline (four intramuscular administrations of a long-acting formulation at a rate of 20 mg/kg body weight at seven-day intervals) and imidocarb (two intramuscular administrations at a rate of 5 mg/kg body weight at an interval of 14 days) eliminates *A. ovis* carrier infections in splenectomized sheep, although these treatment regimens do result in a transient suppression of parasitaemia to a level where parasites are no longer microscopically detectable in blood smears.[59]

In practice, sheep and goats are not usually vaccinated against anaplasmosis. Animals could be immunized by artificially infecting them with *A. ovis*-infected blood[35] possibly followed by tetracycline treatment to control the infection. Care should be taken when selecting a suitable donor animal to avoid the accidental transmission of other blood-borne diseases. One or two administrations (at an interval of seven days) of a long-acting oxytetracycline formulation, administered during the prepatent period in experimentally induced *A. ovis* infection in sheep, has been shown to prolong the incubation period and to reduce the severity of the clinical reaction.[50]

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Infectious Diseases of Livestock

VOLUME ONE

This revised, updated and expanded second edition of the prize-winning textbook *Infectious Diseases of Livestock* is an essential reference text for veterinary researchers, veterinarians in farm practice, veterinary laboratory diagnosticians, and under- and postgraduate students studying infectious diseases – in short, for all those charged with the promotion of animal health.

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